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Microbiological pollution of soils and surface waters of the Pokuttia-Bukovyna Carpathians

Andrij Masikevych[1], Yurij Masikevych[2], Myroslav S. Malovanyy[3] ✉, Mykola Blyzniuk[4]

[1] Bukovinian State Medical University, Department of Hygiene and Ecology, Chernivtsi, Ukraine
[2] Bukovinian State Medical University, Department of Physiology, Chernivtsi, Ukraine
[3] Lviv Polytechnic National University, Viacheslav Chornovil Institute of Sustainable Development, Department of Ecology and Sustainable Environmental Management, S. Bandera St, 12, 79013, Lviv, Ukraine
[4] Poltava V.G. Korolenko National Pedagogical University, Department of Production and Information Technologies and Life Safety, Poltava, Ukraine

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Abstract: Based on the analysis of a number of studies, it was found that to assess the state of the environment (including surface waters and soils) it is advisable to use indicators of microbiological pollution, which in general integrally reflect the state of the ecosystem. To assess the dynamics of changes in the pollution of the studied areas, a comparison of monitoring data with the corresponding level of pollution in protected areas (Vyzhnytsia National Nature Park) was used. Research methods included soil and surface water sampling, inoculation on appropriate nutrient selective media, counting of colony forming units (CFU) and other microbiological indicators. To assess the biological activity of soils, urease activity was determined by a method generally accepted in biochemistry. It is established that within the protected areas, despite some existing annual fluctuations, the relative stability of the studied indicators of the hydrosphere is preserved. Studies have shown that soils of anthropogenically altered landscapes are characterised by a high content of sanitary-indicative bacteria. As our research shows, according to the colony forming units (CFU), total microbial count, and titer of *Escherichia coli*, the soils selected in the protected area of the Vyzhnytsia National Nature Park correspond to the "pure" level. The soils of the territories out of the National Nature Park are characterised by high biological capacity, as evidenced by the level of activity of the enzyme urease and the ratio of the main forms of nitrogen compounds.

Keywords: bacteria, ecosystem, hydrosphere, microbiological pollution, national nature park, soils

INTRODUCTION

Pokuttia-Bukovyna Carpathians are specific in terms of landscape, climate, and socio-economic conditions, and are insufficiently studied in terms of the region's environmental safety of the Eastern Carpathians (Fig. 1).

This is the outer strip of the Ukrainian (Eastern) Carpathians within the Ivano-Frankivsk and Chernivtsi regions, stretching from the northwest to the southeast to the border with Romania for almost 75 km [Karta ... 2016]. The relief is dominated by lowlands (up to 800 m) and mid-mountainous (altitude up to 1483 m) landscapes. The slopes are covered mainly with beech and beech-fir-spruce forests. Above 1150 m of sea level, there are mountain meadows, many hayfields, pastures, agricultural lands, and a fairly high degree of ploughing (15–18%), the area is densely populated, and the climate is cool, humid (up to 1000 mm of rainfall per year). These features have created a basis for intensive economic activity, which in combination with natural conditions, contribute to reducing forest cover, soil destruction, surface erosion, the formation of mudslides and pollution of watercourses, etc. in the Cheremosh and Siret River basins. Making regulatory and technical decisions on wastewater and surface water purification from ammonium ions [Malovanyy *et al.* 2013; Sakalova *et al.* 2019], heavy metals

Fig. 1. Pokuttia-Bukovyna Carpathians on the map of Eastern (Ukrainian) Carpathians; source: own elaboration

[Kostenko *et al.* 2017; Malovanyy *et al.* 2019a], organic and biological contaminants [Nykyforov *et al.* 2016; Popovych *et al.* 2020] is impossible without constant monitoring of the state of water bodies [Odnorih *et al.* 2020]. According to some researchers, to assess the state of the environment (including surface waters and soils) it is advisable to use indicators of microbiological pollution, which in general integrally reflect the state of the ecosystem. A number of studies (for example Gauthier *et al.* [2001], Pekarova *et al.* [2009]) show the possibility of assessing the quality of surface waters by studying the microbiota of aquatic ecosystems. According to Pekarova *et al.* [2009], the assessment of surface and groundwater quality is still a major public interest in developed countries. Identification of pathogenic bacteria in water, according to a number of researchers [Fey *et al.* 2004; Straub *et al.* 2003], is one of the main problems in assessing environmental safety for human health and the ecosystem in general. The study of the bacterial density of water can provide an approach to assessing the reliability of monitoring data [Straub *et al.* 2003]. Faecal indicator bacteria, such as total coliform forms, faecal coliforms (thermotolerant coliform forms), *Escherichia coli* and intestinal enterococci (faecal streptococci), are released by humans and warm-blooded animals into wastewater in large quantities, and these bacteria retain their long life and have been used successfully to monitor soil and aquatic ecosystems [Besemer *et al.* 2005; Ishii, Sadowsky 2008]. However, the suitability of these bacteria as common indicators is increasingly being questioned and it is suggested to use *Clostridium perfringens* as an additional indicator, especially when we mention the contamination of wastewater by human faeces [Li *et al.* 2021; Vierheilig *et al.* 2013].

