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Bacterioplankton of the Dniester River and its right tributaries on the territory of the Republic of Moldova in the 2024 vegetation period

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Abstract. The article presents the results of research of bacterioplankton communities in the lotic ecosystems of the Dniester River basin on the Republic of Moldova territory during the vegetation period (spring - summer - autumn) of 2024. The quantitative parameters of bacterioplankton physiological groups in the right tributaries of the Dniester - the rivers Răut, Bâc and Botna - were studied in comparison with the state of these communities in the Dniester upstream and downstream of the confluence. Water quality, assessed by the presence of representatives of physiological groups and their numerical composition in the studied ecosystems, ranged from class I (Dniester upstream of the confluence) to class III (Dniester downstream of the confluence with the River Bâc) and classes IV-V (the River Bâc).

Keywords: the rivers Dniester, Răut, Bâc and Botna, water quality, physiological groups of bacterioplankton.

Bacterioplanctonul fluviului Nistru și afluenților de dreapta de pe teritoriul Republicii Moldova în perioada de vegetație 2024

Rezumat. Articolul prezintă rezultatele cercetărilor comunităților de bacterioplancton din ecosistemele lotice ale bazinului fluviului Nistru pe teritoriul Republicii Moldova în perioada de vegetație (primăvară - vară - toamnă) a anului 2024. Parametrii cantitativi ai grupurilor fiziologice de bacterioplancton din afluenții de dreapta ai Nistrului, râurile Răut, Bâc și Botna, au fost studiați în comparație cu starea acestor comunități din Nistru în amonte și în aval de confluență. Calitatea apei, evaluată prin prezența reprezentanților grupelor fiziologice și componența lor numerică în ecosistemele studiate, a variat de la clasa I (Nistru în amonte de confluență) la clasa III (Nistru în aval de confluența cu râul Bâc) și clasele IV-V (râul Bâc).

Cuvinte-cheie: râurile Nistru, Răut, Bâc și Botna, calitatea apei, grupe fiziologice ale bacterioplanctonului.

1. Introduction

In temperate climates, the vegetation season is the period from the last frost in spring to the first frost in autumn. As for the Eastern Plains Ecoregion, where the Dniester

River basin study area is located, the hydrological vegetation period is associated with the attainment of water temperatures of 3-5°C [1].

The transboundary Dniester River, flowing from the Carpathian Mountains, is one of the main sources of water in the Republic of Moldova and is essential for water supply to the population and economic agents of the country. The largest right-bank tributaries of the Dniester in the Moldovan part of the basin are the rivers Răut, Bâc and Botna, which are 286, 155 and 152 kilometres long, respectively. Under current climate change conditions, when hydrological droughts in the Republic of Moldova occur not only in summer, but also in spring and autumn, the flow of these rivers is significantly reduced and sometimes completely disappears. In the summer-autumn expeditions of 2024, carried out by the Laboratory of Hydrobiology and Ecotoxicology at the mouth of the Botna River, the absence of water flow was recorded - the riverbed was dry.

The water of the Dniester tributaries is intensively used by the population, including for fishing and irrigation of household lands [2], despite the fact that their ecological status does not correspond to the water quality for these purposes. For example, in terms of oxygen saturation, the water of the River Bâc in March 2024 was worse than water quality class V. According to the concentration of biodegradable organic substances (BOD_5), the water of this tributary at the sampling point Gura-Bâcului was qualified as a heavily polluted wastewater effluent [3]. The Răut River also has a significant impact on the ecological state of the Dniester River [4]. Given that the mouth of the Răut is located 19 km upstream from the pumping station for the water supply of the Chisinau municipality, it is obvious that the water quality of this tributary makes it difficult to treat it to drinking water standards [5].

The main role in the process of water self-purification in surface aquatic ecosystems belongs to bacterial communities, whose composition is formed under the influence of several factors, including the allochthonous microflora of tributary runoff. The aim of this work is to study the seasonal dynamics of bacterioplankton communities in the right tributaries of the Dniester, the Răut, Bâc and Botna rivers, in comparison with the state of these communities in the Dniester River upstream and downstream of the confluence.

