

A research on coliform bacteria in the Golden Horn Estuary (Sea of Marmara, Turkey)

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ABSTRACT

Spatio-temporal variation of the total and fecal coliform groups as bacteriological indicators in the marine environment was investigated in surface water of the Golden Horn Estuary in monthly intervals between June 2012 and May 2013. Temperature, salinity, Secchi depth, pH, dissolved oxygen and oxygen saturation were measured at 10 stations simultaneously with bacteriological analyses. Highest total coliform counts in surface water was 940×10^3 CFU/100 ml at the Alibeykoy Creek in November and highest fecal coliform was 178×10^3 CFU/100 ml at the same area in October. The lowest coliform counts were observed during summer, while the highest counts were in autumn. During rainy days, some domestic wastewater overflowing from sewerages are discharged to the estuary, which those water adversely affect water quality. The total and fecal coliform counts in surface water increased gradually from the lower to upper section of the estuary. Although the Black Sea water pumping from the Strait of Istanbul to the Golden Horn by Kagithane Creek is an important factor in the decrease of pollution and better water quality, there are still some runoff materials including uncontrolled discharges particularly in the Alibeykoy Creek. The results of this study showed that the bacteriological pollution increased due to municipal wastewater resulting from overflow after the precipitation and the surrounding waters of the Golden Horn basin mix with surface waters. Thus, the most important factor in order to decrease the bacteriological pollution is to provide a full control of the surface discharges.

Keywords: Coliform bacteria; Bacteriological pollution; Water quality; Golden Horn Estuary

1. Introduction

Land based microorganisms included in marine ecosystems can be from natural (rain, erosion *etc.*) or artificial causes (wastewaters of domestic, agricultural and industrial). The most frequently cited groups are from domestic waste. Approximately 20–30% of human waste is undigested food waste, with water and bacteria forming

the remainder [1]. Almost 1×10^{11} unit bacteria, including approximately 400 species, can be found in 1 g of human pollution [2]. Therefore, it is inevitable that contamination of water bodies with human domestic waste microorganisms causes many diseases.

Coliform bacteria are commonly used as indicators of water pollution in environmental microbiology. In 1885, the German-Austrian bacteriologist and pediatrician Theodor Escherich concluded that *Escherichia coli* was not only present in very large quantities in human feces, but was also associated with typhoid females, suggesting that fecal

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contamination could be used as a pollutant indicator for water [3]. The Enterobacteriaceae group are broadly called coliforms, and include fecal coliforms. These bacteria can produce lactic acid by fermenting carbonic acid and gas [4].

Microorganisms require favorable environmental conditions, including sufficient water and nutrients, i.e., carbon, nitrogen, macro and micro nutrients, and some vitamins. While the pH should generally be between 6.5 and 7.5, bacteria can live in a wide temperature range [5].

The Golden Horn Estuary (GHE), fed by the Alibeyköy and Kagithane Creeks, was inundated at the end of the Glacier Age geological period. This formation is one of the most beautiful examples of deep gulf or sea invaded valleys, often called ria-type in the physical geography field. The area has extensively settled by humans throughout history [6].

Written records of erosion materials coming from the Golden Horn slopes have survived from the Byzantine period. They were noted even then as being contaminated with domestic wastes [7]. In the 1960s, household waste was drained into the Golden Horn with several hundred small discharges. Inadequate sewage infrastructure meant many domestic residues were discharged into the Golden Horn through various channels and infiltration. The first study on sewage facilities was performed between 1965 and 1970 [8], and extended in 1975 as Istanbul continued to grow [7].

The Water Quality Monitoring Study in the GHE between 1998 and 2000 selected the fecal coliform group as an indicator for microbiological pollution. There have been evaluated the distribution of microbiological pollution including fecal coliform levels in a few studies carried out in the GHE [9–11].

