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SPATIAL VARIATION OF MICROBIAL COMMUNITIES IN THE BAHLUI RIVER ACROSS THE URBAN AREA OF IASI

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Abstract

The Bahlui River, flowing through the urban area of Iași, is subjected to significant anthropogenic pressures that influence its microbial communities. This study assessed the structure and temporal dynamics of microbial populations along a 24 km urban stretch of the river, using six sampling sites distributed from the river's entry into the city to its exit. Water samples collected in April and July 2025 were analyzed for total bacterial counts, Gram-positive bacteria (G^+), and micromycetes. Results revealed significant spatial and temporal variations: total bacterial and Gram-positive abundances increased from April to July, with point 3 consistently exhibiting the highest counts, while micromycete abundance was lower but also displayed site-specific and seasonal variation. Two-way ANOVA confirmed that both sampling location and period significantly influenced microbial abundance, with interactions indicating non-uniform temporal changes along the river. These findings highlight the combined effects of urbanization and seasonal dynamics on microbial community structure and provide valuable insights for monitoring and managing urban aquatic ecosystems.

Key words*: aquatic microbiology, Bahlui river, DPSIR, microbial communities, spatial variation, urban river.*

INTRODUCTION

Water bodies, such as rivers that traverse urban areas, constitute vulnerable aquatic ecosystems, as they are subjected to significant anthropogenic pressures resulting from direct or indirect human activities (Teurlincx et al., 2019). Urban rivers are subjected to several categories of anthropogenic pressures (Castro et al., 2021), including chemical pollution, resulting from the input of nutrients, heavy metals, organic compounds, pharmaceuticals, antibiotic residues, and microplastics (Mandaric et al., 2018; Sekudewicz et al., 2021; Tiwari et al., 2022; Mansor et al., 2024); microbiological contamination, associated with wastewater discharges (Olds et al., 2018; Cui et al., 2019; Jing et al., 2024); hydromorphological alterations, caused by channel modifications, embankments, and bank impermeabilization (Ollero et al., 2015; Borek et al., 2022); and thermal pollution, arising from industrial warm-water effluents (Briciu et al., 2020; Chakraborty, 2021).

These types of pressures need to be carefully investigated, as they can cause significant changes in the structure, diversity, and dynamics of aquatic microbial communities. Given the essential role of the watermicrobiota in organic matter decomposition (Liu et al., 2023), nutrient recycling (Ávila et al., 2019), and the regulation of biogeochemical cycles (Pan et al., 2024), its disruption can lead to functional imbalances in the ecosystem, a reduced natural self-purification capacity of the rivers, and impaired health of the aquatic ecosystems (Xie et al., 2021; Singh et al., 2025).

In the urban area of Iași Municipality, the Bahlui River, the municipality's main hydrographic collector, exhibits significant changes in the physico-chemical and biological parameters of the water (Zaharia, 2015; Marcoie et al., 2025). These transformations represent the cumulative result of the expansion of urban infrastructure, wastewater discharges, and inputs originating from industrial, residential, and agricultural activities carried out within the basin corresponding to the urban sector.

The ecological status assessment of the Bahlui River should be conducted through the integration of data within the DPSIR framework (Drivers–Pressures–State–Impact–Response), facilitating the correlation between socio-economic driving forces, the

pressures exerted on the ecosystem, and the associated impacts. Previous studies have demonstrated that the DPSIR framework provides a holistic approach for evaluating the overall ecological effects of polluted urban rivers (Skoulikidis et al., 2009; Lu et al., 2019).
In this context, the Bahlui River represents a relevant case study for the systematic application of the DPSIR framework in an urban environment.
Within this framework, the ecological dynamics of the urban river sector can be structured as follows: **Drivers** are represented by positive demographic growth and the accelerated urban expansion of Iași and its surrounding areas (Holobâcă et al., 2022), reflected in intensified economic activities and the expansion of impervious surfaces, which have significantly altered the hydrological regime. These processes generate **Pressures**, including increased domestic and industrial wastewater discharges (Marcoie et al., 2024), nutrient inputs (nitrates and phosphates) from urban runoff and diffuse agricultural sources (Neamtu et al., 2009; Oiște & Breabăn, 2011), microbiological contamination associated with insufficient or malfunctioning sewage systems, as well as the occurrence of emerging contaminants such as pharmaceutical residues, antibiotics, and microplastics (Apostol, 2008; Zaharia, 2015). Consequently, the **State** of the river ecosystem is altered, particularly through shifts in microbial community structure along the pollution gradient, characterized by reduced diversity and functional modifications of key biogeochemical cycles (e.g., nitrogen and carbon cycling). These changes lead to **Impacts** manifested as ecosystem imbalance, degradation of ecological integrity, and potential public health risks. Therefore, appropriate **Responses** should include continuous microbiological and physicochemical monitoring at the watershed scale, modernization of wastewater treatment infrastructure, and the implementation of integrated urban water management strategies aimed at improving the ecological status of the Bahlui River within the broader framework of sustainable urban development.
The present study investigates changes in microbial communities of the Bahlui River along the sector that crosses the city of Iași. While numerous studies have addressed hydrological, physicochemical, and general water quality aspects of the river, research specifically focused on the dynamics and structure of microbial communities remains limited. This gap in the literature underscores the need for an integrated microbiological approach capable of revealing functional and structural alterations in the river ecosystem. Accordingly, the present research provides a scientific basis for future studies aimed at assessing hydrological sustainability in anthropized environments, contributing to the development of monitoring and management strategies tailored to urban river systems.