2. Materials and Methods

During the 2024 hydrological vegetation period (March-October) comprehensive expeditions were organized in the Dniester River basin, when water samples and biological material were collected from the rivers Dniester, Raut, Bâc, and Botna. The water samples for microbiological studies were collected in sterile 500 mL bottles from the water stratum at 0.25 m depth. This paper presents the results of 27 samples in which the following parameters were determined: total number of bacteria (N_{tot}, million cells mL^{-1}); number of saprophytic microorganisms (N_{sap}, thousand CFU[1] mL^{-1}); bacterial destruction of organic matter by heterotrophs (R, cal L^{-1} 24 h^{-1}); and number of representatives of bacterioplankton physiological groups, such as ammonifiers, denitrifiers, solubilizers of phosphorus compounds, and microorganisms oxidizing starch, cellulose, phenolic compounds and petroleum products.

The total number of bacteria (N_{tot}) was determined at the cellular level by the Razumov method, which consists of direct microscopy of bacteria fixed on a filter membrane (pore size 0.2 µm) and stained with 5 % erythrosine solution. The number of saprophytic microorganisms (N_{sap}) was determined at colony level by the incorporation method. The presence in the analyzed sample of microorganisms from other ecophysiological groups was also determined at the colony level by inoculating a natural sample (or its decimal dilutions) on elective culture media (Figure 1). Determination of bacterial destruction is based on two procedures: determination of the total number of bacteria by the Razumov method and determination of the oxygen consumed by bacterioplankton (after 24 h of exposure under conditions similar to the water body under study) by the Winkler method [6]. The counting of microorganisms at colony level was performed according to national standards SM EN ISO 8199:2015 [7] and SM SR EN ISO 6222:2014 [8].

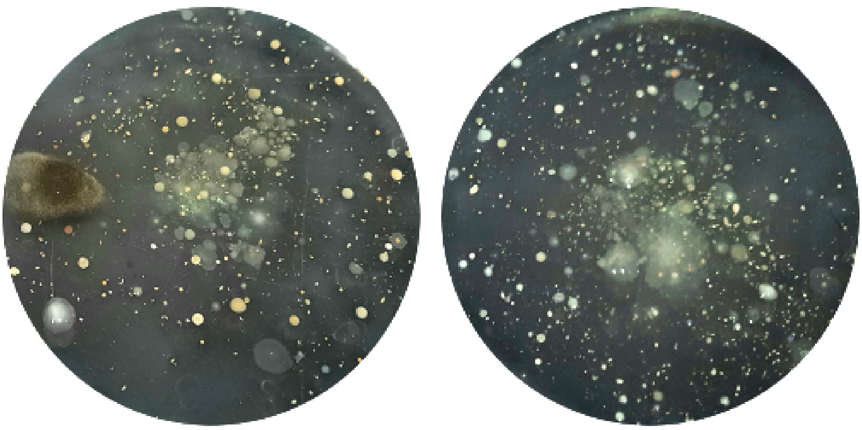

Figure 1. (black background). Colonies of heterotrophic microflora on the solid elective nutrient media inoculated by the pour-plate technique and incubated inverted at 25ºC: the left sample is from the River Botna, the right one is from the River Răut

[1]CFU – colony forming units

The quality indicators for bacterioplankton (Table 1) are included in Annex 1 of the *Regulation on quality requirements for surface waters* [9], which contains the limit values of five water quality classes for 90 indicators, including hydrobiological parameters.

Table 1. Limit values of the quality indicators of bacterioplankton.

