

Comprehensive ecological and microbiological assessment of water quality in selected rivers of Azerbaijan

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ABSTRACT

Assessment of the ecological status of river waters is of great importance for the protection of water resources, their efficient management, and the identification of anthropogenic impacts. The aim of this study was to assess the water quality of selected rivers belonging to the Kura and Araz River basins based on physicochemical and microbiological parameters, as well as to determine the relationships among these parameters using statistical methods. Within the framework of the study, water samples collected from the Lower Kura, Ganjachay, Kurakchay, Tartarchay, Araz River, Hakarichay, Bargushadchay, and Okhchuchay were analyzed for temperature, pH, dissolved oxygen, concentrations of ammonium, nitrite, nitrate, and phosphate ions, total microbiota, saprotrophic bacteria, bacteria involved in the nitrogen cycle, as well as the quantitative abundance of oil- and phenol-oxidizing microorganisms using standard physicochemical and microbiological methods. Pearson correlation analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were applied to evaluate the relationships among the parameters. The obtained results demonstrated that the physicochemical and microbiological parameters of the studied rivers varied both seasonally and among rivers. Higher levels of nutrients and microbiological parameters were observed in the Ganjachay, Araz River, Lower Kura, and Okhchuchay, whereas the Hakarichay, Kurakchay, and Tartarchay were characterized by relatively favorable ecological conditions. A strong positive correlation was identified between total microbiota and saprotrophic bacteria ($r = 0.709$; $p < 0.001$). PCA and HCA revealed that the rivers were grouped differently according to nutrient loading, microbiological activity, and the level of anthropogenic impact. The results of the study demonstrate that the integrated application of physicochemical, microbiological, and multivariate statistical approaches provides a reliable framework for the comprehensive assessment of the ecological status of river waters, the identification of river basins associated with ecological risks, and the scientifically based development of long-term monitoring programs.

Keywords: river water quality, ecological assessment, microbiological indicators, nutrient pollution, transboundary rivers, anthropogenic impact, PCA, HCA.

INTRODUCTION

Water is one of the fundamental components of life on Earth and plays a crucial role in maintaining ecological balance and ensuring socioeconomic development. The uneven spatial distribution of freshwater resources, climate change, and increasing anthropogenic

pressures have made their protection and efficient management one of the major global environmental challenges (UNESCO WWAP, 2024). In this context, transboundary river basins are of particular importance. Covering approximately half of the Earth's land surface, these basins provide water to approximately 40% of the world's population and contain a

substantial proportion of global freshwater resources (FAO, 2024; UN-Water, 2021).

However, water use and other anthropogenic activities carried out in upstream countries directly affect both the quantity and quality of water in downstream countries. Climate change, population growth, industrialization, and the intensification of agriculture further exacerbate these problems, making the management of transboundary water resources increasingly complex.

Transboundary pollution refers to pollution originating within the territory of one state but crossing national borders through air currents, rivers, seas, or other natural pathways and adversely affecting the environment of another country (UNECE, 1992). This type of pollution can deteriorate the condition of atmospheric air, water bodies, soils, and ecosystems, damage biodiversity, and pose serious risks to human health. The principal characteristic of transboundary pollution is that its effects are not confined to the country in which the pollution originates but may extend to neighboring and even distant states.

In addition, transboundary pollution is characterized by its ability to spread over considerable distances. Through river systems, marine currents, and atmospheric circulation, pollutants may travel hundreds or even thousands of kilometers and reach areas far from their original sources. This characteristic demonstrates that pollution is not restricted by national borders and may acquire a global dimension. For this reason, transboundary pollution is often described as “pollution without borders.” A classic example of such an event is the Baia Mare accident that occurred in Romania in 2000, when 100,000 m³ of cyanide-contaminated wastewater entered watercourses and spread into Hungary and Serbia, causing a transboundary environmental disaster (UNECE, 2004).

Another important aspect of transboundary pollution is that its ecological impacts are not limited to the state in which the pollution originates. On the contrary, these impacts may also be transferred to countries with relatively low levels of emissions. Consequently, the burden of environmental damage may fall on states that are not the primary sources of pollution, raising important legal and political issues concerning environmental justice and the international responsibility of states.

Furthermore, because ecosystems are interconnected, intensive pollution originating in one region may cross geographical boundaries and

adversely affect the ecological condition of distant and even relatively unpolluted areas. This phenomenon confirms that the environment constitutes an integrated and interdependent system and demonstrates that the prevention of transboundary pollution is possible only through coordinated cooperation among states.

Thus, the prevention and effective management of transboundary pollution are regarded as major global environmental issues requiring strengthened international cooperation among states, effective information exchange, the implementation of joint monitoring mechanisms, and compliance with international environmental law. Solid waste generated by industrial activities may contain various chemical elements that can act as potential sources of environmental pollution; therefore, the application of modern analytical techniques for the characterization of such waste is essential for assessing its potential environmental impacts (Jafarova et al., 2026).

The water balance of Azerbaijan is formed predominantly by the waters of the Kura and Araz rivers, both of which are transboundary rivers. This characteristic highlights the crucial role of these rivers and their basins in the country’s hydrographic network. The river network of the Republic of Azerbaijan consists of more than 8,350 rivers. Their total length is approximately 33,665 km, while the total area of their drainage basins is approximately 85,500 km². Classification according to river length indicates that only two rivers are longer than 500 km, 22 rivers have lengths ranging from 101 to 500 km, and 324 rivers range from 11 to 100 km in length (Azerbaijan Geographical Society, n.d.). The vast majority of the country’s rivers are less than 10 km long. All rivers in Azerbaijan belong to the Caspian Sea basin and, according to their hydrographic characteristics, are divided into three main groups:

1. Rivers of the Kura basin – including the left and right tributaries of the Kura River;
2. Rivers of the Araz basin – consisting mainly of the left tributaries of the Araz River;
3. Rivers flowing directly into the Caspian Sea – rivers that do not belong to the Kura or Araz basins and discharge directly into the Caspian Sea.

Among the most polluted rivers in the country are the Kura, Araz, Okhchuchay, Aghstafachay, Tovuzchay, and Gargar River; in particular, Okhchuchay has been described as a “dead river” due to waste originating from the mining industry in

the Kapan region of Armenia (Azerbaijan Geographical Society, n.d.).

The investigations were conducted in the Kura and Araz rivers and several of their tributaries. Within the framework of this study, the Ganjachay, Kurakchay, and Tartarchay are right-bank tributaries of the Kura River, whereas the Hakarichay, Bargushadchay, and Okhchuchay are left-bank tributaries of the Araz River, together forming part of an integrated hydrological system. Therefore, ecological changes and anthropogenic impacts occurring in these rivers may propagate downstream and directly affect the ecological status and water quality of the Kura and Araz rivers. Consequently, assessment and regular monitoring of the ecological status of rivers belonging to the Kura and Araz River basins are of considerable scientific and practical importance (Karr, 1999). Similar ecological assessment approaches have also been successfully applied to other water bodies in Azerbaijan, such as Lake Boyukshor (Hajiyeva et al., 2020). Considering that the Kura River is one of Azerbaijan's major water sources and that its right-bank tributaries—the Tartarchay, Ganjachay, and Kurakchay – play an important role in regional water supply, protecting these rivers from anthropogenic impacts, maintaining their water balance, and investigating seasonal variations in their hydrochemical and microbiological parameters are essential for an objective assessment of their ecological status. The microbiological composition and self-purification potential of Azerbaijan's transboundary river waters have also been partially investigated in previous studies. In particular, the role of micromycetes involved in the degradation of organic matter in transboundary river waters has been investigated (Hasanova et al., 2023), and the bioremediation potential of microorganisms isolated from river waters has been evaluated (Hasanova et al., 2025). These studies indicate that the microbial community of river ecosystems is not only an indicator of pollution but also a functional component actively involved in the self-purification of water. Similarly, studies conducted in the southern sector of the Caspian Sea belonging to Azerbaijan have confirmed that the genetic diversity and distribution of macrozoobenthic species are also important indicators reflecting the biological status of aquatic ecosystems (Huseynov et al., 2026).

Although the hydrochemistry of the Kura and Araz basins has been described in general terms, previous assessments of Azerbaijani surface

waters have addressed either physicochemical variables or individual microbial groups in isolation, and have typically been restricted to a single river, a single season, or a single functional group of microorganisms (Hasanova et al., 2023; Hasanova et al., 2025; Babashli et al., 2022; Babashli et al., 2023). No study has so far combined seasonally resolved hydrochemical monitoring with the simultaneous quantification of several functional microbial guilds – saprotrophic bacteria, nitrogen-cycle bacteria, and hydrocarbon- and phenol-oxidizing microorganisms – across rivers of both the Kura and the Araz basins, nor has such a combined dataset been interpreted by multivariate ordination. This lack of an integrated, basin-scale and seasonally resolved dataset constitutes the knowledge gap addressed here.

The present study was therefore designed to quantify seasonal and inter-river variation in the physicochemical characteristics and nutrient content of eight rivers of the Kura and Araz basins, to determine in the same samples the abundance of total microbiota, saprotrophic bacteria, nitrogen-cycle bacteria and oil- and phenol-oxidizing microorganisms, and to classify the rivers by their combined physicochemical and microbiological characteristics using multivariate statistics, thereby identifying the basins in which anthropogenic pressure is most clearly expressed.

