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Research Article

Bio-indicator Bacteria Levels in Riva Stream, an Important Stream in İstanbul, Türkiye

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ABSTRACT

Bacterial pollution is the presence of harmful bacteria in aquatic ecosystems, which can cause health problems if ingested. These bacteria originate from a variety of sources, including human and animal waste, agricultural runoff, and untreated sewage. This study aimed to assess the levels of bioindicator bacteria and their associations with various environmental parameters in surface water samples. The samples were collected seasonally from five stations along Riva Stream between March 2018 and January 2019. Indicator bacterial levels were determined using membrane filtration. Temperature, salinity, pH, dissolved oxygen, and conductivity values of the stations were measured in situ during sampling using a multiparameter (YSI). As a result of the sampling, maximum total coliform, fecal coliform, and intestinal enterococci levels were recorded as 24 x 10^3 CFU/100ml, 18 x 10^3 CFU/100ml and 24 x 10^2 CFU/100 ml, respectively. During sampling, the indicator bacterial levels were above the limit at all stations. When the FC/IE ratios were analyzed, the maximum FC/IE ratio was recorded as 52.10 during the winter sampling period at the station. The detection of bioindicator bacteria levels above the standard limit throughout the sampling period (especially during the summer period) was associated with a high level of domestic waste in the region. The results showed that more frequent and detailed monitoring studies should be conducted in this region.

Keywords: Total coliform, Fecal coliform, Intestinal enterococci, Environmental parameters, Riva stream

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INTRODUCTION

Microbial communities have a significant impact on the regulation of water quality and biogeochemical cycling in freshwater ecosystems. It is well-known that bacteria play a pivotal role in the decomposition and stabilization of organic matter in both natural environments and biological sewage treatment processes. Comprehending the ecology and evolution of microbial communities and the implications of these changes for public health, it is imperative to evaluate physical and chemical parameters, including salinity, temperature, and pH (Ibekwe et al., 2012).

Accelerating urbanization, increased population density, and ineffective sewerage infrastructure systems have important implications for public health (Mallin, 2000; 2001; Garbossa, 2017). Water sources contaminated with sewage and human fecal contamination are related to the occurrence of infectious waterborne diseases (McKee and Cruz, 2021; Manetu and Karanja, 2021; Kahn et al., 2018).

Coliform bacteria, especially enteric bacteria, serve as indicators of the presence of bacterial pathogens. Although fecal coliforms themselves are generally not directly harmful to human health, their detection indicates the presence of fecal waste that may harbor harmful pathogens (Farrell, 2013). The microbial water quality standard includes the evaluation of bacterial indicator organisms, which often includes the coliform group, fecal streptococci, enterococci, and *Escherichia coli* (E. coli) (Wen et al., 2020; Davene et al., 2020; Augustyn et al., 2016; APHA, 2013). When pollution originates from human waste, the fecal coliform/fecal streptococcal ratio (FC/IS ratio) is greater than 4, between 0.1 and 4.0 when it originates from domestic animals such as cattle, and below 0.1 in wild animals (Clinton et al., 2006, Benhassane et al., 2019, Gerba, 2009).

The Marmara Sea, an inland sea between Asia and Europe and the gateway to the Aegean and Black Seas through the Bosphorus (Bosphorus) and the Dardanelles (Dardanelles), is important for the fisheries sector, maritime transport, and ecosystem health (Altuğ et al., 2009). The Riva Stream flows through Riva village, Istanbul and it is approximately 33 km long. It is located between the Black Sea and the exit of the Marmara Sea and is one of the largest rivers in the region. Ömerli Dam, the largest drinking water reservoir on Riva Stream, was established by the General Directorate of State Hydraulic Works (DSİ) in 1972 (Tarkan, 2010; Özuluğ et al., 2005). The area around Riva Stream and Riva Beach offers opportunities for a variety of outdoor activities such as hiking, picnics, camping and water sports. Due to these features, it has become a popular touristic and recreational destination.

