

Sanitary, Microbiological Condition, and Ecological State of Surface Water Quality in the Upper Siret River Basin (Ukraine)

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ABSTRACT

This study aimed to spatially assess the ecological state of the upper Siret River basin in Ukraine, along with its main tributaries, using physicochemical and microbiological indicators. Additionally, we evaluated the impact of anthropogenic activities on surface water quality in the region. The research spanned one year, with nine sampling points ranging from the Siret River sources in the Pokutsko-Bukovynian Carpathians to the Romania border crossing area. Notably, a significant portion of the upper basin lies within the Vyzhnytskyi National Nature Park. Physicochemical analysis involved measuring pH, water turbidity, and concentrations of NH_4^+ , NO_3^- , and Cl^- ions. Microbiological analysis focused on total and fecal bacteria, specifically *E. coli* species. Results revealed a significant correlation between population density, the absence of centralized sewage treatment facilities in large settlements, and the level of surface water pollution in the Ukrainian segment of the Siret River basin. The upper part of the river network, where the Vyzhnytskyi National Nature Park is located, displayed the cleanest waters. Downstream, surface water pollution increased, particularly near certain points outside large settlements and tourist complexes (Myhove, Berehomet, and Storozhynets). Our findings highlight the importance of using microbiological indicators to monitor the ecological state of Danube sources in the Eastern Carpathians.

Keywords: Siret River basin, water quality, coliforms *E. coli*, anthropogenic influence.

INTRODUCTION

Recent research emphasizes the importance of studying the microbiota of aquatic ecosystems to assess surface water quality. Coliform bacteria, particularly *E. coli*, have proven reliable indicators of fecal pollution and potential risks to public health (Gauthier et al., 2001; Pekarova et al., 2009). Additionally, these bacteria serve as indicators of fecal contamination in pulp and paper production effluents, highlighting their significance for risk assessment (Gauthier et al., 2001). Water quality assessment and the implementation

of innovative treatment technologies remain significant public interests in developed countries (Pekarova et al., 2009; Kostenko et al., 2017; Malovanyy et al., 2019; Malovanyy et al., 2020). Pathogenic bacteria identification, according to numerous researchers (Fey et al., 2004; Tymchuk et al., 2020), remains a major concern for assessing environmental safety and its impact on human health and the ecosystem. In tropical and temperate regions, microbiological *E. coli* contamination serves as a specific indicator of fecal pollution. Monitoring bacterial density aids in evaluating data reliability (Besemer et al., 2005). Faecal

indicator bacteria, including total coliforms, faecal coliforms, *Escherichia coli*, and intestinal enterococci, are excreted by humans and warm-blooded animals into wastewater, maintaining viability and pathogenicity for extended periods (Besemer et al., 2005).

Anthropogenic activity has led to an increase in fecal bacteria in the river network of the Carpathians, particularly in the Danube basin. Despite efforts to preserve its ecosystem, the Danube faces severe physical, chemical, and biological pollution, especially in its upper part, the Pokutsko-Bukovynian Carpathians (Pall et al., 2013; Kolarevich et al., 2011). Researchers have classified the microbiological quality of water in the Danube basin into different classes based on standard indicators of fecal and organic pollution (Eiler et al., 2022; Kavka et al., 2006; Winter et al., 2007; Kirschner et al., 2009; Kirschner et al., 2017). The Siret and Prut rivers, originating in the Ukrainian Carpathians, were categorized as critical and strongly fecal pollution classes, respectively. In Romania, studies have focused on priority pollutants in the Siret River Basin, originating from municipal, industrial, and agricultural wastewater discharges (Zait et al., 2022). The assessment included monitoring inorganic (e.g., As, Cd, Hg, Ni, Pb) and organic (e.g., naphthalene, anthracene, phenanthrene, fluoranthene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(hy)perylene, indeno(1,2,3-cd)pyrene, α , β , and γ -hexachlorocyclohexane and di-2-ethylhexyl phthalate) priority pollutants in the Siret River Basin from 2015 to 2020. Findings revealed that the water quality generally corresponds to the 2nd or 3rd class and is mostly unsuitable for drinking water supply, influenced by main tributaries' quality and treatment facility effluents. The sanitary and microbiological state of the Siret River in Romania has been comprehensively studied.