Quality indicators	Water quality classes				
	I	II	III	IV	V
Total bacterioplankton, million cells/mL	1.0	2.0	5.0	7.0	>7.0
Saprophytic Bacterioplankton, thousand CFU/mL	0.5	2.5	5.0	7.5	>10

3. Results and Discussions

The structure and abundance of microbial communities in fluvial aquatic ecosystems are closely related to the natural factors of the habitat (climatic conditions, hydrological and temperature regimes, presence of biogenic elements and organic matter, consumption of microorganisms by other hydrobionts ("top-down limitation"), as well as anthropogenic factors such as wastewater discharge, presence of toxic substances, etc.). The temperature of the habitat determines the normal course of metabolic processes and, through them, the growth and multiplication of microbial biota [10]. Considering the fact that the beginning of the vegetation is associated with reaching the water temperature of 3-5 °C, the vegetation period in the Dniester River basin on the territory of the Republic of Moldova in 2024 has already started in February [3]. The spring expeditions were carried out in the end of March - beginning of April, when the water temperature in the studied watercourses varied between 8 and 16 °C. In August, this variation was 23-26 °C; in October, the water temperature decreased to 16–18 °C (ECOTOX project database, 2024). The seasonal dynamics of N_{tot} and N_{sap} parameters directly correlate with seasonal variations in water temperature. The results obtained during the vegetation period of 2024 are presented in Table 2 with the indication of the water quality class (WQC) of the studied water bodies according to the *Regulation* [9].

The presented data demonstrate that the total number of bacteria in the Răut River varied between 0.6 and 1.4 (mln cells/mL) during the vegetation period, with the highest values in the summer season, which corresponds to the water quality classes I-II. The determination of saprophytic microorganisms showed a wider range of fluctuations, i.e. 1.0-8.8 (thousand CFU/mL), which corresponds to the WQC II-V. After confluence with the Răut River, the values of these quantitative parameters of bacterioplankton in the Dniester water increase by 1.2-1.5 times. Before the confluence with the Bâc River, these

Table 2. The 2024 seasonal dynamics of bacterioplankton quality indicators in the water of the rivers Răut, Bâc, Botna and Dniester upstream and downstream of the confluence.

Location	Season	N_{tot}, mln cells/mL	WQC	N_{sap}, thous. CFU/mL	WQC	R cal/L/24 h
Dniester upstream of the Răut River	Spring	0.8	I	0.60	I	2.21
	Summer	1.1	II	2.86	III	2.64
	Autumn	0.5	I	1.73	II	1.70
Răut River, confluence zone	Spring	1.1	II	1.00	II	1.30
	Summer	1.4	II	8.80	V	3.92
	Autumn	0.6	I	2.12	II	1.38
Dniester downstream of the Răut River	Spring	0.9	I	0.90	II	1.30
	Summer	1.3	II	3.20	III	1.75
	Autumn	0.6	I	2.00	II	1.38
Dniester upstream of the Bâc River	Spring	0.6	I	2.60	II	2.91
	Summer	1.7	II	4.50	III	2.36
	Autumn	0.6	I	1.34	II	3.80
Bâc River, confluence zone	Spring	9.6	V	2.40	II	0.01
	Summer	10.0	V	42.5	V	8.42
	Autumn	6.2	IV	45.0	V	3.80
Dniester downstream of the Bâc River	Spring	0.8	I	2.40	II	1.33
	Summer	4.0	III	10.87	V	4.65
	Autumn	1.0	I	3.92	III	0.92
Dniester upstream of the Botna River	Spring	0.8	I	2.22	II	0.88
	Summer	1.6	II	4.84	III	1.98
Botna River, confluence zone	Spring	1.1	II	2.52	II	8.70
	Summer	There was no water in the riverbed				
	Autumn	There was no water in the riverbed				
Dniester downstream of the Botna River	Spring	0.5	I	1.61	II	0.01
	Summer	1.6	II	4.84	III	1.98
	Autumn	1.0	I	1.50	II	1.93

indicators in the Dniester correspond to WQC II and III. After the confluence with the Bâc River (whose water quality throughout the vegetation period varies between classes IV and V), the water quality of the Dniester in terms of bacterioplankton indicators decreases to classes III and IV. The study of bacterioplankton in the Botna River was assessed only in spring, because there was no water in the riverbed when the August and October expeditions were carried out. The water quality of the Dniester upstream and downstream of the Botna River, in terms of bacterioplankton indicators, varied between classes I-II (N_{tot}) and II-III (N_{sap}).

Table 3. Quantitative values (thousand CFU/mL) of the main physiological groups of bacterioplankton in the water of the rivers Raut, Bâc, Botna and Dniester in the 2024 vegetation season.