Many factors have affected water quality in the GHE, including domestic and industrial wastewater, solid wastes, garbage from the hills and slopes, etc. sewerage infrastructures before 1990 allowed more than 200 large and small discharges and domestic waste to flow into the Golden Horn Estuary. Various industrial establishments on the sides of the Alibeyköy and Kagithane Creeks have also played a major role in pollution of the GHE in the past years [12].

The Golden Horn Rehabilitation Project was initiated in 1997 to remove the heavy pollution. The surface discharges were gradually taken under control, connected to collector systems. A total of $4.25 \times 10^6 \text{ m}^3$ anoxic sediment was removed from the completely filled upper section and at least 5 m depth was gained in this region. Moreover, the opening of the floating Valide Sultan Bridge in May 2000 resulted in rapid renewal and oxygenation at the upper estuary. But, the GHE started to get polluted again since towards the end of 2000s and still affected by some discharges of untreated sewage in Alibeyköy Creek. Therefore, a new project was planned to prevent pollution and to increase biodiversity. For this purpose, Black Sea surface water from the Strait of Istanbul started to pump with $260.000 \text{ m}^3 \text{ d}^{-1}$ through Kagithane Creek to the GHE waters in October 2012. The plant was recently opened, and pumping operations were interrupted from time to time. Pumping was also paused during rainy months due to the risk of overflowing the creeks. In particular, Black Sea water was continuously pumped through Kagithane Creek level during February. Consequently, Golden Horn surface salinity was

significantly increased in February. During the sampling, the test results of Black Sea water cannot be pumped to the Golden Horn through Kagithane Creek on a regular basis. Since Kagithane Creek area is shallow and narrow, salinity was more affected by whether or not Black Sea water was pumped during rainy months.

This study aimed to examine the spatio-temporal variations of the total and fecal coliform groups in surface waters of the GHE from June 2012 to May 2013 together with some environmental parameters and the effect of Black Sea water which was pumped to the GHE during the study period.

2. Materials and methods

2.1. Study area and sampling stations

The Golden Horn Estuary (GHE) is located in the north-east of the Sea of Marmara, extending in a northwest-southeast direction, approximately 7.5 km long and 700 m wide, with a surface area of 2.6 km² (Fig. 1). The depth of the study area decreases from the lower section to the upper. The depth is 40 m in the lower section (St. GK), it decreases rapidly to 14 m in the middle section (St. CA), and to 4 m in the upper section (St. AS) (Fig. 1). The Creeks (Alibey and Kagithane) were the main sources of freshwater input before the construction of series of dams at the end of the 1990s. Hence, the amount of freshwater inflow decreased considerably and nowadays, rainfall is the main source of freshwater flowing into the study area [13]. Water sampling was performed at monthly intervals at 10 stations along the Golden Horn estuary (41° 01.32' N; 28° 58.45' E and 41° 04.06' N; 28° 56.36' E) (Fig. 1).

Water samplings were made from the 10 stations in the last week of every month throughout the study period. Eight of the 10 sampling stations (from 1 to 8) were employed in previous project, Water Quality Monitoring Project at the Golden Horn conducted by Istanbul University, Institute of Marine Sciences and Management supported by General Directorate of Istanbul Water and Sewerage Administration (İSKİ), as noted in Table 1. The other stations, Alibeyköy Creek (St. 9) and Kagithane Creek (St. 10) were included to investigate the effects of creeks on Golden Horn surface waters. A GARMIN GPS map 76CS instrument was used to identify specific points at the sampling stations, and fixed the boat position while samples were taken.

2.2. Seawater sampling and analyses

Seawater samples were taken from surface (0.2 m) using a surface sampler device and transferred directly into a sterile amber bottle. They were then placed into ice packed bins. Temperature, salinity, dissolved oxygen (DO), and pH measurements were taken simultaneously at the sampling site using a multi-parameter probe (Multi 350i / SETWTW), and water transparency was measured using a Secchi disc in diameter of 30 cm.

Coliform bacteria were determined using the membrane filter method (S.M 9222B). The seawater samples were brought to the laboratory in the ice bins, and analyzed [14]. Samples filtered through sterile filter kit (Sartorius), 0.45 µm filters (Sartorius 13906 50-AJN), were placed on sterile deionized water soaked dehydrated media in aseptic



Fig. 1. Sampling stations in the study area.

