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Original Research

Assessment of *Escherichia coli* Levels in Vjosa River using Standard Microbiological Indicators

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Abstract

The Vjosa River one of the few wilde large European rivers is increasingly exposed to human-driven pressures may put at risk its microbial quality. This study estimate the surface-water microbiological status of the Vjosa using Escherichia coli (E. coli) as a primary indicator of faecal contamination. Sampling was performed between October 2024 and October 2025 at 13 locations (seven on the main channel and six on tributaries) in accordance with ISO 19458 for water-quality microbiology. E. coli concentrations were determined by using the Most Probable Number (MPN) method following ISO 9308-1:2014. The results showed contamination in different areas of the river. Average E. coli levels resulted between Class I-II (slight to moderate pollution), yet the several sites repeatedly reached Class III levels (severe pollution), especially in the catchments of Sites 03, 11, 13, 15, and 16. These hotspots are ralated to sites impacted by untreated wastewater discharges, agricultural runoff, and animal activities. A strong positive correlation is between E. coli and intestinal enterococci confirmed that the contamination is originated from faecal sources. Vjosa River generally maintains a moderate microbiological quality, episodic episodes of intense faecal input create public-health concerns. High concentrations of E. coli, total and faecal coliforms, and enterococci render the water unsuitable for recreation or drinking. The study underscores the value of E. coli as a rapid proxy for overall microbial safety and recommends sustained monitoring together with improved catchment-area management to curb faecal pollution in the river's surface waters.

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Keywords: *Vjosa river, Escherichia coli, MPN, Faecal coliform, Heterotrophic bacteria, water quality status.*

Introduction

1. The Vjosa River originates in the mountainous regions of Greece and flows to the Adriatic coast of Albania, representing an important freshwater system both ecologically and socio-economically (Ahmed et al., 2018; Brunkard et al., 2011; Farnleitner et al., 2010). In recent decades, population growth within the watershed and the expansion of agricultural activities have increased its vulnerability to faecal pollution (. Dassenakis, et al. ,1998; Field&Samadpour, 2007 ; ISO .2014). The river supports rich biodiversity, contains valuable natural habitats, and is widely used for irrigation, recreation, and household water needs(Boehm & Soller, 2019 ; Byamukama et al., 2005; EEA. 2018). Microbiological monitoring of surface waters is commonly based on the detection of faecal indicator bacteria, mainly Escherichia coli and intestinal enterococci (EF), which are strongly associated with human and animal faecal contamination (APHA, 2017 ; Bartram & Rees, 2000).

Higher concentrations of these indicators are related to increased public health risks, especially for recreational and agricultural water use. In this context, E. coli is mostly considered the primary indicator of faecal pollution because it persists in freshwater environments and is closely related to the possible presence of enteric pathogens (Boehm & Soller , 2019).

The microbiological quality of waters is based on standardized international regulations, on which this study is supported. For field sampling, the ISO 19458 standard method was applied, while the determination of E. coli was carried out according to ISO 9308-1. In addition, the Most Probable Number (MPN) technique was used to estimate the total number of E. coli (Borrego et al., 2002; Brunkard et al., 2011). There are very few studies, if any, on the water quality of the Vjosa River. Previous studies of Albanian freshwater systems often confirm localized faecal contamination, which is most evident where waters flow downstream from populated urban areas and intensive domestic animal operations (Byamukama et al. ,2005). Since there is a lack of systematic monitoring of microbiological parameters across the entire Vjosa River, this study provides more comprehensive information on its microbiological pollution (Byamukama et al. ,2005). Over a one-year period, E. coli and faecal enterococci concentrations will be measured at thirteen strategically selected sampling stations along the river's main course and its primary tributaries (Field & Samadpour , 2007) This spatial and temporal dataset will reveal patterns of faecal contamination by highlighting the most polluted areas, which can become a primary focus for intervention by institutions responsible for developing water management strategies in Albania (Coffey et al., 2020).

Material and Methods

Study area and sampling design.

Sampling was taken every month from October 2024 to October 2025 at 13 stations distributed along the main channel and 6 major tributaries of Vjosa river (Figure 1), by following recommendations for assessing the microbiological water quality in different areas of the river (Dassenakis et al., 1998). The sampling for this study was based on 13 monitoring points, which included locations from the upper part of the river as well as areas near agricultural and urban zones, providing a comprehensive overview of microbiological pollution over a full year (EPA, 2012).

