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SOIL AND WATER MICROBIOTA AS BIOINDICATORS FOR THE ASSESSMENT ECOLOGICAL STATUS OF ECOSYSTEMS

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ABSTRACT

The article presents the results of monitoring the area adjacent to the Carpathian Biosphere Reserve for investigation of unauthorized landfills and examines the ecological status of these ecosystems. Four unauthorized landfills for solid waste in Pidgirna, Stanislav, Steryshory, and Feresok tracts with an area of 0.15 to 1.5 ha with a waste accumulation period of 12-22 years and different morphological composition were identified, which had a significant impact on the soil and water microbiota. Bioindication methods have shown changes in the microbial communities of soil and water under the direct influence of unauthorized landfills. Increased the number of organotrophic bacteria and micromycetes and decreased content of nitrogen-fixing microorganisms. The highest number of bacteria using mineral forms of nitrogen (25.36–28.61 million CFU/g. d. soil) and micromycetes (51.8-76.8 thousand CFU/g. d. soil) was in the soils of Pidgirna and Feresok with an advantage of 1.5-1.7 times and 2.5-3.8 times compared to the soil of the protected area. Results of the analysis of water microbiome showed that it is a sensitive indicator. The high number of anaerobic bacteria of the genus Clostridium was in the tract Feresok, so above the flow of the study area 3.69 thousand CFU /ml of water, in a place close to the landfill 6.22 thousand CFU/ml of water, below the flow of 9.35 thousand CFU/ml of water. Gram-positive bacteria of the genus Staphylococcus varied from the most polluted area in Feresok to the least polluted in Steryshory: 3.89 thousand CFU/ml of water, downstream 7.35 thousand CFU /ml of water. A close relationship was established between the duration of solid waste storage at a certain site and the level of soil phytotoxicity ($r = 0.92$). In the soil of landfills in Pidgirna, Steryshory, and Feresok tracts, the phytotoxicity index is significant (over 50%), which indicates a high level of soil ecosystem pollution and increased environmental risks in the area of unauthorized accumulation of solid waste.

Key words: soil, water, bioindication, ecosystem, microorganisms, landfills.

INTRODUCTION

Pollution of ecosystems can cause changes in structure and functions of soil and water microbime. The number of organisms can be reduced due to the toxicity of the contaminants, but also changes can be observed at community level in that tolerant or resistant organisms will benefit compared to those sensitive to the contaminants. It has also been observed that the exposure to some trace elements can induce resistance in soil microorganisms and also promote resistance to antimicrobials (Chen, 2015; Symochko et al, 2018). In addition, soil contaminants can enter the food chain and cause disease and mortality in soil-dwelling, terrestrial (including humans) and aquatic organisms. Polluted soils in turn become a source of pollution for groundwater, through leaching of contaminants. The Framework Convention for the Protection and Sustainable Development of the Carpathians defines a number of priorities in ensuring the preservation and restoration of unique natural complexes of the Carpathians, including conservation and sustainable use of biological and landscape diversity, prevention of negative impacts on mountain ecosystems, etc. (Rybak, 2019).

However, despite a number of environmental documents and international initiatives, the Carpathian region has an unfavorable environmental situation due to significant uncontrolled anthropogenic impacts, among which unauthorized landfills pose a great threat to the environment. Toxic compounds such as salts of iron, lead, zinc, mercury, pesticides and other bioorganic compounds are often found in the soil (Tetenyova, 2017). This type of pollution negatively affects all components of the ecosystem, in particular the functioning of the soil microbiome, because during the decomposition of waste toxic compounds are released, landfills become the habitat and reproduction of many pathogenic microorganisms.