Table 1
Significant one-way analysis of variance (ANOVA) results of spatiotemporal pattern in some environmental and coliform data

Parameter	Factor loading	ANOVA result
Temperature	Season	$F_{3,39} = 1107.88, p < 0.001$
Salinity	Season	$F_{3,39} = 3.681, p < 0.05$
	Station	$F_{9,39} = 0.015, p < 0.005$
Secchi depth	Station	$F_{9,39} = 23.147, p < 0.001$
pH	Season	$F_{3,39} = 7.738, p < 0.001$
Dissolved Oxygen	Season	$F_{3,39} = 9.207, p < 0.001$
Total Coliform	Season	$F_{3,39} = 19.505, p < 0.001$
Fecal Coliform	Season	$F_{3,39} = 19.500, p < 0.001$

conditions without air bubbles. Endo FC-NKS (Sartorius, 14068-50-N) and mFC-NKS (Sartorius, 14068-50-N) were used as feedlots for total and fecal coliform, respectively. Prepared samples were incubated for 24 hours at $44.5 \pm 0.2^\circ\text{C}$ and $35 \pm 0.5^\circ\text{C}$ for fecal and total coliform, respectively. After incubation, the volume of blue colony on the mFC culture (fecal coliform) and golden whitening colloid on the endo FC broth (total coliform) were counted.

2.3. Statistical analysis

The relationship between environmental variables and total coliform (TC), fecal coliform (FC) throughout the study period and for each season were analyzed by Spearman rank correlation, following transformation to natural logarithms using SPSS Statistics 21.0 software. Spatiotemporal patterns and alterations in environmental variables and coliform data were investigated among stations and seasons by one-way analysis of variance (ANOVA). Prior to ANOVA all data were normalized by logarithmic transformation using SPSS Statistics 21.0 software.

3. Results and discussion

3.1. Temperature

The surface water temperature in the study area was the highest (27.2°C) in June at Alibeykoy Creek (St. AD) and the lowest in January (5.7°C) at Kagithane Creek (St. KD). Short time effects of seasonal weather conditions were evident due to the weak water currents, and surface water temperatures at the same time intervals differed along the study area. Temperatures were significantly higher in January at the Galata Bridge (St. GK), which interacts with the Strait of Istanbul (Bosphorus) and has a heavy urban ship traffic, whereas temperatures were

lower than the others stations in July. Thus, the lower estuary showed the characteristics of the neighboring Strait of Istanbul Strait water during the study period, as previously stated by some researchers [19]. The mean seasonal temperatures (7.8 to 25.8°C) showed that the upper estuary is warmer in summer and spring than the lower estuary, while it is colder than the lower estuary in winter (Fig. 2). Surface water temperatures will tend to have characteristics of Black Sea water by mixing of Black Sea water to the Golden Horn Estuary from the Kagithane Creek, i.e. colder in summer and warmer in winter than previous days. Temperature values displayed a strong seasonal variations in the study area according to ANOVA results ($p < 0.001$) (Table 1). The effect of temperature on coliform groups was the highest in summer, when considered a significant positive correlation between temperature and coliform data ($p < 0.01$). No correlation was found between temperature and coliform values in the other seasons (Table 2).

3.2. Salinity

Salinity values in the study area decreased considerably from the lower to the upper section and it was the lowest (3.6) at the upper section in December and the highest (19.7) at the lower section in February. Mean seasonal salinity values (7.4–18.6) showed that the salinity difference between the lower and the upper section was the highest in winter and autumn, and it was the lowest in summer (Fig. 2). Sur-

face salinity varies with wind, currents, evaporation, precipitation, and the runoff water carrying by two Creeks. The lowest values in salinity were observed in winter, probably due to high precipitation.