Pathogens themselves are normal components of natural ecosystems, but the growth of faecal bacteria due to anthropogenic activity is a very important problem in the river network of the Carpathians. Thus, the search for simple and affordable technologies for detecting minor amounts of pathogens remains relevant today. It should be noted that the use of sanitary-microbiological indicators to assess the state of regions and protected areas is now episodic and does not apply to specific functional zones of these areas [Mudrak *et al.* 2012; Patyka *et al.* 2013].

Soils are important components of mountain ecosystems. Excessive anthropogenic impact leads to soil depletion, changes the activity of their enzymatic complex, composition and number of microorganisms, also leads to their degradation and violates the environmental safety of natural and semi-natural complexes. The use of sanitary-microbiological indicators of the soil to assess the condition of the studied objects is a promising tool for determining the general state of pollution in specific functional areas of indicated regions [Mudrak *et al.* 2012].

The feasibility of using indicators of urease enzyme activity in determining the soils' ecological status is noted in the studies by Kravkaz Kuşcu and Karaöz [2015], and Utobo and Tewari [2015].

Endowing with appropriate environmental status and land zoning of protected areas are kind of standards for monitoring studies of changes in ecosystems under the influence of anthropogenic activities. Investigating their condition makes it possible to predict changes in the environment in the long run. Therefore, to assess the dynamics of changes in the pollution of the studied areas, it is rational to use a comparison of monitoring data with the corresponding level of pollution of protected areas that are in close proximity to the investigated region or are part of it and play the role of background pollution values.

Such a protected area of the Pokuttia-Bukovyna Carpathians is the Vyzhnytsia National Nature Park (hereinafter NNP), which was established in 1995 and for more than two decades on its territory formed specific ecosystems associated with environmental protection [Malovanyy *et al.* 2019b]. Indicators of microbiological contamination of soils and waters of the Vyzhnytsia National Nature Park were accepted as background indicators for these studies.

MATERIALS AND METHODS

Soil sampling was performed by the method of "envelope" with a size of 5×5 m in four repetitions according to the current standards [DSTU 2006]. Samples were taken in the first half of August for the periods 2013–2020 (for soils), and 2016–2020 (for flood waters) from 5–10 points of 12 representative research sites. The combined soil sample was about 0.5 kg. Isolation of microorganisms from soil samples and accounting for colony forming units (CFU) was performed according to the methods described [Hrytsaienko *et al.* 2003]. The resulting soil suspension was seeded on meat-peptone agar (1.5% MPA). The results of the analysis were expressed in colony forming units (CFU) in 1 g of completely dry soil. The method of determining the titer of *Escherichia coli* involved the selection and seeding of 1 cm^3 of different dilutions of soil in the glucose-peptone medium. The titer of enterococci was determined by seeding the appropriate dilutions on Kalyna selective milk medium with polymyxin at a temperature of 37°C, the perfringens titer was determined by seeding dilutions on Wilson–Blair medium. To determine the titer of thermophilic bacteria, the dilution of the soil suspension was filled with cooled MPA, incubated for 24 h at 60°C, and the number of colonies per 1 g of soil was counted [Hrytsaienko *et al.* 2003]. To assess the biological activity of soils, urease was determined by methods generally accepted in soil biochemistry [Haziev 2005]. Urease activity is expressed in mg of ammonia formed per 1 g of sample per minute. Water sampling was carried out in the Cheremosh and Siret River basins, which flow into the Pokuttia-Bukovyna Carpathians and belong to the Ukrainian part of the Danube basin. The research was conducted during the summer season (2013–2020). Water sampling (23–25°C) was carried out in watercourses of various functional zones of the protected object of national importance (Vyzhnytsia National Nature Park) and territories of landscapes with intensive economic activity. Coli index, the total microbial count, was determined by generally accepted methods in accordance with methodological guidelines [Haziev 2005].

To confirm the morphological and other properties of the culture of microorganisms, the method of microscopy with subsequent identification according to the determinant classifier of Bergey was used [Hoult *et al.* 1997].

