

Original Research

**MICROBIOLOGICAL QUALITY OF THE VELIKA MORAVA RIVER:
ECOLOGICAL AND PUBLIC HEALTH ASPECTS**

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Abstract

*Microbiological parameters represent important indicators of water quality and ecological status in river systems. This study evaluates the microbiological quality of the Velika Morava River (Serbia) at its confluence with the Danube during the period 2015–2020, with particular emphasis on seasonal variability. Key parameters included heterotrophic bacteria, aerobic mesophilic bacteria, total and fecal coliforms, *Escherichia coli*, and *Enterococcus* spp., analyzed according to ISO and standard microbiological methods. The results indicate that the river predominantly exhibits moderate ecological status (Class III), observed in 67% of samples, while poor status (Class IV) was recorded in 17% of cases. Elevated levels of fecal indicators, particularly *E. coli*, confirm continuous anthropogenic pressure from untreated wastewater and diffuse sources. Seasonal analysis shows that higher variability and maximum values of microbiological parameters occur during the spring period, primarily due to surface runoff and increased pollutant transport, while during low-flow conditions in late summer, reduced variability but sustained elevated values reflect the dominant influence of temperature and limited dilution capacity. Despite significant organic and fecal pollution, the dominance of oligotrophic bacteria ($FO/H > 1$) indicates a moderate self-purification potential of the river system.*

Keywords: microbiological indicators, Velika Morava, seasonal variability, fecal pollution, self-purification.

INTRODUCTION

Surface waters in intensively used catchments are increasingly exposed to microbiological contamination, primarily originating from untreated wastewater and diffuse agricultural sources. Unlike physicochemical parameters, microbiological indicators provide direct insight into the origin, intensity, and dynamics of pollution, particularly in relation to organic and fecal contamination. The presence of indicator organisms such as *Escherichia coli* and intestinal *enterococci* is widely used to detect recent fecal pollution and to assess potential health risks associated with water use. Their occurrence implies the possible presence of pathogenic microorganisms, including bacteria, viruses, and parasites, and therefore represents an important component in evaluating the suitability of surface waters for drinking water supply, irrigation, and recreational purposes. In addition to fecal indicators, heterotrophic and mesophilic bacteria provide valuable information on organic pollution and

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ecosystem functioning [1]. Their abundance is closely controlled by nutrient availability, temperature, and hydrological conditions, reflecting both external pollution pressures and internal biogeochemical processes. The relationship between heterotrophic and oligotrophic bacterial populations, often expressed through indices such as FO/H, enables the assessment of the self-purification capacity and mineralization potential of aquatic systems [2]. The Velika Morava River represents one of the most important river systems in central Serbia, with significant ecological, agricultural, and socio-economic importance. However, it is continuously exposed to anthropogenic pressures that influence the level and seasonal dynamics of microbiological contamination.

The Velika Morava is formed by the confluence of the West and South Morava near Stalać and flows into the Danube between Smederevo and Kostolac. The catchment area of the Velika Morava is approximately 6,126 km², while the entire Morava river system exceeds 37,000 km², covering more than 40% of the territory of Serbia. Hydromorphologically, the river is classified as a lowland river (Type 1), characterized by fine sediment dominance, pronounced meandering, and high hydrological variability. Its flow regime includes frequent spring floods and reduced discharge during summer low-flow periods, which directly influence water quality through dilution during high-flow events and increased contaminant concentrations during low-flow conditions.

The basin, located within the fertile Pomoravlje region, is subject to multiple anthropogenic pressures. Although physicochemical parameters generally correspond to Class I–II, the river is sensitive to organic and nutrient pollution, particularly during low-flow periods when dissolved oxygen concentrations may decline. Microbiological contamination is especially pronounced near urban areas such as Jagodina, Čuprija, and Paraćin, where untreated municipal wastewater represents a dominant pollution source. Additional pressures include agricultural runoff enriched with nutrients, as well as diffuse pollution and solid waste inputs, resulting in pronounced spatial and temporal variability of water quality, which generally deteriorates in the downstream direction.

The aim of this study is to assess the microbiological quality of the Velika Morava River and to evaluate its ecological status based on standard microbiological indicators, with particular emphasis on seasonal variability and the influence of hydrological and temperature-related factors on microbial dynamics.

MATERIALS AND METHODS

Water samples were collected at a monitoring profile located approximately 2 km upstream from the confluence of the Velika Morava River with the Danube (rkm 1103) during the period 2015–2020 (Figure 1). Sampling was conducted twice per year, in early spring (April) and late summer (August–September), to capture seasonal variability under contrasting hydrological conditions. Microbiological analyses were performed in accordance with national regulations (Official Gazette RS 50/2012 and 74/2011) and internationally accepted methods. Heterotrophic bacteria (HPC) were enumerated according to ISO 6222:1999/2024 at two incubation regimes: (i) 22 ± 2°C for 5–7 days (psychrophilic/autochthonous bacterial population), and (ii) 35 ± 2°C for 48 h (aerobic mesophilic bacteria, indicating allochthonous