As for the upper part of the Siret River (Ukraine), it should be noted that the issue of the ecological state of this part of the basin has not been sufficiently studied. Siret is a river that flows in Ukraine (Chernivtsi region) and Romania. Siret is a left tributary of the Danube. The length of 513 km, including the upper part of 110 km flows through the territory of Ukraine, then through Romania to the confluence with the Danube near the city of Galati. In the upper part of the stream (Ukraine), the channel of the Siret is moderately winding, with a width of 7–10 m and a depth of 0.2–0.7 m, the current speed varies

from 1.5 to 2–3 m/s. It crosses the Ukrainian-Romanian border in the area of Novy Vovchynets village. More than twenty tributaries flow into the Siret on the territory of Ukraine, among them: right tributaries Lustun, Myhyvka, Maly Siret, Siretul, etc., left tributaries Lapushna, Lekechi, Stebnyk, Sukhyy, Hlybochok, Molnytsia, etc. Due to the lack of centralized sewage treatment facilities, there are large settlements in the upper part of the basin: the village of Berehomet, the city of Storozhynets, the villages of Ropcha, Panka, Yordaneshti, Karapchiv, Kamianka, Petrichanka, etc. are potential polluters of the Siret Basin (Baseinove upravlinnia ..., 2022).

Control over the quality of water resources of the Prut and Siret in Ukraine at the state level is carried out by the basin management of this river network (BUVR), which publishes the results of research on the corresponding website (Baseinove upravlinnia..., 2022; Derzhavne ahenstvo..., 2022). However, there are no sanitary-microbiological indicators among the indicators by which the certified laboratories of BUVR assess the condition of surface, underground, return (wastewater) and drinking water. Therefore, scientifically based bioindication of the upper part of the Siret River (Ukraine) has not been carried out until now.

Separate studies on the study of phytoplankton to establish anthropogenic regression of water ecosystems of the Siret River basin are known (Karavan et al., 2013; Karavan et al., 2019). The authors show that algae are a convenient display object for bioindication of the water environment of mountain and foothill rivers. Our earlier research made it possible to find out the sanitary and microbiological state of the sources of the Siret River (Masikevych et al., 2018; Masikevych et al., 2022). It was shown that there is a progressive pollution of watercourses by discharges of an organic nature downstream, as evidenced by BSK5 indicators, an increase in indicators of the total microbial number and Coli index, etc. These studies are aimed at assessing the sanitary and microbiological state of the Siret River from its source in the Pokutsko-Bukovynian Carpathians to the border with Romania.

MATERIALS AND METHODS

The material was prepared on the basis of joint research carried out in the laboratories of Bukovynian State Medical University, Zaporizhzhia

National University. As a result of the conducted research, the sanitary and ecological condition was analyzed in terms of a number of microbiological, as well as the content of surface-active substances and Ukrainian oil products in 9 sampling points in the upper part (territory of Ukraine) of the Siret River basin. Below, Figure 1 presents water sampling points in the Siret river basin.

Based on the data in Table 1, taking into account the indicator of the area of the basin, it is

possible to calculate the average population density of the river basin. It is 37.5 people per km², while the average rate in Chernivtsi region is 109.9 people per km² and 72 people per km² in Ukraine. Therefore, the basin of the Siret River within the borders of Ukraine is characterized by rather low average indicators of population density. This territory is inhabited mainly by the rural population, which is approximately 60% and is mainly engaged in cattle breeding, logging and