Soil pollution is also closely related to groundwater and surface water pollution, including and those used for drinking purposes. Biota (plants, fauna, microorganisms) and soil under natural conditions have undergone a long path of coevolution. Today, their close relationship is maintained at different hierarchical levels of the structural and functional organization of this system. Soils that are at the climax level of evolution have a stable multicomponent grouping of biota, the diversity of species, life forms and physiological functions of which reflect their properties. However, the evolutionarily formed unity of soil and biodiversity is quite vulnerable and can function in a balanced way only if the integrity of all its components and natural landscapes in general is preserved.

The modern approach to assessing the quality of environmental objects is based on the principle of balanced functioning of ecosystems and takes into account the relationship of components of the biocenosis and their interaction with the soil environment (Demyanyuk, 2017). Diagnosis and assessment of the ecological condition of the soil is an integral part of a comprehensive study of the state of the environment. Soil as a habitat for living organisms contains very complex biocenoses, which causes complex processes at the biophysical, biochemical levels and at the level of interpopulation interaction. Therefore, it is not always possible to predict the response of the biotic component to contamination. Promising integrated methods for studying the state of the environment are methods of biotesting and bioindication, based on the feedback of living organisms to the negative effects of pollutants, they are able to provide reliable information about the quality of environmental components, including soils (Symochko, 2017).

Biotesting methods make it possible to obtain an integrated assessment of soil toxicity in a relatively short time, which should be used in monitoring studies. According to many leading scientists, ecologists, microbiologists, soil scientists (Blakely, 2002; Borowik, 2017; Delgado-Baquerizo et al, 2018; Jiao et al, 2019; Symochko, 2020) microbiological indicators of soil, as the most informative and sensitive, are a necessary components in comprehensive environmental assessment of soils. Therefore, the assessment of environmental risk to natural ecosystems and human health from the effects of soil pollution due to unauthorized landfills is relevant for determining the level of environmental hazards and the development of appropriate environmental measures. The aim is to study the ecological condition of soils and water in the area of unauthorized landfills around the Carpathian Biosphere Reserve by bioindication method.

MATERIALS AND METHODS

The study was performed at Uzhhorod National University and the Institute of Agroecology and Nature Management of NAAS during 2018-2020. The study materials were soil and water samples taken from various transformed ecosystems (unauthorized landfills) around the Carpathian Biosphere. Soil samples were taken in September-October by the method of squares (10 × 10 m) at a depth of 0–25 cm in accordance with DSTU ISO 10381–6: 2015 and determined its ecological status by bioindication and biotesting:

• The number of microorganisms of the main ecological groups - according to DSTU 7847: 2015 and standart methods in soil and water microbiology (Zvyagintsev, 1991; Tepper, Shilnikova, Pereverzeva, 2004)

• Direction of microbiological processes in the soil - according to the relevant coefficients (mineralization-immobilization (Cm), oligotrophic (Col.), pedotrophic (Kped.) By the calculation method (Zvyagintsev, 1991);

• Phytotoxicity of soil - using soil plates and test culture according to DSTU ISO 11269–2: 2002 and generally accepted methods.

The results of the experimental studies were statistically analyzed using the Microsoft Excel program package. Results were expressed as means (±) standard deviation (SD) and (SSD_{05}) smallest significant differences of experiments conducted in quadruplicating. The level of significance selected for the study was $P < 0.05$ (Bailey, 1995).

RESULTS AND DISCUSSION

Carpathian Biosphere Reserve with an area of 8035.8 hectares is located in Transcarpathia region. Among a number of factors that have a negative impact on the ecological condition of the reserve and surrounding areas are solid waste, the reason for the accumulation of which is the local population and the tourism industry. It is worth noting that in the entire mountainous area of the upper reaches of the Tisza River basin there are no landfills for solid waste storage and disposal system. Only a small part of the waste is taken out for processing and disposal, and the rest is dumped by locals and tourists in inappropriate places, including on the river bank. In general, the Tisza and its tributaries serve as permanent transboundary waste pipelines to Romania and Hungary. The survey of the territory adjacent to the Carpathian Biosphere Reserve showed the presence of a significant number of 9 landfills, which were formed as a result of unauthorized dumping of solid waste, among which four garbage centers with the largest area (0.15-1.50 ha) and significant danger to natural ecosystems in tracts Pidgirna, Stanislav, Steryshory and Feresok. A visual inspection showed that the oldest and largest landfill in the Feresok tract has accumulated significant amounts of a wide variety of household waste, including a significant proportion of construction materials, household appliances, used batteries, plastic containers for household chemicals and other waste, including those that are not recyclable.

