

## RESEARCH OF MICROBIOLOGICAL INDICATORS OF QUALITY OF SURFACE WATERS OF NATURAL ENVIRONMENTAL TERRITORIES OF THE DANUBE BASIN

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### Abstract

A comparative analysis of the sanitary and ecological state of surface watercourses in the upper part of the Danube basin (on the territory of Ukraine) was carried out according to microbiological indicators. Similar hygienic studies were previously conducted in the middle and lower Danube in Austria, Slovakia, Hungary and Romania. In Ukraine, the river network of the Danube River basin was not studied by microbiological indicators.

The object of research is the watercourses on the territory of various zones of the nature protection object, which are different in function. This approach makes it possible to use hygienic indicators of water in protected areas of nature conservation areas as a reference for conducting background monitoring.

The original design of the treatment plant based on the use of “Viya” fibrous carrier and “paste” technical structure is proposed. It was established that as the transition from the reserve to the economic zone occurs, the nitrate content in the water increases, the BOD increases in water, and the dissolved oxygen in the water decreases for all the watercourses studied. Significant differences in microbiological indices of watercourses of various functional zones of the protected object have been revealed.

The possibility of using the sanitary-microbiological indicators of the river network as a reliable rapid test for assessing the state of environmental safety of nature conservation areas is shown.

**Keywords:** nature protection areas, functional zones, surface waters, Danube basin, sanitary-microbiological indicators.

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## 1. Introduction

Sanitary-microbiological indicators are successfully used as indicators of environmental pollution: water, air, soil [1–3], as well as to monitor the status of ecosystems, forecasting and modeling of development. In connection with the rapid reaction to environmental changes, bacteria proved to be ideal markers of microbial contamination of surface waters [4]. This approach is especially valuable for protected areas. Protected areas serve as background sites for assessing the ecological status and changes in the environment. Studying their condition makes it possible to predict changes in the environment to a distant future.

The object of research is the sanitary and epidemiological indicators of the water network of the Siret River. This river enters the upper part of the Danube basin and forms the basis of the hydrological network of the protected nature reserve “Vyzhnytsia National Nature Park”. The Danube is one of the most important rivers of Europe and the world and, simultaneously, according to a number of authors [4], is one of the most vulnerable aquatic ecosystems. For monitoring the water quality of the Danube River, the coastal countries currently use different approaches and methods of sanitary and ecological analysis, which does not allow the formation of a holistic picture of the state of the ecosystem of an important waterway in Europe. That is why the use of microbiological analysis as a universal approach for water quality monitoring by the coastal countries of the international river Danube is relevant at this time.

## 2. Literature review and problem statement

To date, there is a strong demand for monitoring water quality. Researches of scientists of the Institute of Hydrometeorology from Bratislava [5] suggest the use of autoregressive models for studying the impact of natural and technogenic changes on the quality of the Danube.

In terms of monitoring the water network, the assessment of the presence of pathogenic bacteria in water is of particular interest, according to the American School of Researchers at the University of California, Davis and Iowa State University. In particular, this concerns the problems associated with the use of indicator organisms for this purpose. This approach is the main problem of protecting human and animal health [6, 7].

The results obtained by Hungarian researchers [8] indicate that the bacteriological properties of water can be a specific indicator of fecal pollution and organic contamination. That is why, according to the authors, microbiological contamination by fecal (pathogenic) bacteria is considered to be the most important issues of surface water quality, and especially in the Danube basin. The accumulation index of *E. coli* is one of the specific indicators of fecal contamination of the aquatic environment, and can be used for express diagnostics of the aquatic environment.

An interesting experience is the assessment of the quality of the surrounding aquatic environment of the Kingdom of the Netherlands [11]. The national program for monitoring surface water in this country, in addition to hydrochemical indicators, including also hydrobiological monitoring (including epidemiological). In a number of EU countries (most of which have their own

biological assessment of surface waters), bacteria, algae, invertebrate hydrobionts, phytoplankton are used as bioindicators [12, 13].