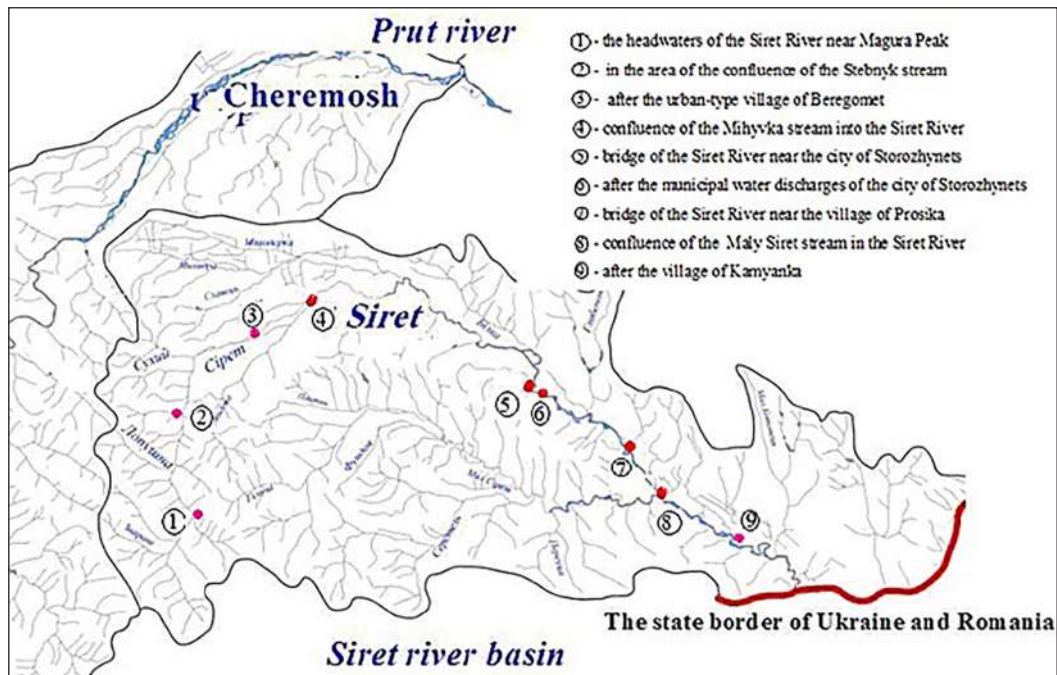


Fig. 1. Hydrological network of the Siret River basin within the territory of Ukraine with the indication of water sampling points

Table 1 Characteristics of population indicators and characteristics of the landscape of the Siret River basin within Ukraine

Range between sampling sites	The number of settlements within the range	Population, in thousands of people	General characteristics of the landscape
1–2	3	2385	mountainous area, mostly covered with spruce-fir forest
2–3	1	7647	foothills, mostly covered with fir-beech forest
3–4	3	6933	flat terrain, sparse forests, deciduous species prevail, meadows
4–5	5	6799	flat area, sparse forests, deciduous species prevail, arable land
5–6	1	14428	urban landscape
6–7	4	8471	flat and rough terrain, deciduous species prevail, arable land
7–8	2	2302	flat and rough terrain, deciduous species prevail, arable land
8–9	1	6284	flat terrain, deciduous species prevail, arable land
In all	20	55 249	

Note: the length of the Siret river basin within Ukraine is 110 km, the area of the basin is 2.07 thousand km² (Baseinove upravlinnia ..., 2022).

agriculture. It should also be noted that centralized sewage treatment facilities are completely absent in this region, and therefore waste from economic activities and communal discharges to a large extent enter the surface waters of the Siret River network. In the land cover of the Siret basin, three vegetation zones can be distinguished: the upper mountain zone (spruce-fir forest), the foothill zone (Carpathian spruce-beech forest) and the cross-plain zone (broad-leaved species prevail).

