International Journal of Applied Environmental Sciences
ISSN 0973-6077 Volume 14, Number 2 (2019), pp. 145-160
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Spatiotemporal Variations of Bacterial Indicators in Coastal Waters of Tripoli, Northern Lebanon

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Abstract

The increased anthropogenic pressure and microbial discharges into the Eastern Mediterranean coasts necessitates continuous water quality assessment studies. The aim of the current study is to assess bacteriological loads in Tripoli's coastal water (northern Lebanon) in samples collected from two locations: site 1 including three distances from a large sewage outlet, and site 2 located in semi-enclosed area next to smaller sewage outlet in the port of Tripoli. 100% of the water samples collected at 10 m from the sewage outlet and from the port area exceeded the *E. coli* and total coliform threshold values set by international foundations for recreational water. However, *E. coli* counts in samples collected at 500 m and 1000 m from the sewer were significantly lower than the acceptable thresholds. Seasonal fluctuation of bacterial indicator in coastal water was evident as revealed by the significantly higher levels of fecal indicator during winter and summer compared to fall and spring. These findings highlight the need for year-round monitoring of fecal coliform, and call on national and local authorities to install signs banning recreational activities such as swimming and fishing next to such contaminated hotspots.

Keywords: Eastern Mediterranean, *E. coli*, Lebanon, seasonal fluctuation, spatial variations, total coliform

INTRODUCTION

The global deterioration of seawater quality and its effect on human health and the environment is of great concern. Developing countries are facing a decline in marine water quality manifested by an increase in microbial contaminants whose occurrence in water has been linked to an increasing risk of waterborne illnesses in swimmers and fishermen [1-4]. This is especially encountered in areas with high recreational activities where aquatic pathogens may be ingested and/or adsorbed during bathing, thus causing many kinds of diseases including skin and intestinal infections [5]. In this regard, standards were developed by several international bodies to determine the quality of recreational water [6-9]. When threshold limits are exceeded, signs should be placed by authorities in unhygienic areas to alert the users to the actual state of the water and restrict activities.

Recreational water safety has been investigated in seawater by continuously measuring the concentration of non-pathogenic indicator bacteria, namely total and fecal coliform, indicating the presence of human waste [10,11]. Levels of Enterococci and *Escherichia coli* (*E. coli*) were recommended as basic bacterial water quality criteria since their presence in water is highly associated with outbreaks of certain diseases [12], especially gastroenteritis [13]. Particularly, *E. coli* was recognized as the favorite indicator of pollution since its levels in water has been correlated with bather illness [14].

Different factors contribute to fecal bacterial survival rates in coastal waters, ranging from natural variations of climate during seasons [15] to anthropogenic influences [16]. Physical factors affecting the survival of fecal microorganisms in seawater include solar radiation, temperature, pH, sedimentation, algal blooms, and salinity [17]. Another key factor contributing to seawater deterioration lies in the discharge of untreated effluent waste from municipal sewers leading to an increase in the occurrence of microbial biomass in coastal water [18].

In Lebanon, an underdeveloped country on the eastern side of the Mediterranean Sea, coastal water has deteriorated over the last 40 years [19]. Lebanon is characterized by its treasured water resource that is crucial for a sustainable life for humans and biomass. However, anthropogenic activities are contributing to the deterioration of both potable and sea water in terms of quality and quantity [20]. Sewage influent is being discharged into the marine environment without primary treatment, although many wastewater treatment plants were built during the past two decades around the Lebanese coastal cities. Unfortunately, some of these plants are still not effectively functioning [21]. People of low income use the public coastal areas for recreational activities such as swimming, fishing, and boating even in areas close to sewage outlets (Figure 1) since they cannot afford pricey coastal resorts. The hazard of deteriorating water quality also extends to include the consumption of potentially contaminated fish sampled from the area by the local population. Testing and disseminating water quality is thus a key issue in the initiation of health awareness campaign inhibiting human activities in polluted marine areas.