Figure 1. Sampling stations along the Vjosa River

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At each site, 200 mL grab samples were collected using sterile glass containers at 30-50 cm depth and transported at 4°C to the laboratory, where analyses were carried out within 6-7 hours (Borrego et al. ,2002). Numerical determination of faecal indicator bacteria (E. coli and intestinal enterococci) followed the Most Probable Number (MPN) approach using serial dilutions interpreted through probability tables ((APHA, 2017). Heterotrophic plate counts were earned via the pour-plate protocol on PCA medium and incubated at 22 °C and 35 °C to quantify psychrophilic and mesophilic communities (European Commission, 2020). Water-quality classes were assigned based on E. coli concentrations expressed in CFU/100 mL in agreement with internationally known microbiological limits. (European Commission, 2020).

Enumeration of faecal indicator bacteria.

Escherichia coli (E. coli) and intestinal enterococci were quantified using the Most Probable Number (MPN) method. Serial dilutions (0, I, II, III) were prepared for all samples, and results were calculated based on standardized MPN statistical probability tables (Dassenakis et al. ,1998). Enumeration and confirmation of E. coli and intestinal enterococci followed the procedures set by ISO 19458 for microbiological sampling and laboratory handling (Borrego et al., 2002) .For heterotrophic determination, the pour plate technique was applied on PCA medium, they were incubated at 22 °C and 35 °C for 48 hours up to 5 days in a biological thermostat, allowing the detection of both psychrophilic and mesophilic bacterial groups (EPA, 2012). Microbiological water status was classified based on E. coli concentrations expressed in CFU/100 mL according to accepted international thresholds for recreational water and surface-water quality classification (European Commission, 2020).

Results

Spatial variation of E. coli concentrations.
Faecal contamination along the entire Vjosa River is reflected in (Table 1), which outlines the mean and maximum E. coli concentrations measured across 13 stations over the monitoring year (Brunkard et al., 2011). The data collected during this year clearly show that the points with the lowest levels of contamination are those located in the upper part of the river, such as Site-01 (MIF) and Site-09 (VJO). The highest levels of pollution were observed in the downstream section of the river, where urban areas and livestock development zones are concentrated, specifically at Site-03, Site-11, Site-13, Site-15, and Site-16 (European Environment Agency, 2018).

Table 1. Summary of mean and maximum E. coli (FC) and intestinal enterococci (EF) concentrations and microbiological water quality class for each station along the Vjosa River (October 2024 - October 2025).

Code	Zone	FC mean	FC max	EF mean	EF max	Class
Site-01	Mifol	136	460	162	1100	**II**

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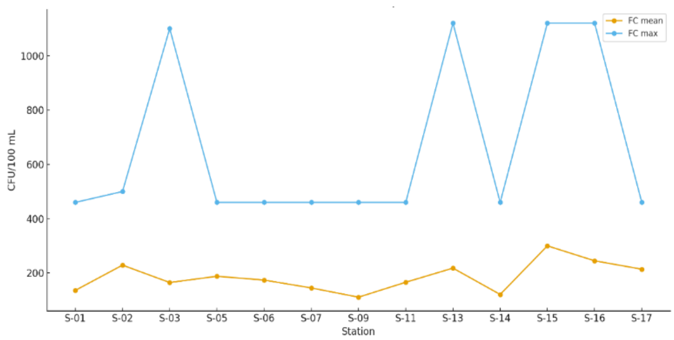
Site-02	Hambare	229	499	81	460	**II**
Site-03	Poçem	165	1100	153	460	**III**
Site-05	Memaliaj	188	460	95	150	**II**
Site-06	Dragot	174	460	153	460	**II**
Site-07	Kelcyre	145	460	106	460	**II**
Site-09	Vjosë	111	460	136	460	**II**
Site-11	Sarandoporo	166	1100	90	240	**III**
Site-13	Gjorm	218	1100	137	460	**III**
Site-14	Bençë	121	460	187	1100	**II**
Site-15	Drino	300	1100	74	150	**III**
Site-16	Kardhiq	245	1100	149	240	**II**
Site-17	Gjirokastër	214	460	145	240	**II**

We used annual mean values as well as maximum values of Faecal Coliforms (FC) as indicators of contamination (Figure 2), where a comparison was made among the 13 sampling points. During this monitoring year, a change between maximum and mean values was observed at all sampling sites. The overall distribution shows that most locations present a moderate contamination (Class II). This suggests that, in most of these points, pollution is not constant throughout the entire year but rather episodic, possibly related to different activities such as climatic variations (for example rainfall) or agricultural discharges occurring at different periods by local farmers (Farnleitner et al., 2010).

