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SANITARY AND MICROBIOLOGICAL STATUS OF SURFACE WATERS OF PROTECTED AREAS AND TRADITIONAL ECONOMIC LANDSCAPES OF THE CARPATHIANS IN POKUTTIA-BUKOVINA REGION

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Abstract. The microbiological parameters of surface water as indicators of the ecological state of the Carpathians in Pokuttia-Bukovina region were studied. The water streams of the National Nature Park "Vyzhnytskyi", where a specific ecosystem has been formed for more than two decades, were used as a standard for comparison. The possibility of using "biofilters" based on a synthetic carrier "Viia" for purification of natural water streams and raising the level of ecological safety of mountain ecosystems has been proved.

Key words: mountain ecosystem, surface waters, microbiological indicators, protected areas.

1. Introduction

As a result of anthropogenic influence in the mountainous part of the Ukrainian Carpathians in recent years, the threat of violation of the ecological safety of the region has become acute. The territory of the Carpathians in Pokuttia-Bykovina region is characterized by ecological problems that are typical for the entire Carpathian region. However, the region is characterized by some specifics due to the trans-boundary situation, the peculiarity of climatic conditions, the conduct of traditional farming, etc. The state of the river network in the region is of particular concern. As you know, the main waterways of the Carpathians in Pokuttia-Bykovina region are the Siret and Cheremosh rivers which are part of the Danube basin, the largest waterway in Europe. The choice of reliable and operational criteria for assessing the sanitary and ecological importance of this region is very important.

Bacteria turned out to be ideal indicators of surface water contamination due to their rapid reaction to environmental changes [1]. Scientists have no single opinion regarding the choice of bacterial indicators for assessing the state of the environment. However, the methodical approach accepted in international practice is most inclined to the thought that such indicators may be fecal bacteria, enterococci and thermophilic bacteria Escherichia coli [2].

Studies [3, 4, 5] show significant organic and bacterial contamination in the middle part of the channel and the Danube Delta. The Danube Delta, where the Biosphere Reserve is located, has become one of the most vulnerable ecosystems. In terms of microbiology, left tributaries – the Prut and the Siret originating in the Eastern (Ukrainian) Carpathians were included in the list of the largest polluters of the Danube. [6]

Several authors indicate the feasibility of the study of sanitary and microbiological parameters as indicators of ecological status and background monitoring of nature reserves [5, 7, 8]. It was established that protected status of the areas affects the bacterial diversity. The importance of microbiological indicators for monitoring protected areas was shown [9]. It should be noted that the use of sanitary-microbiological indicators to assess the state of protected objects nowadays is sporadic and are not applied to specific functional areas of these territories. Previously, we [10] investigated the sanitary-

microbiological state of the water network of protected areas of the Carpathians in Pokuttia-Bykovina region.

The purpose of this study was to conduct a comparative analysis of the sanitary-microbiological state of the water network of protected areas and territories of intensive anthropogenic loading of the Pokuttia-Bykovina Carpathians as a prerequisite for the ecological safety of the mountain region and preservation of the aquatic ecosystem of the Danube River.

2. Methodology and research methods

For research we have chosen the Pokuttia-Bykovina Carpathians - the region of the Eastern Carpathians which is specific in terms of landscape, climatic and socio-economic conditions, insufficiently studied in the aspect of environmental safety, (Fig. 1). This is an outer strip of the Ukrainian (Eastern) Carpathian Mountains within Ivano-Frankivsk and Chernivtsi regions, extending from the north-west to the south-east to the border with Romania for almost 75 km. The research area included traditional economic landscapes and the National Nature Park "Vyzhnytskyi" (sampling points Nos. 1-8) in the Siret and Cheremosh basin (Fig. 1). Investigations outside the NNP were conducted within the 10-kilometer zone of traditional landscapes (sampling points Nos. 9-15).

