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MICROBIAL PATTERNS IN URBAN AND PERI-URBAN LAKES FROM SOUTHERN ROMANIA: IMPLICATIONS FOR WATER QUALITY AND ECOSYSTEM HEALTH

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Abstract

Urbanisation strongly influences water quality and the structure of microbial communities in aquatic ecosystems. This study investigates seasonal variations in physicochemical parameters and microorganisms in three urban lakes in Bucharest (Tineretului, Titan 1 and Titan 2) and one peri-urban lake (Comana). Samples were collected from five stations per lake during summer and autumn. Physicochemical parameters (temperature, pH, ORP, dissolved oxygen and TDS) were measured in situ, and bacterial communities were assessed by determining heterotrophic bacteria, total and faecal coliforms, and faecal streptococci, respectively.
The results revealed clear differences between urban and peri-urban lakes. The lakes in Bucharest presented increased TDS values (up to 738 ppm) and an alkaline pH (up to 10 in Titan 2), reflecting anthropogenic pressure. Bacterial abundance was higher in Titan 1 and Titan 2, where heterotroph density exceeded 10^3 CFU·mL^{-1}. Faecal coliforms were sporadically detected in urban lakes, but were absent in Comana, indicating point sources of contamination. The findings suggest that urbanisation causes significant changes in water quality and microbial load, highlighting the need for integrated monitoring for sustainable management of urban lakes.

Key words*: anthropogenic impact, microbial contamination, physicochemical parameters, urban lakes, water quality.*

INTRODUCTION

Freshwater ecosystems, especially urban and peri-urban lakes, are critical components of regional biodiversity and provide essential ecosystem services, but are increasingly threatened by anthropogenic activities (Kapetanović et al., 2024). These aquatic environments are highly susceptible to environmental change and serve as indicators of landscape health due to their vulnerability (Roguet & Lucas, 2012). Their ecological integrity is frequently compromised by pollution, nutrient enrichment and habitat degradation, often resulting from human activities in adjacent areas (Lazăr et al., 2017; Mititelu-Ionuș et al., 2020).
The microbial communities in these lakes are particularly sensitive to such disturbances, reflecting changes in water quality and overall ecosystem health (Kiersztyn et al., 2019). Microorganisms play a central role in the functioning of aquatic ecosystems, being involved in the biogeochemical cycles of carbon, nitrogen and phosphorus, as well as in the degradation of organic matter. Bacterial communities in lakes respond rapidly to environmental changes, making them important indicators of ecological status and water quality (Marcondes et al., 2024). Added to this is the escalating impact of global change and land-use changes, emphasising the need for comprehensive studies focused on bacterial populations in urban lake environments to identify reliable indicators of ecological status and emerging pathogens in water (Orel et al., 2022).
Classical microbiological indicators, such as heterotrophic bacteria, total coliforms, faecal coliforms and faecal streptococci, are widely used to assess water quality and identify sources of faecal contamination. The presence of these microorganisms is often associated with risks to human health and deterioration of

ecosystem health, especially in recreational lakes (Lazăr et al., 2017; Lew et al., 2023).

Globally, extensive research has focused on the application of these microbiological indicators to assess water quality in diverse aquatic environments (Loucif & Chenchouni, 2024). For example, studies in lakes in Turkey have correlated bacterial diversity with environmental factors, indicating the presence of wastewater and agricultural runoff even when coliform counts were not exceptionally high (Mercan & Arslan, 2023). Similarly, in Brazil, investigations in urban aquatic systems have used these bacterial indicators to assess the efficiency of wastewater treatment plants and to identify sources of pollution affecting recreational waters, highlighting their broad utility in environmental monitoring. In the Romanian context, research has similarly used these microbial indicators to assess water quality in various freshwater bodies, revealing contamination patterns influenced by urban development and agricultural practices (Lazăr et al., 2017).

In Romania, information on the structure of microbial communities and their relationships with water physicochemical parameters in urban lakes remains limited, especially in the southern region. Given the accelerated expansion of urban areas and the importance of lakes in Bucharest for recreation and local ecological balance, an integrated assessment of water quality from this perspective was of interest.

This study aims to address this knowledge gap by investigating microbial patterns in urban and peri-urban lakes in southern Romania, focusing on how these patterns reflect water quality and ecosystem health under varying degrees of anthropogenic pressure.

Therefore, the main objectives were: (i) assessing seasonal variations of physicochemical parameters and microbiological indicators in urban and peri-urban lakes in southern Romania, (ii) evaluating the relationship between microbiological indicators and physicochemical factors contributing to a better understanding of the impact of urbanisation on the health of aquatic ecosystems.