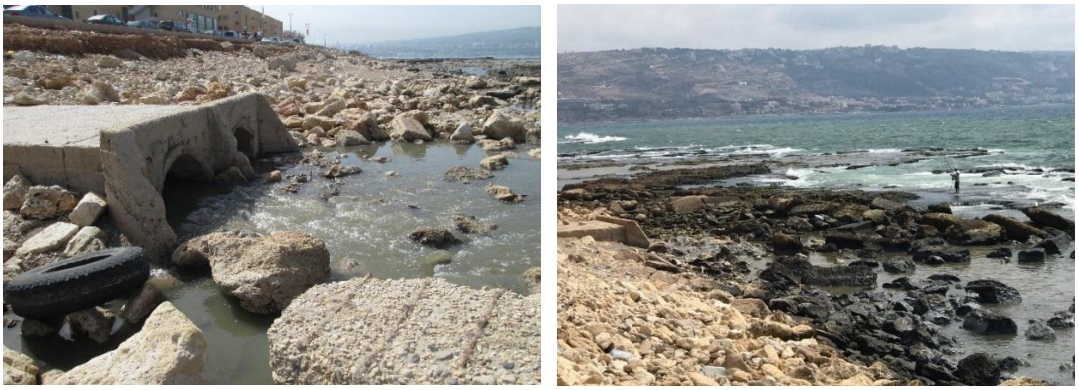

Figure 1. Sewage outlet releasing non-treated effluents into the sea in Tripoli, North Lebanon. A fisherman can be noticed fishing in close proximity to the outlet.

Therefore, this study aimed at i) quantifying the microbial load in the marine coastal area of Tripoli, North Lebanon, currently used for recreational and fishing activities; ii) evaluating the potential health risk for swimmer/fishermen/boaters, by comparing levels of total coliform (TC) and *E. coli* in marine water to standards adopted by EPA and EC guidelines for recreational water quality; iii) assessing the effect of both distance and season on the microbial load; iv) supplying local authorities/municipalities with evidence to support decision making (such as ban of activities) on health issues related to water quality in Tripoli marine environment, and to complete the gap of other studies previously done to the south and to the north of the sites evaluated in the current study. This study did not use any reference area around Tripoli as the entire coast there might be suffering from fecal pollution.

MATERIAL AND METHODS

Study area characteristics and sampling sites

Tripoli is a city in North Lebanon covering an area of 45 Km^2 and classified as the second largest city in Lebanon [22] (Figure 2). In the coastal zone of Tripoli, wastewater is released untreated (Figure 1) because of the suspension in operation of the wastewater treatment plant. In addition to the untreated sewage discharged into the sea, coastal water is affected by the large seafront dumpsite in Tripoli [19]. Consequently, with the probable incidence of microbial pathogens contaminating water, potential health hazards increase [23]. The extent of bacterial contamination in the study area was evaluated over 10 months to account for seasonal variations. The first site include set distances (10 m, 500 m and 1000 m) from a large sewage outlet releasing effluents without treatment into the sea (Figure 1, Figure 2), and site 2 is located in the port of Tripoli, near a smaller sewage outlet in an semi enclosed area (Figure 2).

Figure 2. Sampling site map. The first site included 3 different distances from a sewage outlet and the second site is located in the Port of Tripoli (Latitude: 34º 25' 59.99" N, Longitude: 35º 50' 59.99" E).

Water Sampling and analysis

Water sampling was performed monthly between May 2015 and March 2016. Water samples were collected nearly 10 cm below water surface in inverted sterile bottles [24]. Tightly sealed, samples were transported in insulated box to the laboratory and were analyzed for total and fecal coliform within 4 hours of collection. The membrane filter technique was used to count TC and *E. coli*, and results are expressed as number of colony forming unit (CFU) in 100 ml of seawater. Water samples were filtered through a 0.45 µm sterile membrane filter aspirated with an attached vacuum pump [25]. Following filtration, filters were transferred to a plate with chromogenic rapid E-coli 2 (BIORAD- France) agar. The agar plates were incubated at 37ºC ± 1ºC and 44ºC ± 1ºC for 24 hours to detect TC and *E. coli*.