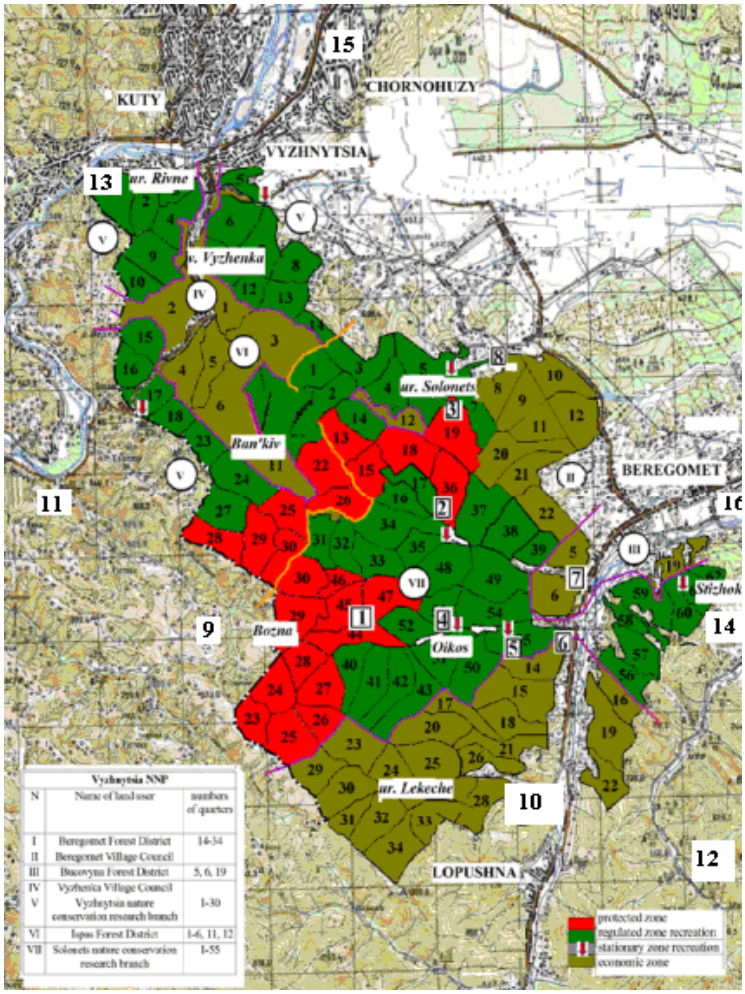

Fig. 1. Map-scheme of the research area. Sampling points:
National Nature Park "Vizhnytsky" (NPP):
1 – the upper part of the Stebnik riverbed; 2 – the upper part of the Sukhyi strumok;
3 – the upper part of the Slavets riverbed; 4 – "OIKOS" recreational zone;
5 – "Myslyvskyi budynok recreational zone; 6 – the lower part of the Stebnik riverbed;
7 – the lower part of the Sukhyi strumok, 8 – the middle part of the Solonets riverbed
The Cheremosh river (outside NPP):
9 – Top Tovarnytsia; 11 – Lower Tovarnytsia; 13 – turbazha "Cheremshina",
15 – Chornogusy;
The Siret river (outside NPP):
10 – Lopushna; 12 – the upper part of of the Migova river;
14 – the lower part of the Migova river; 16 – pension "Yunist"

The National Nature Park "Vyzhnytskyi" (NNP) is selected as a standard for comparing the impact of anthropogenic activities on the state of mountain ecosystems. NNP was established in 1995 and for more than two decades a specific ecosystem has been formed on its territory due to observance of the nature protection regime in various functional zones of the object of the nature reserve fund. The Pokuttia-Bykovina Carpathians is a cross-border area.

The linking of the research region to the map of the Eastern Carpathians is shown in Fig. 2.

In order to assess the quality of the surface water of the water streams of the NNP, we used a fibrous carrier of the "Viia" type (TU (995990) made of textured plait yarn (TU6-06-C116-87, tex 350). Previously, a number of authors [11, 12, 13] established that the fibrous carrier "Viia" can be successfully used to construct "bioreactors" for surface water purification. Using the approach described by the authors, we have mounted a "bioreactor" based on special wooden structures – "casings" that have long been used by the locals to enrich the streams with oxygen. This design is presented in Fig. 3.

The research was conducted during the summer season of 2014-2017. The sampling of water (23–25 ºC) was carried out immediately before and after the "biofilters" from different functional zones of the water streams of the NNP and adjacent territories of the traditional economic landscapes indicated in the scheme (Fig. 1), in accordance with the existing standard methods. The explored water streams are a very convenient model for studying the quality of surface waters, since they include territories of different environmental status and anthropogenic loading. The content of nitrates and chlorides was determined according to DSTU 4080-2001 [14]. Biological oxygen consumption, dissolved oxygen, coli-index and total microbial number were determined by generally accepted methods in accordance with the methodological guidelines [15]. The statistical processing of the results was carried out using the computer program Excel.