Location	Season	N_1	N_2	N_3	N_4	N_5	N_6	N_7
Dniester upstream of the Răut River	Spring	0.60	0.03	0.50	0.70	0.08	0.094	0.023
	Summer	1.98	0.20	0.95	1.50	0.20	0.308	0.395
	Autumn	1.58	4.80	10.00	2.80	0.10	0.029	1.472
Răut River, confluence zone	Spring	1.00	0.12	0.80	2.00	0.13	0.047	0.078
	Summer	5.90	1.40	7.20	10.00	1.00	0.505	0.560
	Autumn	3.00	6.40	13.00	3.50	0.28	0.309	1.046
Dniester downstream of the Răut River	Spring	0.90	0.03	1.20	2.20	0.09	0.147	0.031
	Summer	2.90	1.00	4.50	3.50	0.50	0.297	0.475
	Autumn	2.00	5.20	12.00	3.00	0.20	0.032	0.676
Dniester upstream of the Bâc River	Spring	1.50	0.45	1.30	0.90	0.15	0.162	0.028
	Summer	3.50	0.75	1.85	4.00	0.80	0.248	0.886
	Autumn	-	-	-	-	-	0.037	0.704
Bâc River, confluence zone	Spring	2.50	2.30	9.00	4.0	1.00	2.990	0.096
	Summer	35.0	6.40	15.50	25.0	1.50	10.074	10.000
	Autumn	25.0	35.00	40.00	25.0	2.00	0.946	1.264
Dniester downstream of the Bâc River	Spring	3.00	0.00	3.50	0.80	0.19	1.470	0.040
	Summer	9.00	1.50	4.80	11.00	1.30	0.236	0.341
	Autumn	3.82	10.00	15.00	4.80	1.50	0.084	0.796
Dniester upstream of the Botna River	Spring	3.20	1.20	2.06	1.10	0.11	0.200	0.037
	Summer	2.80	1.30	2.00	2.30	0.10	0.036	0.948
Botna River, confluence zone	Spring	6.52	3.00	5.75	1.90	0.10	0.050	0.012
	Summer	There was no water in the riverbed						
	Autumn	There was no water in the riverbed						
Dniester downstream of the Botna River	Spring	2.50	1.40	5.10	0.80	0.06	0.153	0.034
	Summer	3.00	1.00	2.00	2.50	0.70	0.162	0.422
	Autumn	1.32	2.90	4.80	3.82	0.21	0.098	0.952

N_1 – ammonifiers, N_2 – denitrifiers, N_3 – phosphate-solubilizing MOs, N_4 – amylolytic MOs, N_5 – cellulosolytic MOs, N_6 – phenol-oxidizing MOs, N_7 – hydrocarbon-oxidizing MOs

Due to the presence of different enzymatic systems, bacterioplankton play a crucial role in the processes of destruction of autochthonous and allochthonous organic matter in aquatic ecosystems, thus ensuring the process of self-purification and the circulation of elements in the habitat. A comparative analysis of bacterial degradation in the Dniester River and its tributaries Răut, Bâc, and Botna reveals a clear trend: higher values of destruction coincide in most cases with a higher number of saprophytic microorganisms. Thus, we can conclude that degradation of organic matter by bacterioplankton is more efficient in more polluted areas. In cases of deviations from this trend, we can assume the influence of interfering factors, e.g., toxic substances.

The quantitative variations in abundance of the physiological groups of microorganisms that provide biochemical transformation of nutrients in the studied aquatic ecosystems are extremely diverse (Table 3). Their abundance is significantly higher in tributaries, which is due to their increase in the Dniester River downstream of the confluence.

As can be seen from the data presented, ammonifiers are present in all processed samples. They ranged from thousands to tens of thousands CFU/mL in the tributaries and from hundreds to thousands in the Dniester upstream and downstream of the confluence. A seasonal trend was observed, showing an increase in numerical values of ammonifiers in warmer months. This is more visible in the tributaries water, where nutrients are constantly available for their development. The process of denitrification in the studied ecosystems is also intense. The number of denitrifiers ranged between 0.12-35.0 thousand CFU/mL in tributaries and 0.03-10.0 thousand CFU/mL in the Dniester. The number of denitrifiers in the Dniester water downstream of the confluence zone was 2-5 times higher than their number upstream of the confluence.