MATERIALS AND METHODS

Study site

Four representative ecosystems were selected in the study: 3 urban lakes in Bucharest (Tineretului, Titan 1 and Titan 2) and a peri-urban lake, as part of the Comana Natural Park (Balta Comana), Giurgiu County in southern Romania, in order to capture how these models reflect water quality and ecosystem health under varying degrees of anthropogenic pressure (Figure 1). Five stations were established for the urban lakes Titan 1, Titan 2, and Tineretului, and three for Balta Comana.

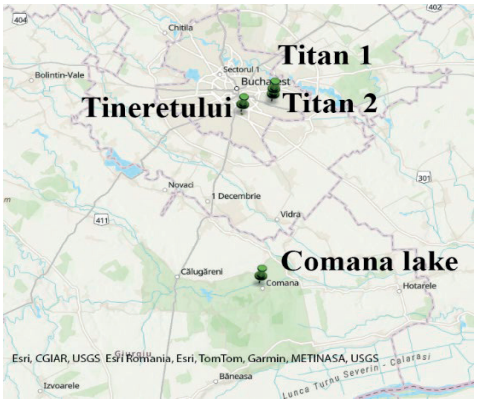


Figure 1. Study site with sampling ecosystems

Water sample collection

Water-column samples were collected in the summer and autumn of 2024. Using GPS, five sampling points were selected for Lake Tineretului, Titan 1 and Titan 2, whereas three sampling points were selected for Lake Comana. The samples collected into sterile containers, stored, and transported at 4°C were analysed within 24 hours of sampling.

Measurement of physicochemical parameters

From the water column, a series of physicochemical parameters (oxidation–reduction potential, temperature, percentage saturation of dissolved oxygen, pH, and turbidity) was measured *in situ* with a HI 9828 multi-parameter probe (Hanna Instruments, Woonsocket, RI, USA).

Microbial analysis

To perform the quantitative analyses, a serial dilution was prepared.

Using the pour plating method to quantify *heterotrophic bacteria*, sterile Petri plates were inoculated with 1 mL aliquots from each dilution for every replicate. After inoculation, 15 ml of nutrient agar medium (sterile broth media with 20 $g \cdot L^{-1}$ agar), molten and cooled at 45°C, was poured onto the plates and incubated at 37°C. After 48 hours, heterotrophic bacteria counts were determined (Jackson et al. 2000; Sanders, 2012).

The most probable number (MPN) technique was used to determine the number of *faecal coliforms* and *faecal streptococci*. For each dilution and each sample, three tubes were inoculated. MacConkey broth (bile lactose bromcresol purple medium) was added for the cultivation of faecal coliforms, while sodium azide broth containing bromcresol purple was added for the cultivation of faecal streptococci. The inoculated tubes were incubated at 45 °C for 24 h for faecal coliforms and 48 h for faecal streptococci (Berg et al., 1978; Maul & Block, 1983; Sinton et al., 1998; Gronewold &Wolpert, 2008).

To determine the colony-forming units (CFU) of total coliforms, 1 mL of each serial dilution for each duplicate was added to eosin-methyl blue media. The plates were incubated at 37°C for 24 hours (An et al., 2002).

Data processing

Violin plots and boxplots were used to display the data distribution. Boxplots were used to highlight the essential statistics of the sample (median and quartiles), while violin plots visualised the full shape of the distribution, including any multimodality and density variations. The width of the violin plot reflects the local density of the data, providing a more detailed picture of the distribution's structure than the quartile representation alone. Combining the two types of graphs provided a more comprehensive characterisation of the data, integrating both central tendency and distributional peculiarities relevant to the interpretation of the results (Hu, 2020).

Heatmaps are powerful visualisation tools for displaying two-dimensional data, effectively revealing patterns shared across subsets of rows and columns. This package excels at connecting multi-source information, making it broadly applicable in diverse data analysis fields, particularly in bioinformatics and genomics (Gu, 2022).

The two-factor ANOVA method examines the simultaneous influence of two qualitative independent variables (factors), in this case, months and lakes, on a quantitative dependent variable, along with their possible interactions. This factorial design allows investigation of the main effects of each factor (month and lake) and detection of a statistical interaction, in which the effect of one factor is modified by the levels of the other factor. A factorial design analyses the effects of two independent variables on a single continuous dependent variable, yielding three results: a main effect for each independent variable and an interaction between the two (Andrade, 2024).

Redundancy Analysis (RDA) is a constrained ordination method used to quantify the relationships between microbial community composition and environmental variables. The technique models variation in abundance data as a function of explanatory factors, allowing the significance of interaction terms to be assessed within a linear framework. RDA extends multivariate regression to multivariate response sets, estimating explained variance and facilitating direct comparison between the community matrix and the environmental variable matrix (Legendre et al., 2010). The software used was Past version 5.2.1, Xlstat add-in 2014 and R-studio R 4.5.2.