The measured data show three water layers in the Golden Horn in terms of salinity: 0–10 m is Black sea water with less saline waters (18–19 psu), 30–44 m is Mediterranean water with saline waters (37–38 psu), and 10–30 m is an intermediate or mixture layer [15]. Black Sea waters enter to the Golden Horn Estuary at the surface depending on the seabed topographical structure. Mediterranean water rises towards the surface, as the overall depth decreases. This saline water mixes with the fresh waters in the mixture layer. Strong northern and north-eastern winds affect the Golden Horn as a whole, which increases the mixture effects [16]. Salinity values displayed a spatiotemporal variations in the study area according to ANOVA results (Table 1). There was a significant negative correlation between salinity and coliform groups particularly in autumn and winter ($p < 0.01$) (Table 2). In a previous study, Aslan et al. (2004) reported that fecal coliform values increased during the periods of low salinity. The results of our study on negative correlation between salinity and coliform groups is consistent with the study performed by Aslan-Yılmaz et al. (2004). No correlation between salinity and coliform values in spring and summer coincided with higher salinity values at these periods (Table 2). As amount of the Black Sea water entering to the surface water of the study area increases, salinity values increase particularly in the upper section, while the coliform values decrease.

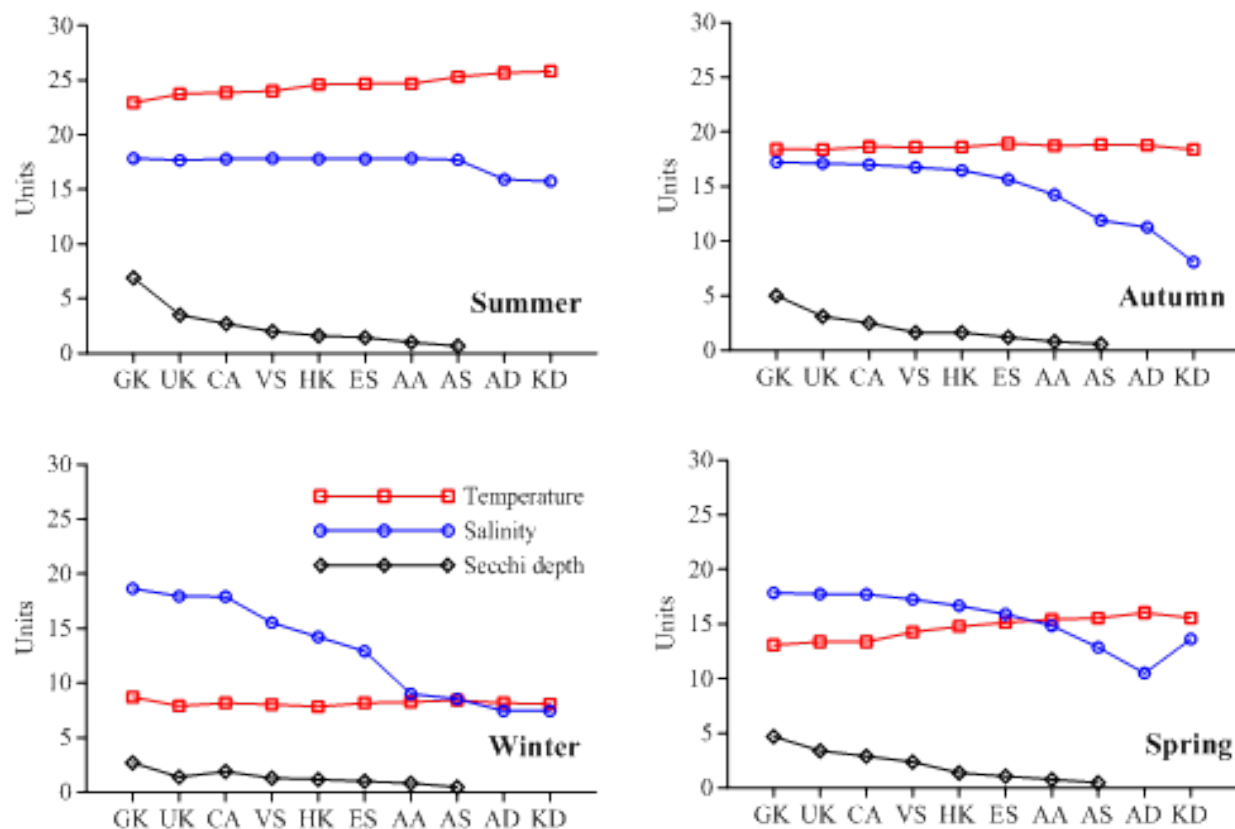


Fig. 2. Seasonal variations in temperature (°C), salinity (psu), and Secchi depth (m) in surface water along the study area

Table 2

Spearman rank correlation coefficients (rho) between environmental variables and total coliform (TC), fecal coliform (FC).

Variables		Rho				
		All year	Spring	Summer	Autumn	Winter
Temperature	TC	-0.415**	–	0.875**	–	–
	FC	–	–	0.723**	–	–
Salinity	TC	-0.668**	–	–	-0.964**	-0.973**
	FC	-0.556**	–	–	–	-0.823**
Secchi depth	TC	-0.470**	–	-0.850**	-0.954**	-0.912**
	FC	-0.240**	–	-0.638*	–	-0.733**
DO	TC	–	–	-0.806**	-0.830**	–
	FC	–	–	-0.806**	–	–
pH	TC	–	–	-0.723*	-0.952**	–
	FC	–	–	–	-0.636*	–

Statistically significant correlations are indicated by symbols: – not significant; * $p < 0.05$; ** $p < 0.01$.

3.3. Secchi depth