Fig. 2. Geographical location of the research area on the map of Eastern Carpathians

Fig. 3. A bioreactor-type structure based on a fibrous carrier "Viia" and wooden structures –"casings"

3. Results and discussion

The results of the studies on the microbiological state of the water streams of various functional zones of NNP (Table 1) showed that the features of the environmental protection regime have a significant impact on the quantitative characteristics of the microbiocenosis of these territories. Thus, according to microbiological indicators, surface waters of the protected area and the zones of stationary recreation do not exceed the San-PiN standards 4630-88 [16] and the normative indicators adopted in the EEC countries (Surface Water Directive: 75/440 EEC) [17]. For the water streams of these functional zones the total microbial number is <5000 KUO / dm3. Closer to the economic zone there is a growth of microbiological indicators above the specified admissible norms for all the investigated water streams. This was especially true for the increase in the number of lactose-positive intestinal sticks (E. coli) per 1 liter of water (coli-index). It should be noted that the E. coli is a sanitary indicator, and it indicates fecal contamination, in this case, that of the environmental water objects. By comparing the indicators of the coli-index in the samples of river water of the protected zone and the selected samples of the water in the economic zone, it was established that coli-index in average increased 2 times and the total microbial number – from 2 to 4 times.

It should be noted that the sanitary-microbiological state of the NNP has been formed over more than 20 years of observance of the nature protection regime in various functional zones of the object of the nature reserve fund.

In most cases, there is a direct correlation ($r = 0.95$) between the magnitude of microbiological parameters and the sanitary and hygienic parameters (BSC, HSC), which we have investigated before [18, 19].

The study of sanitary-microbiological indicators of surface waters of the economic landscapes adjacent to the NNP territories showed (Table 2) that the majority of the investigated areas are characterized by a significant level of bacterial contamination of surface waters which

significantly exceeds the permissible standards of SanPin 4630-88. Only a small number of territories, basically the upper part of the streams of the Mihova River and the Tovarnytsia, contain the water that meets the requirements of the standards.

Comparison of the microbiological indicators of the water streams in the areas where traditional economic activity is conducted and which differ only in the nature protection status are given in Table 3.

Table 1

The value of the basic sanitary-microbiological indicators of surface waters of various functional zones of NNP "Vyzhnytskyi"

Sample collection points	Coli-index (dm^3)	Coli-titer (dm^3)	Total microbial number (CFO/dm^3)
protected area			
1	85 ± 10,5	8,7 ± 0,40	1500 ± 85,4
2	80 ± 8,0	9,0 ± 0,42	1700 ± 90,2
3	82 ± 12,2	8,9 ± 0,45	1600 ± 65,5
X mid	82,3 ± 10,2	8,9 ± 0,42	1600 ± 80,4
zone of stationary recreation			
4	90 ± 11,5	8,5 ± 0,35	2300 ± 92,5
5	100 ± 9,4	8,0 ± 0,38	3500 ± 150,4
á mid	95,0±10,5	8,25± 0,37	2900 ± 121,5
economic zone			
6	120 ± 5,3	7,1 ± 0,33	5800 ± 250,5
7	105 ± 5,2	7,5 ± 0,36	5200 ± 280,3
8	110 ± 5,6	7,3 ± 0,30	5500 ± 255,2
á mid	111,7± 5,4	7,3 ± 0,33	5500 ± 262,0

- CFO – colony-forming organisms;
- the difference is significant at $p < 0,05$;
- SanPiN 4630-88, total microbial number <5000 CFO/dm^3

Table 2

The value of the basic sanitary-microbiological indicators of surface water of economic landscapes adjacent to the territory of the NNP "Vyzhnytskyi"

Sample collection points	number point	Coli-index (dm^3)	Coli-titer (dm^3)	Total microbial number (CFO/dm^3)
Top Tovarnytsia	9	96 ± 4,40	7,8 ± 0,30	4100 ± 205,0
Lopushna	10	108 ± 5,50	7,2 ± 0,35	5750 ± 250,0
Lower Tovarnytsia	11	112 ± 4,50	7,0 ± 0,42	5840 ± 260,5
the upper part of of the Migova rive	12	98 ± 5,00	7,6 ± 0,32	4200 ±215,6
turbazha "Cheremshyna"	13	136 ± 6,00	5,2 ± 0,20	6800 ± 250,2
the lower part of the Migova rive	14	145 ± 6,00	4,7 ± 0,28	7250 ± 350,0
Chornogusy	15	142 ± 6,30	4,9 ± 0,25	7200 ± 350,5
pension "Yunist"	16	139 ± 7,00	5,0 ± 0,22	6900 ± 325,0
á mid		122 ± 5,58	6,2 ± 0,29	6005 ±275,9