The Steryshory tract is dominated by food waste, as well as their packaging, wraps for sweets, disposable plastic containers, plastic and glass bottles and more. Plastic that is difficult to identify. As tourist facilities are being actively built in the vicinity of the tract, the remains of construction materials, plastic, cardboard are brought here. In the Stanislav tract, the landfill consists of several fragments and is located near the river, which increases the ecological danger. It has accumulated a significant amount of waste, which is subject to sorting and recycling. There are many glassware and plastic and polyethylene, food and beverage packaging, cardboard products and more. Pidhirna tract is the closest to the settlement. There is a constant and systematic storage of household waste, including dangerous. The typical composition of solid waste in this landfill is quite diverse. There is a large amount of glass, plastic, paper, cardboard, rubber, medicines, wet wipes, containers of household chemicals and more. Most waste is sorted and recycled. Vegetation changes were recorded around all landfills surveyed, and weeds and invasive species completely displaced the aboriginal flora. Also, in the warm period of the year due to the decomposition of organic waste, the release of an unpleasant odor was observed. Clusters of insects, rodents and reptiles, which are potential carriers of various infectious diseases, were observed in landfills, which is especially dangerous for humans and livestock.

The study of the microbiological condition of landfill soils showed a change in the structure of the soil microbiome and the redistribution of the main ecological-trophic and taxonomic groups of microorganisms. In particular there increased number of organotrophic bacteria and micromycetes. The highest number of bacteria using nitrogen of organic compounds (25.36-28.61 million CFU/g. of soil) and micromycetes (51.8–76.8 thousand CFU/g. of soil) was recorded in soils in Pidhirna and Feresok tracts with an advantage of 1.5-1.7 times and 2.5–3.8 times compared to the soil of the protected area (Table 1). The high number of micromycetes can be explained by the peculiarities of their physiology, which are characterized by more economical metabolism in combination with higher biochemical activity.

Table 1. The number of microorganisms of main ecological groups in the soil

Place of sampling	Bacteria using		Pedotrophic	Streptomycetes	Oligotrophic	Nitrogen -fixing, %	Micro-mycetest husand CFU /g. of soil
	mineral nitrogen	organic nitrogen					
	mln. colony-forming units per gram soil (CFU/g. of soil)						
Protected area	17,91	16,48	7,12	,67	4,88	83,24	20,4
Steryshory	25,24	20,61	11,34	6,34	9,96	68,22	24,5
Pidgirna	35,12	25,36	22,64	4,22	13,88	47,90	51,8
Feresok	39,29	28,61	27,34	2,34	20,43	33,60	76,8
Stanislav	29,88	23,31	15,56	5,22	11,12	59,20	31,8
SSD 05	0,13	0,21	0,12	0,14	0,11	1,15	0,24

Therefore, it is natural that micromycetes proved to be more competitive with high adaptation to environmental conditions. At the same time, representatives of these groups of microorganisms have a high degree of toxicity, which determines the toxicity of the soil. There was also a rapid development of bacteria that use nitrogen mineral compounds in 1.4–2.2 times and a decrease in the number of streptomycetes by 8–59% and nitrogen-fixing microorganisms by 18–60% compared to the soil of the natural ecosystem. These changes were especially significant in the tracts of Feresok and Pidhirna, where the duration of solid waste accumulation was 22 and 16 years, respectively. We found that the number of pedotrophic and oligotrophic microorganisms and microorganisms that assimilate organic forms of nitrogen in the soil of landfills increased by an average of 2.70, 2.84 and 1.48 times, which, respectively, influenced the direction of the main microbiological processes.