RESULTS AND DISCUSSION

Studies have shown that the values of sanitary-microbiological indicators increase downstream in all studied watercourses. Of particular concern was the increase in the number of lactose-positive *Escherichia coli* (*E. coli*) per 1 dm^3 of water (coli index). It should be considered that *Escherichia coli* is a sanitary indicator and indicates faecal contamination of water bodies. To establish the spatial dynamics of changes in microbiological contamination of surface waters in the various zones (protected – regulated recreation – stationary recreation – business), the watercourse, which consistently flows through all these areas (the Siret River with tributaries), was studied. The research results are presented in Figure 2.

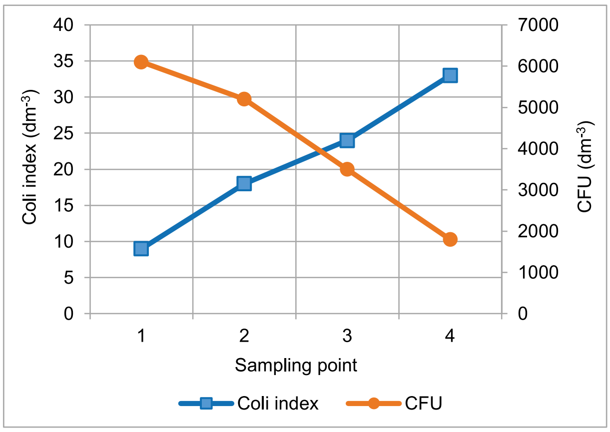

Fig. 2. Microbiological indicators of the Syret River water from different functional zones of Vyzhnytsia National Nature Park (NNP); 1 = protected area, 2 = regulated recreation area, 3 = stationary recreation area, 4 = business area; source: own study

Comparing the coli index in the river water samples of the protected area and the selected water samples in the business area, we found an average increase of the coli index by 2 times (Fig. 2). The total microbial count (CFU) was 2–4 times higher than the normative indicators, adopted in EU countries [FAO, FAOLEX 1975] and amounted to 1500–1700 dm^{-3} (for protected area), 2300–3500 dm^{-3} (for stationary recreation area) and more than 5000 dm^{-3} (for business area).

Figure 3 shows the dynamics of the main microbiological indicators of the aquatic environment different in functional load and conservation status of the Pokuttia-Bukovyna Carpathians over the past seven years.

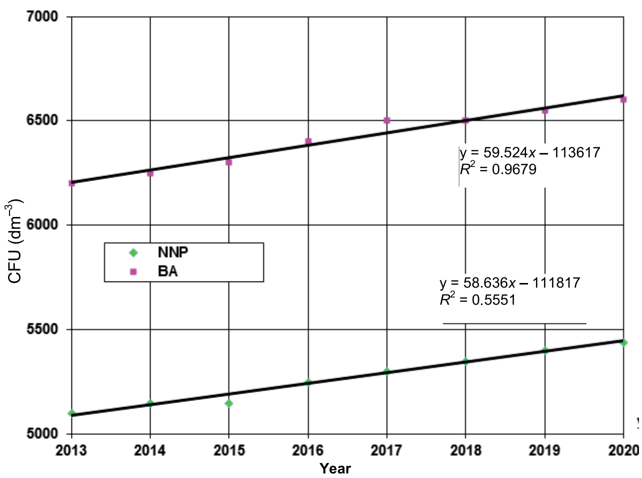

Fig. 3. Dynamics of change of the total microbial number of surface waters of the river network of the Pokuttia-Bukovyna Carpathians in National Nature Park "Vyzhnytsia" (NNP) and in the business area (BA); source: own study

Studies of the soils of the protected area of the NNP have shown that no samples of *Clostridium perfringens* and gram-positive cocci were detected in the selected and analysed samples, as evidenced by the corresponding parameters of perfringens and enterococci titer. It is known that the content of these microorganisms in the soil is a sign of fresh or old faecal contamination. Therefore, it can be argued that in the protected area of the nature reserve, the soil is free of faeces. According to the content of thermophilic gram-positive bacilli, the soils of the protected area can be called clean. Estimation of the microbiological condition of the soils of the stationary recreation zone indicates an increase in the studied samples by almost an order of magnitude of CFU and a corresponding decrease in the value of titer of *Escherichia coli* [MASIKEVYCH *et al.* 2018].