- CFO – colony-forming organisms;
- the difference is significant at $p < 0,05$;
- SanPiN 4630-88, total microbial number <5000 CFO/dm^3

Table 3

Comparative analysis of the mean values of sanitary-microbiological indicators of surface water of the economic zone of NPP "Vizhnitsky" and economic landscapes adjacent to the NPP

№	Sanitary-microbiological Indicators	Territory of NPP (economic zone), á mid	Adjacent territories, á mid*	Divergence (±), %	Td
1.	Coli-index (dm^3)	111,7 ± 5,4	122 ± 5,58	+ 13,5	< 3,18
2.	Coli-titer (dm^3)	7,5 ± 0,6	6,2 ± 0,29	- 21,0	< 3,18
3.	Total microbial number (CFO/dm^3)	5350 ± 270	6005 ± 276	+ 12,5	< 3,18

- * average data on 8 sampling points in the territories adjacent to the NPP
- the difference is significant at $p < 0,05$;
- Td – Student's criterion.

The comparison of the averaged microbiological indicators of surface waters of the territories characterized by active human activity and differing only by the regime of protection has shown the proximity of surface water streams of the territories of two types by coli-index, coli-titer and total microbial number. However, it should be noted that the comparison of the average indicators is not sufficiently correct, since the sanitary and microbiological indicators of a number of watercourses of traditional economic landscapes (sampling points Nos. 9 and 12) do not fall under the law of normal distribution and go beyond the 3 sigma. That is why we have also compared the maximum values of microbiological indicators of economic territories of different status (Table 4).

The analysis showed that by the maximum microbiological parameters, surface water streams of traditional economic landscapes are significantly higher (Td > 3.18 with a credibility level of $p < 0.05$) more than 30 % of the water streams of the economic zone of the NNP. Thus, observance of the environmental protection requirements in protected areas for two decades had a positive ecological effect on the mountain ecosystem.

Our previous studies have shown [10] that, in our research scheme, the fibrous carrier "Viia" used in our research scheme is capable of accumulation of bacterial organisms and invertebrate hydrobionts on a large scale on its surface. It was discovered [21] that the greatest difficulty in detecting pathogenic microorganisms is the negligible concentration of the latter at the early stages of incubation. The use of a fibrous carrier "Viia" makes it possible to solve the problem of detection of the microorganisms that are found in reservoirs in a small amount and carry out an early diagnosis of the reservoir. Table 5 presents the data on the process of biological treatment of surface water in artificially created "biofilters".

In the water which has passed through "biofilters" the amount of suspended substances, chlorides and compounds of nitrogen decreases. The water quality in the studied water streams is improved, as evidenced by the increase in the content of dissolved oxygen in the range of 26–33 %, as well as the decrease in the parameters of BOC and COC. Accordingly, there is a decrease in the total microbial number and the index of the studied water samples. It deserves attention to the sharp reduction of nitrites in the reservoirs of traditional economic landscapes after passing through the "biofilter". Probably, this is due to the involvement of the microorganisms adsorbed on the carriers in the processes of transformation of nitrogen compounds.

Nowadays, the technology of multiculturalism of anaerobic and aerobic beneficial microorganisms which is characterized as ecologically clean and safe, becomes particularly popular. Microorganism technology (EM) uses the ability of natural microorganisms to purify and restore nature. Studies [21, 22] show the possibility of using activated microorganisms solutions to restore the quality of degraded (contaminated) waters.

In our situation, a specific "biofilter" is formed on a fibrous carrier in the form of an artificially created micro-ecosystem. In this micro-ecosystem, the fibrous carrier serves as a kind of "home" for microorganisms, plants and invertebrate animal organisms that inhabit this reservoir. On an artificial carrier, these organisms are able to accumulate and form food chains. Under these circumstances, part of the bacteria becomes an element of the nutritional chain and serves as food for invertebrate hydrobionts. Thus, purification of reservoirs is observed in two stages: due to the adsorption of bacterial organisms on the synthetic carrier at the first stage and by the trophic chains at the second one. Due to the balance of such food chains, there is no need to regenerate "biofilters".