MATERIALS AND METHODS

Study area

The study covered the Lower Kura and its right-bank tributaries – the Ganjachay, Kurakchay and Tartarchay – as well as the Araz River and its left-bank tributaries – the Hakarichay, Bargushadchay, and Okhchuchay (Figure 1).

The Kura River, one of the study sites, is the largest transboundary river in the Caucasus. It originates from Mount Kizilgedik (2740 m) in Türkiye, flows through Georgia, enters Azerbaijan, and crosses the Kura-Araz Lowland before discharging into the Caspian Sea (length: 1515 km; basin area: ~188,000 km²). Its lower reach, extending from the Mingachevir Reservoir to the Caspian Sea (Lower Kura), was selected as the study area. Among the right-bank tributaries of the Lower Kura, the Tartarchay (200 km; 2650 km²) flows through the Kalbajar, Aghdara, Tartar, and Barda districts; the Kurakchay (186 km; 2080



Figure 1. Hydrographic characteristics of the studied rivers

km²) flows through the Goranboy, Goygol, and Yevlakh districts; and the Ganjachay (99 km; 752 km²) originates in the Murovdag Range of the Lesser Caucasus (2814 m), flows through Dashkasan, Goygol, and the city of Ganja, and discharges into the Mingachevir Reservoir. The Araz River (1072 km; 101,900 km²), the largest tributary of the Kura and the second-largest river in the South Caucasus in terms of discharge, originates in the Bingol Mountains (2990 m) in Türkiye. It flows through the territories of Türkiye, Armenia, and Azerbaijan, forms part of the international borders between Azerbaijan, Türkiye, and Iran over a considerable distance, and, after flowing for its final 80 km through Azerbaijan, joins the Kura River near Sabirabad. Among its left-bank tributaries, the Hakarichay (113 km; 2570 km²) flows through the Lachin, Gubadli, and Zangilan districts; the Bargushadchay (Bazarchay) (178 km; 2711 km²) originates from Lake Zalkha in the Zangezur Range, flows through Armenia, and enters Azerbaijan in the Gubadli District; while the Okhchuchay (85 km; 1140 km²) originates in the Kapan region of Armenia and flows through the Zangilan District. Before joining the Araz River, all three rivers enter Azerbaijan from Armenia as transboundary watercourses (Azerbaijan Geographical Society, n.d.).

Seasonal monitoring was conducted to investigate the microbiological and hydrobiological status of the studied rivers. Water samples were collected from different locations along each river, and the physicochemical properties of the water, quantitative and qualitative variations in microorganisms, biotic and abiotic factors, saprotrophic bacteria, oil- and phenol-utilizing

microorganisms, bacteria involved in the nitrogen cycle, and concentrations of biogenic elements were determined.

Sampling procedure

Water samples were collected seasonally during four campaigns: December 2024 (winter), April 2025 (spring), July 2025 (summer) and October 2025 (autumn). Monitoring stations were established on each of the studied rivers. On the Lower Kura, twelve stations were located at the upstream and downstream boundaries of six settlements – Mingachevir, Yevlakh, Zardab, Shirvan, Salyan and Neftchala - in order to capture the influence of each settlement on water quality. On the tributaries, sampling was carried out on the Ganjachay downstream of the city of Ganja, on the Kurakchay near Goranboy, on the Tartarchay near Tartar, on the Araz River near Sabirabad, and on the Hakarichay, Bargushadchay and Okhchuchay within the Gubadli and Zangilan districts. Coordinates were not recorded during the campaigns; station positions are therefore reported by river and locality. Three replicate water samples ($n = 3$) were collected from each station and transported to the laboratory under refrigerated conditions.

Physicochemical analysis

All physicochemical determinations were carried out in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 2017; 23rd edition). Water temperature (°C) was measured in situ with a mercury thermometer. The pH was determined in situ using

a portable pH-200 analyzer, and dissolved oxygen (DO, mg L⁻¹) with an MW 600 Dissolved Oxygen Meter, both calibrated before each field campaign. The concentrations of the biogenic elements - ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻) and phosphate (PO₄³⁻) – were determined photometrically following the corresponding standard procedures of the same reference. The same analytical protocol has been applied in parallel monitoring of Azerbaijani transboundary and internal rivers (Hasanova et al., 2026).

Collection and transportation of samples for microbiological analysis

Water samples for microbiological analysis were collected aseptically in sterile polyethylene containers, transported to the laboratory in refrigerated containers at +4 °C, and analyzed within 6–24 hours of collection. This approach complies with international standards for preventing changes in microbial abundance during transportation and ensuring the reliability of the results.

Microbiological analysis

The total abundance of microorganisms was determined by direct microscopic counting on membrane filters according to Razumov's method (Razumov, 1932; Jannasch and Jones, 1959), and the results are expressed as million cells per millilitre (×10⁶ cells mL⁻¹).

Saprotrophic bacteria were cultivated on meat-peptone agar using the pour-plate technique according to the Koch method. Plates were incubated at 28 ± 2 °C and the resulting colonies were counted after 3–5 days. Smears were prepared from the dominant colonies and examined microscopically to determine the morphological characteristics of the strains.

Bacteria involved in the nitrogen cycle were enumerated as follows. Aerobic nitrogen-fixing bacteria (*Azotobacter*) were cultivated on Ashby's medium; the resulting colonies were counted after incubation and expressed as colony-forming units per millilitre (CFU mL⁻¹). Anaerobic nitrogen-fixing bacteria (*Clostridium pasteurianum*) sample were inoculated into the corresponding elective media, with three replicate tubes per dilution level. Tubes were incubated for 7–14 days depending on the group and were then scored as positive on the basis of visible growth and the characteristic metabolic response of each group, and abundance

was estimated as the most probable number per millilitre (MPN mL⁻¹) using McCrady's statistical tables. Because this technique yields a categorical estimate rather than a direct count, the results are reported as orders of magnitude.

Oil- and phenol-oxidizing microorganisms were cultivated in elective (selective) media and quantified using the O.G. Mironov method. This methodology has previously been applied to characterize oil- and phenol-degrading microbial communities in coastal and river ecosystems of Azerbaijan (Babashli et al., 2022; Babashli et al., 2023; Veliev et al., 2013). These studies demonstrated that bacteria belonging to the genera *Pseudomonas* and *Arthrobacter*, isolated from similar aquatic environments, actively degraded aromatic and halogenated hydrocarbons (Babashli et al., 2022; Babashli et al., 2025). Counts of oil- and phenol-oxidizing microorganisms were likewise obtained by ten-fold serial dilution in the respective elective media (three replicate tubes per dilution) and are expressed as the most probable number per millilitre (MPN mL⁻¹), reported as orders of magnitude.

The role of micromycetes and heterotrophic microbiota in the degradation of organic matter in transboundary river waters, as well as the bioremediation potential of river ecosystems, has previously been documented for Azerbaijani rivers using similar sampling and enumeration approaches (Hasanova et al., 2023; Hasanova et al., 2025), thereby providing methodological continuity with the present study.

Statistical analysis

Seasonal and spatial variations in the physicochemical and microbiological parameters were summarized by descriptive statistics, expressed as minimum–maximum ranges by river and season. The relationship between total microbiota abundance and the number of saprotrophic bacteria was assessed by Pearson correlation analysis for n = 32 paired observations (eight rivers × four seasons); because microbial counts may deviate from normality, the coefficient was interpreted as a measure of monotonic association. Two multivariate techniques were then applied to classify the rivers according to their combined physicochemical and microbiological characteristics and to identify the principal sources of variance within the dataset: hierarchical cluster analysis (HCA), using Ward's method with Euclidean distance,

and principal component analysis (PCA). For both analyses the seasonal values of each river were averaged, and all variables were autoscaled (z-score standardization) so that parameters expressed in different units contributed equally to the ordination. This approach is consistent with established methodologies for the multivariate assessment of water quality (Muniz and Oliveira-Filho, 2023). All computations were performed in Python 3.11.5, and the level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Seasonal variation in the physicochemical parameters of river waters

Seasonal variations in water temperature, dissolved oxygen (DO), and pH in the studied rivers are presented in Tables 1–3 and Figure 2.

The obtained results indicate that these physicochemical parameters were shaped by the combined effects of seasonal climatic conditions, hydrological regimes, and anthropogenic pressures.

Water temperature exhibited clear seasonal variability in all rivers. The lowest temperatures were recorded during winter (3–10 °C), whereas the highest values were observed during summer (22–28 °C) (Table 1). During the summer months, the highest temperatures were recorded in the Lower Kura (27–28 °C), while the lowest were observed in the Hakarichay (22–24 °C). The relatively low temperature of the Hakarichay can be attributed to its recharge from high-altitude mountainous areas, intensive groundwater inflows, and rapid flow regime. The obtained results are consistent with findings reported in previous studies. The observed seasonal temperature variations are

also consistent with previous research demonstrating that the thermal regime of river waters is closely associated with air temperature, solar radiation, flow characteristics, and the geomorphological structure of the river basin (O’Sullivan et al., 2019; Tokuda et al., 2019; Caissie, 2006).