Perfringens titers, enterococci titers and the number of thermophilic bacteria vary within the range corresponding to the moderately pure state of the soil. With the transition to the economic (business) zone, there is a significant increase (by two orders of magnitude) in the number of thermophilic bacteria. An increase in the number of thermophilic microorganisms indicates the introduction of humus or compost into the soil and therefore may be the result of intensive agriculture and the use of local organic fertilisers of animal origin for these purposes [MASIKEVYCH 2019].

A long-term study of the total microbial population in soils in areas of different anthropogenic loads is presented in Figure 4.

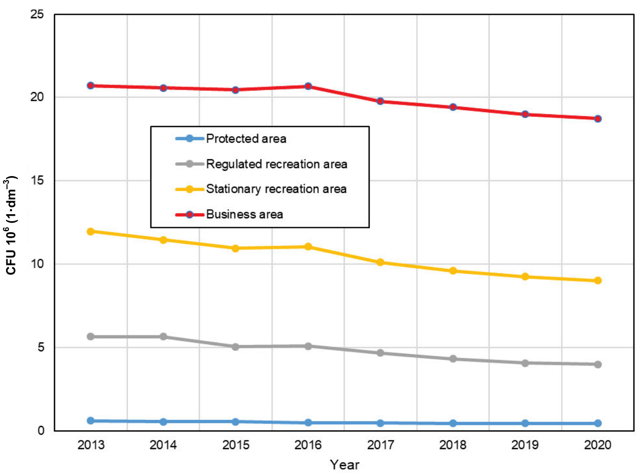

Fig. 4. Dynamics of change of the total microbial population, colony forming units (CFU), of soils of the research object; source: own study

According to Figure 4, over the past 10 years, there has been a tendency to significant microbiological contamination of soils, which are subject to the intense economic impact of the local population in the region.

To assess the biological activity of soils, we determined their urease activity. Our results are consistent with the study by LIU *et al.* [2008], which indicates a direct link between enzyme activity and the stability of the soil ecosystem. Thus, with the transition to the economic zone (agricultural land zone), the stability of natural ecosystems undergoes further changes due to more intensive livestock farming and the development of agricultural lands. Such changes are accompanied by a sharp increase in urease activity in human-transformed ecosystems [MASIKEVYCH *et al.* 2018], which has a direct influence on the metabolism of nitrogen's basic forms in soils (Tab. 1).

Table 1. The content of nitrogen compounds and urease activity in the soils of the study region

Indicators of soil condition	NNP "Vyzhnytsia"		Traditional economic landscapes
	protected area	economic area	
Ammonium (mg·kg^{-1})	1.80	2.90	5.50
Nitrates (mg·kg^{-1})	3.70	5.40	6.50
Urease activity (μg NO_3–NH_4·g^{-1} of soil)	1.50	2.37	2.40

Explanations: NNP = National Nature Park.
Source: own study.

It is known that the enzyme urease belongs to the class of hydrolases, which play a significant role in the hydrolytic cleavage of organic matter and its conversion into nutrients available to plants. As a result, the amount of nitrates in the soils of the economic zone increases and the soil fertility progressively decreases. The increase in urease activity with the transition from the protected area to the economic zone indicates significant changes in the activity of the soil biocenosis for more than two decades since the creation of the protected area.

CONCLUSIONS

Based on the data of monitoring observations, it can be stated that there is a progressive pollution trend of the river network in the territories of traditional economic landscapes, which are out of the protected areas and where active anthropogenic activity exists. Within the protected areas, despite some existing annual fluctuations, the relative stability of the studied indicators of the hydrosphere is preserved. As for our studies, according to total microbial count (expressed in CFU) and titer of *Escherichia coli*, the soils selected in the NNP protected area correspond to the level of "pure", according to the scale.

Studies have shown that soils of anthropogenically altered landscapes are characterised by a high content of sanitary-indicative bacteria. The soils of the territories out of the NNP are characterised by high biological capacity, as evidenced by the level of activity of the enzyme urease and the ratio of the main forms of nitrogen compounds.

A comparative analysis of soils of the economic activity development areas, which differ only in terms of conservation status, showed that the soils of the economic zone of the NNP contain fewer nitrates and ammonium and are inferior to the content of sanitary bacteria. As for the activity of the enzyme urease, it is approximately the same in different areas of economic activity.

Thus, it can be argued that the number of studied microorganisms and biological activity of soils of different functional zones of the nature reserve fund depends on the level of anthropogenic load. Studies have proved that the activity of soil microorganisms is a highly sensitive indicator of soil biological activity, which is especially important for monitoring the condition of reference (protected) areas – centres (cores) of the ecological network, centres of conservation, reproduction of landscape-biological diversity and environmental protection.

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