Table 4

Comparative analysis of maximum values of sanitary-microbiological indicators of surface waters of the economic zone of NNP "Vyzhnytskyi" and economic landscapes adjacent to the NNP

№	Sanitary-microbiological Indicators	Territory of NPP (economic zone), á max.	Adjacent territories, á max.	Divergence (±), %	Td
1.	Coli-index (dm^3)	120 ± 5,3	145 ± 6,00	+ 31,8	
2.	Coli-titer (dm^3)	7,3 ± 0,30	4,7 ± 0,28	- 64,4	> 3,18
3.	Total microbial number (CFO/dm^3)	5500 ± 255,2	7250 ± 350,0	+ 31,2	> 3,18

Table 5

Assessment of surface water quality after passing through "biofilters"

№	Indicators	Zone of stationary recreation			Economic zone		
		before the "biofilter"	after the "biofilter"	± in %	before the "biofilter"	after the "biofilter"	± in %
1.	Sanitary-hygienic Indicators						
1.1.	suspended matter	1,6 ± 0,06	1,3 ± 0,05	- 18,8	3,63 ± 0,12	2,80 ± 0,10	-23,9
1.2.	dissolved oxygen (mg O_2/dm^3)	5,84 ± 0,25	7,4 ± 0,35	+ 26,7	4,00 ± 0,22	5,32 ± 0,25	+33,0
1.3.	BOC_5(mg O_2/dm^3)	5,23 ± 0,27	3,75 ± 0,15	- 28,3	7,57 ± 0,45	5,3 ± 0,20	-30,0
1.4.	COC(мг O_2/dm^3)	12,9 ± 0,53	10,0 ± 0,42	-22,5	25,30 ± 1,17	17,9	- 29,3
1.5.	chlorides (mg/dm^3)	0,50 ± 0,018	0,41 ± 0,02	- 18,0	0,53 ± 0,04	0,40 ± 0, 02	- 24,6
1.6.	nitrites (mg/ml)	0,003 ± 0,0003	0,002 ± 0,0001	- 33,4	0,13 ± 0,011	0,05	- 61,5
2.	Sanitary-microbiological Indicators						
2.1.	coli-index (dm^3)	90,9 ± 4,1	78,3 ± 3,2	- 13,9	111,7 ± 5,4	96,0 ± 4,5	-14,1
2.2.	coli-titer (dm^3)	6,8 ± 0,30	7,9 ± 0,35	+ 14,0	6,6 ± 0,25	7,4 ± 0,33	+ 10,9
2.3.	total microbial number (CFO/dm^3)	3019 ± 128	2600 ± 105	- 13,5	5350 ± 270	4800 ± 205	- 10,3

BOC_5 – biochemical oxygen consumption; COC – chemical oxygen consumption

Conclusions

A comparative analysis of surface waters of the Carpathians in Pokuttia-Bykovina region using microbiological indicators was carried out. The specificity of formation of microbiocenosis of the river network of the mountain region is shown, depending on the energies of anthropogenic activity. It was established that the nature conservation status of the territories has an impact on microbiocenosis and the degree of organic contamination of the water network. The importance of the use of microbiological indicators for monitoring of the protected areas and background monitoring of mountain regions is shown. The expediency of the use of artificially created "biofilters" based on a synthetic carrier "Viia" for purification of natural waters from organic pollutants that are consequences of economic activity is discussed.

Referens

[1] Kavka G. G. & Poetsch E.: Microbiology [in:] Péter Literáthy, Veronika Koller Kreimel, Igor Liska (Ed.), Technical Report of the International Commission for the Protection of the Danbue River. Eigenverlag, ICPDR 2002, 138-150.

[2] Lenore S. Clesceri: American Public Health Association Standard. Standard methods for the Examination of Water and Wastewater,21. American Public Health Association, 2005.

[3] Kavka G. G., Kasimir D., Farnleitner A. H. **(**2006**).** Microbiological water quality of the River

Danube (km 2581 – km 15): longitudinal variation of pollution as determined by standard parameters. In Proceedings of the 36th International Conference of the IAD, pp. 415–421 International Association of Danube Research. https: // www.oen-iad.org/conference/docs/8_ microbial/kavka.pdf citeseerx.ist.psu.edu/viewdoc/download. doi=10.1.1.