Secchi depth decreased markedly from the lower to the upper section, probably due to high concentration of suspended solids. Mean Secchi depth was the lowest (0.5 m) in winter at the upper estuary (St. AS) and the highest (6.9 m) in summer at the lower estuary (St. GK) (Fig. 2). Natural factors (rainfall, water currents, suspended solids, etc.) affecting Secchi depth measurements have more effect at the upper section. Secchi depths vary with suspended solids carrying by Creeks. Another important factor that maximizes this effect is the topography of the study area, which varies significantly towards upper section.

Secchi depth displayed a strong spatial variation (ANOVA, $p < 0.001$) that evidenced decreasing water transparency towards the upper section (Table 1). Coliform values are quite high during periods of low water transparency in February, probably due to high suspended particulate matters (SPM) and other pollutants. Water transparency was higher at the study area during summer, due to little or no precipitation for some time and pumping of Black Sea water from Kagithane Creek into the Golden Horn. Secchi depth were strong negatively correlated with coliform groups during the study year except spring ($p < 0.01$) (Table 2). High coliform values in surface water were found generally at the upper section, where Secchi depths were lower. Deteriorating water quality particularly at the upper section based on the very low water transparency may cause to the increase in coliform values.

3.4. DO and pH

DO concentrations decreased generally from the lower to the upper section as it was Secchi depths and salinity (Fig. 3). However, this trend may have changed by transfer of Black Sea water to the GHE by the Kagithane Creek. Mean DO concentrations varied between 1.19 mg L⁻¹ (St. KD, summer) and 9.2 mg L⁻¹ (St. KD, winter) and tended to rising during winter and spring, while they decreased during summer and autumn (Fig. 3). In general, salinity and DO values will be higher in the upper section, when the sea waters in the lower section enter the interior of the

estuary with the effect of the southerly winds. Furthermore, an increasing trend in DO values was followed in the upper section (except St. AD) by the pumping of the Black Sea water regularly e.g. DO values in February was found high at the upper section (Fig. 3). DO values displayed a strong seasonal variations in the study area according to ANOVA results ($p < 0.001$) (Table 1). This indicates that there may be the effect of the Black Sea waters pumping to the GHE.