Studies indicating the presence of pollutants and including topics such as fishing, mollusks, indicator bacteria and eutrophication have emerged in previous studies on the Riva Stream (Dökümcü and Koşal Şahin, 2022; Altuğ et al., 2016; Garipağaoğlu et al., 2015; Tarkan, 2010; Özuluğ et al., 2005; Dinseven and Çurgunlu, 1988).

This study monitoried the level of indicator organisms associated with bacterial pollution at determined stations in Riva stream and determined the relationship of bio-indicators with variable environmental parameters.

MATERIALS AND METHODS

Surface water (0-30 cm) was collected seasonally from five stations between March 2018 and January 2019. The samples were delivered to a laboratory under cold chain conditions and were analyzed on the same day as the sampling. The stations are shown in Figure 1 and listed in Table 1.

The membrane filtration method was used to determine indicator bacteria levels. The100ml samples filtered through sterile filters were placed in Endo-NKS (sartorius) medium for total coliform bacteria, m-FC - NKS (Sartorius) for fecal coliform and azide - NKS (Sartorius) medium for intestinal enterococci. The media were incubated for 24 hours at 37±0.1°C for total coliforms and intestinal enterococci, and at 44±0.1°C for fecal coliforms. The colonies grown at the end of the incubation were counted and recorded as CFU/100ml. The pink-red colonies exhibiting a yellow-green metallic sheen in the m-Endo medium were considered as potential indicators of coliforms. The Cytochrome oxidase test (API Strep, BioMereux) was performed on the pink-red colonies with a yellow-green metallic sheen, which were evaluated as suspicious for coliform, and oxidase-negative colonies were taken into numerical evaluation. The

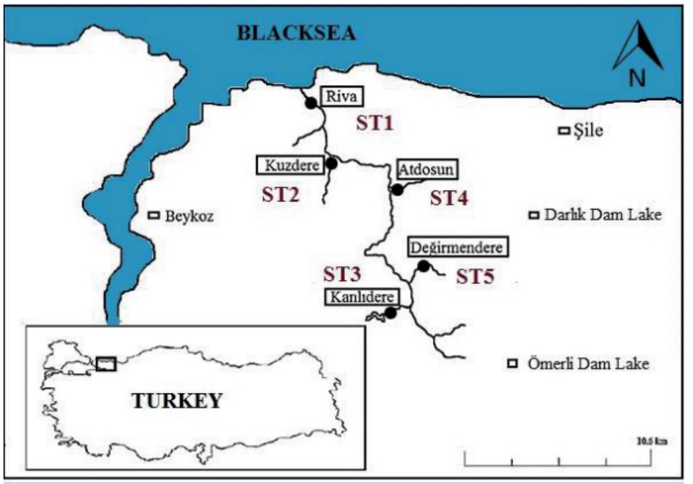

Figure 1. The positioning of Riva Stream and the designated sampling sites.

Table 1. The geographical coordinates of the sampling stations along Riva Stream.

Station No	Station Name	Coordinate
S 1	Riva Stream	N 41.12763° E 29.13521°
S 2	Kuzdere	N 41.12763° E 29.13521°
S 3	Kanlıdere	N 41.07127° E 29.16175°
S 4	Atdosun Stream	N 41.10403° E 29.16333°
S 5	Değirmendere	N 41.08222° D 29.17393°

blue colonies observed in the m-FC medium were considered as potential indicators of fecal coliforms. Cytochrome oxidase (API Strep, bioMérieux) and indole tests were performed on these colonies with suspected fecal coliform. Oxidase negative and indole positive colonies were evaluated numerically (APHA, 2013). To determine if the bacteriological contamination was of human or animal origin (such as cattle), the ratio of fecal coliform bacteria levels (FC) to intestinal enterococci (IE) bacteria levels was evaluated. Saturated oxygen (mg/L), water temperature (°C), salinity (‰), conductivity (µS/cm) and pH values were measured in situ during sampling using multiparameters (YSI). Spearman's rank correlation statistical analysis was performed to determine the relationship between environmental parameters and indicator bacterial levels. The significance level for differences between the parameters was set at 0.05. The differences between the measured environmental parameters and bacterial levels were examined by two-way ANOVA test. Statistical analyses were performed using SPSS 28.0.