RESULTS AND DISCUSSION

In order to determine the microbiological indicator of minor components of surface waters, a fibrous carrier of the „Viya” type was used, made of textured plait thread (Hvozdiak, 2003). It is shown (Fig. 2) that bacteria are able to accumulate on fibrous material, which facilitates their identification. Figure 2 shows the coefficient of bacterial accumulation on the fibrous carrier, ranging from 6.5 to 8.8 units. The enumeration of total coliforms and thermotolerant (fecal) coliforms, represented by purple-red colonies with a metallic sheen on Endo agar, was conducted using the membrane filtration method. For total and thermotolerant *E. coli* identification, blue-green colonies were observed on TBX - Tryptone Bile X agar, incubated at 37°C and 44°C for 48 hours, respectively (Sanitarno-virusolohichnyy control..., 2007). The morphology and other properties of the microbial cultures were confirmed through microscopy, followed by identification based on Bergey’s determinant (Khoult et al., 1997). Physico-chemical indicators of water were

determined in accordance with (Voda pytna..., 2014; Pro vidpovidnist..., 2005). The results of the study were processed statistically at the significance level of $p < 0.05$ (Herych et al., 2021). The data presented in Table 2 reflect the average value of the physico-chemical and microbiological indicators of the flood waters of the Siret River basin (within the territory of Ukraine) at 9 investigated sampling points. The average value of the pH indicator was 6.5–6.8 units, which indicates slight acidification of flood waters of the river network.

In the upper part of the basin (sampling points 1–4), the water of the river network was characterized by a high degree of transparency, which subsequently undergoes changes (acquires turbidity). As for microbiological indicators, the water intake points we studied are characterized by quite diverse values. Thus, the average concentrations of fecal *E. coli* range from 150 CFU/100 ml (in the main part of the Siret River basin, in the headwaters) to 10,800 CFU/100 ml (at intake point 6, after discharges in the city of Storozhynets). The range of differences is even greater when comparing the total number of BGCP and ranges from 360 CFU/100 ml in the upper reaches of the Siret River to 25,650 CFU/100 ml after the river passes through large settlements (Berehomet and Storozhynets). It should be noted, that the maximum values of physico-chemical indicators (NH_4 , NO_3 , CI) were also recorded for sampling point No. 6 (Storozhynets). The obtained result can be explained by the lack of centralized sewage treatment facilities and a significant number of people in this settlement, as evidenced by the data of Table 1. On the other hand, the minimum value of the studied physico-chemical and microbiological

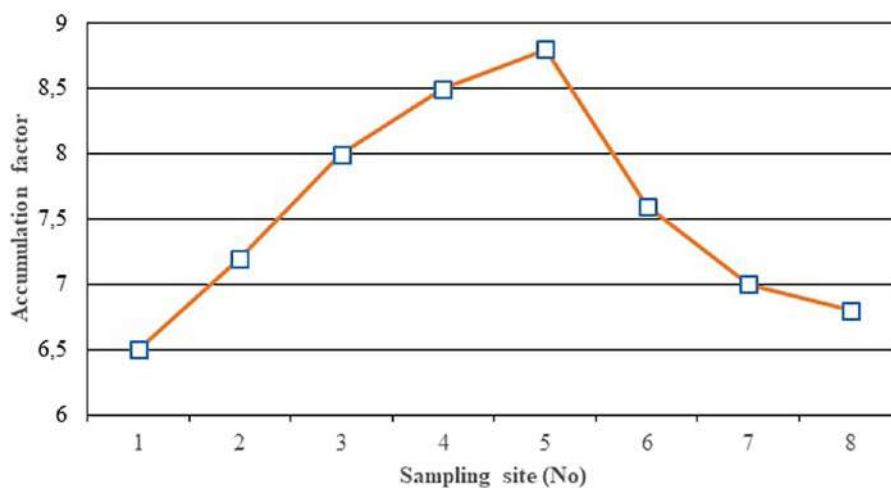


Fig. 2. Bacterial accumulation coefficient on the fibrous carrier “VIYA”

Table 2. Mean values of physico-chemical and microbiological indicators of water quality in the examined catchments River Siret