RESULTS

Physicochemical characterisation of urban and peri-urban lakes

The temperature boxplots showed a relatively wide distribution of temperatures across all lakes (Figure 2 – Temperature). The water temperature varied across the lakes, with mean values of approximately 17.58-21.74°C, indicating a moderate-to-warm thermal regime during the study period. The minimum values recorded ranged from 6.23°C (Titan 2) to 14.76°C (Comana), and the maximum values ranged between 28.52°C (Titan 2) and 32.23°C (Comana). The means were 21.74°C (Comana), 21.74°C (Tineretului), 17.58°C (Titan 1) and

18.75°C (Titan 2). Also, the boxplots of the lakes showed distinct thermal regimes in Tineretului and Comana, compared to the two Titan lakes, indicating that, in the dynamics of summer and autumn temperatures, the two ecosystems showed lower exposure to extreme values. The two lakes exhibited a stable thermal regime, characterised by progressive warming and cooling of the water without sudden fluctuations.

Regarding pH, the values generally indicated an alkaline range, with minimum values between 8.63 (Titan 1) and 8.91 (Tineretului), and maximum values between 9.53 (Titan 1) and 10.30 (Comana). The averages were 9.58 (Comana), 9.41 (Tineretului), 9.27 (Titan 1) and 9.43 (Titan 2). The differences in the profile of the violin-type boxplots (Figure 2 – pH) suggested that Comana and Tineretului presented a more homogeneous and stable pH, while the Titan lakes, especially Titan 1, exhibited more pronounced chemical fluctuations, reflected by the asymmetry and shape of the distributions. A stable alkaline pH may reflect intense photosynthetic activity and a relatively well-buffered system, whereas more pronounced pH fluctuations may indicate temporary chemical imbalances, with potential implications for microbial community structure and water quality.

The redox potential (ORP) showed very high variability (Figure 2 – ORP), with a general trend towards more oxidising conditions in the urban lakes than in Comana. Negative minima ranged between -82.90 mV (Comana) and -33.50 mV (Titan 1), and positive maxima up to 138.20 mV (Titan 1). The averages were 21.65 mV (Comana), 48.25 mV (Tineretului), 52.82 mV (Titan 1) and 54.15 mV (Titan 2). The boxplots indicated in Comana a frequent alternation between oxidative and reductive conditions, suggesting a more unstable redox dynamic. The relatively broad distribution shape reflected the influence of natural processes, such as the input of organic matter and microbiological activity, characteristic of a less anthropized system. In contrast, urban lakes showed a tendency to exhibit higher ORP values, with higher averages (48.25 mV - Tineretului < 52.82 mV in Titan 1 < 54.15 mV in Titan 2). Thus, the distributions were more concentrated in the oxidative range, with fewer negative values and less extensive negative values. This profile suggested dominant oxidative conditions, associated with better oxygenation and frequent water mixing, typical of managed urban lakes.

Dissolved oxygen (%DO) presented wide ranges, with minimums between 45.10% (Titan 1) and 64.10% (Tineretului) and maximums between 92.90% (Titan 2) and 156.50% (Comana). The averages were 90.48% (Comana), 97.56% (Tineretului), 70.63% (Titan 1) and 65.51% (Titan 2), suggesting generally good to very good levels of oxygenation, with distributions slightly elongated towards high values in Comana. Differences between urban and natural lakes were also observed in oxygen levels. In Lake Comana, the variations were greater, indicating pronounced fluctuations in oxygenation, including frequent episodes of supersaturation. In contrast, in urban lakes, the distributions were more compact and better centred, which explains moderate levels of dissolved oxygen and a lower variability (Figure 2 – DO).

TDS values revealed pronounced differences between urban lakes and Lake Comana, as clearly reflected in the boxplot (Figure 2 – TDS). In Lake Comana, TDS ranged from 381 to 619 mg/L, with an average of 500.33 mg/L, and the relatively wide distribution indicated moderate variability in water mineralisation. The boxplot of the lake highlights seasonal fluctuations in dissolved salt supply, typical of natural ecosystems. In contrast, in urban systems, the boxplots indicate high values, within much lower limits. Lake Tineretului presented the highest values (614-738 $mg \cdot L^{-1}$), with an average of 672.90 $mg \cdot L^{-1}$, and was characterised by a high and constant mineralisation, typical of urban areas. In Titan 1, TDS ranged from 512 $mg \cdot L^{-1}$ to 591 $mg \cdot L^{-1}$, with an average of 547.10 $mg \cdot L^{-1}$ and a relatively compact distribution. In Titan 2, although the average was lower (474.70 $mg \cdot L^{-1}$), the wide range (258-558 $mg \cdot L^{-1}$) suggested greater variability, likely associated with episodic water intakes with varying mineralisation.