The highest contamination peaks, reaching up to 1100 CFU/100 mL, were recorded at four locations: Site-03 (Poçem), Site-11 (Sarandoporo), Site-15 (Drino), and Site-16 (Kardhiq). These locations indicate significant localized contamination, which is most likely the result of untreated wastewater discharge, intensive agricultural runoff, and livestock operations (A gradient in FC levels was also observed. The lowest concentrations were found at Site-01 (Mifol), Site-09 (Vjosë), and Site-14 (Bençë), which may be associated with upstream locations or minimal anthropogenic influence (Haller et al., 2009). Mean FC concentrations varied between 110-300 CFU/100 mL, with the highest averages mostly recorded at Site-15 (Drino). The consistently high levels at Site-15 identify it as the most impacted site throughout the study. From Figure 2, differences between the maximum and minimum values of pollution distribution can be observed, showing high intensity and clear localization near populated areas and agricultural zones along the Vjosa River
(Hlavsa et al., 2015).

Collectively, these findings reveal that FC pollution along the Vjosa River is not diffuse but is strongly driven by specific local anthropogenic sources, including livestock, wastewater effluents, and agricultural runoff (ISO, 2006).

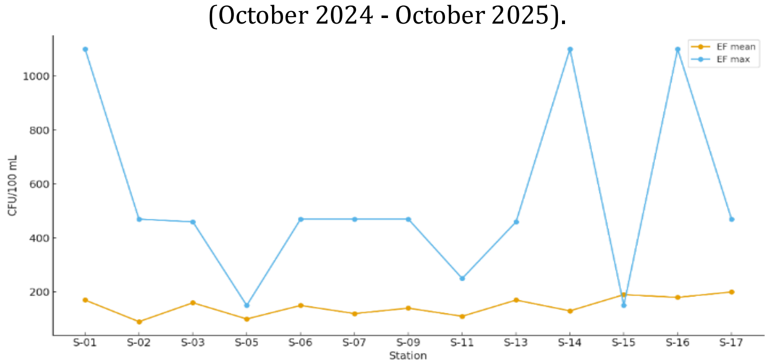
Figure 2. Average and maximum Escherichia coli (FC) concentrations across sampling stations along the Vjosa River (October 2024–2025).



Spatial variation of intestinal enterococci

Looking the pattern in fecal coliforms (FC), the lower mean concentrations compared to maximum EF values across all sites (Figure 3) it means that contamination run by sporadic, acute pollution events rather than continuous, background faecal loading during the monitoring period (ISO, 2014). Peak enterococcal pollution was intense, reaching 1100 CFU/100 mL at the most locations: Site-01 (Mifol), Site-14 (Bençë), and Site-16 (Kardhiq). The most, Site-15 (Drino) presented a microbiological exception: it showed the lowest mean EF concentration despite having previously shown the highest FC mean. This difference observed between each site provides important information about pollution sources as well as variations in the survival rates of microorganisms (Kavka et al., 2006). Overall EF mean concentrations were gathered, ranging between 80-200 CFU/100 mL (with Site-14, Bençë, recording the highest average). This tight range suggests that while EF contamination peaks dramatically in specific locations, its average distribution is particulary more uniform than that of FC (Field & Samadpour, 2007). Contrasts between average and peak E. coli levels vividly reveal episodic contamination events (EEA,2018). These spikes are most showed at stations situated close to farming areas, urban zones, and domestic animal operations (Byamukama et al., 2005). This geographical distribution of E. coli contamination clearly indicates that pollution mainly originates from occasional sources, such as human or animal waste, rather than from continuous sources, which could become concerning at high levels for the population living around the Vjosa River (Kay et al., 1994).

Figure 3. EF mean and EF max concentration across stations of the Vjosa River (October 2024 - October 2025).