DO values were negatively correlated with coliform groups during summer and autumn ($p < 0.01$) (Table 2). Coliform values increased particularly at the stations with low DO. Our results are similar to the results reported by Aslan-Yilmaz et al., (2004) from the same area. Aslan et al., (2004) suggested that decreasing values in fecal coliform were detected during high DO concentrations. The regions with very low DO values depending on water pollution caused to the high coliform values in the study area.

Mean pH values varied between 7.2 (St. AD, summer) and 8.9 (St. HK, spring) during the study period (Fig. 3). Estuarine surface waters were slightly basic with pH generally between 7.8 and 8.9, and some low pH (6.0) are observed immediately after rainfall. It is understood that the pH value is generally lower at the stations of KD and AD. This was due to the acidic character of rainfall in Istanbul, and delayed pH balancing by the lack of water transport from the source. pH values displayed a strong seasonal variations in the study area (ANOVA, $p < 0.001$) (Table 1). This shows the effect of the rain in winter on the low pH particularly at the upper section. pH values were negatively correlated with total coliform in summer and autumn ($p < 0.05$ and $p < 0.01$), with fecal coliform in autumn ($p < 0.05$) (Table 2). Our results are consistent with the earlier study performed by Aslan-Yilmaz et al. (2004) which they stated that increasing values in fecal coliform were observed during periods of low pH. It is revealed that high coliform values were mostly observed at the area with lower pH.

3.5. Total and fecal coliforms

The distribution of total and fecal coliforms in the study period differed among the stations and seasons

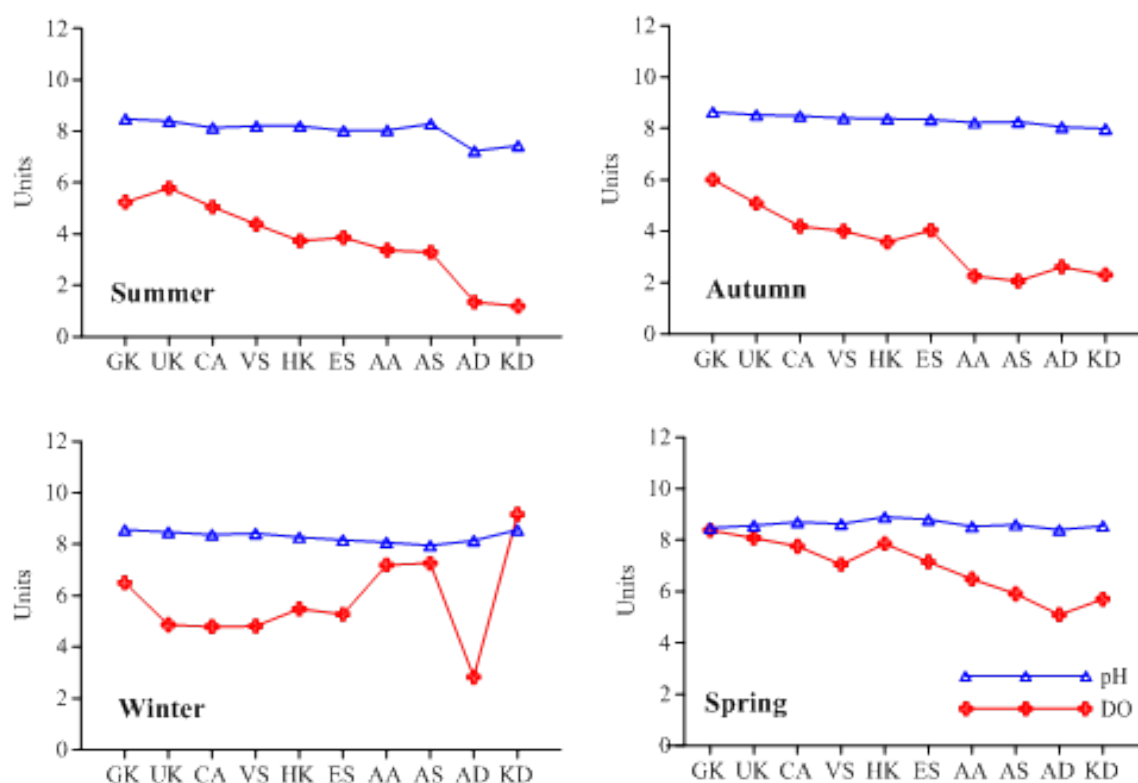


Fig. 3. Seasonal variations in DO (dissolved oxygen, mg L⁻¹) and pH in surface water along the study area.