MATERIALS AND METHODS

Study area

The Bahlui River, the main watercourse traversing the city of Iași, has a total length of approximately 119 km. It originates at an altitude of around 500 m in Tudora commune, Botoșani County, and flows into the Jijia River near the village of Chiperești, Iași County (Figure 1).



Figure 1. Hydrographical overview of the Bahlui River, showing the source (red circle), river course (orange line), and confluence with the Jijia River (blue triangle) (map generated from www.openstreetmap.org)

Along its course, the Bahlui River passes through a series of settlements, the most significant of which are Hârlău, Podu Iloaiei, and the municipality of Iași. Within Iași and its surrounding localities, the river spans approximately 28 km, between Lețcani and Chiperești. This stretch represents the focus

area of the present study. Along this segment, the population density is high, economic activities are intensive, and extensive impervious surfaces exist, all of which have increased the river's susceptibility to anthropogenic pressures (Figure 2).



Figure 2. Geographical positioning of the sampling points (1-6) along the Bahlui River (map generated from www.openstreetmap.org)

The sampling points were distributed along a 24 km river course, following the longitudinal course of the Bahlui River through the urban area of Iași. The inter-site distances varied between 2.8 km and 6.5 km, reflecting differences in urban pressure intensity and hydromorphological conditions along the river continuum. This spatial configuration allows for the evaluation of cumulative anthropogenic effects and the identification of potential shifts in microbial community structure along the upstream–downstream gradient (Table 1).

Table 1. Gheographic coordinates of the sampling points sites along the Bahlui rivers and distances between consecutives sites

Sampling points	Coordinates		Distance	
	Latitude (N)	Longitude (E)	Distance from previous sampling points (km)	Cumulative distance (km)
1	47°11'05.6"	27°24'58.3"	-	0.0
2	47°10'06.4"	27°28'13.1"	≈4.5	≈4.5
3	47°10'10.4"	27°32'20.9"	≈6.1	≈10.6
4	47°09'10.8"	27°35'46.1"	≈4.1	≈14.7
5	47°09'02.8"	27°38'31.1"	≈2.8	≈17.5
6	47°08'26.5"	27°41'30.2"	≈6.5	≈24.0

Sampling

Water sampling was performed twice in 2025. The first sampling moment took place on April 21, 2025 (T1), followed by a second one conducted approximately three months later, on July 14, 2025 (T2). Water samples were collected from each of the six sampling points for microbiological analyses. At each site, a total of 108 mL of surface water was obtained (six tubes × 18 mL per tube) from approximately 15 cm below the water surface (Figure 3). To ensure spatial representativeness across the river cross-section, subsamples were collected as follows: two near the left bank, two from the mid-channel, and two near the right bank.



Figure 3. Water sampling procedure

Sterile tubes were used for sample collection. After sampling, the tubes were stored in a refrigerated container to ensure optimal transport conditions to the laboratory. Sample processing was performed on the same day as collection.