and potentially anthropogenic input). Total and fecal (thermotolerant) coliforms, as well as *Escherichia coli* and *Enterococcus spp.*, were determined using the Most Probable Number (MPN) method based on defined substrate technology (Colilert-18 and Enterolert E/Quantitray®, IDEXX, USA) [3,4]. Additionally, the abundance of facultative oligotrophic bacteria was determined using R2A medium, and their proportion relative to total heterotrophic bacteria was expressed as the FO/H index, representing an indicator of self-purification potential and organic matter mineralization [2]. Water quality classification was performed according to Serbian regulatory criteria (Official Gazette RS 50/2012 and 74/2011), based on the most unfavorable value among the analyzed microbiological parameters [5,6]. For the purposes of this study, the results are presented as seasonally aggregated datasets (spring and late summer periods). Descriptive statistical analysis was applied to evaluate data variability, including median (Q2), lower (Q1) and upper (Q3) quartiles, interquartile range (IQR), and identification of extreme values using standard box-plot criteria ($Q1 - 1.5 \times IQR$ and $Q3 + 1.5 \times IQR$). These statistics were used to illustrate seasonal differences in microbiological water quality



Figure 1 Location of sampling sites

RESULTS AND DISCUSSION

The variability and distribution of the analyzed microbiological parameters are presented in Figure 2, showing a wide range between minimum and maximum values for all indicators. The highest variability was observed for heterotrophic bacteria and coliform groups, confirming their sensitivity to changes in environmental conditions and pollution inputs. Elevated average values of these parameters indicate a persistent influence of organic and fecal contamination in the Velika Morava River. Seasonal differences are clearly reflected in the results. As shown in Figure 3, microbiological parameters during the spring period (April–June) exhibit increased variability and generally higher maximum values. This pattern is primarily associated with intensified surface runoff, soil erosion, and resuspension processes, which contribute to the transport of microorganisms and organic matter into the river system. The elevated values of coliform bacteria, *Escherichia coli*, and *enterococci* further indicate a stronger influence of diffuse pollution sources during high-flow conditions.

In contrast, Figure 4 shows that during the late summer–early autumn period (August–September), the variability of microbiological parameters is reduced, but the values remain

elevated. This reflects more stable hydrological conditions combined with limited dilution capacity. Under low-flow conditions, the influence of temperature becomes more pronounced, enhancing microbial activity and favoring the dominance of mesophilic bacteria.

The comparison between Figures 3 and 4 highlights a clear seasonal shift in controlling factors: hydrological processes dominate during the spring period, while temperature-driven processes become more significant during late summer. These results indicate that microbiological water quality in the Velika Morava River is governed by the combined effects of hydrological variability and thermal conditions, which together control the intensity and dynamics of microbial contamination.

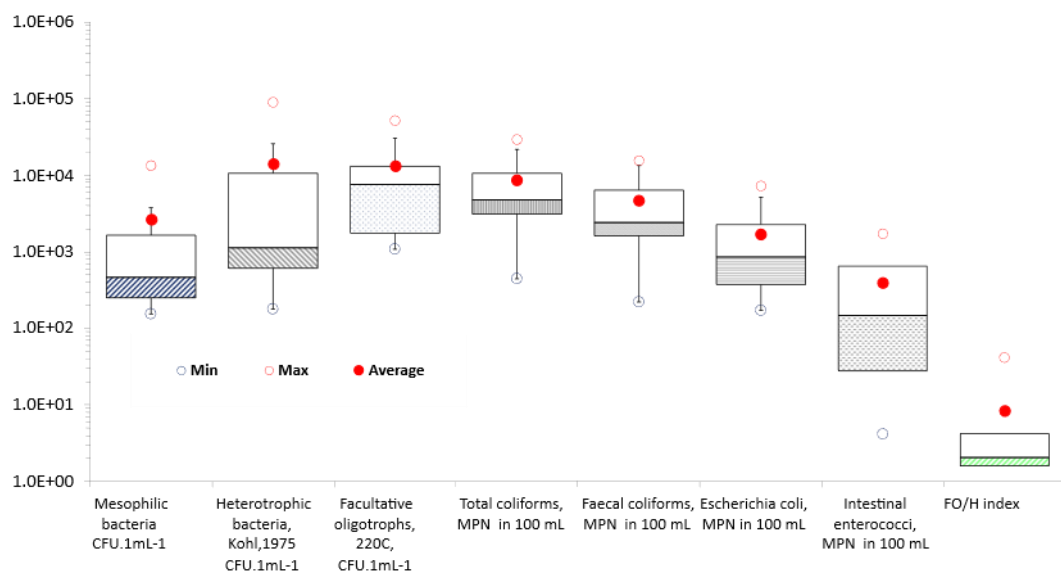


Figure 2 Distribution of microbiological indicators in the Velika Morava River (2015–2020), presented as minimum, maximum, and mean values