Statistical analysis

IBM SPSS statistics 21 and Excel version 2013 were used to statistically treat the data. Results are presented as CFU mean ± SD and median with range (min-max) for all sampling sites. For statistical and figure representation, data was log transformed to obtain normal distributions. Association between TC and *E. coli* values in water samples was done using Spearman's rank correlation. This correlation was performed to verify the relationships between these bacterial indicators. When evaluating the potential health risk on bathers, one sample t-test was used to assess whether the evaluated concentration of *E. coli* and TC in sampled water is significantly higher than threshold levels adopted for recreational water safety (For *E. coli*: 100 CFU/100 ml [8]; for TC: 500 CFU/100 ml [6]). In order to account for location and seasonal factors affecting the variability in TC and *E. coli* abundance in marine water, two-way analysis of Variance (ANOVA) was performed. *E. coli* and TC counts were compared among seasons and locations using Bonferroni post-hoc test.

RESULTS

Concentrations of bacteria fluctuated from season to season and by location (Table 1). The mean *E. coli* ranged from 8 ± 10.408 CFU in the spring at a distance of 1000 m from the sewage outlet to 106400 ± 64863.7 CFU recorded in the fall in the port area. The mean of TC varied from 33 ± 25.166 CFU in samples collected at 1000 m during spring to 212600 ± 131809.7 CFU during fall in the port. As expected, mean and median concentrations for TC and *E. coli* were higher in water samples collected at 10 m distance from the sewage outlet compared to those at 500 m and 1000 m. However, the highest *E. coli* and TC were found in the port area in all seasons (Table 1).

Table 1. Mean ± SD and median with range (min-max) concentrations of TC and *E.coli* in water samples collected from sites 1 (10 m, 500 m and 1000 m from the sewage outlet) and site 2 (Port) during the four seasons of years 2015-2016; N: the number water samples; SD: Standard deviation.

Locations	Seasons	N	Mean *E. coli* ± SD	Median *E. coli* (range)	Mean TC ± SD	Median TC (range)
1000 m	Fall	5	23 ± 12.042	20 (10-40)	94 ± 20.179	90 (70-115)
	Spring	3	8 ± 10.408	5 (0-20)	33 ± 25.166	30 (10-60)
	Summer	5	196 ± 145.362	110 (80-400)	328 ± 210.879	250 (120-600)
	Winter	5	72 ± 24.9	70 (40-110)	108 ± 14.484	105 (90-125)
10 m	Fall	5	1100 ± 1007.472	400 (300-2300)	2820 ± 3024.401	700 (600-6700)
	Spring	3	5133 ± 1677.299	6000 (3200-6200)	7200 ± 1946.792	7900 (5000-8700)
	Summer	5	3580 ± 2264.288	3700 (1300-6500)	5940 ± 3628.774	7500 (2000-10000)
	Winter	5	6040 ± 4624.716	3600 (1200-11000)	14660 ± 7901.139	15000 (2300-22000)
500 m	Fall	5	58 ± 8.367	60 (50-70)	106 ± 18.166	110 (80-130)
	Spring	3	36 ± 9.292	40 (25-42)	70 ± 13.229	65 (60-85)
	Summer	5	268 ± 225.1	150 (70-600)	542 ± 240.977	450 (310-800)
	Winter	5	94 ± 12.942	90 (80-110)	126 ± 10.257	126.2 (113-140)
Port	Fall	5	106400 ± 64863.7	150000 (25000-160000)	212600 ± 131809.7	280000 (60000-350000)
	Spring	3	23000 ± 13076.7	29000 (8000-32000)	39667± 27153.8	43000 (11000-65000)
	Summer	5	9760 ± 3985.975	7800 (6000-15000)	16300 ± 5333.9	13500 (11000-23000)
	Winter	5	14280 ± 8970.897	15000 (4100-24000)	24000 ± 2000	25000 (21000-26000)