Microbiological analyses

Microbiological analyses of the water samples were performed using conventional culture-based methods (plate technique). For each sample, five replicates were analyzed. For microbial enumeration, 1 mL of river water, as well as 1 mL from serial decimal dilutions (up to 10^{-3}), was inoculated onto the appropriate culture media using the pour plate method. Total bacterial counts were determined using the 10^{-1} dilution, as undiluted samples resulted in overgrown plates (>400-500 bacterial colonies per plate), whereas higher dilutions (10^{-2} and 10^{-3}) produced low colony numbers (1-20 colonies per plate), outside the optimal counting range. Bacterial counts were determined on Plate Count Agar (PCA, Scharlau, Spain, 23.5 g L^{-1}) after incubation at 37°C for 24 h. Colonies were enumerated using an automatic colony counter (Interscience, Scan 1200), and results were expressed as colony-forming units per milliliter (CFU mL^{-1}). Selective media were used to differentiate microbial groups.

Gram-positive bacteria (G^+) were enumerated on PCA supplemented with streptomycin (35 mg L^{-1}) using undiluted river water samples.
Filamentous fungi were determined by inoculating undiluted samples onto Potato Dextrose Agar (PDA, Scharlau, Spain, 39 g L^{-1}) supplemented with Rose Bengal (35 mg L^{-1}) to limit colony overgrowth and facilitate counting. Fungal plates were incubated at 28°C for 3-5 days and monitored periodically according to colony growth rate.

Statistical analyses

Experimental data were analyzed using two-way analysis of variance (ANOVA) to evaluate the effects of sampling point (Sample), sampling moment (Moment), and their interaction on microbial counts. Microbial counts were log-transformed prior to analysis to improve normality. Homogeneity of variances was tested using Levene's test. Statistical analyses were performed using IBM SPSS Statistics 26, and differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Microbiological analyses revealed significant variations in the total number of bacteria, Gram-positive bacteria (G^+), and total number of micromycetes along the six sampling points established on the Bahlui River course, as well as between the two investigation periods.
To further quantify these spatial and temporal variations, the total bacterial abundance at each sampling point and investigation period was determined (Table 2).

Table 2. Total bacterial counts across sampling points and investigation periods on the Bahlui River

Sampling point	Time	Total number of bacteria (mean±SD)
1	T1	$1.0 \times 10^3 \pm 0.17$
	T2	$2.1 \times 10^3 \pm 0.36$
2	T1	$1.2 \times 10^3 \pm 0.16$
	T2	$2.3 \times 10^3 \pm 0.35$
3	T1	$4.4 \times 10^3 \pm 0.05$
	T2	$5.7 \times 10^3 \pm 0.43$
4	T1	$0.9 \times 10^3 \pm 0.07$
	T2	$2.2 \times 10^3 \pm 0.33$
5	T1	$1.2 \times 10^3 \pm 0.06$
	T2	$2.5 \times 10^3 \pm 0.35$
6	T1	$2.4 \times 10^3 \pm 0.13$
	T2	$4.0 \times 10^3 \pm 0.45$

The results show that total bacterial counts increased at all sampling points from T1 (April) to T2 (July). At T1, counts ranged from 0.9×10^3 CFU mL^{-1} at sampling point 4 to 4.4×10^3 CFU mL^{-1} at sampling point 3. By T2, counts rose across all locations, reaching a maximum of 5.7×10^3 CFU mL^{-1} at sampling point 3 and a minimum of 2.1×10^3 CFU mL^{-1} at sampling point 1. This indicates a clear temporal increase in bacterial abundance along the river. Two-way ANOVA confirmed that these differences were statistically significant. Significant variation was observed among sampling points ($F(5.48) = 180.70$, $p < 0.001$) and between sampling moments ($F(1.48) = 418.58$, $p < 0.001$). A significant interaction between sampling point and moment ($F(5.48) = 9.44$, $p < 0.001$) further indicates that the magnitude of temporal changes differed across locations (Table 3).

Table 3. Two-way ANOVA results for total bacterial counts across sampling points and investigation periods

Source	df	F	*p*
Sampling point	5	180.70	<0.001
Sampling moment	1	418.58	<0.001
Point x Moment	5	9.44	<0.001

Gram-positive (G^+) bacterial abundance was also analyzed at the six sampling points and two investigation periods (Table 4).