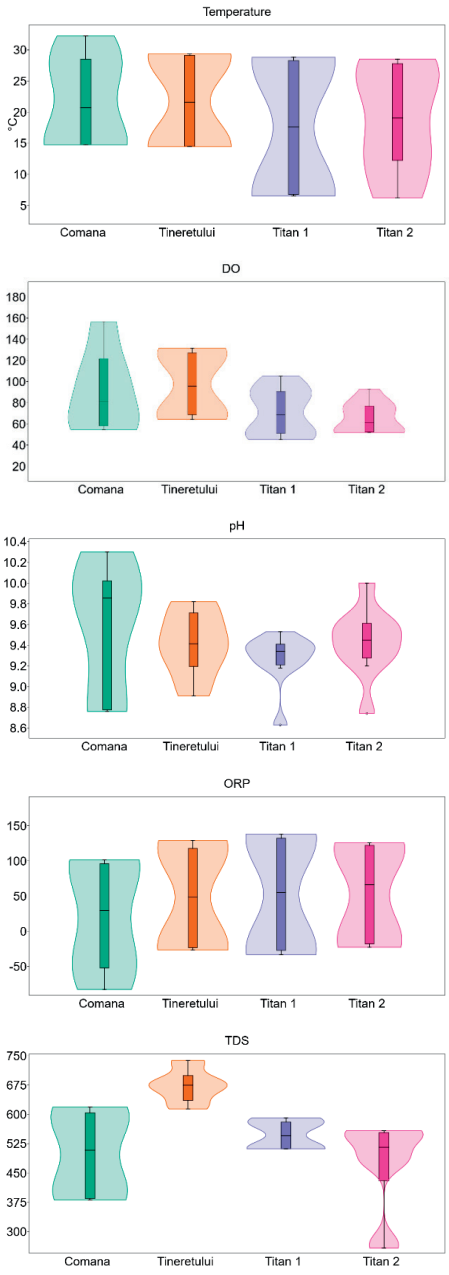


Figure 2. In situ variations of measured physicochemical parameters

Features of microbial communities in the studied ecosystems

Microbiological evaluation using general indicators of microbial load (heterotrophic bacteria - HB, total coliforms - TC) as well as specific indicators of faecal contamination (faecal coliforms - FC, faecal streptococci - FS) highlighted varying degrees of anthropogenic pressure on urban lakes.

In Lake Comana, faecal contamination was absent, whereas in urban lakes it was present at varying intensities, indicating episodes of faecal pollution (Figure 3). In Lake Comana, established as a reference system for anthropogenically unaffected conditions, the absence of faecal indicators (FS and FC) and the high productivity of HB confirm the characteristic resilience of these systems. Microbiological values recorded in Comana revealed a stable natural bacterial background, with HB ranging between 2400–8300 CFU·mL^{-1} (mean 5033±1959), low TC (range 2–6, ± 2.53 CFU mL^{-1}) and the complete absence of FC and streptococci (0 in all samples), indicating optimal conditions of relatively stable aquatic biota, possibly related to the productivity and organic matter specific to the natural lake (Figure 3).

This ecosystem contrasts sharply with urban lakes, where anthropogenic pressures have produced much more variable microbiological patterns. The high variability in urban lakes underscores the stark contrast between the stability of the natural Comana ecosystem and the disturbed dynamics of urban lakes under anthropogenic pressure.

In Tineretului, the overall microbial load was the highest among all urban ecosystems, with TC reaching 28 CFU·mL^{-1} (mean 7.2 ± 7.87), while faecal contamination remained low (FC 0-0.4, FS average 0.9-0.98), suggesting non-faecal organic inputs (from typical park sources - leaves, sediments, human activity).

Titan 1 and Titan 2 presented the most intense episodes of faecal contamination, with maximum FC of 25 CFU·mL^{-1} (means 4.8 and 4.1, ± 7.7) and FS with maxima between 2.5-4.5 CFU·mL^{-1}, indicating irregular inputs of faecal origin.

From the perspective of heterotrophic bacteria, Titan 1 showed intermediate values (300-2600), while Titan 2 showed the greatest ecological instability, with HB ranging from 109 to 8500 CFU·mL^{-1} (average 2550.9), reflecting episodes of intense bacterial proliferation and possible local eutrophication.

The high variability of HB in Titan 2 indicated a pronounced ecological instability, possibly

related to irregular nutrient inputs and unfavourable conditions.

In these ecosystems, the development of coliforms, associated with high levels of heterotrophic bacteria, highlighted differences in the microbiological features of urban lakes compared to those of the natural Lake Comana.