Correlation between TC and *E. coli* in water samples

Spearman rank correlation (r-value) was performed between log-transformed concentrations of TC and *E. coli* (log TC/EC). TC and *E. coli* counts were strongly correlated during fall, winter, spring and summer with r- values of 0.856, 0.888, 0.966 and 0.967, respectively (p=0.000).

Comparison with standards

Acknowledging that some recreational activities are practiced in specific seasons (such as swimming in spring and summer), the percent of water samples failing the EPA standards for recreational water (100 CFU *E. coli*/100 ml) during the four seasons of collection are presented in table 2. In site 1, 100% of the water samples obtained at 10 m distance from the sewage outlet exceeded the EPA 2012 standards for *E. coli* in all seasons of collection. However, samples collected at 500 m and 1000 m distance from the outlet during fall and spring did not exceed the standards for *E. coli*. During the summer, the proportion of samples exceeding *E. coli* standard was 80% and 60% for water collected at 500 m and 1000 m distance, respectively. However, during winter, 40% of the water samples collected at 500 m distance violated the *E. coli* standards compared to 20% failure for water sampled at 1000 m distance from the sewer. On the other hand, all water samples collected from the port exceeded *E. coli* standards adopted by the EPA 2012 for recreational water regardless of the seasons.

Table 2. Percentage of samples failing EPA, 2012 standards for *E. coli* in recreational water.

Location	1000 m	500 m	10 m	Port
Fall	0%	0%	100%	100%
Winter	20%	40%	100%	100%
Spring	0%	0%	100%	100%
Summer	60%	80%	100%	100%

As some recreational activities are practiced all year long such as fishing and boating, data were then pooled based on the location of sampling irrespective of seasons, and means of *E. coli* and TC in water samples were compared to standards (Table 3). Mean *E. coli* in samples obtained at 10 m distance from the sewage in site 1 was significantly higher than the EPA standards. Samples harvested at 500 m distance exhibited mean *E. coli* that is not significantly higher than the EPA standards (t-test, p>0.05). In addition, mean *E. coli* in samples collected at 1000 m from the sewage was significantly lower than the standards. As for samples collected from the port area (site 2), mean *E. coli* in water samples was found to be significantly higher than the EPA standards. Furthermore, mean TC in samples collected at 10 m distance from the

sewage and from the port significantly exceeded the European Commission standards [6] for TC in recreational water. However, water samples harvested from the 500 m and the 1000 m distance from the sewage exhibited TC means significantly lower than the regulation.

Table 3. One sample t-test comparing mean *E. coli* and mean TC to standards; t-test was performed on log-transformed means.

		E. coli			**TC**		
Sampling locations	N	log mean	t-test sig.	Comparison with EPA, 2012 standards	log mean	t-test sig.	Comparison with EC (2003) standards
10 m	18	3.391±0.475	0.000	> 2	3.680±0.503	0.000	> 2.699
500 m	18	1.931±0.337	0.395	Not sig	2.201±0.346	0.000	< 2.699
1000 m	18	1.651±0.454	0.02	< 2	2.023±0.397	0.000	< 2.699
Port	18	4.583±0.485	0.000	> 2	4.583±0.485	0.000	> 2.699