An increase in water temperature is important not only as a physical parameter but also as one of the major ecological factors determining the intensity of biogeochemical processes in river ecosystems. Increasing temperature accelerates the metabolic activity of microorganisms, intensifies the decomposition of organic matter, and increases biological oxygen demand. Consequently, nutrient cycling and microbiological processes become more intensive during the summer season (Dodds and Whiles, 2020).

Dissolved oxygen concentrations exhibited an inverse pattern in relation to temperature (Table 2 and Figure 3).

The highest DO values were generally recorded during autumn and winter, whereas the lowest values were observed during the summer months. The maximum dissolved oxygen concentration was recorded in the Kurakchay during autumn (9.9–11.2 mg/L), whereas the minimum values were observed in the Lower Kura during summer (5.5–6.0 mg/L). The Hakarichay maintained consistently high and stable DO levels throughout the year, which can be explained by its low water temperature, high flow velocity, and natural aeration.

The inverse relationship between DO and temperature is a well-established pattern in limnology. As temperature increases, the solubility of oxygen in water decreases, while microbial respiration and the oxidation of organic matter intensify, resulting in increased biological oxygen demand (Wetzel, 2001). Furthermore, reduced

Table 1. Seasonal variation of water temperature in the studied rivers. Values are presented as minimum–maximum (°C)

River	Winter	Spring	Summer	Autumn
Lower Kura	6–9	14–17	27–28	15–26
Ganjachay	5–9	16–23	25–27	14–21
Kurakchay	6–10	17–18	24.5–26	16–20
Tartarchay	3–8	14–19	24–27	14–22
Araz River	7–9	16–19	25–26	15–17
Hakarichay	4–7	15–17	22–24	13–19
Bargushadchay	5–8	16–18	25–27	14–17
Okhchuchay	4–9	15–18	23–25	15–16

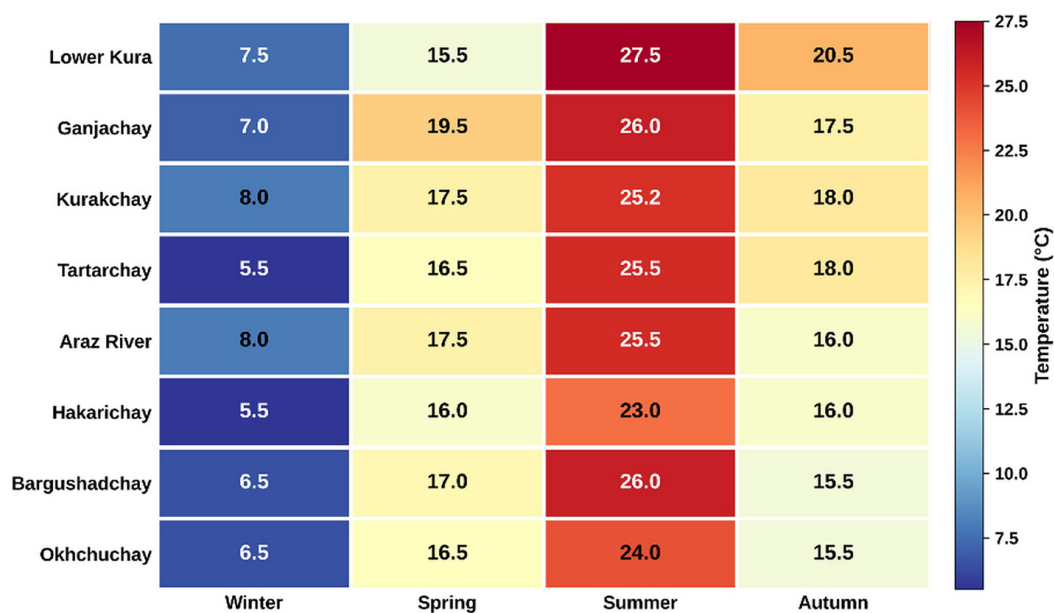


Figure 2. Seasonal water temperature of rivers

Table 2. Seasonal variation of dissolved oxygen (DO) concentrations in the studied rivers. Values are presented as minimum-maximum (mg/L)

River	Winter	Spring	Summer	Autumn
Lower Kura	7.4–8.0	6.5–7.5	5.5–6.0	6.5–7.0
Ganjachay	9.1–9.5	8.0–9.5	6.8–7.3	9.7–10.1
Kurakchay	8.0–8.5	8.5–9.0	7.8–8.5	9.9–11.2
Tartarchay	8.6–10	9.5–10.5	6.7–7.0	8.0–8.1
Araz River	7.9–8.2	8.9–9.2	7.8–8.4	9.1–10.3
Hakarichay	8.0–8.5	8.0–8.5	8.0–8.9	9.3–10.2
Bargushadchay	7.9–8.1	7.1–7.3	6.7–7.3	7.8–7.9
Okhchuchay	7.2–8.0	7–7.9	6.4–6.9	7.7–7.9



Figure 3. Seasonal variation of dissolved oxygen (DO) concentrations

river discharge and increased water stagnation during the summer months may limit atmospheric oxygen diffusion and lead to a further decline in DO concentrations (Dodds and Whiles, 2020).

Nevertheless, in most of the studied rivers, dissolved oxygen concentrations remained above the critical threshold of 5 mg/L generally considered necessary for the normal functioning of aquatic organisms. Although this indicates that the overall oxygen regime of the rivers was satisfactory, the relatively low values observed in the Lower Kura suggest a potentially greater influence of organic pollution, agricultural runoff, and settlements in this area. Vörösmarty et al. (2010) reported a similar tendency in river systems exposed to anthropogenic pressures.

Compared with temperature and dissolved oxygen, pH exhibited less pronounced seasonal variability (Table 3; Figure 4).

In all rivers, pH values generally ranged from neutral to slightly alkaline (6.5–8.6), which is considered favorable for freshwater ecosystems. The highest pH value was recorded in the Araz River during summer (8.2–8.6).

The relatively higher pH values observed during the summer months can be attributed to increased photosynthetic activity of phytoplankton and other aquatic plants. During photosynthesis, intensive uptake of carbon dioxide alters the equilibrium of the carbonate-bicarbonate system, thereby increasing the alkalinity of the aquatic environment (Wetzel, 2001). In addition, the widespread occurrence of carbonate rocks in certain river basins may contribute to the stability of pH values and the development of slightly alkaline conditions.

Overall, the obtained results indicate that seasonal climatic factors play a decisive role in shaping the hydroecological status of the studied rivers. Increasing temperature resulted in lower dissolved

oxygen concentrations, which, in turn, was associated with increased microbiological activity and accelerated decomposition of organic matter. At the same time, the observed differences among the rivers can be explained by the geomorphological characteristics of their basins, hydrological regimes, and levels of anthropogenic pressure.

From an ecological perspective, the Lower Kura, Ganjachay, and, to some extent, the Araz River exhibited stronger indications of anthropogenic impact compared with the other studied rivers. The combination of higher summer temperatures and relatively lower dissolved oxygen concentrations in these rivers may indicate the influence of organic and nutrient pollution. In contrast, the greater year-round stability of physicochemical parameters in the Hakarichay, Kurakchay, and Tartarchay indicates relatively more favorable ecological conditions in these basins. Similar findings have been reported in studies of river systems located in different climatic zones, demonstrating that increasing anthropogenic pressure negatively affects water temperature, oxygen regimes, and overall ecological quality (Vörösmarty et al., 2010; UNEP, 2021).

Thus, the seasonal patterns of physicochemical parameters provide a reliable basis for assessing nutrient cycling, microbiological processes, and the functional status of aquatic ecosystems in the studied rivers. The biogenic elements and microbiological parameters presented in the following sections developed against the background of these physicochemical conditions.