Sampling site (N ₀)	Parameter									
	BOC ₅ mg O ₂ / dm ³	O ₂ mg/ dm ³	PP mg/ dm ³	NH ₄ ⁺	NO ₃ ⁻	Cl ⁻	Fecal <i>E. coli</i>	Fecal coli-forms	Total <i>E. coli</i>	Total coli-forms
				mg l ⁻¹			CFU/100ml			
1	1.5	10.5	-	0.015	1.55	2.95	150	260	210	360
2	1.9	9.8	-	0.018	2.77	2.93	178	285	240	450
3	6.2	4.6	0.012	0.055	6.80	13.44	1020	1470	2300	3800
4	6.1	4.0	0.027	0.057	6.84	14.22	2600	3430	3100	6900
5	7.0	4.5	0.055	0.051	6.95	15.21	8200	9100	6350	11800
6	12.8	2.2	0.090	0.204	25.22	20.43	10800	10300	12460	25650
7	6.4	5.3	0.015	0.030	7.20	14.68	2450	6200	8300	5130
8	3.5	5.8	0.013	0.031	6.91	12.71	2120	3900	2200	3950
9	3.2	7.0	0.010	0.028	6.65	8.55	840	950	1145	2210

Note: PP – petroleum products; BOC₅ – biochemical oxygen consumption; CFU – Colony-forming units.