Spatial and temporal variation in seawater quality

The log mean for *E. coli* and TC in water samples collected during the four seasons of sampling and from the three different distances from the sewage outlet are summarized in Table 4. Water samples collected at 10 m from the sewage outlet exhibited highest means of TC and *E. coli* (log CFU/100 ml) (Table 4). The perceived spatial and temporal variations for both bacterial communities were statistically analyzed using the two-way ANOVA (Table 5) and Bonferroni post- hoc testing (Figure 3). Both location and seasons have significant effect on water quality. Indeed, there was a statistically significant difference in log mean *E. coli* between the three locations from the outlet (10 m, 500 m, 1000 m) (ANOVA: df = 2, F= 189.613, p= 0.000) and between the four seasons of collection (ANOVA: df= 3, F= 16.895, p= 0.000) (Table 5). Likewise, log mean TC differed significantly between seasons (ANOVA: df= 3, F= 13.356, p= 0.000) and among distances from the sewage outlet (ANOVA: df= 2, F= 201.787; p= 0.000) (Table 5). In addition, there was a statistically significant interaction between the effect of location and season on the load of *E. coli* and TC in water samples (Table 5).

Table 4. Log (mean *E. coli*) and log (mean TC) in water samples. N: number of water samples; SD: Standard deviation.

Season	Locations	N	log (Mean TC) ± SD	log (Mean *E. coli*) ± SD
Spring	10	3	3.845±0.129	3.692±0.162
	500	3	1.840±0.079	1.541±0.124
	1000	3	1.418±0.392	1.00±0.426
	Average± SD		2.368±1.142	2.078±1.233
Summer	10	5	3.684±0.335	3.468±0.321
	500	5	2.699±0.195	2.296±0.384
	1000	5	2.437±0.299	2.196±0.321
	Average± SD		2.940±0.614	2.653±0.677
Fall	10	5	3.194±0.54	2.873±0.431
	500	5	2.020±0.077	1.760±0.062
	1000	5	1.964±0.095	1.311±0.238
	Average± SD		2.393±0.657	1.981±0.73
Winter	10	5	4.063±0.404	3.650±0.405
	500	5	2.100±0.035	1.970±0.059
	1000	5	2.032±0.059	1.836±0.156
	Average± SD		2.732±0.999	2.485±0.886
Total	10	18	3.68±0.503	3.391±0.475
	500	18	2.201±0.346	1.931±0.337
	1000	18	2.023±0.397	1.651±0.454
	Average± SD		2.635±0.856	2.349±0.875

Table 5. Two-Way ANOVA calculation for the two bacterial communities.

Bacterial community	Variation	df	Type III sum of squares	Mean square	F	*p*
E. coli	Season	3	4.243	1.414	16.895	0.000
	Location	2	31.744	15.872	189.613	0.000
	Season * location interaction	6	2.242	0.374	4.464	0.001
TC	Season	3	3.060	1.020	13.356	0.000
	Location	2	30.820	15.410	201.787	0.000
	Season * location interaction	6	2.756	0.459	6.015	0.000

Bonferroni post-hoc test (Figure 3) indicated that in all four seasons, there was a statistically significant difference in mean *E. coli* and TC between samples collected from 10 m and each of the other two distances from the outlet (500 and 1000 m) (p<0.05), while no significant difference was observed between samples collected at 500 m and 1000 m (p>0.05). Furthermore, water samples collected in winter at 10 m showed the highest log mean of TC compared to the significantly lowest records in the fall. At 500 m and 1000 m, water samples exhibited highest means of TC in summer and significantly lower records in spring. Highest means of *E. coli* were observed in water samples collected at 10 m distance from the outlet in winter, spring and summer, compared with the fall season where water showed significantly lower mean values. Water samples collected at 500 m and 1000 m from the outlet exhibited highest means of *E. coli* in the summer and winter compared to significantly lowest records in spring and fall.