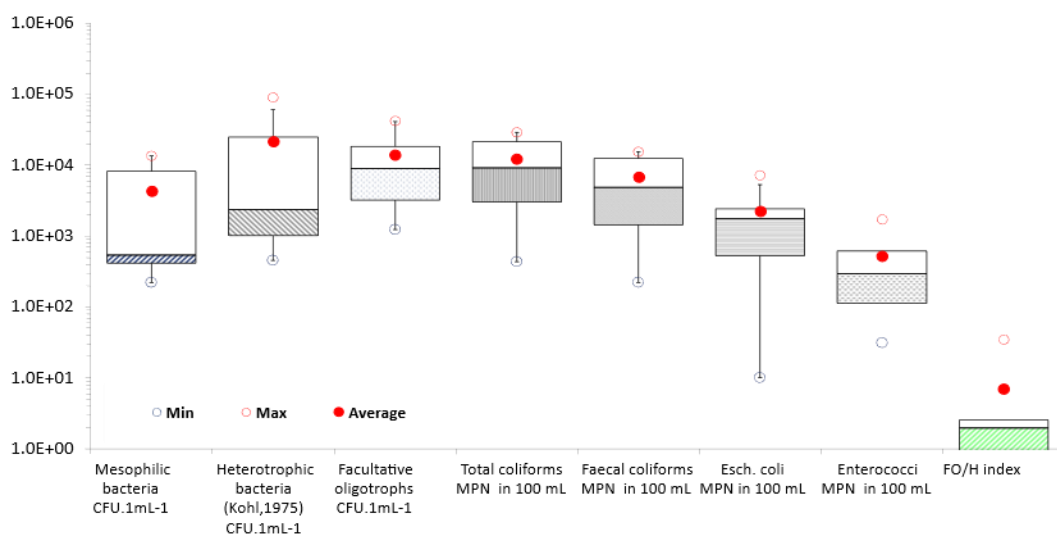


Figure 3 Seasonal variation of microbiological indicators in the Velika Morava River during the spring period (April–June 2015–2020), presented as minimum, maximum, and mean values

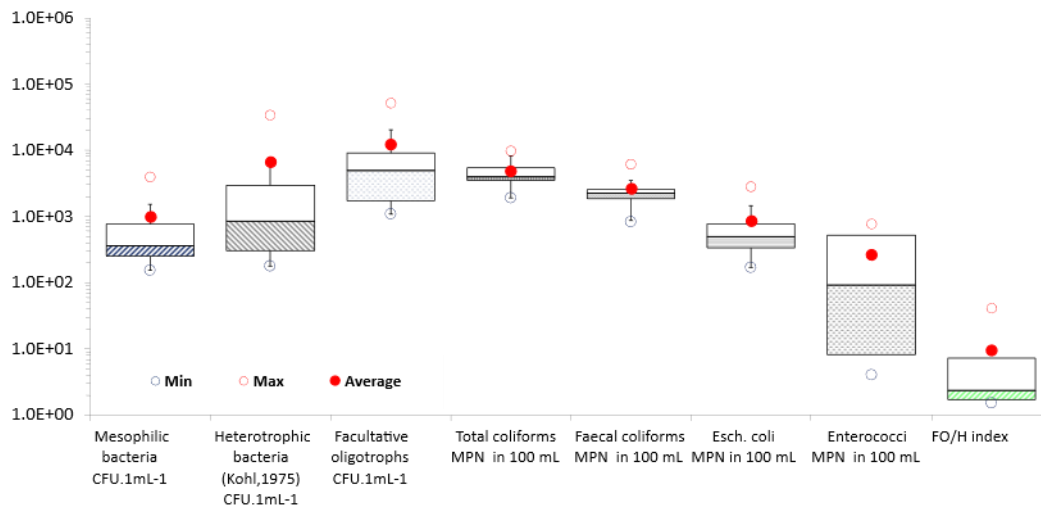


Figure 4 Seasonal distribution of microbiological indicators in the Velika Morava River during low-flow conditions (August–September 2015–2020), presented as minimum, maximum, and mean values

CONCLUSION

The results of this study indicate that the Velika Morava River is predominantly characterized by moderate ecological status, accompanied by persistent organic and fecal contamination. Elevated concentrations of indicator bacteria, particularly *Escherichia coli*, confirm continuous anthropogenic pressure and highlight potential sanitary risks associated with water use. The findings further demonstrate that microbiological water quality is strongly influenced by hydrological conditions and seasonal variability. During low-flow periods, increased water temperature and reduced dilution capacity enhance microbial activity, leading to higher abundances of mesophilic bacteria and indicating an elevated risk of the presence of potentially pathogenic microorganisms [1]. Under such conditions, the use of river water for recreational purposes or irrigation of crops consumed without thermal processing may be limited. Although the dominance of oligotrophic bacterial communities suggests a moderate self-purification potential, this natural capacity is insufficient to counterbalance continuous pollutant inputs from both point and diffuse sources. These results emphasize the need for adaptive water management strategies, including the improvement of wastewater treatment infrastructure and the adjustment of treatment processes for drinking water production in accordance with seasonal and hydrological variations. Such measures are essential for achieving a better ecological status and ensuring the safe and sustainable use of water resources in the Velika Morava basin.

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