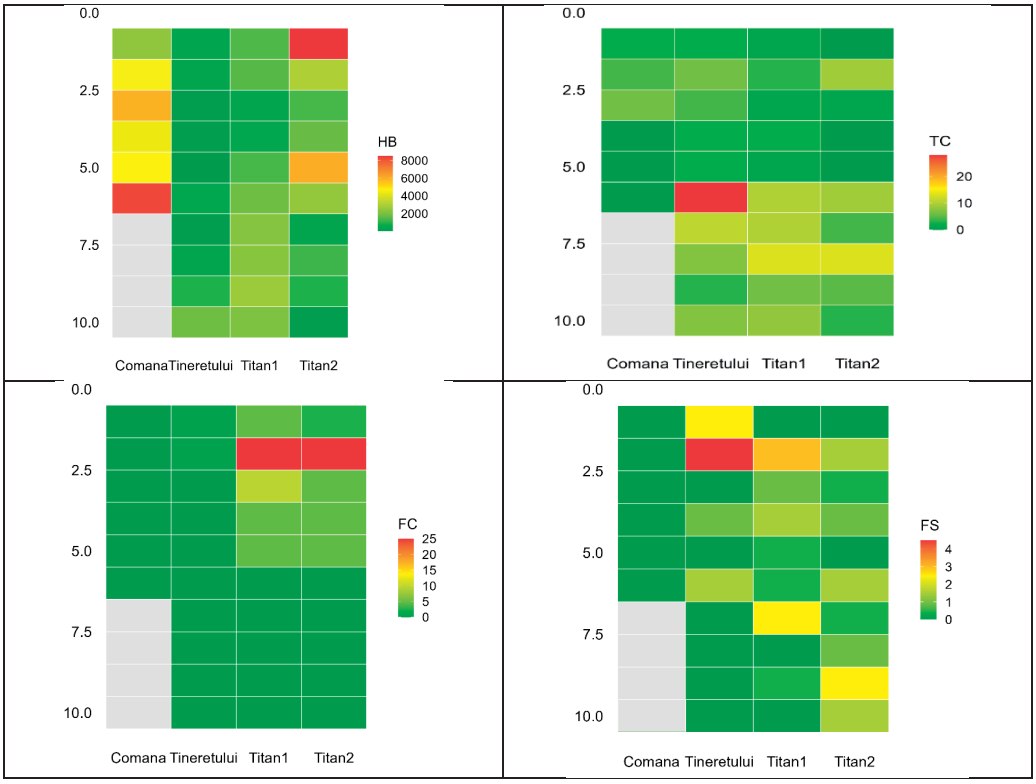


Figure 3. Heatmaps of the abundance of the investigated microbial communities

Seasonal dynamics and interaction with anthropogenic pressure

HB bacteria showed distinct spatial and seasonal variations (Table 1), so during the summer the abundances were higher (mean values 5.77×10^3 CFU·mL^{-1} - Comana > 2.20×10^3 CFU·mL^{-1}- Titan 1 > 1.00×10^3 CFU·mL^{-1} - Titan 2 > 7.00×10^2 CFU·mL^{-1} - Tineretului), compared to the autumn values (mean values - 4.30×10^3 CFU·mL^{-1} - Comana > 4.10×10^3 CFU·mL^{-1} - Titan 2 > 9.80×10^2 CFU·mL^{-1} - Titan 1> 1.77×10^2 CFU·mL^{-1} - Tineretului).

TC were absent in Comana in summer (0.00), but were detected in autumn (4.00 CFU·mL^{-1}), while in Tineretului the values were higher in summer (11.20 CFU·mL^{-1}) compared to autumn (3.20 CFU·mL^{-1}).

In Titan 1 and Titan 2 lakes, total coliforms also recorded a decline from summer (9.40 and 6.80 CFU·mL^{-1}, respectively) to autumn (means values 1.60 and 2.00 CFU·mL^{-1}) (Table 1).

FC, absent in both seasons in Comana, were detected in urban ecosystems, in high abundances in autumn in Titan 1 (9.60 CFU·mL^{-1}) and Titan 2 (8.20CFU·mL^{-1}).

FS were also present in urban ecosystems, with distinct trends across the three ecosystems investigated.

They were characterised by increases from summer to autumn in Tineretului and Titan 1, whereas in Titan 2, the values were higher in summer (1.36 CFU·mL^{-1}) than in autumn (0.56 CFU·mL^{-1}) (Table 1).