Seasonal dynamics of biogenic elements

Biogenic elements are among the principal regulators of nutrient cycling and biological productivity in river ecosystems. The concentrations of nitrogen and phosphorus compounds are

Table 3. Seasonal variation of pH in the studied rivers. Values are presented as minimum-maximum

River	Winter	Spring	Summer	Autumn
Lower Kura	6.9–7.3	7.2–7.5	7.9–8.4	7.0–7.2
Ganjachay	6.8–7.1	7.0–7.3	7.8–8.0	7.1–7.4
Kurakchay	7.5–8.1	7.2–8.1	8.0–8.3	7.5–7.9
Tartarchay	7.6–7.9	7.3–8.6	7.9–8.3	7.0–7.9
Araz River	6.6–8.0	7.1–7.9	8.2–8.6	7.2–7.6
Hakarichay	7.7–8.0	7.3–7.5	7.6–8.0	7.0–7.1
Bargushadchay	7.2–7.4	7.9–8.0	8.2–8.4	7.9–8.0
Okhchuchay	6.5–7.8	7.8–8.1	7.6–7.8	7.5–7.6

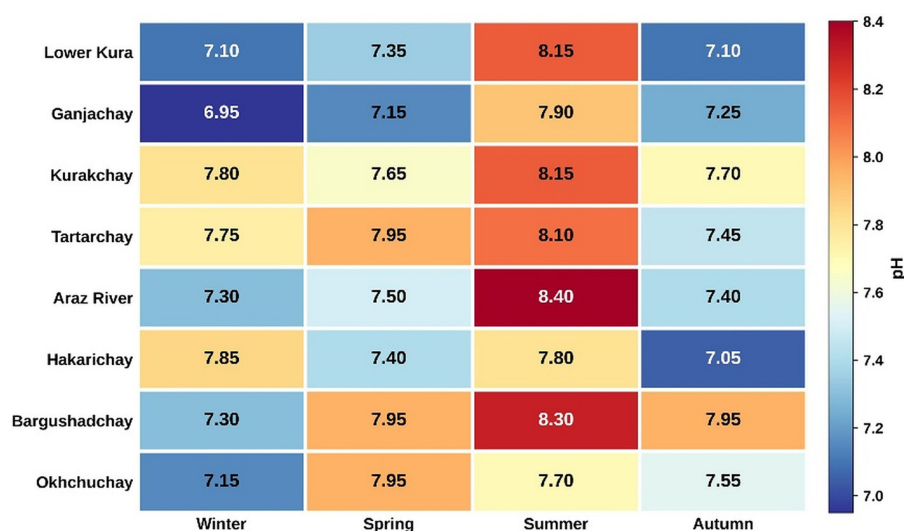


Figure 4. Seasonal variation of pH in the studied rivers

considered important indicators for assessing the trophic status of water bodies, the level of anthropogenic impact, and the functional stability of ecosystems. These elements are essential nutrients required for the growth and development of phytoplankton, algae, and other aquatic plants. Under natural conditions, their concentrations support the normal functioning of aquatic ecosystems; however, increases in nitrogen and phosphorus compounds resulting from anthropogenic activities, particularly domestic, agricultural, and industrial discharges, may lead to water pollution, accelerate eutrophication, promote excessive algal growth, reduce dissolved oxygen concentrations, and ultimately disrupt the biological balance of aquatic ecosystems. In this context, determination of the concentrations of biogenic elements, particularly ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) ions, is regarded as one of the key approaches for assessing river water quality and ecological status. These parameters make it possible to evaluate the level of anthropogenic pressure on river waters, characterize potential pollution sources, and assess nutrient migration.

Figure 5 presents the seasonal variation in ammonium ion concentrations. According to the obtained results, NH_4^+ concentrations increased in most rivers during the summer and autumn seasons. The highest values were recorded in the Ganjachay during summer (1.82 mg/L), in the Okhchuchay during autumn (1.72 mg/L), and in the Araz River during autumn (1.38 mg/L). The increase in ammonium concentrations during the summer-autumn period is primarily associated

with the intensive mineralization of organic matter under high-temperature conditions and the acceleration of ammonification processes. During this period, increased metabolic activity of microorganisms promotes the conversion of organic forms of nitrogen into ammonium. On the other hand, an increase in water temperature accompanied by a decrease in dissolved oxygen may limit nitrification and thereby promote the accumulation of NH_4^+ ions in the water. This pattern has also been confirmed in numerous studies conducted in freshwater ecosystems (Wetzel, 2001; Dodds and Whiles, 2020).

The high ammonium concentrations observed in the Ganjachay and Okhchuchay may be associated with the discharge of domestic wastewater, agricultural activities, and more intensive organic pollution within these basins. In particular, untreated wastewater originating from settlements is considered one of the major anthropogenic sources of ammonium. In this respect, these rivers may be regarded as ecosystems that are more vulnerable to organic pollution compared with the other studied basins (Camargo and Alonso, 2006). It should be stressed, however, that no discharge inventory, wastewater-treatment data or land-use information were compiled in this study, so this interpretation remains hypothetical.

The seasonal variation in nitrate concentrations (Figure 6) showed that nitrate was the dominant form of nitrogen in the studied rivers. The highest NO_3^- concentrations were recorded during autumn in the Ganjachay (19.8 mg/L), Araz River (18.5 mg/L), Okhchuchay (14.3 mg/L), Lower Kura (10.5 mg/L), and Kurakchay (10.2 mg/L).

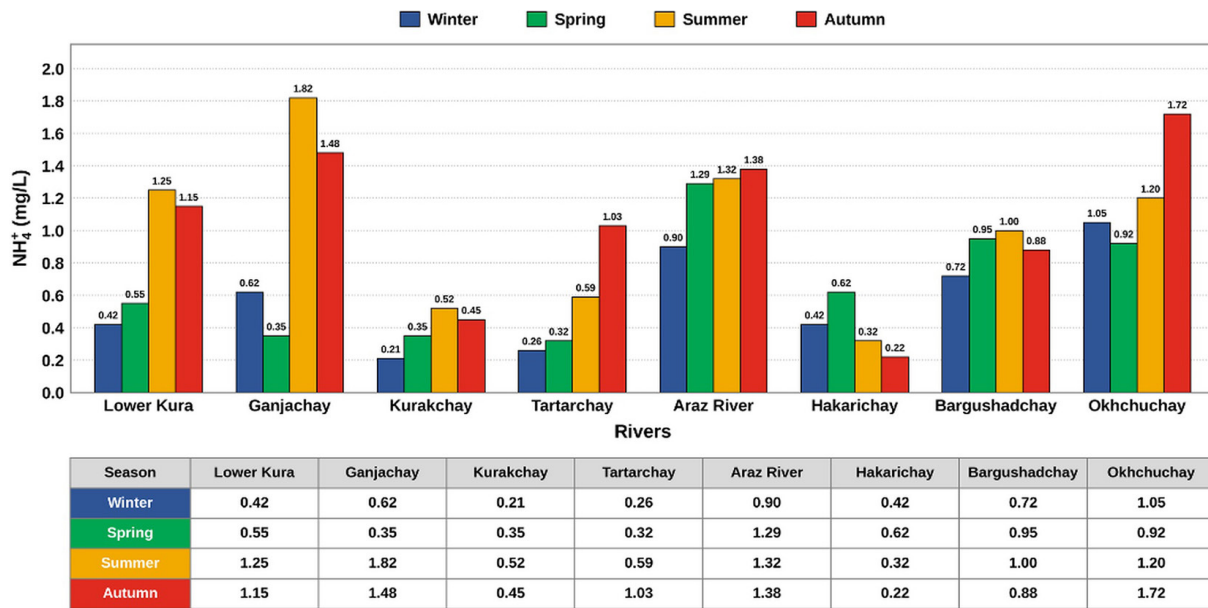


Figure 5. Seasonal variation in ammonium (NH_4^+) concentrations in the studied rivers

The maximum nitrate concentrations observed in autumn may be explained by intensified surface runoff caused by seasonal precipitation and the transport of nitrogen fertilizers from agricultural areas into river systems. In addition, the leaching of nitrates accumulated in soils during summer into rivers during periods of intensive rainfall may also play an important role in this process. Similar tendencies have been observed in river systems of Europe and the Caucasus region (EEA, 2019). Comparable results have also been reported in studies on the quality of irrigation water resources in the northeastern coastal region of Azerbaijan, where the influence of agricultural activity on biogenic element concentrations was identified (Mehdiyeva et al., 2026). Agricultural inputs and river discharge were not quantified in the present work, and this explanation is therefore proposed as a plausible rather than a demonstrated mechanism.

High nitrate concentrations are among the most important indicators of anthropogenic nitrogen loading. In particular, the elevated values recorded in the Ganjachay and Araz River reflect the intensity of agricultural activities and the influence of settlements in these basins. Prolonged exposure to high nitrate concentrations may increase the risk of eutrophication, promote excessive phytoplankton growth, and reduce dissolved oxygen reserves (Smith et al., 1999; Carpenter et al., 1998).

As shown in Figure 7, nitrite ion concentrations were considerably lower than those of the other mineral forms of nitrogen at all monitoring

stations. Nevertheless, localized increases were observed in some rivers during the summer and autumn months. The highest values were recorded in the Lower Kura during autumn (0.68 mg/L), in the Ganjachay during summer (0.60 mg/L), and in the Okhchuchay during summer (0.39 mg/L).

Because nitrite is an intermediate product in the conversion of ammonium to nitrate within the nitrogen cycle, elevated nitrite concentrations may indicate incomplete nitrification. This condition is primarily associated with oxygen deficiency, high organic matter loads, and intensive microbiological processes. The increase in temperature and decrease in DO levels observed during summer may have promoted the short-term accumulation of nitrite.

The higher nitrite concentrations recorded in the Ganjachay and Lower Kura compared with the other rivers indicate stronger anthropogenic pressure in these basins. These results are consistent with the ammonium and nitrate data and confirm that nitrogen transformation processes are more intensive in these rivers.

As shown in Figure 8, the seasonal variation in phosphate ion concentrations also exhibited considerable differences among the rivers. The highest PO_4^{3-} concentration was recorded in the Araz River during autumn (0.80 mg/L), followed by the Okhchuchay during spring (0.60 mg/L), the Bargushadchay during autumn (0.41 mg/L), and the Hakarichay during spring and autumn (0.40 mg/L).