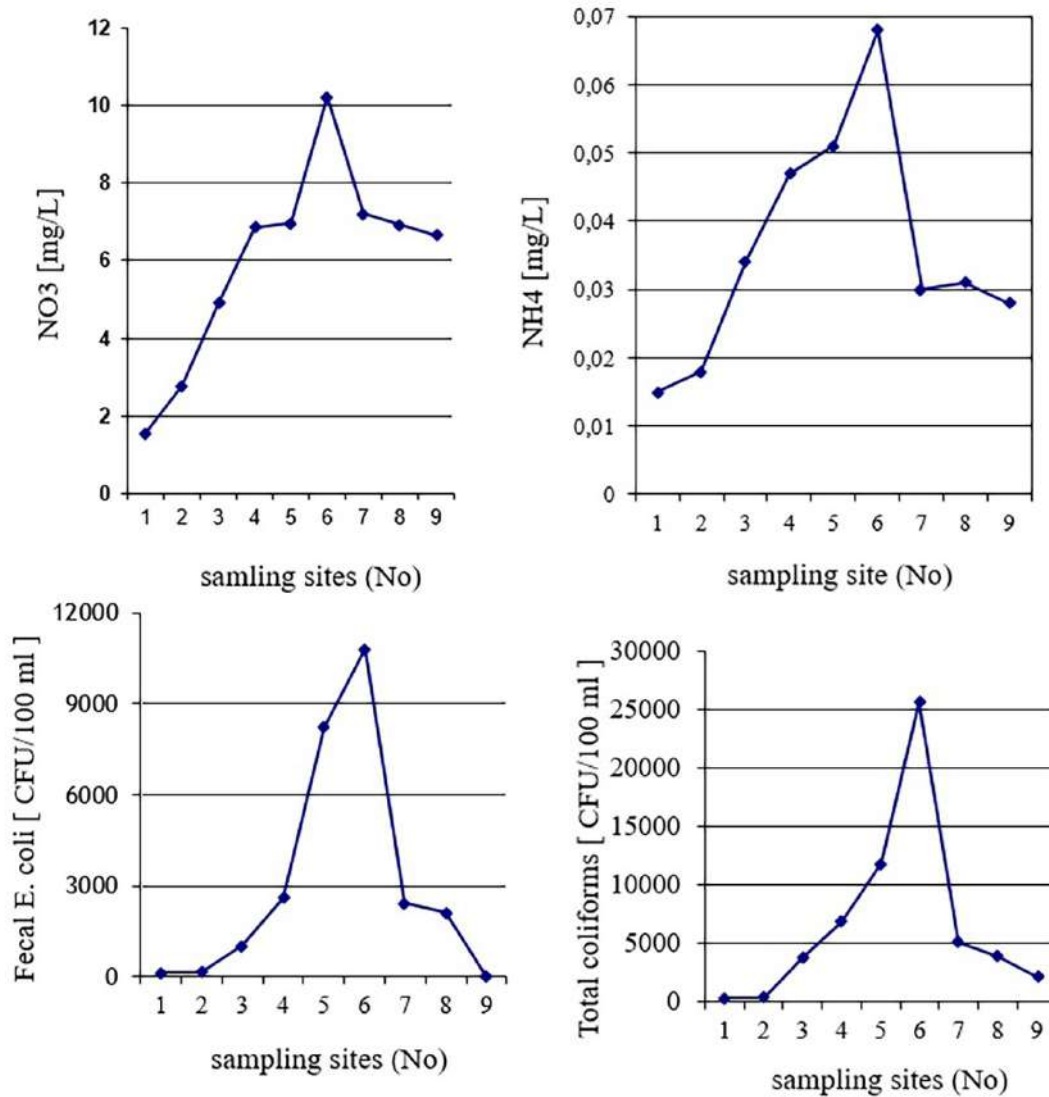


Fig. 3. Changes in the physico-chemical and microbiological indicators of water quality along the course of the Siret River within the territory of Ukraine

indicators was registered in points 1-2 of the water intake of the Siret river basin. These points are located in the upper part of the stream, where surface water enters mainly from the protected territories of the national natural park „Vyzhnytskyi”, and where there is a slight impact from economic activity. The studied territories are characterized by a sparse population of approximately 2,385 people. Previous research has demonstrated a strong positive correlation ($r = 0.95$) between biochemical oxygen demand (BOC), humic organic carbon (HOC), and microbiological indicators. Moving downstream, the river shows an increase in suspended matter content, leading to reduced dissolved oxygen levels and elevated BOC5 values. Additionally, there is an increase in chloride and nitrite concentrations (salts of hydrochloric and nitric acids). These findings suggest a rise in organic pollution in the river network, potentially indicating the presence of fecal compounds, particularly in areas with economic landscapes lacking functional treatment facilities. The observed differences in water quality indicators are primarily attributed to variations in land use, management practices of subsidiary farms, and the absence of centralized treatment facilities within the Siret River basin. The data are presented in Fig. 3. testify to the existence of spatial diversity of physico-chemical and microbiological indicators along the Siret River bed.

Thus, in the upper reaches of the basin (sampling points No. 1-2), where the population density is insignificant and the territories of the nature reserve fund prevail, the lowest concentrations of nitrogen compounds in the water occur and the minimum number of coliforms bacteria is recorded. The surface waters of this region are characterized by high transparency. Along the course, the value of these indicators increases. Deterioration of water quality at sampling points No. 3-4 is most likely caused by the placement of a developed network of private „green” tourism in the basin of the Sukhyy, Solonets, and Myhivka tributaries without the presence of appropriate cleaning spores. The maximum values of most of the investigated indicators were recorded below the sources of communal discharges in the city of Storozhynets,

where about 15,000 people live (Table 1). In this way, the differences in the values of water quality indicators obtained by us are mainly the result of land use, management of subsidiary farms and the absence of centralized treatment facilities in the Siret River basin. The classes of microbiological quality of water in the investigated bodies were determined on the basis of (Directive 2006/7/EC..., 2006), which regulates the quality of water for domestic purposes (Table 3).

Of the 9 water sampling points analyzed, only 2 points (1, 2) were characterized by the best microbiological condition in terms of the level of *E. Coli* contamination. These are the territories of the headwaters of the sources of the Siret River, which are located mainly in the mountain and forest part of the protected zone of the Vyzhnytskyi National Nature Park, which can be compared to the reference ones. The worst microbiological quality of water occurs in densely populated areas (items 3-7), where there is no centralized system of municipal sewage treatment. Downstream, the microbiological quality slightly improves (item 9), which can be explained most likely by the reduction of anthropogenic pressure on the water ecosystem and the process of its spatial self-renewal.