Table 1. Descriptive statistics and seasonal changes in microbiological indicators (s - summer; a - autumn)

Type		Minimum	Maximum	Mean	SD (n-1)
Heterotrophic bacteria (CFU·mL^{-1})					
Comana	s	4.30×10^{3}	8.30×10^{3}	5.77×10^{3}	2.20×10^{3}
	a	2.40×10^{3}	5.90×10^{3}	4.30×10^{3}	1.77×10^{3}
Tineretului	s	1.00×10^{2}	1.80×10^{3}	7.00×10^{2}	6.82×10^{2}
	a	4.50×10^{1}	3.30×10^{2}	1.77×10^{2}	1.33×10^{2}
Titan 1	s	1.80×10^{3}	2.60×10^{3}	2.20×10^{3}	2.92×10^{2}
	a	3.00×10^{2}	1.50×10^{3}	9.80×10^{2}	5.81×10^{2}
Titan 2	s	1.09×10^{2}	2.50×10^{3}	1.00×10^{3}	9.47×10^{2}
	a	1.30×10^{3}	8.50×10^{3}	4.10×10^{3}	3.07×10^{3}
Total coliforms (CFU·mL^{-1})					
Comana	s	0.00	0.00	0.00	0.00
	a	2.00	6.00	4.00	2.00
Tineretului	s	3.00	28.00	11.20	9.81
	a	2.00	6.00	3.20	1.79
Titan 1	s	6.00	13.00	9.40	2.61
	a	1.00	3.00	1.60	0.89
Titan 2	s	3.00	13.00	6.80	4.15
	a	0.00	9.00	2.00	3.94
Faecal coliforms (CFU·mL^{-1})					
Comana	s	0.00	0.00	0.00	0.00
	a	0.00	0.00	0.00	0.00
Tineretului	a	0.00	0.40	0.16	0.22
	s	0.00	0.00	0.00	0.00
Titan 1	s	0.00	0.00	0.00	0.00
	a	4.50	25.00	9.60	8.88
Titan 2	s	0.00	0.00	0.00	0.00
	a	2.50	25.00	8.20	9.43
Faecal streptococci (CFU·mL^{-1})					
Comana	s	0.00	0.00	0.00	0.00
	a	0.00	0.00	0.00	0.00
Tineretului	s	0.00	1.50	0.38	0.65
	a	0.00	4.50	1.58	1.93
Titan 1	s	0.00	2.50	0.66	1.05
	a	0.00	3.00	1.16	1.17
Titan 2	s	0.40	2.50	1.36	0.79
	a	0.00	1.50	0.56	0.64

Two-way ANOVA analysis revealed significant differences in microbial community structure by ecosystem type and season, with effect sizes varying across the analysed microbiological indicators (Table 2).

For HB, the model explained a high proportion of the total variation ($R^2 = 0.71$) and showed a strong overall effect ($F = 10.02$, $p < 0.0001$). Spatial differences were dominant, with values being significantly more different between urban and natural lakes ($F = 17.09$, $P < 0.0001$). The lake × season interaction was also significant ($F = 6.11$, $p = 0.00$), suggesting that HB seasonal dynamics were influenced by ecosystem type, with distinct responses in urban versus natural lakes.

For TC, the ANOVA model explained 69% of the total variation ($R^2 = 0.69$), indicating a significant overall effect ($F = 9.02$, $p < 0.0001$). TC showed both spatial ($F = 5.00$, $p = 0.01$) and seasonal ($F = 7.57$, $p = 0.01$) differences, also reflected in the lake×season interaction (F

= 10.57, $p < 0.0001$). The results show that seasonal amplitude varied across ecosystems, highlighting the indicator's high sensitivity to environmental changes.

For FC, the model had the highest explanatory power ($R^2 = 0.8$, $F = 26.84$, $p < 0.0001$). Both the ecosystem factor ($F = 18.21$, $p < 0.0001$) and the period ($F = 60.63$, $p < 0.0001$) significantly influenced FC concentrations, indicating a pronounced variability also in the lake×season interaction (Table 2). In contrast, FS showed reduced variability, with the model explaining only 30% of the total variation ($R^2 = 0.30$) and no significant overall effect ($F = 1.73$, $p = 0.14$). No significant spatial or temporal differences in the FS distribution were found, highlighting a uniformity of FS across ecosystems and seasons, reflecting a good ecological stability or reduced sensitivity to the analysed factors.

Table 2. Two-Way ANOVA results for microbiological indicators by lake and season

	HB	**TC**	**FC**	**FS**
R^2	0.71	0.69	0.87	0.30
F	10.02	9.02	26.84	1.73
p	< 0.0001	< 0.0001	< 0.0001	0.14
Lake	17.09	5.00	18.21	2.23
	< 0.0001	0.01	< 0.0001	0.11
Season	0.59	7.57	60.63	0.23
	0.45	0.01	< 0.0001	0.64
Lake×Season	6.11	10.57	18.21	1.70
	0.00	< 0.0001	< 0.0001	0.19