and the highest coliform values were detected in autumn (Fig. 4). The highest total coliform value in surface water was 940×10^3 CFU/100 ml at the Alibeykoy Creek (St. AD) in November, while fecal coliform was 16×10^3 CFU/100 ml. The highest fecal coliform value in surface water was 178×10^3 CFU/100 ml in October at the same station, while total coliform was 230×10^3 CFU/100 ml. The lowest total coliform was 167 CFU/100 ml at the St. GK in April and the lowest fecal coliform was 5 CFU/100 ml at the St. UK in September. The reason of higher total coliform values at the stations VS and HK in spring was the high values in March, probably due to a coastal effluent discharge (Fig. 4).

In 1 ml of untreated domestic wastewater, there were 10^5 – 10^6 and 10^4 – 10^5 total and fecal coliform bacteria, respectively [17]. Hence, the ratio between the two bacteria groups ranges from 10^{-1} – 10^{-2} (Fecal coliform/Total coliform). The wastewater with the domestic character is generally around Alibeykoy and Kagithane Creek. This is confirmed by salinity value at the same sampling time and stations, where total coliform was the highest. It was measured very low salinity (1.12) during the highest total coliform (94×10^4 CFU/100 ml) at the St. AD in November with coliform ratio of 0.017. Salinity was 16.38 at the St. AD in October, when fecal coliform was 178×10^3 CFU/100 ml and total coliform was 230×10^3 CFU/100 ml with coliform ratio of 0.774. In this case, the salinity rate (1.12/16.38) is 0.068. This shows that the Golden Horn surface water was considerably diluted by domestic wastewater, particularly when total coliform count was high. The fecal coliform values confirm that there is significant domestic pollution, but the pollution load generally does not affect salinity.

Total coliform at the St. GK ranged from 167 to 234×10^2 CFU/100 ml in January and April, whereas fecal coliform 5 to 13.5×10^2 CFU/100 ml in November and September. The increase in total number of coliforms presents in the receiving environment. Fecal coliform in coastal waters depends on bacterial loading from streams and rivers, water mixing and dispersion in receiving waters, and bacterial losses due to death [18].

In a long-term bacteriological study (from 1998 to 2002) performed by Aslan-Yilmaz et al. (2004) in the Golden Horn has been reported that surface fecal coliform was above 10^6 CFU/100 ml at the upper section in 1998, when the Golden Horn extremely polluted [11]. Following the recovery of water quality, fecal coliform counts decreased below 10^3 CFU/100 ml in the summer of 2002 and the data of fecal coliform and fecal streptococci indicated that pollution drastically decreased in five years [11].

Total and fecal coliform displayed a strong seasonal variation according to the ANOVA results ($p < 0.001$) (Table 1). This result was supported by the high coliform values in autumn (November), due to high amount of inputs by creeks during rainy periods. Similarly, in the previous study performed by Aslan-Yilmaz et al. (2004) was reported that coliform counts significantly increased in rainy periods depending on runoff with domestic discharges. Very low dissolved oxygen (1.24 mg L⁻¹) measured during highest total coliform counts at the St. AD in November indicates the very poor water quality. Thus, the pollution load carrying by the creeks during the rainy periods is the main factor influencing the water quality and coliform values particularly in the upper section of the estuary.