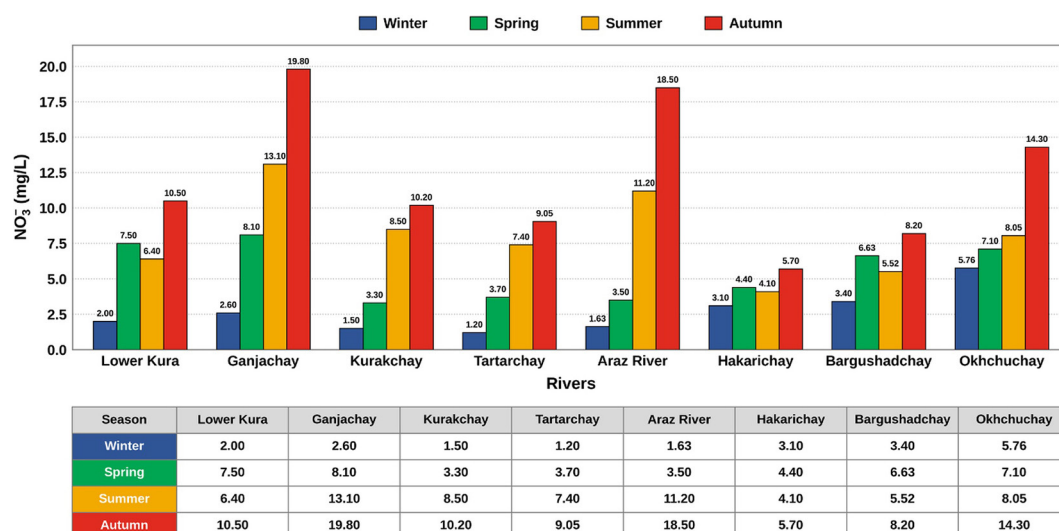


Figure 6. Seasonal variation of nitrate (NO_3^-) concentrations in the studied rivers

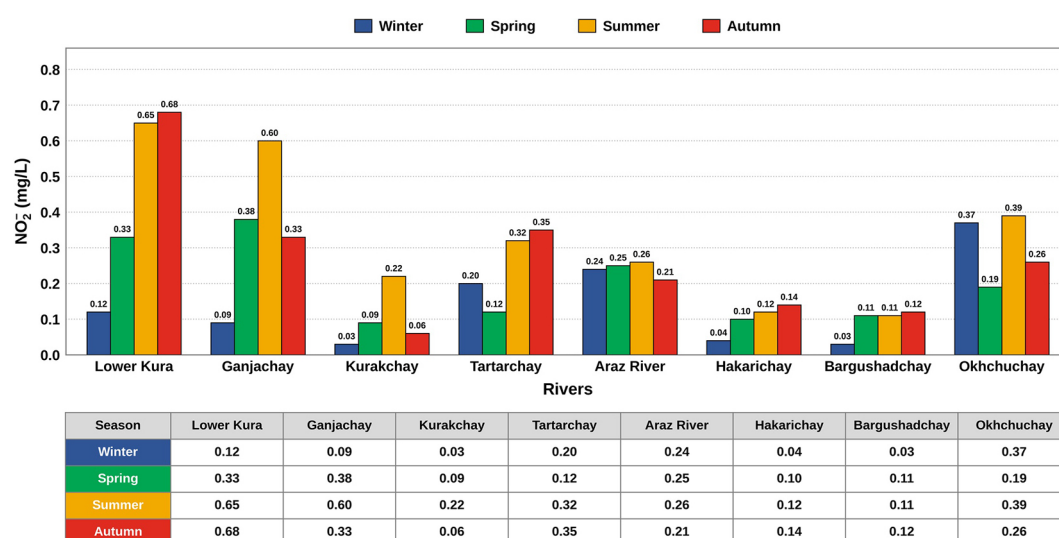


Figure 7. Seasonal variation of nitrite (NO_2^-) concentrations in the studied rivers

High phosphate concentrations are mainly associated with the leaching of phosphorus fertilizers used in agriculture, soil erosion, and the influence of domestic wastewater. Since phosphorus is generally considered a limiting nutrient in freshwater ecosystems, an increase in its concentration is one of the principal factors accelerating eutrophication processes (Schindler et al., 2008). In the absence of source-apportionment data, the relative contribution of each of these sources cannot be determined here.

The high phosphate concentrations observed in the Araz River indicate more intensive anthropogenic pressure within this basin. The elevated concentrations recorded in the Okhchuchay and Bargushadchay may be explained not only by

the geological characteristics of their basins but also by agricultural activities and the influence of nearby settlements.

The integrated analysis of biogenic elements indicates that the distribution of nitrogen and phosphorus compounds in the studied rivers was shaped by the combined influence of natural seasonal processes and anthropogenic activities. The increase in NH_4^+ and NO_2^- ions mainly during the summer months is associated with intensified decomposition of organic matter and accelerated microbiological processes. In contrast, the increase in NO_3^- and PO_4^{3-} concentrations during autumn reflects enhanced surface runoff caused by precipitation and the transport of nutrients from agricultural areas into river systems.

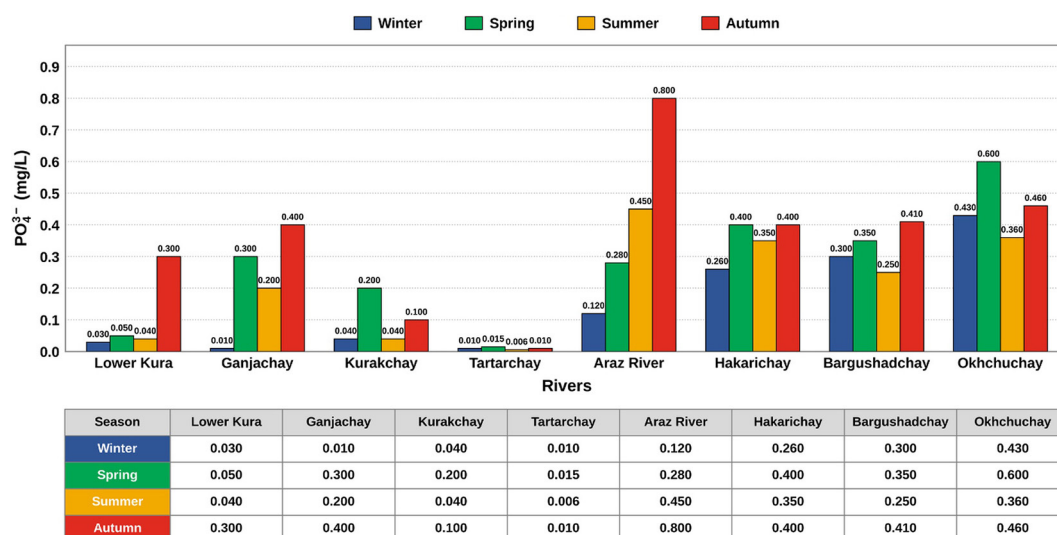


Figure 8. Seasonal variation of phosphate (PO_4^{3-}) concentrations in the studied rivers

According to the obtained results, the Ganjachay, Araz River, and Okhchuchay had higher biogenic loads compared with the other studied basins. When evaluated together with the observed changes in physicochemical parameters, the elevated concentrations of nitrogen and phosphorus compounds in these rivers indicate stronger anthropogenic pressure. In contrast, the Hakarichay, Kurakchay, and Tartarchay were characterized by lower nutrient concentrations and relatively stable hydroecological parameters, indicating more favorable ecological conditions in these basins.

Overall, the obtained results demonstrate that monitoring biogenic elements provides highly informative indicators for assessing the ecological status of river ecosystems, while their combined analysis with physicochemical parameters enables the identification of potential sources of anthropogenic pollution.

Comparison with water quality reference values

To place the measured concentrations within a formal water quality framework, the results were compared with the national maximum allowable concentration (MAC) applied in the Azerbaijani river monitoring network and with the guideline values of EU Directive 78/659/EEC, later codified as Directive 2006/44/EC, on the quality of fresh waters needing protection or improvement in order to support fish life. Although these Directives have since been repealed and superseded by the Water Framework Directive, their threshold

values remain a widely used reference framework and have previously been applied to Azerbaijani rivers (EEA, 2019). Because the studied reaches are lowland watercourses, the cyprinid class was applied. Nitrate was additionally compared with the 50 mg L^{-1} guideline value of the EU Nitrates Directive (91/676/EEC) and the WHO drinking-water guidelines (Table 4).

Nitrate remained well below 50 mg L^{-1} in every river and season, the maximum recorded value being 19.80 mg L^{-1} in the Ganjachay in autumn. Nitrogen enrichment in the studied basins is therefore not expressed as nitrate accumulation to levels of direct concern for water use.