RDA analysis indicates a separation of microbial communities based on physicochemical water conditions (Figure 4). RDA results indicated an R^2=0.23 (23% total explained variation) and R^2adj=0.10 (10%), indicating a moderate effect of physicochemical factors on microbial communities, but significant (F=1.85, p=0.03 at 999 permutations). Axis 1 dominates (69% of the explained variation), explaining the clear separation Titan/Tineretului vs. Comana; Axis 2 (21%) details the differences TC vs. FC/FS/HB. FS and FC were associated with Axis 1 and were influenced by ORP and pH in the anthropogenic conditions of Titan 1 and Titan 2 lakes, indicating recent faecal inputs. In contrast, HB was positioned in the opposite plot to FC and FS, favoured by oxygenated conditions (% DO) with lower temperatures, indicating distinct niches of heterotrophic bacteria compared to faecal indicators. On the other hand, TC, separated from the other microbial communities, was associated with Axis 2, influenced by increases in temperature and oxygen, and, to a lesser extent, by TDS, indicating distinct ecological conditions. The analysis also shows that, depending on environmental conditions and trends in microbial communities, the studied ecosystems were clearly separated. Thus, urban ecosystems are found in the upper part of the plot, whereas Lake Comana is located in the opposite part; faecal indicators (FC/FS) were the main drivers of ecosystem separation and also validate sources of anthropogenic pollution in urban sites.

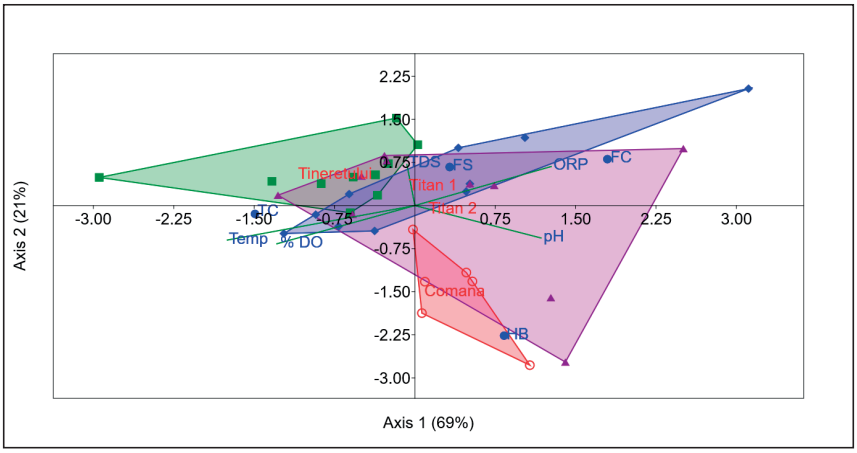


Figure 4. Redundancy analysis (RDA) reveals the relationships between microbial communities and environmental parameters in the investigated aquatic systems

DISCUSSIONS

The results of our study highlighted a close relationship between the structure and dynamics of microbial communities and environmental conditions in the investigated lakes, demonstrating the impact of the urbanisation gradient and seasonal variability on these communities. Unlike classical descriptive microbiological approaches, the integrated analysis of environmental parameters and microbiological indicators highlighted the ecological mechanisms that control the dynamics of HB communities, as well as those of TC, FC, and FS. Higher values in the warm season were associated with increased abundances of HB across all ecosystems (Figure 2 and Table 1), highlighting the role of temperature in intensifying organic matter degradation. However, the response of microbial communities to this factor varied across ecosystem types. At the peri-urban Lake Comana, HB growth in summer was relatively uniform and correlated with the natural availability of organic matter, indicating a system predominantly controlled by internal processes. In contrast, in urban lakes, especially Titan 2, the high variability of HB suggests an amplified response to episodic nutrient inputs, probably associated with urban runoff and sediment resuspension. Thus, water temperature has been shown to be a primary factor regulating microbial activity, directly influencing metabolic processes and bacterial growth rates (White et al., 1991; Nedwell, 1999). In addition, the alkaline and relatively stable pH created a favourable framework for the development of bacterial communities, but differences in variability between lakes indicate distinct control mechanisms (Wetzel, 2001; Dippong et al., 2023). The stability of pH in Comana and Tineretului suggests a well-buffered system, whereas the more pronounced fluctuations in Titan lakes may reflect temporary imbalances induced by anthropogenic processes, affecting the selection of bacterial taxa tolerant to chemical variations. These differences may contribute to more homogeneous microbial structures in peri-urban lakes and more heterogeneous in urban ones. Instead, redox potential and dissolved oxygen played a central role in the differentiation of microbial niches (Baqué, 2006; Fenchel et al., 2012). In Lake Comana, the alternation of oxic and suboxic conditions (Figure 2 - ORP and DO) favoured the coexistence of microbial communities adapted to variable micro-niches, which explains the general stability of the communities and the absence of faecal indicators. The pronounced fluctuations in dissolved oxygen in the peri-urban Lake Comana reflect a natural ecological control dominated by the circadian cycles of photosynthesis and respiration, amplified by algal and macrophytic biomass and by the mineralisation of organic matter in sediments (Cristini et al., 2017). This dynamic indicates both an intrinsic self-purification capacity and