Researchers [9] have summarized existing methods of microbiological bioindication of the aquatic environment. It should be noted that the standards of the USSR in the field of protection of the hydrosphere operating in Ukraine do not take into account bacteriological indicators of water quality. This was, in all probability, the reason that data on the microbiological state of the upper part of the Danube basin (in the Ukrainian part: Tisza, Prut, Siret rivers) are practically absent.

Since water quality assessment is expensive [14], the development of rapid assessment methods remains an urgent problem. The introduction of this approach in many developed countries can reduce the cost of monitoring.

It should also be noted that the use of sanitary and microbiological indicators of the river network for assessing the status of protected sites, to date, is episodic [4, 10]. The question of assessing the sanitary and hygienic quality of surface waters of different purpose and status of functional zones of nature protection territories remains to be fully investigated.

The analysis of literature data confirms the need to use methods of sanitary-microbiological analysis of surface waters for assessing the ecological status of nature reserves and monitoring of aquatic ecosystems as a whole.

### 3. The aim and objectives of research

The aim of research is studying the epidemiological state of the surface waters of the river network of the Siret River (the upper part of the Danube basin, the Ukrainian territory) of various functional zones of the nature reserve object.

To achieve the aim, the following tasks are set:

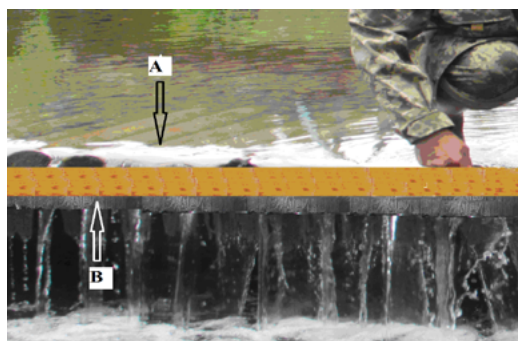
- to determine the main sanitary and microbiological indicators (nitrite content, biological oxygen consumption, dissolved oxygen, coli-index, total microbial number), determine the quality of surface waters;
- to conduct a comparative analysis of sanitary and microbiological indicators, various in terms of the functional purpose of the zones, a nature reserve site;
- to study the possibility of using the proposed “biofilter”, created on the basis of “Viya” artificial fibrous carrier and “paste” technical design, for assessing the quality of surface waters of various functional zones of a nature reserve site.