In the presence of organic waste the mineralization-immobilization increased by 12.4–27.1%, due to the rather active reproduction of microorganisms that assimilate mineral forms of nutrients and perform the immobilizing function of microbial community (Table 2).

Table 2. The direction of microbiological processes in the soil in the area of unauthorized landfills

Place of soil sampling	Coefficient		
	mineralization - immobilization	oligotrophicity	pedotrophicity
Protected area	1,09	0,14	0,43
Ur. Sterishori	1,22	0,22	0,55
Ur. Pidgirna	1,38	0,23	0,89
Ur. Feresok	1,37	0,30	0,96
Ur. Stanislav	1,28	0,21	0,67

The oligotrophic index varied in the range of 0.21–0.30, the coefficient of mineralization-immobilization - 1.22–1.38 with a maximum in the soil of the landfill in the tracts of Feresok and Pidgirna, which indicates the strengthening of microbiological processes of mineralization of soil organic matter, as well as narrow ratio of carbon to nitrogen in the soil. In the soil in the Steryshory tract, these indicators were lower by an average of 5–36%.

High values of the oligotrophic coefficient in the soil of landfills indicate on reducing the content of nutrients in the soil. Similarly, was increased the pedotrophic coefficient (C ped. = 0.55–0.96). In the soil of landfills was recorded by 28–23% compared to the soil of the reserve, which indicates about activation of decomposition of soil organic matter, including humic compounds.

An important indicator of the ecological condition of the soil is its phytotoxicity - an informative indicator that is recommended for use in assessing the anthropogenic impact on the soil environment (Symochko, Dombay, 2007). The obtained results show that the level of soil phytotoxicity in the area of accumulation is quite significant and directly depends from morphological composition and duration of storage (Table 3).

Table 3. Phytotoxicity of soil in the area of unauthorized landfills

Place of soil sampling	Number of germinated seeds		Length		Phyto-toxicity,%
	pks	%	roots	sprouts	
			mm		
Control	26 ± 0,8	87	12 ± 0,4	9 ± 0,8	—
Protected area	25 ± 0,7	83	11 ± 0,4	8 ± 0,5	7,3
Sterishory	23 ± 0,6	77	11 ± 0,8	8 ± 0,8	11,5
Pidgirna	12 ± 0,4	40	9 ± 0,9	6 ± 0,7	53,6
Feresok	5 ± 0,3	2	7 ± 0,4	4 ± 0,3	80,8
Stanislav	13 ± 0,9	43	9 ± 0,4	7 ± 0,5	50,0

A close relationship was found between the level of soil phytotoxicity and the duration of waste storage ($r = 0.92$) and the morphological composition of solid waste. The lowest level of phytotoxicity of the soil in comparison with the control and the soil of the reserve was found on the territory of the smallest landfill and with the shortest term of solid waste storage in Steryshory tract. It is dominated by recyclable household waste, which indicates low environmental stress in the landfill area and is probably due to the distance from settlements and periodic waste disposal. Whereas in the soil of landfills in other tracts the phytotoxicity index is significant (50–81%) and indicates a high level of soil ecosystem pollution and increased environmental risks in the area of unauthorized accumulation of solid waste. The level of soil phytotoxicity in the Stanislav tract is defined as above average. This landfill was established a little later, but there is a systematic storage of solid waste and a significant amount of hazardous waste, including those that are not recyclable. The number of germinated seeds of the test culture was 50% less than in the control, and the average length of the seedlings was 2 mm less, the roots - 3 mm compared to the control. In the Pidhirna tract, which is closest to the settlement, there is a constant and systematic storage of household waste, including and dangerous, the level of phytotoxicity of the soil is above average. The number of germinated seeds of the test culture is almost 46% less than the control. The length of the shoots of seedlings is on average 3 mm, and the roots are 2 mm less than the control. The highest level of soil phytotoxicity was recorded in the Feresok tract, where the landfill is the largest in area and has been operating for almost 23 years and the processes of decomposition of organic matter, decay and half-life of mixed waste under the influence of natural factors are active. The number of germinated seeds of the test culture was almost 8% less than in the control. The length of roots and seedlings of test plants was almost twice less than the control and was 7 and 4 mm, respectively. This can be caused by direct contamination of the soil with filtrate and accumulation of phytotoxic substances - products of metabolism of soil microorganisms.