The reduced forms of nitrogen behaved very differently. Ammonium exceeded the national MAC of $0.4 \text{ mg NH}_4\text{-N L}^{-1}$ (equivalent to $0.51 \text{ mg NH}_4^+ \text{ L}^{-1}$) in 22 of the 32 river-season combinations, the highest value being 1.82 mg L^{-1} in the Ganjachay in summer, that is 3.6 times the MAC. Nitrite exceeded the cyprinid guideline value of $0.03 \text{ mg NO}_2^- \text{ L}^{-1}$ in 30 of the 32 combinations and in all eight rivers, reaching 0.68 mg L^{-1} in the Lower Kura in autumn, more than twenty times the guideline value. Since nitrite is an intermediate of nitrification, this widespread exceedance is consistent with incomplete oxidation of ammonium under the summer oxygen minima described above, and it identifies the reduced nitrogen fraction – rather than nitrate – as the principal water quality concern in these basins.

Phosphate exceeded the indicative cyprinid value of $0.4 \text{ mg PO}_4 \text{ L}^{-1}$ in six of the 32 combinations, restricted to the Araz River, Okhchuchay and Bargushadchay, with a maximum of 0.80 mg

Table 4. Comparison of the measured concentrations with national and international water quality reference values

Parameter	Range in this study	Reference value	Source	Exceedances (of 32 river–season combinations)
NH ₄ ⁺ (mg L ⁻¹)	0.21–1.82	0.51 (= 0.4 mg NH ₄ - N L ⁻¹)	National MAC	22
NO ₂ ⁻ (mg L ⁻¹)	0.03–0.68	0.03 (cyprinid)	78/659/EEC	30
NO ₃ ⁻ (mg L ⁻¹)	1.20–19.80	50	91/676/EEC; WHO	0
PO ₄ ³⁻ (mg L ⁻¹)	0.006–0.80	0.4 (cyprinid, indicative)	78/659/EEC	6
DO (mg L ⁻¹)	5.5–11.2	≥ 6	National MAC framework	1

L⁻¹ in the Araz River in autumn. Dissolved oxygen fell below 6 mg L⁻¹ only in the Lower Kura in summer (5.5 mg L⁻¹).

On this basis the Ganjachay, Lower Kura, Araz River and Okhchuchay can be assigned to a lower quality class with respect to nitrogen and phosphorus compounds, whereas the Kurakchay, Tartarchay and Hakarichay remained within the phosphate guideline throughout the year and showed the smallest and least frequent exceedances for the reduced nitrogen forms. This classification is independent of, and consistent with, the grouping subsequently obtained by cluster analysis.

Seasonal variation in microbiological parameters

Microbiological indicators are considered among the most sensitive bioindicators for assessing the ecological status of river ecosystems, alongside physicochemical parameters. Changes in the structure of microbial communities reflect the trophic status of water bodies, the level of organic pollution, and the intensity of biogeochemical processes. In this context, the seasonal dynamics of total microbiota and saprotrophic bacteria in the studied rivers were evaluated comprehensively (Figure 9).

The conducted investigations showed that the abundance of total microbiota was significantly higher than the number of saprotrophic bacteria at all monitoring stations. This result is expected because total microbiota includes not only heterotrophic bacteria but also microorganisms belonging to various functional groups. Saprotrophic bacteria, in contrast, represent a specific functional component of this community involved in the decomposition of organic matter.

Similar seasonal dynamics were observed for both total microbiota and saprotrophic bacteria. Minimum values were recorded during winter, whereas maximum values were observed during the summer and autumn months. During summer, increased water temperatures, more rapid

mineralization of organic matter, and intensified enzymatic processes created favorable conditions for the development of heterotrophic microorganisms. Consequently, the abundance of saprotrophic bacteria also increased significantly.

This pattern is consistent with findings from previous studies. Servais et al. (2009) demonstrated that the abundance of heterotrophic bacteria in river waters is primarily determined by water temperature and the concentration of dissolved organic carbon. Similarly, Newton et al. (2011) reported that the metabolic activity of bacterial communities increases substantially during warmer seasons, thereby accelerating the decomposition of organic matter.

Observations showed that increases in microbiological indicators were particularly pronounced in the Ganjachay, Lower Kura, and Okhchuchay basins. The elevated concentrations of biogenic elements in these rivers also indicate a close relationship between microbiological activity and nutrient availability. High concentrations of organic matter and biogenic elements stimulated the development of heterotrophic microorganisms and resulted in an increase in the abundance of saprotrophic bacteria.

In contrast, microbiological indicators remained more stable throughout the year in the Hakarichay and Kurakchay. Lower temperatures, high dissolved oxygen concentrations, and relatively weak anthropogenic pressure in these basins contributed to the maintenance of microbiological equilibrium. These results are fully consistent with the findings of the preceding physicochemical analyses and indicate that the intensity of microbiological processes is directly dependent on hydroecological conditions.

Thus, the seasonal variation in total microbiota and saprotrophic bacteria indicates that the intensity of microbiological processes is mainly determined by water temperature, dissolved oxygen concentrations, and the levels of biogenic elements. The higher microbiological activity

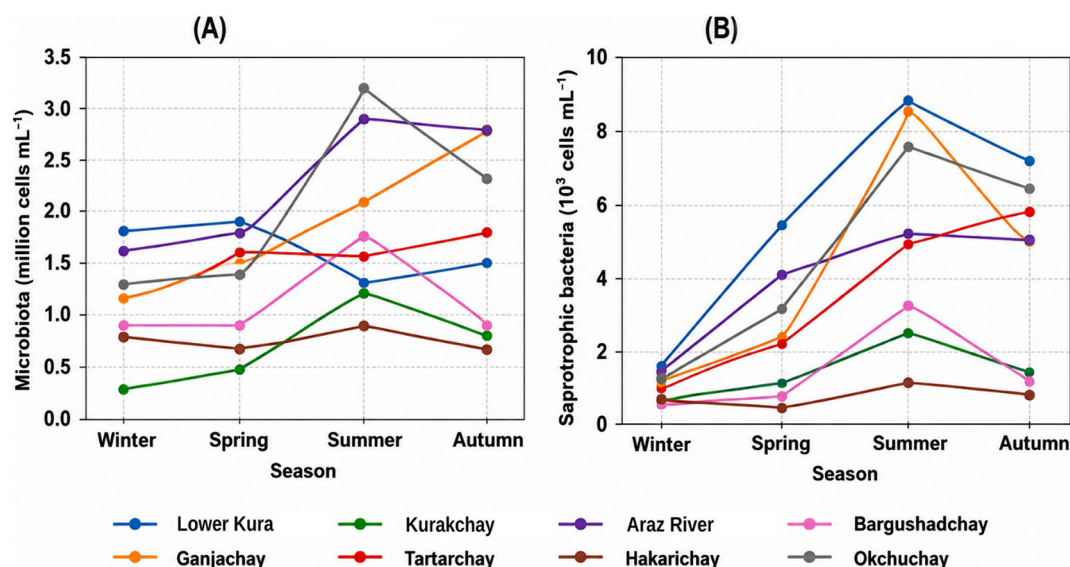


Figure 9. Seasonal variation in the abundance of (A) total microbiota (10⁶ cells/mL) and (B) saprotrophic bacteria (10³ CFU/mL) in the studied river waters

observed during the summer and autumn months is associated with more intensive decomposition of organic matter and accelerated nutrient cycling. Nevertheless, for a more detailed assessment of biogeochemical processes occurring in river ecosystems, analysis of the distribution of functional bacteria involved in the nitrogen cycle is of particular importance.

Distribution of bacteria involved in the nitrogen cycle

Microorganisms involved in the nitrogen cycle play an important role in nitrogen fixation, ammonification, nitrification, and denitrification processes in river ecosystems. In addition to mediating the transformation of nitrogen compounds in aquatic environments, these functional bacteria significantly influence ecosystem productivity and self-purification potential. Their distribution patterns are considered important microbiological indicators for assessing the trophic status of rivers and the level of anthropogenic impact. The distribution and quantitative characteristics of bacteria involved in the nitrogen cycle in the water column of the studied rivers are presented in Table 5.

The results presented in Table 5 demonstrate that the distribution of bacteria involved in the nitrogen cycle was characterized by considerable differences among both rivers and seasons. The abundance of nitrogen-fixing and denitrifying

bacteria varied mainly in relation to the amount of organic matter, dissolved oxygen concentrations, and the levels of mineral nitrogen compounds.

During the summer and autumn seasons, increases in temperature and microbiological activity resulted in higher abundances of bacteria involved in nitrogen transformations in most of the studied rivers. During this period, intensive decomposition of organic matter accelerated ammonification and subsequent nitrification processes, thereby increasing the overall intensity of the nitrogen cycle. The obtained results are consistent with the seasonal variations in ammonium, nitrate, and nitrite concentrations identified in the previous sections.

The differences observed among the rivers may be explained by their hydrological characteristics and levels of anthropogenic loading. Rivers with higher concentrations of biogenic elements also exhibited greater abundances of bacteria involved in the nitrogen cycle, indicating a close relationship between nutrient cycling and microbiological processes. In contrast, the abundance of these bacteria remained more stable in rivers exposed to relatively weak anthropogenic pressure.