The minimum physical and chemical indicators of watercourses are also recorded for these territories. The water pollution index (*WPI*) was calculated on the basis of physical and chemical parameters using six indicators (BOC_5 , O_2 , PP-petroleum products, NH_4^+ , NO_2^- , Cl^-) according to the formula (Snizhko, 2001).

$$WPI = \frac{1}{6} \sum_{i=1}^n \frac{C_i}{MPK_i} \quad (1)$$

where: C_i – the arithmetic average value of the water quality indicator, MPK – the maximum permissible concentration of the indicator. For O_2 , the MPK is divided by the average value of its concentration. The criteria for assessing the quality of surface water are presented in Table 4.

So, according to physical and chemical indicators, the surface waters of the Siret River basin, in accordance with the existing requirements

Table 3. Classes of microbiological quality of water

Sampling sities (N_0)								
1	2	3	4	5	6	7	8	9
Excellent quality		Poor quality					Sufficient	

Table 4. Criteria for assessing surface water quality (Snizhko, 2001)

Water quality class	I	II	III	IV	V	VI	VII
Characteristics of the class	Very clean	Clean	Moderately polluted	Polluted	Dirty	Very dirty	Extremely dirty
Value of the water pollution index (WPI)	< 0.30	0.31-1.00	1.01-2.50	2.51-4.00	4.01-6.00	6.01-10.00	> 10.00

Table 5. Classes of physico-chemical quality of water

Sampling site (N_p)	1	2	3	4	5	6	7	8	9
Value of the water pollution index (WPI)	0.28	0.37	1.01	1.04	1.09	2.66	1.01	0.78	0.70
Quality classes of water	I	II	III	III	III	IV	III	II	II

of Ukrainian standards [24, 25, 27], belong to the 4th class of water quality. The waters of the upper reaches of the basin and sparsely populated border areas belong to the first I and II classes. The water pollution index varies for these areas in the range of 0.28–0.78, which corresponds to the national classification of very clean and clean water classes. Sampling points (3, 4, 5, 6, 7), located in the zone of active anthropogenic activity and urban landscapes, belong to III and II water quality classes (polluted and dirty water). It should be noted that the existing floodwater monitoring system in Ukraine does not provide for the determination of microbiological indicators (Pro zatverdzhennia Poriadku..., 2018). The above assessment of the Siret River basin according to microbiological indicators, in accordance with the requirements of the EU Water Framework Directive (Directive 2006/7/EC..., 2006), made it possible to compare the results obtained according to physico-chemical and microbiological indicators. The results of the conducted research indicate that water sampling points 3–8 are characterized by an unsatisfactory ecological condition, both in terms of physico-chemical and microbiological indicators.

CONCLUSIONS

For the first time, the sanitary and ecological condition of the upper reaches of the Siret River (Danube basin) on the territory of Ukraine was analyzed. 6 physico-chemical and 4 microbiological indicators were studied in 9 sampling sites. The conducted studies showed that the main cause of physical, chemical and microbiological pollution of the Siret River basin within the territory of

Ukraine is the lack of centralized treatment facilities in the region. The greatest pollution was observed in urbanized regions. The lowest values of the studied indicators were observed in the upper reaches of the Siret River basin, located mostly in the territory of the Vyzhnytskyi National Nature Park and mountainous sparsely populated areas with little anthropogenic influence. The obtained microbiological indicators indicate significant water pollution in a larger number of sampling sites than according to physical and chemical indicators. A comparative analysis of the obtained results with the results of research conducted in the lower part of the Siret River basin in the area of Galati (Romania) was carried out. Taking into account the cross-border nature of the studied territory, the use of sanitary and microbiological indicators in the monitoring system of the upper part of the water ecosystem of the Siret River is urgent.

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