The rivers and streams of the Carpathian Biosphere Reserve are mostly clean and unpolluted. Their waters have a favorable sanitary-biological regime, hydrochemical and microbiological indicators, oxygen regime (over 100% oxygen saturation), a low degree of saprobity (putrefaction). Often water meets the drinking qualities, so it is used for drinking, although sanitary epidemiologists do not recommend it. However, constant pollution has a negative impact on the aquatic ecosystems in general. Results of microbiological investigation of water microbiota showed that water microorganisms are very sensitive to the pollution (Table 4). The genus Clostridium includes psychrophilic, mesophilic, and thermophilic species. The major role of these organisms in nature is in the degradation of organic material to acids, alcohols, CO_2, H_2, and minerals. Frequently, a butyric acid smell is associated with the proliferation of clostridia. The ability to form spores that resist dryness, heat, and aerobic conditions makes the clostridia ubiquitous (Rainey, Hollen, Small, 2009). Most species are obligate anaerobic, although tolerance to oxygen occurs. Oxygen sensitivity restricts the habitat of the clostridia to anaerobic areas or areas with low oxygen tensions. Growing and dividing clostridia will, therefore, not be found in air saturated surface layers of lakes and rivers or on the surface of organic material and soil. Clostridial spores, however, are present with high probability in these environments, and will germinate when oxygen is exhausted and when appropriate nutrients are present. In particular, the number of gram - positive bacteria of the genus Clostridium is most recorded in the tract Feresok, so above the flow of the study area 3.69 thousand CFU / ml of water, in a place close to the landfill 6.22 thousand CFU / ml of water, below the flow of 9.35 thousand CFU / ml of water, slightly less in Pidhirna tract 1.31 thousand CFU / ml of water - downstream, close to the landfill 2.02 thousand CFU / ml of water, upstream 4.03 thousand CFU / ml of water. Stanislav tract, respectively, 1.59 thousand CFU / ml of water - downstream, close to the landfill -2.67 thousand CFU / ml of water, 3.89 thousand CFU / ml of water downstream.

The best ecological status of water by this indicator was in the Steryshory tract. The numer of anaerobic bacteria was lower in compare with another point of investigations: 1.33 thousand CFU/ml of water - upstream, close to the landfill -1.82 thousand CFU / ml of water, 2.02 thousand CFU/ml of water - downstream.

Table 5. The results of the analysis of the water microbiota (CFU/ml.)

Place of water sampling	Samples	Meat peptone agar (MPA) $*10^4$	ENDO $*10^3$	Anaerobic bacteria $*10^3$	Staff. (agar) $*10^3$	Bilya selective medium $*10^4$	Pure agar $*10^4$
Protected area	1	2,01	1,87	0,8	0,11	0,61	1,98
Ur. Sterishori	1*	2,17	2,36	1, 33	0,63	2,87	1,78
	2*	3,10	2,85	1, 82	0,33	1,36	3,67
	3*	4,31	5,27	2,02	0,12	1,11	2,13
	SSD 05	0,68	1,20	0,95	0,48	0,55	1,06
Ur. Pidgirna	1*	2,49	3,39	1, 31	1,03	1,17	1,89
	2*	3,12	4,83	2, 02	1,69	1,88	4,67
	3*	5,21	7,22	4,03	2,34	3,24	6,19
	SSD 05	0,66	1,22	0,94	0,44	0,68	1,12
Ur. Feresok	1*	3,34	3,84	3,69	2,06	1,40	5,34
	2*	5,12	6,45	6,22	3,89	2,56	7,78
	3*	8,90	9,39	9,35	7,35	5,68	10,88
	SSD 05	0,61	1,03	0,92	0,59	0,75	1,34
Ur. Stanislav	1*	2,56	2,39	1, 59	1,45	0,97	2,05
	2*	3,88	3,73	2, 67	2,03	1,97	3,96
	3*	6,24	6,72	3,89	3,02	2,89	6,77
	SSD 05	0,38	0,67	0,90	0,65	0,59	1,08