[4] Hosam E.A.F. Bayoumi Hamuda and Istvan Patko: American Journal of Environmental Sciences, 2012, 8(3), 202.

[5] Emőke Páll, Mihaela Niculae, Timea Kiss, Carmen Dana Şandru, and Marina Spînu: J Med Microbiol., 2013, 62(11), 1635. Doi: 10.1099/jmm.0.055749-0.

[6] Alexander K. T. Kirschner, Gerhard G. Kavka, BrankoVelimirov, Robert L. Mach, ReginaSommer, Andreas H. Farnleitner: Water Research, 2009, 43(15), 3673. Doi.org/10.1016/j. watres. 2009.05.034.

[7] Mudrak, O. V.: Zbalansovanyy rozvytok ekomerezhi Podillya: stan, problemy, perspektyvy. SPD Glavatska R. V., Vinnytsya 2012. (in Ukrainian)

[8] Paty`ka V. P., Sy`mochko L. Yu.: Mikrobiologichny`j zhurnal, 2013, 75(2), 21. (in Ukrainian)

[9] Valentina Catania, Gianluca Sarà, Luca Settanni, Paola Quatrini: (2017). Bacterial communities in sediment of a Mediterranean marine protected area. Canadian Journal of Microbiology, 2017, 63(4), 303**.** Doi. org/ 10.1139/cjm-2016-0406**.**

[10] An. Masikevych, M. Kolotylo, V. Yaremchuk, Yu. Masikevych, V. Myslytsky, I. Burdeniuk, K. Dombrovskyi: EURIKA: Physics and Engineering, 2018, 2, 3.

[11] Gvozdyak, P. I.: Visnyk NAN Ukrayiny, 2003, 3, 29. (in Ukrainian)

[12] Ryl's'kyi, O. F., Masikevych, Yu. G.: Visnyk Zaporiz'koho natsional'noho universytetu, 2012, 3, 139. (in Ukrainian)

[13] Masikevych, A., Masikevych, Yu., Myslytsky, V., Burdeniuk, I.: Valuation hydroecological and sanitary-hygienic condition of the river network of Pokutsko-Bukovinisn Carpathians policy [in:] Water Security. Mykolaiv: PMBSNU, Bristol: UWE 2016, 98-108.

[14] DSTU 4080-2001 - Yakist` vody`. http://normativ.com.ua/types/tdoc16015.php. (in Ukrainian)

[15] Sanitarno-virusolohichnyy kontrol' vodnykh ob"yektiv: metod. vkazivky MV 10.2.1-145-2007. http://www.moz.gov.ua/docfiles/8203_dodatok.rar. (in Ukrainian)

[16] SanPiN 4630-88. Sanitarni pravy`la i normy` oxorony` poverxnevy`x vod vid zabrudnennya. http://zakon.rada.gov.ua/laws/show/v4630400-88. (in Ukrainian)

[17] Council Directive of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States. Surface Water Directive: 75/440 EEC. https: // www.eea. europa.eu/.../council-directive-75-440-eec.

[18] Masikevych A., Kolotylo M., Yaremchuk V., Masikevych Yu., Myslytskyi V., Burdenyuk I.: Danish Scientific Journal, 2017, 7, 57.

[19] Masikevych A**.,** Malovanyy M., Masikevych Yu. G., Kolotylo M., Yaremchuk V., Myslytsky V. F., Burdenyuk I. P.: Characteristics of the main components of ecological safety of the Pokutsko-Bukovynian Carpathians [in:] Henryk Sobczuk, Beata Kowalska (Ed.), Water Supply and Wasterwater Disposal. Monografie. Lublin University of Technology, Lublin 2018,132–151.

[20] Thompson D. E, Rajal V. B, De Batz S., Wuertz S.: J. Water Health, 2006, 4(1), 67. https: //www. ncbi. nlm. nih.gov/pubmed/16604839.

[21] Zuraini Zakaria, Sanjay Gairola & Noresah Mohd Shariff. Effective Microorganisms (EM): International Congress on Environmental Modelling and Software Modelling for Environment's Sake. http://www. iemss.org/iemss2010/index. php?n=Main.Proceedings.

[22] Krupyey K. S., Skokova A. O., Rylskyy O. F., Dombrovskyy K. O., Hvozdyak P. I.: Pytannya bioindykatsiyi ta ekolohiyi, 2014, 19(2), 224. (in Ukrainian)