an increased susceptibility to episodes of benthic hypoxia or anoxia, with direct effects on the distribution and activity of microbial communities and on redox-dependent biogeochemical processes (Tolu et al., 2017). In contrast, the relative stability of dissolved oxygen in urban lakes suggests a more strongly anthropogenically controlled system, influenced by active management, shallow depth, and frequent water-column mixing, which limit stratification and the accumulation of hypoxic zones (Carnohan, 2021; Maier et al., 2021). However, during warm periods, local persistence of low oxygen in the lower layers can favour the release of phosphorus from sediments and intensify eutrophication, with implications for microbial community structure (Radu et al., 2019). In contrast, the predominantly oxidative and more uniform conditions in urban lakes favoured aerobic processes and the persistence of bacteria associated with recent contamination, reflected by the presence of FC and FS (Figure 3). The high and constant TDS values in urban lakes indicate a persistent chemical pressure, which may act as an ecological filter, favouring bacterial communities adapted to increased mineralisation and osmotic stress (Paul & Meyer, 2001; Ghervase et al., 2011). In Comana, moderate TDS variability suggests natural control of mineralisation, without inducing major changes in microbial community structure.

The spatial distribution of microbial communities (Figure 3) clearly reflects the urbanisation gradient, as reported in other urban and peri-urban aquatic ecosystems (Newton et al., 2011; Staley et al., 2013). Lake Comana presented a microbiological profile characteristic of natural ecosystems, dominated by indigenous HB and lacking indicators of faecal contamination. This structure suggests a predominance of autochthonous organic matter and an efficient control of self-purification processes. In contrast, urban lakes showed much more heterogeneous spatial patterns. Titan 1 and Titan 2 were characterised by recurrent episodes of faecal contamination, indicating active or intermittent local sources. The differences between these two lakes, especially the extreme variability of HB in Titan 2, suggest a higher degree of ecological instability, possibly related to morphometry, water residence time and intensity of anthropogenic inputs. Lake Tineretului occupied an intermediate position, with high overall microbial loads but low faecal contamination, indicating a dominance of non-faecal organic sources.

Seasonality amplified differences between ecosystems (Table 1), in agreement with patterns observed in temperate lakes under anthropogenic pressures (Sommer et al., 2012; Lazăr et al., 2017). In urban lakes, increasing temperatures and warm-season rainfall events were followed by higher total coliform levels, and, in some cases, by an increase in faecal contamination until autumn. This seasonal lag suggests the accumulation and persistence of faecal bacteria in sediments, followed by resuspension and remobilisation. In Comana, seasonal variations were less pronounced and were not associated with the appearance of faecal indicators, indicating a more resilient system to seasonal fluctuations. The presence of TC only in autumn can be interpreted as due to sediment resuspension or natural inputs, with no direct link to faecal pollution.

The ANOVA results (Table 2) confirm that ecosystem type is the main determinant of microbial community structure, with season having a secondary but significant role in urban ecosystems. The lake × season interaction indicates that the response of microbial communities to seasonal variations is modulated by the degree of urbanisation. Here, the RDA biplot (Figure 4) integrates these observations, highlighting the clear separation of urban lakes from Comana based on the association of faecal indicators with ORP, pH and TDS, a pattern frequently encountered in aquatic ecosystems influenced by urbanisation (Borcard et al., 2018; Dimpor et al., 2024). The positioning of HB relative to dissolved oxygen and temperature suggests that natural microbial communities follow ecological trajectories distinct from those influenced by anthropogenic factors. Overall, our results indicate that urbanisation not only influences the level of microbiological contamination but also the stability and predictability of microbial communities. Urban ecosystems are characterised by more dynamic communities, with pronounced fluctuations and rapid

responses to environmental changes, whereas peri-urban ecosystems exhibit more stable communities, driven by internal ecological processes. A relevant observation is the potential role of sediments as a microbiological reservoir, especially in urban lakes, where seasonal remobilisation can significantly contribute to the dynamics of bacterial communities (Jamieson et al., 2005; Pachepsky & Shelton, 2011). Also, differences between urban lakes suggest that geomorphological features of ecosystems can significantly modulate microbial responses to anthropogenic pressure.

CONCLUSIONS

The natural lake Comana presents stable physicochemical parameters and balanced microbial communities, without faecal contamination, while the urban lakes (Tineretului and Titan) record higher and more variable TDS, ORP, and pH values, associated with the presence of FC and fluctuating number of HB, reflecting anthropogenic pressure. The distribution of microbial communities is correlated with the physicochemical conditions, highlighting clear ecological differences between natural and urban ecosystems and underlining the need for an adapted management of water quality.

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