The microbial cycling of phosphorus is carried out by numerous bacteria and fungi. The number of phosphate solubilizing bacteria varied in the tributaries from 0.5 to 40.0 thousand CFU/mL. The most intense phosphorus transformation processes take place in the Bâc River, which leads to an increase in their number in the Dniester River. Amylolytic microorganisms hydrolyze starch accumulated in plant and animal detritus. Their content in the studied tributaries is quite high, namely 0.6-50.0 thousand CFU/mL. The maximum number was recorded in the summer and autumn samples from the Bâc River.

Phenol- and hydrocarbon-oxidizing bacteria were recorded in all processed samples, which indirectly indicates the presence in the water of phenol compounds (formed by the decomposition and mortality of hydrobionts and aquatic vegetation), and petroleum products, which come with stormwater runoff from urban areas, as well as wastewater. The variation range of phenol-oxidizing MOs is quite wide: from tens in the Dniester water to thousands of CFU/mL in the Bâc River. According to *Vinogradov's* classifier

[6], the water quality of the Dniester tributaries in terms of the numerical content of phenol-oxidizing MOs varies in the range of classes III and V. The Dniester water quality downstream of the confluence varies between classes II and IV. Regarding the presence of hydrocarbon-oxidizing MOs in the analyzed samples, a similar trend is observed (Figures 2-3).

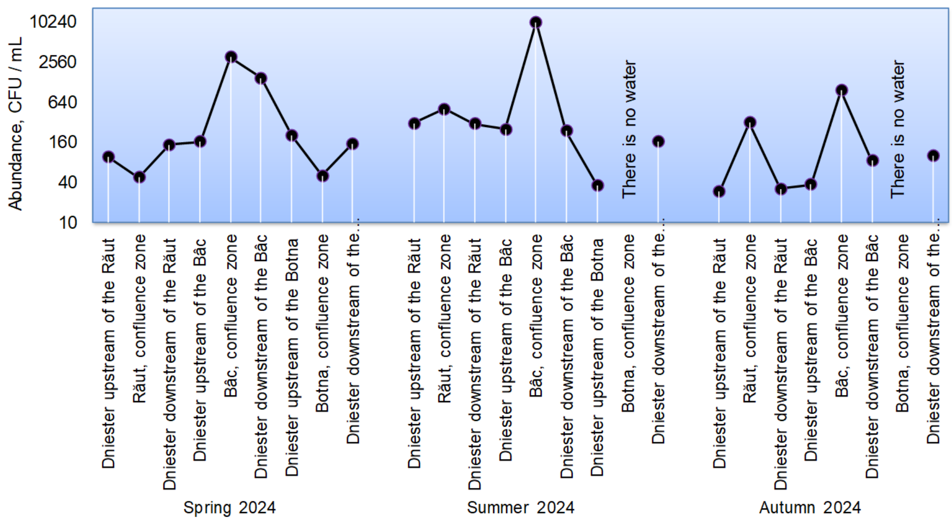

Figure 2. Abundance of phenol-oxidizing microorganisms in the water of the rivers Raut, Bâc, Botna and Dniester upstream and downstream of the confluence.