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It is observed that faecal contamination in the Vjosa River shows strong seasonal variation (Figures 1 and 2) and also indicates consistent pollution. During the monitoring period, this contamination did not meet the recommended limits required to classify the river as a low-pollution and safe aquatic environment (Kavka et al., 2006). The data collected over this year revealed a predictable annual pattern: a slight decline in early summer was followed by a sharp increase in mid-summer. Contamination reached its highest values in August 2025 (Figure 4), recording the highest monthly average of the study (~301 MPN/100 mL). This peak in contamination was not random; it coincided with the peak of tourist activity and intensive recreational use of Vjosa River areas (Boehm & Soller, 2019). Additionally, agricultural activity is another factor influencing microbial growth (Byamukama et al., 2005). Another important factor to consider is the increase in temperatures during the summer months, which contributes to enhanced microbial growth and is accompanied by a reduction in water discharge (Ulanovsky et al., 2002). Together, these conditions maximized both bacterial growth and the concentration of existing contaminants (Hlavsa et al., 2015).

The minimum average value was recorded earlier, in June 2025 (~216 MPN/100 mL). This minimum observed in spring is related to the wet season effect, during which increased river flow leads to water dilution and a reduction in bacterial levels. A decrease in faecal coliforms is observed after August, and this decline continues throughout the autumn months. Based on Figures 1 and 2, Figure 3 clearly shows that faecal contamination in the Vjosa River is sporadic and related to climatic conditions. It appears to be localized and exhibits seasonal variation linked to the hydrological regime (EEA,2018). Based on these results, continuous monitoring is considered necessary during the summer season, since contamination levels increase significantly during these months and may pose a risk to water users, as well as cause negative effects on downstream ecosystems (Ulanovsky et al., 2002).

Figure 4. Monthly average Fecal Coliform (FC) concentrations in the Vjosa River (October 2024 – October 2025).

As shown in Figures 1, 2, and 3, the comparison of the mean concentrations of FC and EF across the 13 stations clearly indicates that microbial pollution along the Vjosa River is not random but is associated with sites located closer to urban areas. This suggests that the contamination mainly originates from human activities (McAllister & Topp, 2012). The stations that consistently recorded the highest FC values also showed elevated

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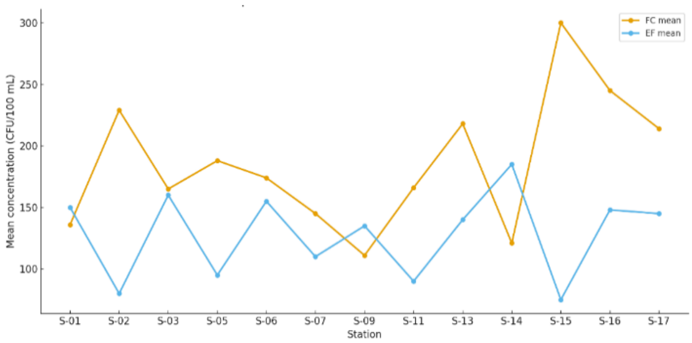
EF values, indicating that both indicators respond similarly to contamination pressure. The highest monthly mean concentration was recorded in August 2025 (~302 MPN/100 mL), suggesting increased contamination during the summer season, likely influenced by higher temperatures, intensified recreational and agricultural activities, and reduced water flow (Leclerc et al., 2001). The lowest concentration was observed in June 2025 (~216 MPN/100 mL), potentially linked to higher river discharge and dilution effects at the peak of the wet season.

During autumn and winter (October-February), average FC values remained relatively stable (275-300 MPN/100 mL), indicating a continuous input of faecal pollution sources independent of seasonal variation (EEA, 2018). After reaching their seasonal minimum in June, FC levels rose sharply again in July and August, followed by a gradual decrease during September-October 2025 (Ulanovsky et al., 2002). Overall, the figures show that FC contamination in the Vjosa River is strongly influenced by weather conditions and human activities, highlighting the need for continuous management and the implementation of mitigation strategies to protect both environmental and public health (McLellan & Eren ,2014).