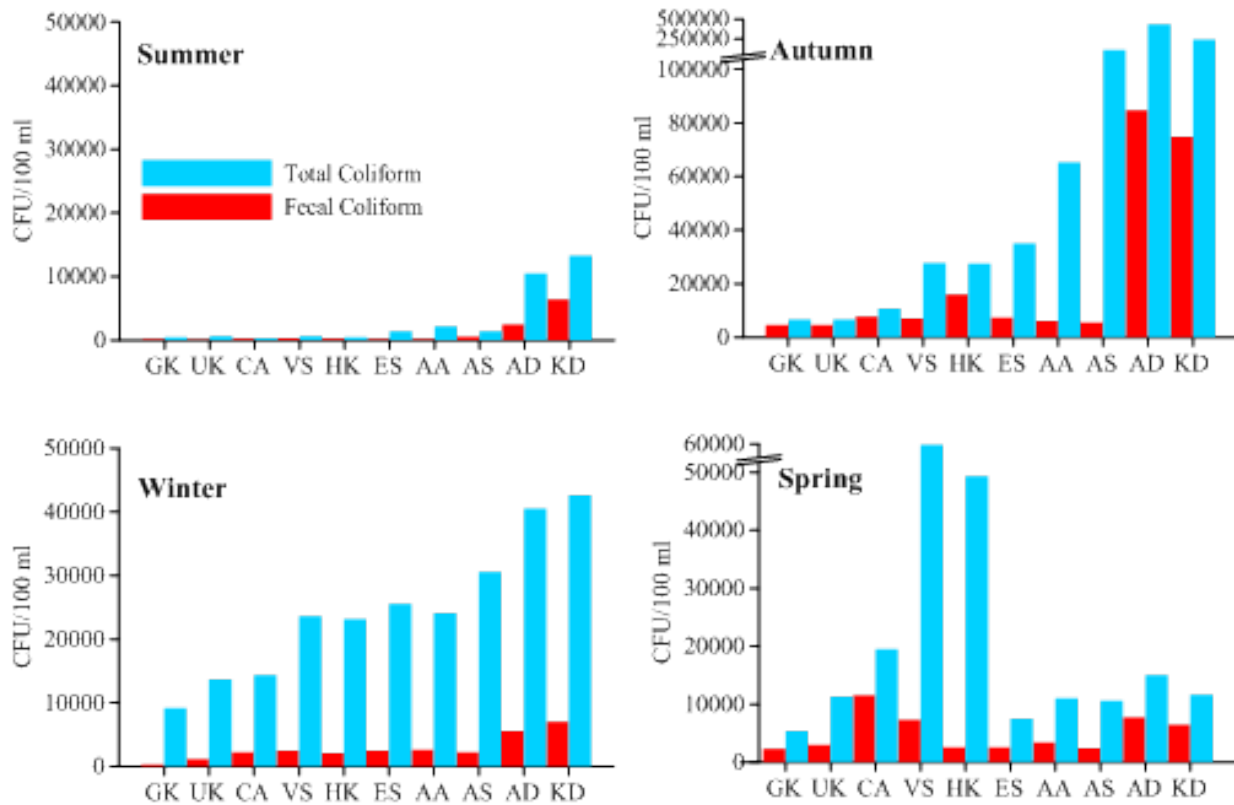


Fig. 4. Seasonal variations in total and fecal coliforms in surface water along the study area.

4. Conclusion

The previous studies on the bacteriological pollution have been showed that the fecal coliform values were very high when the GHE was an extremely polluted area [10,11]. In the earlier study was reported that there was a strong relationship between the bacteriological data and the water pollution in the GHE, indicating drastically decreased coliform values after recovery of water [11]. Although the Black Sea water pumping from the Strait of Istanbul to the Golden Horn through Kagithane Creek is an important factor in the decrease of pollution and better water quality, there are still some runoff materials including uncontrolled discharges particularly in the Alibeykoy Creek. On the other hand, coliform values still significantly increase in rainy periods, probably due the pollution load coming from the Creeks. The results of our study showed that the bacteriological pollution increased due to municipal wastewater resulting from overflow after the precipitation and the surrounding waters of the Golden Horn basin mix with surface waters. Thus, the most important factor in order to decrease the bacteriological pollution is to provide a full control of the surface discharges.

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