Table 4. Gram-positive bacterial counts across sampling points and investigation periods on the Bahlui River

Sampling point	Time	Gram-positive bacteria (mean±SD)
1	T1	$3.0 \times 10^1 \pm 0.55$
	T2	$5.2 \times 10^1 \pm 0.48$
2	T1	$1.3 \times 10^1 \pm 0.35$
	T2	$3.0 \times 10^1 \pm 0.27$
3	T1	$18.6 \times 10^1 \pm 3.54$
	T2	$23.5 \times 10^1 \pm 1.02$
4	T1	$2.9 \times 10^1 \pm 0.73$
	T2	$6.4 \times 10^1 \pm 0.47$
5	T1	$5.7 \times 10^1 \pm 0.45$
	T2	$8.6 \times 10^1 \pm 0.31$
6	T1	$10.6 \times 10^1 \pm 1.08$
	T2	$14.9 \times 10^1 \pm 0.76$

At T1, counts ranged from 1.3×10^1 CFU mL^{-1} at sampling point 2 to 18.6×10^1 CFU mL^{-1} at sampling point 3. By T2, all sampling points showed increases, with a maximum of 23.5×10^1 CFU mL^{-1} at sampling point 3 and a minimum of 3.0×10^1 CFU mL^{-1} at sampling

point 2. Sampling point 3 consistently showed the highest abundance, while sampling point 2 exhibited the lowest, indicating strong spatial heterogeneity. Two-way ANOVA revealed significant differences among sampling points (F (5.48) = 337.02, $p < 0.001$) and between investigation periods (F (1.48) = 196.83, $p < 0.001$). A significant interaction (F (5.48) = 7.14, $p < 0.001$) suggests that temporal changes in Gram-positive abundance differed depending on location (Table 5).

Table 5. Two-way ANOVA results for Gram$^+$ bacterial counts across sampling points and investigation periods

Source	Df	F	*p*
Sampling point	5	337.02	<0.001
Sampling moment	1	196.83	<0.001
Point x Moment	5	7.14	<0.001

To assess the fungal component of the microbial community, total micromycete abundance was determined at the six sampling points and two investigation periods (Table 6).

Table 6. Total micromycete counts across sampling points and investigation periods on the Bahlui River

Sampling point	Time	Total number of micromycetes (mean±SD)
1	T1	$1.2 \times 10^1 \pm 0.15$
	T2	$1.2 \times 10^1 \pm 0.15$
2	T1	$1.3 \times 10^1 \pm 0.05$
	T2	$1.4 \times 10^1 \pm 0.18$
3	T1	$1.2 \times 10^1 \pm 0.25$
	T2	$1.6 \times 10^1 \pm 0.10$
4	T1	$1.1 \times 10^1 \pm 0.09$
	T2	$1.3 \times 10^1 \pm 0.36$
5	T1	$1.4 \times 10^1 \pm 0.13$
	T2	$1.5 \times 10^1 \pm 0.23$
6	T1	$0.7 \times 10^1 \pm 0.15$
	T2	$1.3 \times 10^1 \pm 0.29$

The results show relatively low but significant spatial and temporal variation. At T1, micromycete counts ranged from 0.7×10^1 CFU mL^{-1} at sampling point 6 to 1.4×10^1 CFU mL^{-1} at sampling point 5. By T2, counts increased at most points, with a maximum of 1.6×10^1 CFU mL^{-1} at sampling point 3 and a minimum of 1.2×10^1 CFU mL^{-1} at sampling point 1. Two-way ANOVA confirmed that these differences were statistically significant (Table 7).

Significant variation was observed among sampling points (F (5.48) = 8.64, $p < 0.001$) and between sampling moments (F (1.48) = 22.09, $p < 0.001$). A significant interaction between sampling point and moment (F (5.48) = 4.25, $p = 0.003$) further indicates that temporal changes in micromycete abundance differed across locations.

Table 7. Two-way ANOVA results for total micromycete counts across sampling points and investigation periods

Source	df	F	*p*
Sampling point	5	8.64	<0.001
Sampling moment	1	22.09	<0.001
Point x Moment	5	4.25	0.003

CONCLUSIONS

The present study demonstrated significant spatial and temporal variations in microbial communities along the Bahlui River. Both total bacterial and Gram-positive bacterial abundances increased from April to July, with point 3 consistently exhibiting the highest counts, while micromycetes showed lower overall abundance but also displayed significant temporal and spatial differences. Two-way ANOVA confirmed that both the sampling location and investigation period significantly influenced microbial abundance, and interactions between these factors indicate that temporal changes were not uniform along the river. These findings highlight the impact of local environmental conditions and anthropogenic pressures on microbial dynamics and underscore the importance of multiple spatially distributed sampling points and repeated monitoring for accurate assessment of river water quality. Continuous monitoring of microbial indicators, combined with physicochemical analyses, is recommended to better understand ecological risks and the drivers of microbial variability along urban river systems.

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