Overall, the distribution patterns of bacteria involved in the nitrogen cycle may be regarded as important bioindicators characterizing the intensity of nitrogen transformations and the ecological status of river ecosystems. These findings are consistent with the integrated analysis of physicochemical and microbiological parameters and

Table 5. Distribution and quantitative characteristics of bacteria involved in the nitrogen cycle in the water column of the studied rivers

River	Winter			Spring			Summer			Autumn		
	<i>Azotobacter</i>	<i>Cl. pasteurianum</i>	Denitrifiers	<i>Azotobacter</i>	<i>Cl. pasteurianum</i>	Denitrifiers	<i>Azotobacter</i>	<i>Cl. pasteurianum</i>	Denitrifiers	<i>Azotobacter</i>	<i>Cl. pasteurianum</i>	Denitrifiers
Lower Kura	53	10 ¹	10 ¹	320	10 ²	10 ³	450	10 ³	10 ³	370	10 ²	10 ³
Ganjachay	80	10 ²	10 ²	450	10 ²	10 ³	500	10 ³	10 ⁵	420	10 ³	10 ⁵
Kurakhchay	51	10 ¹	10 ¹	280	10 ¹	10 ²	310	10 ²	10 ³	270	10 ²	10 ²
Tartarchay	60	10 ¹	10 ¹	360	10 ¹	10 ²	450	10 ³	10 ³	290	10 ³	10 ³
Araz River	85	10 ²	10 ²	470	10 ²	10 ³	510	10 ³	10 ³	380	10 ³	10 ³
Hakarichay	43	10 ¹	10 ¹	230	10 ²	10 ²	290	10 ²	10 ²	155	10 ²	10 ²
Bargushadchay	55	10 ²	10 ²	270	10 ²	10 ²	390	10 ³	10 ³	290	10 ²	10 ³
Okhchuchay	75	10 ²	10 ²	340	10 ²	10 ³	480	10 ³	10 ³	410	10 ³	10 ⁵

Note: *Azotobacter* is expressed as CFU mL⁻¹; *Cl. pasteurianum* and denitrifying bacteria are expressed as MPN mL⁻¹ (orders of magnitude).

further confirm the importance of functional microorganisms in assessing the ecological condition of rivers.

In addition to bacteria involved in the nitrogen cycle, oil- and phenol-oxidizing microorganisms that participate in the natural biodegradation of organic pollutants also have considerable ecological importance in river ecosystems. The distribution and quantitative characteristics of these functional groups are presented in the following section.

Distribution of oil- and phenol-oxidizing microorganisms

In river ecosystems, oil- and phenol-oxidizing microorganisms represent one of the major functional groups responsible for the biological degradation of organic pollutants. By participating in the biodegradation of petroleum-derived hydrocarbons and phenolic compounds, these microorganisms make an important contribution to the natural self-purification processes of aquatic environments. Their abundance and distribution patterns are considered important bioindicators for assessing the level of anthropogenic pollution and the biodegradation potential of ecosystems. The distribution and quantitative characteristics of oil- and phenol-oxidizing microorganisms in the water column of the studied rivers are presented in Table 6.

The results presented in Table 6 show that the distribution of oil- and phenol-oxidizing microorganisms varied among both rivers and seasons. The observed changes in their quantitative characteristics were closely associated with the hydrological

properties of the rivers, the amount of organic matter, and the intensity of anthropogenic pollution.

Higher abundances of hydrocarbon-oxidizing microorganisms were recorded in the Ganjachay, Okhchuchay and Araz River. Because petroleum hydrocarbons and phenolic compounds were not quantified in the present study, these counts cannot be attributed directly to hydrocarbon or phenol input; they indicate only that a microbial community capable of utilizing such substrates is present and metabolically active in these rivers. The capacity of these organisms to use hydrocarbons as a carbon source and to proliferate rapidly under favourable conditions is well documented, and elevated abundances of hydrocarbon-degrading bacteria have elsewhere been reported to co-vary with measured hydrocarbon concentrations; this relationship, however, remains to be verified for the rivers studied here. The concurrent determination of total petroleum hydrocarbons and phenols is therefore recommended for future monitoring.

From a seasonal perspective, increases in the abundance of these functional microorganisms were observed during the summer and autumn months. Rising temperatures and intensified microbiological processes accelerated biodegradation reactions, resulting in higher abundances of oil- and phenol-oxidizing bacteria. During winter, however, the reduced metabolic activity of microorganisms under low-temperature conditions led to a decline in their abundance.

Comparative analysis of the results showed that rivers with higher abundances of oil- and phenol-oxidizing microorganisms also tended to exhibit relatively high levels of total microbiota and saprotrophic bacteria. This pattern indicates

Table 6. Distribution and quantitative characteristics of oil- and phenol-oxidizing microorganisms in the water column of the studied rivers.

River	Winter		Spring		Summer		Autumn	
	Oil	Phenol	Oil	Phenol	Oil	Phenol	Oil	Phenol
Lower Kura	10 ¹	10 ¹	10 ²	10 ²	10 ³	10 ³	10 ²	10 ²
Ganjachay	10 ²	10 ²	10 ⁴	10 ⁴	10 ⁵	10 ⁵	10 ⁵	10 ⁵
Kurakchay	10 ¹	10 ¹	10 ¹	10 ²	10 ²	10 ³	10 ²	10 ²
Tartarchay	10 ¹	10 ¹	10 ¹	10 ²	10 ³	10 ³	10 ³	10 ³
Araz River	10 ²	10 ²	10 ²	10 ³	10 ³	10 ⁴	10 ³	10 ³
Hakarichay	10 ¹	10 ¹	10 ²	10 ²	10 ²	10 ²	10 ²	10 ²
Bargushadchay	10 ²	10 ²	10 ²	10 ²	10 ³	10 ³	10 ²	10 ³
Okhchuchay	10 ²	10 ²	10 ²	10 ³	10 ³	10 ⁵	10 ³	10 ⁵

Note: Values are expressed as MPN mL⁻¹ (orders of magnitude)

that organic pollution stimulates the development of various functional groups of microorganisms and is consistent with the microbiological indicators identified in the previous sections.

Overall, the distribution of oil- and phenol-oxidizing microorganisms may be considered an important ecological indicator characterizing the natural self-purification potential of river ecosystems. Evaluating the quantitative characteristics of these functional groups together with other physicochemical and microbiological parameters allows for a more objective assessment of the level of anthropogenic impact and the intensity of biodegradation processes in river basins.

To assess more precisely the relationships between the observed distribution patterns of functional microorganisms and the physicochemical and microbiological parameters, Pearson correlation analysis was performed.

Correlation analysis between total microbiota and saprotrophic bacteria

Pearson correlation analysis was performed to assess the relationship between total microbiota and saprotrophic bacteria in river ecosystems. The results of the analysis are presented in Figure 10.

The results of Pearson correlation analysis showed a strong positive correlation between total microbiota and the abundance of saprotrophic bacteria ($r = 0.709$, $p < 0.001$). The obtained correlation coefficient confirms the existence of a close functional relationship between these two indicators.

As shown in the figure, the abundance of saprotrophic bacteria increased with increasing total microbiota. This pattern indicates that

saprotrophic bacteria represent one of the major functional components of the overall microbial community and play an important role in the decomposition of organic matter. In particular, the high abundance of saprotrophic bacteria in samples with elevated total microbiota reflects the intensive biological transformation of organic matter in river ecosystems.

The observed positive correlation also indicates that the development of saprotrophic bacteria is closely associated with overall microbiological activity. Increased temperature and elevated concentrations of biogenic elements enhanced the metabolic activity of microorganisms and created favorable conditions for the development of heterotrophic bacteria. Consequently, a parallel increase was observed in both total microbiota and saprotrophic bacterial abundance.

From a statistical perspective, the value of $p < 0.001$ indicates that the identified correlation is highly significant and confirms that the observed relationship is unlikely to be due to chance. These results demonstrate the existence of an ecologically important functional relationship between total microbiota and saprotrophic bacteria.

Overall, the correlation analysis confirmed that saprotrophic bacteria are reliable bioindicators of organic matter cycling in river ecosystems and demonstrated their important role in assessing the overall microbiological status of river waters.

To complement the bivariate correlation analysis with a broader statistical assessment, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were subsequently performed. These analyses enabled a comprehensive evaluation of the ecological characteristics of the studied rivers.

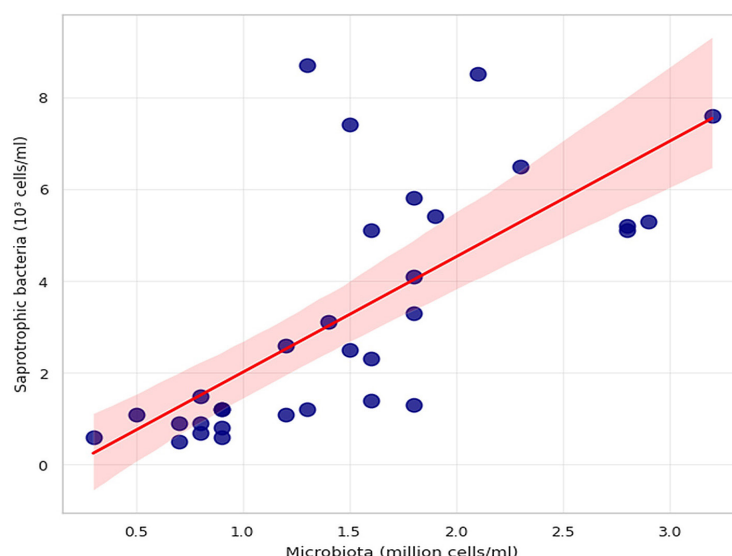


Figure 10. Correlation between microbiota abundance and saprotrophic bacteria in the studied river waters

Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

PCA and HCA were performed to comprehensively assess the ecological status of the river ecosystems and to determine the relationships among physicochemical parameters, biogenic elements, and microbiological indicators (Figure 11).