RESULTS AND DISCUSSION

The variable environmental parameters of the sampling areas, including dissolved oxygen, temperature, salinity, pH, and conductivity, are shown in Table 2.

Table 2. Physicochemical parameters on Riva Stream.

Stations	Months	Temp (°C)	Salinity (‰)	pH	O_2 (mg/L)	Conductivity (μS/cm)
Station 1	Mar-18	11.47	1.25	8.67	4.82	2.44
	Aug-18	24.50	2.40	7.70	3.46	1.12
	Oct-18	18.10	1.40	7.14	3.47	3.07
	Jan-19	5.40	0.20	7.91	11.50	0.97
Station 2	Mar-18	10.47	0.34	8.82	9.70	0.41
	Aug-18	21.50	0.00	7.69	5.82	0.60
	Oct-18	17.70	0.00	7.07	4.70	0.44
	Jan-19	5.80	0.00	8.04	13.76	0.32
Station 3	Mar-18	11.46	0.17	8.88	10.08	0.31
	Aug-18	16.6	0.00	6.32	6.78	0.04
	Oct-18	15.7	0.00	7.16	6.80	0.02
	Jan-19	6.5	0.00	9.63	12.39	0.15
Station 4	Mar-18	11.0	0.16	8.59	10.34	0.58
	Aug-18	21.6	1.20	7.57	8.83	0.01
	Oct-18	15.9	0.00	7.20	6.02	0.04
	Jan-19	4.4	0.00	8.96	12.65	0.25
Station 5	Mar-18	11.97	0.27	7.92	7.58	0.55
	Aug-18	24.9	0.40	7.51	5.52	0.39
	Oct-18	16.2	0.30	7.40	6.76	1.10
	Jan-19	5.4	0.00	8.10	12.57	0.48

pH values 6.32 to 9.63, dissolved oxygen values 3.46 to 13.76mg/L, temperature values 4.4-24.9°C, salinity values and ppt values 0 to 2.4 recorded as the maximum and minimum, respectively.

The detected total coliform levels in the samples are shown in Figure 2.

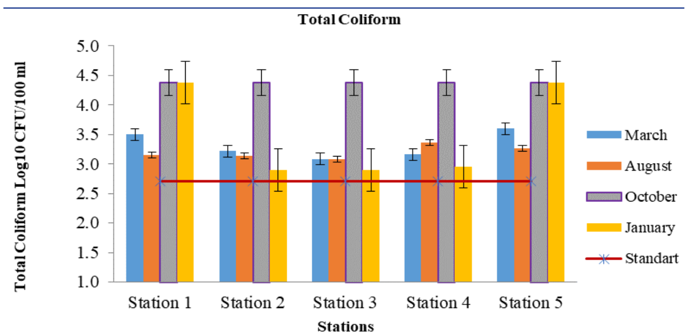

Figure 2. Total coliform bacteria levels are determined in water samples by seasons and stations.

The levels of coliform bacteria in samples from all stations were found to exceed national limit values. The highest total number of coliform bacteria was detected as 24x10³ CFU/100ml in October and January 2018.

The detected fecal coliform levels in the samples are shown in Figure 3. The fecal coliform levels were higher than the recommended national limit values. Fecal coliform counts were recorded at a minimum of 10 CFU/100ml and a maximum of 18 × 10³ CFU/100ml during the study period.