### 4. Materials and methods for research of surface water quality indicators

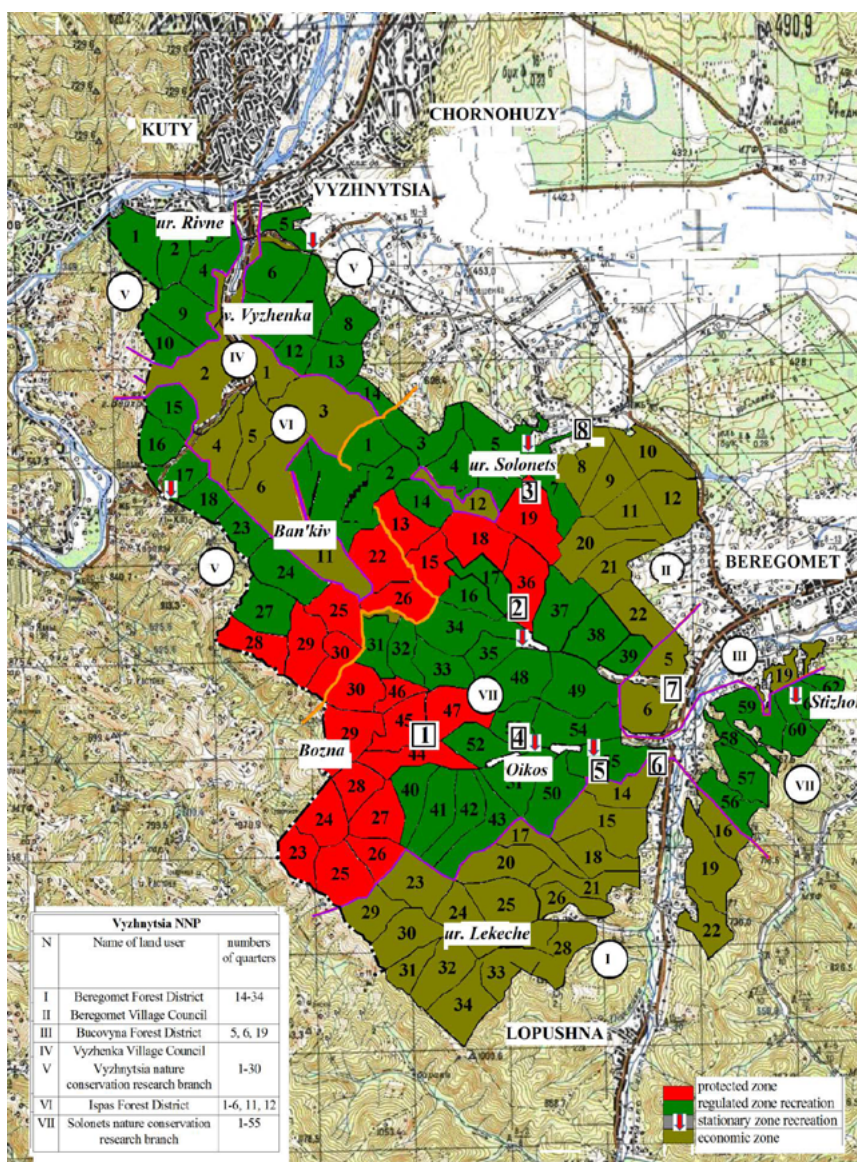
The watercourses have been studied: rivers Stebnyk, Sukhyi Strumok, Solonets entering the Ukrainian part of the Danube Basin. To assess the quality of the surface waters of the waterways of the Vyzhnytsia national nature park (NNP), Viya fibrous carrier (TU 995990) was used. It made from a textured filament yarn (TU 6-06-C116-87, text 350) is described in [15].

Earlier [15, 16] it was established that a fibrous carrier of this type can be successfully used to construct “biofilters” for cleaning surface water. In particular, it was shown that the effectiveness of the “bioreactor” depends on the temperature, the concentration of microorganisms and that the accumulation of phytoplankton and heterotrophic microorganisms on carriers is most effective in the summer period (July-August). Using the approach described by the authors, a “biofilter” was mounted on the basis of special wooden structures – “paste”, have long been used by local residents to saturate the streams with oxygen (**Fig. 1**). “Biofilter” is intended for adsorption on its surface of bacteria on hydrobionts.

The studies were carried out during the summer season 2016 and 2017. Sampling of water (23–25 °C) was carried out immediately before and after the “biofilters” from different functional zones of the waterways of the NNPs indicated in the scheme (**Fig. 2**), in accordance with the existing standard techniques. The choice of water sampling points was determined by their belonging to the functional zones of the protected object. The linking of the research region to the map of Europe is shown in **Fig. 3**.

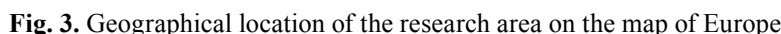


**Fig. 1.** When installing a cleaning structure using “Viya” fibrous carrier: A – a «paste» wooden structure; B – “Viya” fibrous carrier



**Fig. 2.** Map-scheme of functional zoning of Vyzhnytsia national nature park: sampling points: 1 – the upper part of the Stebnik riverbed, 2 – the upper part of the Sukhyi Strumok canal, 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 canal, 8 – the middle part of the Solonets riverbed





The hydrobiological material (biocenosis of the periphyton overgrowth of the Viya" fibrous carrier) was selected in the second decade of August. The material was delivered to the laboratory in an open vessel. The infusoria, rotifers and turbolium of the fouling biocenosis were studied in a living state under the Biola R-14 microscope, with an increase of 150–600 times. Other organisms (larvae of amphibiotic insects and nematodes) were fixed with 70° ethanol and determined their species affiliation. The species were determined according to determinants and scientific works [18, 19]. The statistical processing of the results was carried out using the computer program Excel.