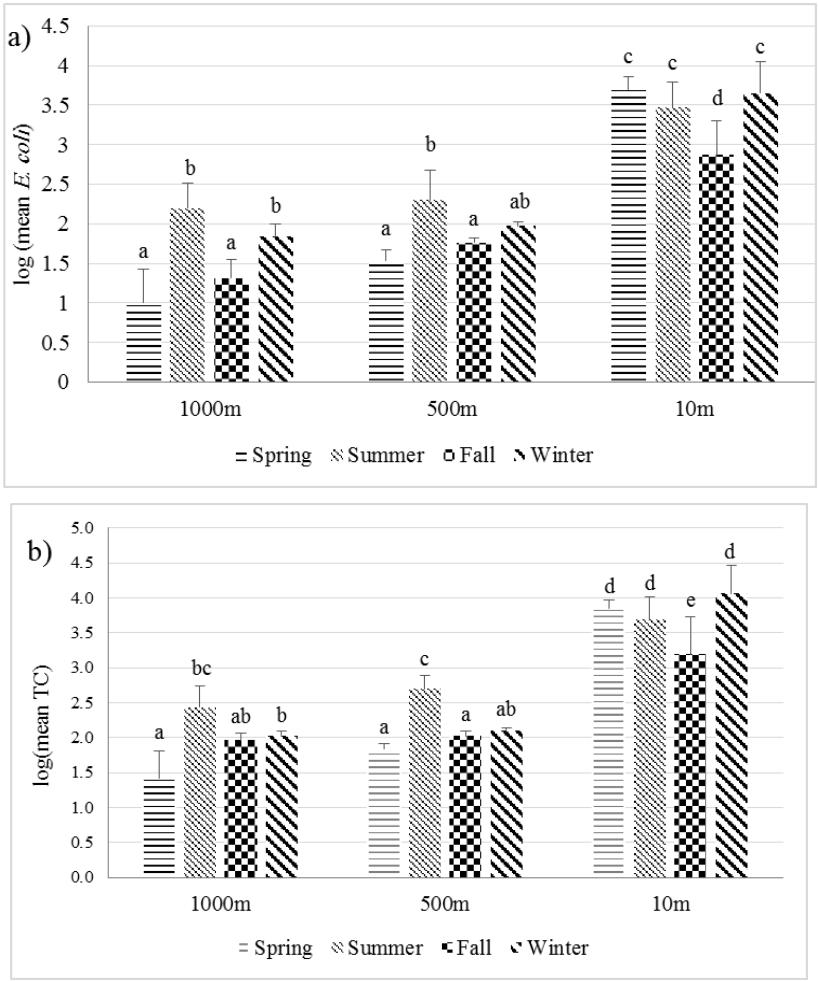

Figure 3. The log-transformed mean of *E. coli* (a) and TC (b) for each location and during each season. Significant differences between the log (mean) are indicated by different letters, whereas same letters indicate non-significant differences (Bonferroni post- hoc).

DISCUSSION

Analysis of the water quality results suggested that the sampling area is contaminated with the fecal coliform discharged from the sewage outlets and requires action from the local authorities. Even though the Spearman's rank correlation revealed a significant positive relationship between TC and *E. coli* during four seasons (r ranging from 0.967 to 0.856), high TC counts in water would not necessarily infer that *E. coli* will also be high and exceed international standards. Fecal coliforms counts typically characterize only a minor percentage of total coliform values, but this percentage could be larger after certain anthropogenic or climatic events [26].

The presence of coliform bacteria, specifically the diarrheagenic *E. coli*, in the environment indicates fecal contamination and correlates to the likelihood that other pathogenic organisms are also present. In fact, the United States EPA encourages the use of *E. coli* as the indicator organism to measure water quality instead of fecal coliform as its presence has been associated with several waterborne illnesses at certain threshold levels [27]. Parameters affecting the fate, transport and persistence of *E. coli* are numerous and interrelated. These factors include exposure to irradiation, nutrient content, salinity, suspended solids concentration, removal by sedimentation, water temperature, pH, and predation [28].