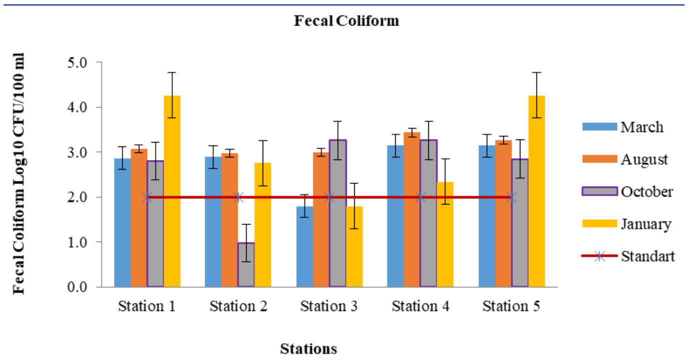

Figure 3. Fecal coliform levels are determined in water samples by seasons and stations.

The detected intestinal enterococci levels in the samples are shown in Figure 4. The levels of intestinal enterococci were higher than the recommended national limit. Intestinal enterococci counts were recorded at a minimum of 38 CFU/100ml and a maximum of 24 × 10² CFU/100ml during the study period.

The fecal coliform/ intestinal enterococci ratios (FC/IE ratio) analyzed in the surface water samples during the study period are shown in Table 3. Data presented in Table 3 shows that the FC/IE counts recorded a minimum 0.04 and maximum 52.10 during the study period.

Values are shown in bold. If the FC/IE ratio is greater than 4, the pollution is considered as human origin, and if the FC/IE ratio is between 0.1 and 4, the pollution is considered as animal origin.

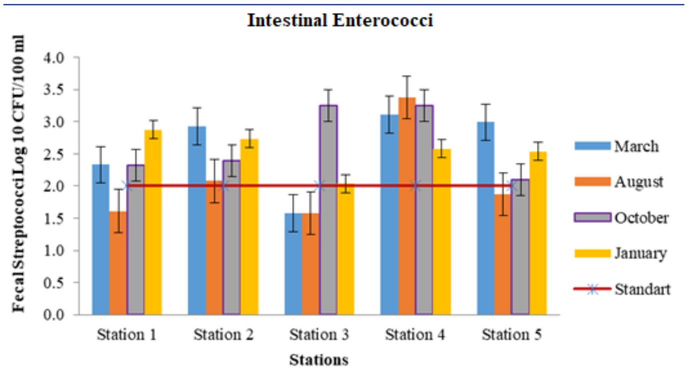

Figure 4. Intestinal enterococci bacteria levels are determined in water samples by seasons and stations.

Table 3. Fecal coliform/Intestinal Enterococci ratio at all stations.

Stations	FC/IE			
	Mar-18	Aug-18	Oct-18	Jan-19
S 1	3.38	**28.34**	2.95	**23.89**
S 2	0.91	**7.78**	0.04	1.05
S 3	1.64	**25.84**	1.00	0.57
S 4	1.08	1.12	1.00	0.58
S5	1.42	**24.21**	**5.60**	**52.10**

Many river and stream studies conducted worldwide pointed out that the pollution rate is above the WHO and EPA standards. Shittu et al. (2008) reported that the total coliform count of Abeokuta, Southwest Nigeria, exceeded the EPA's maximum contamination standards for coliform bacteria in drinking water. Viau et al. (2011) reported that Enterococci were associated with bacterial pathogens from the five indicators tested in Hawaiian Oahu coastal streams. Musyoki et.al. (2013) reported that the microbiological contamination of the Nairobi and Athi rivers was unacceptably high, according to Kenya standards and WHO guidelines for drinking water and agricultural use. Akkan et al. (2019) reported that the Total Coliform (TC), Fecal Coliform (FC) and Fecal Streptococcus (FS) rates of surface water samples in Yağlıdere were 45%, 71.66% and 56.66%, respectively. Altug et al. (2016) reported that the total coliform and fecal coliform counts were higher than advised national limit values in Riva stream.