These multivariate statistical methods are widely applied to identify relationships among environmental variables, group rivers with similar characteristics, and evaluate the major factors influencing ecological processes (Shrestha and Kazama, 2007; Singh et al., 2004).

The HCA results showed that the studied rivers were grouped into two main clusters. The first cluster mainly included the Hakarichay, Kurakchay, and, to some extent, the Tartarchay. These rivers were characterized by low biogenic loads, high dissolved oxygen concentrations, and relatively stable microbiological indicators. This grouping reflects the similarity of their hydro-ecological characteristics, their lower exposure to anthropogenic pressures, and their relatively favorable ecological conditions.

The second cluster included the Lower Kura, Ganjachay, Araz River, and Okhchuchay. These rivers were characterized by higher concentrations of ammonium, nitrate, and phosphate, greater abundance of saprotrophic bacteria and total microbiota, and relatively lower dissolved oxygen levels. These results indicate that nutrient inputs from agricultural activities, settlements, and

other anthropogenic sources have a significant influence on the ecological status of these basins.

According to the PCA results, the first two principal components explained 76.1% of the total variance, indicating that a large proportion of the variation in the ecological variables could be represented by two components. The first principal component (PC1) explained 45.4% of the total variance, while the second principal component (PC2) explained 30.7%. These values confirm the high explanatory capacity of the multivariate statistical model and indicate that the selected variables adequately reflected the ecological status of the river ecosystems.

High positive loadings on PC1 were mainly associated with total microbiota, saprotrophic bacteria, ammonium, nitrate, and phosphate. The positioning of these variables in the same direction indicates that they varied in a coordinated manner and were mainly associated with anthropogenic nutrient loading. Previous studies have likewise reported that high nutrient concentrations stimulate the development of heterotrophic microorganisms and consequently increase microbiological activity (Varol et al., 2012; Shrestha and Kazama, 2007).

The second principal component (PC2) mainly characterized temperature, dissolved oxygen, and pH. The positive loading of temperature and the opposite positioning of dissolved oxygen further confirm the negative relationship between these parameters. This pattern is explained by the temperature dependence of oxygen solubility in

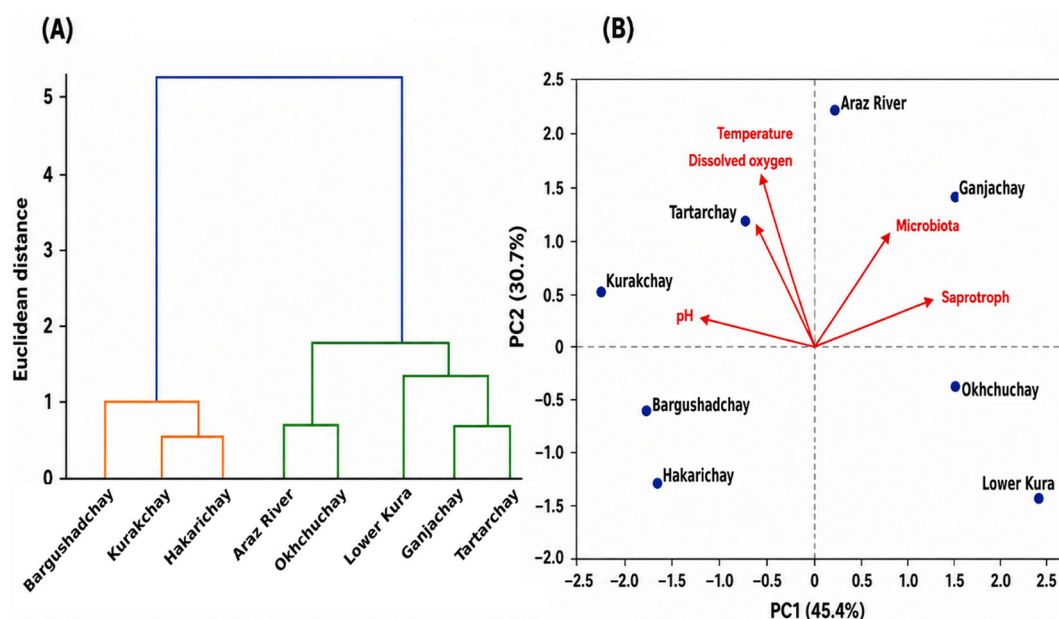


Figure 11. Hierarchical cluster analysis (HCA) (A) and principal component analysis (PCA) (B) based on the microbiological and ecological parameters of the studied rivers

water and is considered characteristic of freshwater ecosystems (Wetzel, 2001).

In the PCA ordination, the proximity of the Ganjachay and Lower Kura to microbiological and biogenic variables indicates higher concentrations of organic matter and nutrients in these basins. In contrast, the Hakarichay and Kurakchay were located in the region associated with relatively lower temperatures and higher dissolved oxygen levels, reflecting their comparatively better ecological status.

The Bargushadchay and Tartarchay occupied an intermediate position in the ordination. Their physicochemical and microbiological indicators were situated between the two main groups, indicating a transitional hydroecological condition. This result suggests that although anthropogenic impacts are present in these basins, they are less pronounced than in the more heavily affected rivers.

Overall, the PCA and HCA results demonstrated that the ecological status of the rivers is determined not by individual physicochemical or microbiological parameters alone, but by their combined interactions. The multivariate statistical approach showed high informative value in identifying basins exposed to stronger anthropogenic pressures and supported the general patterns observed in the physicochemical and microbiological results presented in the previous sections.

Thus, the PCA and HCA results showed that the Ganjachay, Araz River, Lower Kura, and Okhchuchay are river systems in which anthropogenic impacts are more clearly expressed and which are therefore more vulnerable in terms of ecological risk. In contrast, the Hakarichay, Kurakchay, and Tartarchay were characterized by more favorable hydroecological conditions and relatively lower anthropogenic loading. The obtained results provide an important scientific basis for planning future monitoring programs, prioritizing high-risk river basins, and ensuring the efficient management of water resources.

Scope of the study and directions for further research

The present study was designed to characterize the seasonal hydrochemical and functional-microbiological status of eight rivers of the Kura and Araz basins, and the conclusions drawn here relate to that scope. Organic micropollutants - in particular petroleum hydrocarbons and phenols - as well as heavy metals and total nutrient fractions were beyond the scope of this survey, and hydrological and land-use data were not compiled alongside the water quality measurements. The relationships identified between nutrient loading, microbial abundance and anthropogenic pressure are therefore interpreted as consistent associations rather than as demonstrated

cause-and-effect links, and the classification proposed here rests on physicochemical and microbiological indicators. Extending the programme to include hydrocarbon, phenol and metal determinations - especially in the Okhchuchay, where mining activity in the upper catchment has been reported-together with the biological quality elements used in the EU Water Framework Directive and GPS referencing of the sampling stations, would allow the present findings to be developed into a fully source-attributed assessment. The dataset reported here provides the baseline against which such an extended programme can be built.

CONCLUSIONS

This study comprehensively assessed the water quality of eight rivers belonging to the Kura and Araz basins of Azerbaijan over four seasons using physicochemical and microbiological parameters. Water temperature and dissolved oxygen exhibited clear seasonal variability in opposite directions, whereas pH remained relatively stable throughout the year in all rivers.

The concentrations of biogenic elements (NH_4^+ , NO_2^- , NO_3^- , PO_4^{3-}) and microbiological indicators-including total microbiota, saprotrophic bacteria, bacteria involved in the nitrogen cycle, and oil- and phenol-oxidizing microorganisms-were higher in the Ganjachay, Araz River, Lower Kura, and Okhchuchay. In contrast, these indicators were both lower and more stable in the Hakarichay, Kurakchay, and Tartarchay.

A strong positive correlation was identified between total microbiota and saprotrophic bacterial abundance ($r = 0.709$; $p < 0.001$). PCA, in which the first two principal components together explained 76.1% of the total variance, and HCA confirmed this grouping and separated the rivers into two main clusters: a relatively less impacted cluster consisting of the Hakarichay, Kurakchay, and Tartarchay, and a more strongly anthropogenically impacted cluster consisting of the Lower Kura, Ganjachay, Araz River, and Okhchuchay. The Bargushadchay occupied an intermediate position between these two groups.

The obtained results indicate that the ecological status of the studied rivers is shaped by the combined effects of seasonal hydroclimatic factors and anthropogenic nutrient loading. The integrated application of physicochemical, microbiological, and multivariate statistical methods

provides a reliable scientific basis for identifying ecologically vulnerable basins and developing long-term monitoring programs for the trans-boundary rivers of Azerbaijan.

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