*1 *- upstream; 2 * - the place of water sampling closest to landfills; 3 *-lower downstream;*

The potential for bacteria present in human and animal wastes to contaminate water in nearby wells needs special attention (Medema, 2003). An important source of contamination of surface and ground waters is runoff water from agricultural and pasture lands, landfills and urban areas. Gram-positive bacteria of the genus Staphylococcus vary from the most polluted area (Feresok) to the least polluted (Steryshory) within the following limits: 3.89 thousand CFU/ml of water, downstream 7.35 thousand CFU/ml of water. Sterishory upstream of the study area -0.63 thousand CFU / ml of water, in a place close to the landfill 0.33 thousand CFU/ml of water, downstream 0.12 thousand CFU / ml of water. Studies conducted using ENDO medium to isolate enterobacteria indicate an increase in the number of enterobacteria. Monitoring of microbiological indicators allows to estimate negative impact of contaminated areas on microbiological communities of spring water, especially in places close to landfills and downstream. This can be caused by leachate, which in large quantities falls from landfills into natural reservoirs during precipitation.

CONCLUSION

Unauthorized landfills are a powerful factor influencing the ecological state of ecosystems. Around the Carpathian Biosphere Reserve, four unauthorized landfills were found in Pidgirna, Stanislav, Steryshora and Feresok tracts with an area of 0.15 to 1.5 ha with a waste accumulation period of 12–22 years and different morphological composition, which had a significant ecological impact on ecosystems. A significant impact on soil and water quality near such areas was determined. Changes in the soil microbiome under the direct influence of unauthorized landfills, namely an increasing number of organotrophic bacteria and micromycetes and a decreasing content of useful nitrogen-fixing microorganisms were fixed. At the same time, the intensity of mineralization-immobilization, oligotrophic and decomposition of soil organic matter increases. A close relationship was established between the duration of solid waste storage in a certain area and the level of soil phytotoxicity (r = 0.92). In the soil of landfills in Pidgirna, Steryshora and Feresok tracts, the phytotoxicity index is significant (over 50%), which indicates a high level of soil ecosystem pollution and increasing environmental risks in the area of unauthorized accumulation of solid waste.

Destabilization of natural landscapes and ecological systems is largely associated with the pollution of water. So it is important to protect all groups of microorganisms that carry out the cycle of substances in nature, including water and soil microbial communities. After all, harmful effects disrupt the ecological balance, and pathogenic microorganisms are able to eventually displace the beneficial microbiota, which is the beginning of the degradation of natural ecosystems.