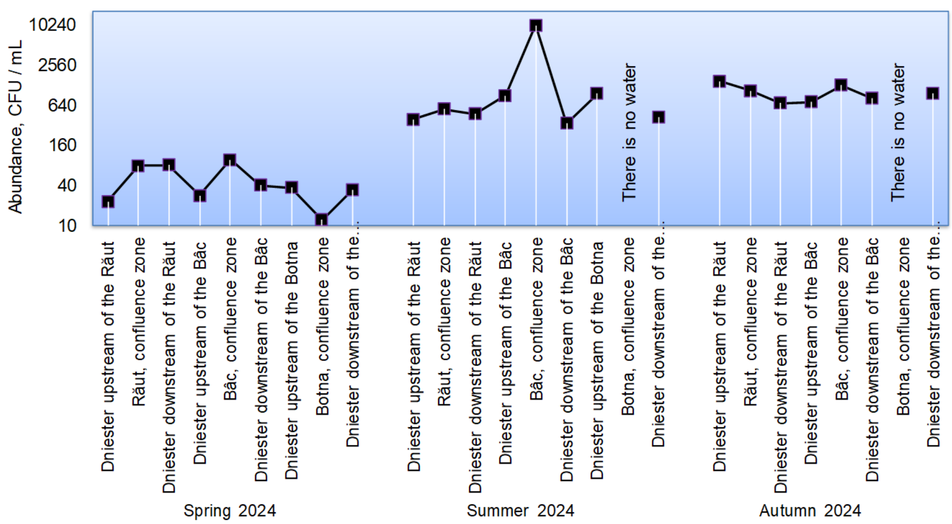

Figure 3. Abundance of hydrocarbon-oxidizing microorganisms in the water of the rivers Raut, Bâc, Botna and Dniester upstream and downstream of the confluence.

The high number of phenol-oxidizing microorganisms indicates that secondary pollution processes are taking place in River Bâc, caused by the decomposition of organic wastes in bottom sediments. The seasonal dynamics of hydrocarbon-oxidizing MOs in the summer months coincide with the dynamics of phenol-oxidizing microflora. But the number of these microorganisms in the fall samples does not reveal a significant difference in the Dniester and its tributaries. This was a consequence of abundant precipitation in September, due to which the allochthonous pollution with petroleum products passed in transit, but in the Răut and Bâc rivers there was secondary contamination with phenolic products as a result of the decomposition of bottom sediments.

4. Conclusions

The current microbiological state of the right tributaries (the rivers Răut, Bâc, and Botna) is unsatisfactory, which significantly affects the water quality of the Dniester River on the territory of the Republic of Moldova. Analyzing the obtained results, the following conclusions can be formulated:

(1) In the 2024 vegetation period (March-October), the total number of bacteria ranged from 0.6 to 1.4 (mln cells/mL) in the bacterioplankton of the Răut River, from 9.6 to 10.0 (mln cells/mL) in the Bâc River, and from 0.6 to 2.3 (mln cells/mL) in the Botna River.

(2) In the Dniester River, the total number of bacteria upstream of the River Bâc averaged 1.0 mln cells/mL, while downstream it was more than twice as high. In other cases, the difference of this parameter in Dniester water upstream and downstream of the confluence with the rivers Răut and Botna was non-significant.

(3) Bacterial destruction in the investigated ecosystems varied over a wide range of 0.01-8.70 (cal/L/24 h), indicating a significant variability of the biodegradable compound concentrations in the Dniester River and its right tributaries.

(4) The abundance of saprophytic bacterioplankton in the Răut, Bâc and Botna rivers varies in a wide range from 0.6 to 45.0 thousand CFU/mL. The maximum value of the parameter - 45.0 thousand CFU/mL - was recorded in the River Bâc. In the Dniester River downstream of the confluence, the number of saprophytic microorganisms increases up to 4.0 thousand CFU/mL.

(5) Water quality in the studied ecosystems varied between classes II-V in terms of quantitative parameters of bacterioplankton.

(6) Phenol- and hydrocarbon-oxidizing microorganisms were recorded in all samples from the Dniester River and its right tributaries. Water quality ranged between

classes II and V for microorganisms oxidizing phenol compounds, and between classes III and V for microorganisms oxidizing hydrocarbons.

(7) The distribution of functional bacterioplankton in the studied ecosystems has a varied dynamic, determined by the content of biogenic substrates of autochthonous and allochthonous origin. The most representative in terms of numbers are ammonifiers, amylolytic, and phosphate-solubilizing microorganisms, capable for decomposition of substances, which include nitrogen, carbon, and phosphorus, derived from plant and animal detritus, corpses, excreta and other residues that are constantly present in aquatic ecosystems. Depending on the number of these representatives in bacterioplankton communities, the surveyed rivers can be arranged as follows: the Bâc River → the Botna River → the Răut River → the Dniester River after confluence.

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