The data obtained within the scope of the study was analyzed in accordance with national and international water quality standards. The standard values for the European Parliament are a total coliform count 500 CFU/100ml (2.69/100 ml based on log10), fecal coliform count 100 CFU/100ml (2.0/100ml based on log10), and intestinal enterococci counts 100 CFU/100ml (2.0/100ml based on log10 value), which are indicated in dark red in the graphs below (European Parliament, 2006). Standard values for sea water used for recreational purposes were determined by the National Regulation published on 31 December 2004. According to this, limit values were established as 1000 cfu/100ml for total coliform, 200 cfu/100ml for fecal coliforms and 100 cfu/100ml for fecal enterococcus (Official Gazette 2004).

When total coliform, fecal coliform and intestinal enterococci data were examined, it was determined that the bacteriological pollution level in the Riva River was above the standard limits in all seasons. During the sampling period, the highest levels of total coliforms were recorded in summer, which was associated with increased human activities. Fecal coliform counts peaked at a maximum of 18 x 10^3 CFU/100ml throughout the study, while intestinal enterococci counts ranged from a minimum of 38 CFU/100ml to a maximum of 24 x 10^2 CFU/100ml. The fact that the levels of total coliforms, fecal coliforms and fecal enterococci consistently exceeded the recommended national limits draws attention to the persistent presence of bacteriological pollution sources in the region.

Environmental variables significantly impact the levels of indicator bacteria in rivers (Herrig et al., 2019; Islam et al., 2017). Indicator bacteria such as *E. coli* and fecal coliforms which are used to measure the presence of fecal contamination in water can indicate the potential presence of other harmful bacteria and viruses (Saxena et al., 2015;Lin and Ganesh, 2013. Temperature is an important factor in bacterial growth in aquatic ecosystems. Warm temperatures can accelerate bacterial growth, leading to higher levels of indicator bacteria in the water. Conversely, cold temperatures can slow bacterial growth and reduce the levels of the indicator bacteria (Kunili and Ateş, 2021). During the summer months, when temperatures are warmer and recreational use of rivers is more common, there is typically an increase in the levels of indicator bacteria owing to higher levels of human and animal waste entering the water. In our study, no significant relationship was found between the total, fecal coliform levels, and water temperature. This situation is due to the fact that there is a continuous input of pollution to the study area. Heavy rainfall washes pollutants, including bacteria, from the surrounding land into the river, leading to increased levels of the indicator bacteria. This is particularly true for urban areas, where rainwater runoff can carry pollutants from streets and parking lots into nearby rivers (Shehane et al., 2005). Higher flow rates dilute the bacteria in water, thereby reducing the levels of indicator bacteria. However, during periods of high flow, bacteria is more easily resuspended from the riverbed and carried downstream, potentially increasing the levels of the indicator bacteria (Crump et al., 2007). Agricultural and urban land use contributes to higher levels of indicator bacteria in rivers. Agricultural runoff contains animal waste and fertilizers, whereas urban runoff contains a variety of pollutants, including pet waste, oil and grease from roads, and chemicals from industrial areas (Gotkowska-Płachta, et al., 2016; Paule-Mercado et al.,2016).

When examining the relationship between measurements of various environmental parameters, a significant correlation was found between the pH values and temperature values ($r = 0.005$, $p < 0.05$). Furthermore, pH values exhibited variations at the different stations (< 0.001), but no seasonal variation was observed. A significant correlation was identified between the dissolved oxygen values, temperature, pH, and conductivity

Table 4. Spearman's rank correlations between environmental parameters and indicator bacteria levels in Riva stream (N 20; marked correlations are significant at p< 0.05).