REFERENCES

1. Bailey N.T.J. (1995). Statistical methods in biology. Third Edition, Cambridg University Press. New York. https://doi.org/10.1017/S0025315400018403;
2. Blakely, J. K., Neher, D. A., and Spongberg, A. L. (2002). Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. Appl. Soil Ecol. 21, 71–88. doi:10.1016/s0929-1393(02)00023-9;
3. Borowik, A., Wyszkowska, J., and Oszust, K. (2017). Functional diversity of fungal communities in soil contaminated with diesel oil. Front. Microbiol. 8, 1862. doi:10.3389/fmicb.2017.01862
4. Chen, C., Wang, Y., Qian, Y., Zhao, X. & Wang, Q. (2015). The synergistic toxicity of the multiple chemical mixtures: Implications for risk assessment in the terrestrial environment. Environment International. Vol. 77. P. 95–105;
5. Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., et al. (2018). A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325. doi:10.1126/science.aap9516;
6. Demyanyuk O.S., Symochko L.Yu., Tertychna O.V. (2017). Modern methodological approaches to assessing the ecological condition of the soil by the activity of the microbiocenosis. Issues of bioindication and ecology. Vol. 22. – № 1. P. 55–68;
7. DSTU 7847: 2015. Soil quality. Determination of the number of microorganisms in the soil by sowing on a solid (agar) nutrient medium. Valid from 2016-07-01. Kyiv: UkrNDNC, 2016. 12 p;
8. DSTU ISO 10381–6: 2015. Soil quality. Sampling. Part 6: Guidelines for the selection, treatment and storage of soil under anaerobic conditions for laboratory assessment of microbiological processes, biomass and diversity. Valid from 2016-04-01. Kyiv: 2017. 12 p;
9. DSTU ISO 11269–2: 2002 Soil quality. Determination of the effect of pollutants on soil flora. Part 2. Influence of chemicals substances for germination and growth of higher plants. Valid from 2003-10-01. Kyiv: State Standard of Ukraine. 14 p;
10. Jiao, S., Wang, J., WeiChen, G. W., Chen, W., and Lu, Y. (2019). Dominant role of abundant rather than rare bacterial taxa in maintaining agro-soil microbiomes under environmental disturbances. Chemosphere 235, 248–259. doi:10.1016/j.chemosphere.2019.06.174;
11. Lyudmyla Symochko, Tamara Meleshko, Vitaliy Symochko, Nadiya Boyko (2018). Microbiological control of soil-borne antibiotic resistance human pathogens in agroecosystems. International Journal of Ecosystems and Ecology Sciences (IJEES) Vol. 8 (3): P. 591-598. https://doi.org/10.31407/ijees8320;
12. Medema GJ, Shaw S, Waite M, Snozzi M, Morreau A, Grabow W. (2003). Assessing Microbial Safety of Drinking Water Improving Approaches and Method. WHO & OECD, IWA Publishing; London, UK: Catchment characteristics and source water quality; pp. 111–158;
13. Rainey FA, Hollen BJ, Small A. (2009). Genus Clostridium. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB, editors. Bergey's Manual of Systematic Bacteriology. 2nd ed. Vol. 3. Springer; New York, NY, USA: pp. 738–828;
14. Rybak M.P., Lukyanova V.V., Pokinchereda V.F., Jonash I.D. (2019). Ecological and recreational activities of the Carpathian Biosphere Reserve as a component of sustainable development. Environmental sciences. 26 (3): P. 88–92;
15. Symochko L. (2020). Soil microbiome: diversity, activity, functional and structural successions. International Journal of Ecosystems and Ecology Sciences. 10 (2). P. 277–284, https://doi.org/10.31407/ijees10.206;
16. Symochko L.Yu., Demyanyuk O.S., Symochko V.V. (2017). Soil bioindication and biotesting as modern methodological approaches. Scientific Bulletin of Uzhhorod National University. Biology. Vol.42. P. 77–81;

17. Symochko L.Yu., Dombay I.V. (2007). Phytotoxic activity of soil in different ecosystems in the lower part of Transcarpathia. Scientific Bulletin of Volyn State University. № 5. P. 254–259;
18. Tepper, E.Z., Shilnikova, V.K. and Pereverzeva, G.I. (2004) Microbiology practicum, Moscow 456p;
19. Tetenyova I.O. (2017). Impact of landfills on the environment and living conditions of the population. Environment and health. № 2. P. 26–30;
20. Zvyagintsev D.G. Methods of soil microbiology and biochemistry. (1991). Moscow: Moscow State University, 304 p;