Figure 5. Comparison of mean Escherichia coli (FC) and intestinal enterococci (EF) concentrations across sampling stations



Discussion

This 12-month monitoring and assessment of microbial contamination in the Vjosa River showed that, in several months and at several sites, the recommended permissible limits for surface waters were exceeded (Dassenakis et al., 1998). Although upstream sections such as Site-01 (Mifol), Site-02 (Hambare) and Site-09 (Vjosë) usually showed lower microbial loads, mid- and downstream segments showed recurrent critical peaks, often surpassing 1,100 CFU/100 mL (Kavka et al., 2006). These results indicate that the Vjosa River experiences measurable faecal pressure, in agreement with previous studies on

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riverine systems affected by agricultural and domestic wastewater inputs (Molina et al., 2014) The most persistent hotspots were identified at Poçem (Site-03), Sarandoporo (Site-11), Gjorm (Site-13), Drino (Site-15) and Kardhiq (Site-16) (Kavka et al., 2006). The above-mentioned areas are associated with residential zones that also have agricultural activities carried out by the local inhabitants, which are factors that contribute to the growth and introduction of faecal bacteria into the river's aquatic environment (Byamukama et al., 2005). The high *E. coli* maxima watched at Poçem, Kardhiq and Gjirokastër indicate major episodic contamination, likely driven by upstream livestock discharge and runoff from agricultural land during rainfall periods (Pinto et al., 2012). Same thing also, with the Sarandoporo and Drino tributaries contributed to elevated values, show us

that the microbiological state of the Vjosa River is firmly influenced by its tributary network rather than the main channel alone (EEA, 2018). The strong relationship between *E. coli* and intestinal enterococci makes it clear that both indicators respond in a directly proportional manner to faecal contaminants, and their combined use provides a broader assessment of surface-water quality, as in this case for the Vjosa River (McAllister & Topp, 2012). Mean values remained mostly within Class II water quality (100-1,000 CFU/100 mL), showing moderate contamination in most parts of the river. However, maximum values in most sites reached Class III, related to critical water quality. This indicates that although the baseline microbial load may remain moderate, episodic pollution pulses represent a significant concern, especially during high-flow seasons or rainfall-driven runoff events (Psomas et al., 2016). If this assessment is viewed from a public-health perspective, some sections of the river cannot be considered safe for use in agriculture, livestock activities, or irrigation. Areas influenced by river tributaries, such as the Gjorm, Kardhiq, and Gjirokastër sites, can be considered to present a high sanitary risk; therefore, improved water management is required in these zones for such uses (Reischer et al., 2008). Overall, the results collected over this one-year period show that the Vjosa River is an important ecological habitat and remains under faecal contamination pressure along its entire course. Continuous microbiological assessments throughout the river are necessary to prevent health and ecological risks (McLellan & Eren, 2014).

Conclusion

This comprehensive one-year assessment of the entire Vjosa River has provided an overall picture of microbial contamination, particularly from Escherichia coli, demonstrating that faecal pollution is widespread along the river's full length and represents a chronic, year-round issue(Ahmed et al., 2018; Boehm & Soller, 2019;Byamukama et al., 2005). In the middle and downstream segments of the river, higher contamination is observed compared to the upstream areas, such as Hambare or Vjosë (Bartram & Rees, 2000;Coffey et al., 2020;EEA, 2018). Critically, persistent hotspots identified repeatedly at Poçem, Sarandoporo, Gjorm, Drino, Kardhiq, showes that this contamination is not a sporadic event but a deeply periodic issue (Boehm & Soller,, 2019; EEA ,2018; Kavka et al., 2006). While the majority of average pollution index remained within Class II (moderate), maximum *E. coli* peaks frequently are into Class III (critical pollution), telling periods of significant sanitary risk
(Bartram & Rees, 2000); Brunkard et al.,2011;Leclerc et al., 2001) .
From a public health point of view, these results show that several parts of the river especially near tributary are not microbiologically safe for recreational use or for irrigating edible crops (Borrego et al., 2002; ISO, 2006; European Commission, 2020). In addition, the consistent agreement between *E. coli* and intestinal enterococci values supports the use of these two indicators together for routine surface-water quality assessments(APHA, 2017;Coffey et al., 2020; Kavka et al., 2006). Generaly, the findings highlight that protecting the ecological habitat of the Vjosa

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River and the health of the communities that depend on it requires coordinated action (EPA, 2012 ; Haller et al., 2009); Kay et al.,1994) . The priority steps should include targeted measures in high-risk areas, rapid investment in improved wastewater infrastructure, stricter control of livestock activities, and continuous, integrated watershed monitoring and management programs [Byamukama et al.,2005; Hlavsa et al., 2015) ;McLellan & Eren,2014)

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