Correlations												
		Depth	**Width**	**FV**	**Temp**	**pH**	**Cond**	**DO**	**Salinity**	**TC**	**FC**	**IE**
D	PC	1										
	Sig. (2-tailed)											
	N	20										
W	PC	.110	1									
	Sig. (2-tailed)	.645										
	N	20	20									
FV	PC	-.133	-.319	1								
	Sig. (2-tailed)	.576	.171									
	N	20	20	20								
T	PC	-.354	.066	-.283	1							
	Sig. (2-tailed)	.126	.782	.227								
	N	20	20	20	20							
pH	PC	.313	-.023	.500*	-.607**	1						
	Sig. (2-tailed)	.179	.924	.025	**.005**							
	N	20	20	20	20	20						
Cond	PC	.188	.802**	-.290	.101	-.034	1					
	Sig. (2-tailed)	.427	**<.001**	.215	.672	.888						
	N	20	20	20	20	20	20					
DO	PC	.149	-.358	.372	-.826**	.600**	-.461*	1				
	Sig. (2-tailed)	.530	.122	.106	**<.001**	**.005**	**.041**					
	N	20	20	20	20	20	20	20				
Salinity	PC	.040	.715**	-.249	.489*	-.080	.591**	-.527*	1			
	Sig. (2-tailed)	.866	**<.001**	.289	**.029**	.737	**.006**	**.017**				
	N	20	20	20	20	20	20	20	20			
TC	PC	-.061	.169	-.349	-.029	-.462*	.219	-.204	-.137	1		
	Sig. (2-tailed)	.799	.477	.131	.904	**.041**	.354	.388	.566			
	N	20	20	20	20	20	20	20	20	20		
FC	PC	.078	.253	-.226	-.381	-.015	-.003	.373	-.131	.453*	1	
	Sig. (2-tailed)	.743	.281	.337	.098	.950	.991	.105	.582	**.045**		
	N	20	20	20	20	20	20	20	20	20	20	
IE	PC	-.238	-.250	-.102	.038	-.137	-.350	.104	-.055	.165	.063	1
	Sig. (2-tailed)	.312	.289	.668	.873	.566	.130	.662	.818	.487	.791	
	N	20	20	20	20	20	20	20	20	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

F.V. Flow Velocity °C; Temperature Cond; Conductivity (μS/cm) D.O; Dissolved Oxygen ‰; Salinity/ TC: Total Coliform levels, FC: Fecal Coliform Levels, IE: Intestinal Enterococci levels

values. Additionally, a significant difference was noted in the dissolved oxygen values among different stations. In the analysis of water temperature values, a disparity was observed between stations ($p < 0.05$), while no significant difference was observed between seasons. The total coliform values displayed significant differences among stations. Overall, environmental variables have a significant impact on the levels of indicator bacteria in rivers. Monitoring these variables and understanding their effects can help water managers and policymakers develop strategies to reduce bacterial pollution in rivers and protect public health.

CONCLUSION

Riva Stream is important due to its ecosystem structure and it is also a popular place for tourism and recreation activities. This study, carried out between March 2018 and January 2019, investigated the seasonal levels of indicator bacteria in Riva stream. It was determined that indicator bacteria levels throughout the study area exceeded national and international standard values throughout the year in every season. Human activities were identified as the primary source of pollution. Seasonal sampling from the Riva stream provided important data for the evaluation of the pollution load carried by the stream and the possible introduc-

Table 5. Two-way ANOVA between bio-indicator bacteria and environmental parameters.

	Stations		Seasons	
	F.	Sig.	F.	Sig.
Depth	1.245	.326	1.645	.215
Width	.003	1.000	4628.520	<.001
Flow Velocity	2.192	.129	2.015	.144
Temperature	75.211	<.001	.071	.990
pH	10.356	<.001	.088	.985
Conductivity	.529	.669	8.256	<.001
Dissolved Oxygen	17.106	<.001	.712	.596
Salinity	1.207	.339	4.494	.014
Total Coliforms	13.309	<.001	.345	.844
Fecal Coliforms	2.056	.147	.804	.542
Intestinal Enterotococci	.288	.834	2.476	.089

tion of bacteria to the Black Sea coast. Bacterial pollution, which was found to flow from both sides of Riva Stream to the Black Sea poses a potential risk for recreational activities in the region. To improve water quality, it is imperative to implement a comprehensive management strategy and adopt measures to address water quality issues. Additionally, improving national awareness of the problems associated with fecal pollution in freshwater sources is crucial to maintaining overall ecosystem health.

Conflict of interests: The authors have no conflicts of interest to declare.

Ethics committee approval: Ethics committee approval is not required.

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