The study of the qualitative species composition of organisms (periphyton) that inhabited the fibrous substrate showed bacteria, protozoa, fungi, algae, worms, crustaceans, bivalves and others to form fouling.

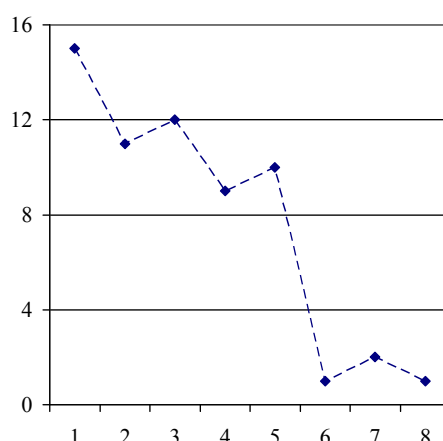
The data are presented in **Fig. 4** indicate that the “Viya” fibrous material is able to accumulate in considerable quantities the bacteria of the *E. coli* group (**Fig. 4**).

**Table 1**

The values of the main sanitary-microbiological indices of various functional zones of the Vyzhnytsia NNP\*

Sampling points	BOD <sub>tot</sub> (mg O <sub>2</sub> /dm <sup>3</sup> )	Dissolved oxygen (mg O <sub>2</sub> /dm <sup>3</sup> )	Coli-index (dm <sup>3</sup> )	Microbial number (CFU/ml)	Nitrogen (mg N/dm <sup>3</sup> )
protected area					
1	1,8±0,10	7,5±0,55	85±10,5	1500±85,4	0,01±0,001
2	2,3±0,12	7,1±0,50	80±8,0	1700±90,2	0,02±0,001
3	2,0±0,08	8,0±0,70	82±12,2	1600±65,5	0,02±0,002
zone of stationary recreation					
4	5,1±0,30	5,2±0,60	90±11,5	2300±92,5	0,02±0,03
5	4,5±0,25	6,0±0,55	100±9,4	3500±150,4	0,05±0,004
economic zone					
6	8,8±0,50	4,2±0,20	120±15,3	5800±250,5	0,15±0,02
7	9,2±0,45	4,0±0,25	105±12,5	5200±280,3	0,10±0,01
8	9,5±0,40	3,8±0,20	110±14,6	5500±255,2	0,14±0,02

Note: \* – the data are reliable for  $p < 0.05$ ; the name of the sampling points corresponds to the notation of **Fig. 2**



**Fig. 4.** The coefficient of accumulation of bacteria on fibrous carriers “Viya”: the name of the sampling points corresponds to the notation in **Fig. 2**; coefficient of accumulation – the multiplicity of excess of content in comparison with the surrounding water environment.

As the data in **Fig. 4** for sampling points 1, 2, 3, included in the NNP protected area is characterized by a high coefficient (from 11 to 14 times) of the accumulation of bacteria on fibrous carriers. In water samples taken in the zone of stationary recreation (points 4–5), this coefficient also has a sufficiently high index in the range of 9–10 units, that is, the carrier accumulates on its surface an order of more bacteria than the river water of the sampling points.

In the periphyton of the “Viya” fibrous carrier, 12 species and subspecies of hydrobionts were found. Most of these hydrobionts belong to amphibiotic insects (8 taxa). Rotifers and infusoria are represented by 2 taxa, respectively. Other systematic groups (ciliated worms, nematodes) were represented by one species, respectively. The species composition of the hydrobionts was supplemented with new species as the transition from the protected zone – 5 species (points 1, 2, 3) to the zone of stationary recreation – 7 species (points 4, 5) and the economic zone – 12 species (points 6, 7, 8).

## 6. Discussion of the research results of surface water quality of various functional zones of the Vyzhnytsia NNP

Comparison of the obtained results (**Table 1**) with the normative indicators adopted in the EU countries (Surface Water Directive: 75/440 EU) give grounds to assert that the surface waters of the river network of the NNP reserve and recreational zone refer to the permissible level of pollution. At the same time, the river water of the economic zone does not meet the sanitary and hygienic requirements and refers to a moderate and high pollution degree. The use of water of this pollution degree, without proper cleaning, can lead to the appearance of a number of diseases in the population.

The conducted studies showed (**Fig. 4**) that in the protected zone and the zone of NNP stationary recreation, the maximum values of the accumulation coefficient of bacteria on fibrous carriers take place. This coefficient characterizes the multiplicity of exceeding the amount of *E. coli* group on a synthetic carrier in comparison with the surrounding aqueous medium. It is known that the presence of *E. coli* group in water indicates fecal contamination and, accordingly, the possible contamination of water by pathogenic microorganisms of the intestinal group (typhoid, paratyphoid, dysentery, etc.) [8]. However, the detection and isolation of pathogens is complicated by the low concentration of bacteria in surface waters, the high financial costs and the length of detection technology [23]. The use of natural adsorbents is well-established in this regard [24]. In order to overcome this methodological obstacle, “biofilter” based on a synthetic material such as “Viya” was used. It was mounted on special «paste» wooden structures. The data in Fig. 3 indicate that the use of the “biofilter” makes it possible to concentrate the *E. coli* group for their identification and possible destruction.

It is also shown that as the transition to the economic zone and the increase in the amount of organic pollution of waterways, there is a sharp increase in the magnitude of the coli-index and the microbial number (**Table 1**). At the same time, the accumulation coefficient of *E. coli* group on the “biofilter” is characterized by minimal indicators. This can be explained by the saturation of the absorption surface by microorganisms, and also by their partial incorporation into nutrient chains.

One of the proofs of this statement is an increase in the species composition of hydrobionts on the “biofilters” of the watercourses of the economic zone. Thus, representatives of rotifers, copepods (and other lithophilic species) prevailed in water samples collected in the protected zone (upper part of the current). At the same time, in the water of the economic zone, with the growth of organic detritus, the species composition of the fouling is replenished at the expense of detritophages, incl. nematodes, oligochaetes, dreissena and others.

Thus, a specific “biofilter” in the form of an artificially created microecosystem is formed downstream on fibrous carriers. In this microecosystem fibrous carrier serves as a kind of “home” for microorganisms, plant and invertebrate animal organisms, they are able to accumulate, which is the basis for cleaning reservoirs. In addition, some bacteria, in all probability, become an element of the feeding chain and serves as food for invertebrate hydrobionts. Thus, water reservoirs are cleaned in two stages: due to adsorption on synthetic carriers in the first stage and trophic chains on the second stage.

In general, in the estuary part of the tributaries of the Siret basin there is a significant contamination of surface waters (III-IV quality class), which is consistent with the results of studies [25], which showed that it is the Siret and Prut rivers that are significant factors in the pollution of the lower part of the Danube riverbed.

## 7. Conclusions

1. It is shown that as the transition from the protected to the economic zone occurs, the nitrate content in the water increases, the BOD index increases and the dissolved oxygen in the water decreases for all the investigated watercourses.

2. “Viya” fibrous carrier is capable of effective accumulation on the adsorbing surface of microorganisms of bacteria of the group of *Escherichia coli* and invertebrate hydrobionts for watercourses with insignificant content of organic pollutants.

3. Significant differences in microbiological indices of watercourses of various functional zones of the protected object have been established. The total microbial number (CFU/ml) was 1500–1700 (for the protected zone), 2300–3500 (for the zone of stationary recreation) and more than 5000 (for the economic zone).

3. It is shown that during the growth of organic pollution of water bodies, especially in the economic zone of nature protection territories, a kind of “biofilters” – artificial microecosystems – are formed on fibrous carriers.

4. The obtained results supplement the idea of the sanitary and ecological state of the Danube basin in its upper part on the territory of Ukraine. Pollution of the upper part of the Siret river basin ultimately determines the level of pollution of the lower part of the Danube riverbed, and actually affects the state of the ecosystem of this international waterway of Europe.

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