In site 1, and at 10 m from the sewage outlet that is accessible to the public, 100% of the water samples exceeded the *E. coli* threshold value set by the EPA 2012 in recreational water (100 CFU/100 ml) regardless of the season when samples were collected. From a health perspective, and given the high number of contaminated water samples, it is crucial to highlight the danger to bathers at 10 m proximity from the sewage outlet where urban runoffs drain directly into the sea without treatment contaminating water with pathogens. Similar outcomes were observed for water samples collected from the port area where 100% of the samples were loaded with *E. coli* exceeding EPA standards. The port represents an area of heavy traffic of ships with some marine vessels releasing their ballast water, thus constituting a potential source of pollution. Means of *E. coli* in samples collected at 1000 m or 500 m distance from the sewer were found to be significantly lower than the regulations. On the other hand, TC exceeded international thresholds only in water samples collected at 10 m distance from the sewer outlet and in the port. Nevertheless, *E. coli* in water constitutes the key indicator for a classification of the area as hazardous even when high total coliform levels are detected. It is evident that as the distance from the sewage outlet increases, the load of pathogens decreases. Thus, recreational activity must be banned in areas in proximity of the sewer outlet in order to decrease the potential health risk of contaminated water for bathers and fishermen. As for the semi-enclosed port area, the high microbial load recorded in water samples (near a smaller sewage outlet) should also force the authorities to restrict any recreational activity there.

The significant spatial (between sampling distances) as well as temporal (seasonal) variations found for *E. coli* and TC suggested that both sampling location and collection season have a significant effect on the load of bacteria in water. Evaluating locational variations revealed that both *E. coli* and TC were highest at 10 m from the

sewage outlet in all seasons probably due to the direct disposal of large amount of untreated sewage. The load of TC and *E. coli* in the samples collected at 500 m was not significantly different from those recorded at 1000 m perhaps due to the dilution effect. As for the assessment of seasonal variation on microbial loads in water samples, different factors that influence the fate of fecal coliforms in water such as sunlight, predation and salinity, might interfere [29,30]. It was documented that seasonal pollution mostly affects fecal coliforms, extremely sensitive to climatic fluctuation [26]. The results of the study revealed significant differences in bacterial levels between different sampling seasons. High bacterial contamination recorded in water samples collected during winter at 10 m distance might be attributed to different environmental factors. In fact, the fate of microorganisms in a water body depends on sunlight, temperature, availability of nutrient, salinity and predation [31].

It has been shown that *E. coli* survives longer in cold temperatures than in warm temperatures, probably due to increased competition for nutrients by other organisms and increased predation in warmer water bodies. Furthermore, it is well documented that solar radiation plays a key role in determining the fate of fecal coliform in water body [32,33], being more lethal in saline water. During winter, UV radiations from sunlight are partially blocked by clouds thus hindering its access to bacteria in seawater and therefore decreasing its mortality rates [34]. It has also been reported that lower water temperatures of winter may contribute to increased microbial survival rates [35]. Another factor that may contribute to the dynamics of coliform in coastal water is the bacterial load from streams and other sources [16]. In fact, soil moisture during spring and high summer temperatures provide optimal conditions that facilitate the growth of coliform bacteria. In winter, runoff from the contaminated soil particularly during heavy rainfalls might spread to the beach rapidly and form a fresh source of bacterial contamination [36,37]. This will also increase water turbidity which leads to less sunlight penetration and eventually longer *E. coli* survival [38].

While lower levels of microbiological pollution during the summer were reported in literature compared with other seasons possibly due to the effect of sunlight that increases microbial mortality in water [37,39], high microbial pollution was recorded in summer in the current study. Several factors may have contributed to this observation as demonstrated in other studies [26]. The increased microbiological pollution can be attributed to the increased human presence in the city of Tripoli during the summer months resulting in an increased wastewater drained into the sea. The release of sewage into the sea might also increase the eutrophication process where plants and algae grow excessively particularly at warmer temperatures. This will reduce solar radiation penetration into water and thus extend the survival of fecal bacteria in the summer season. Furthermore, the phototrophic algae can be a source of organic compounds necessary for the growth of fecal bacterial indicators [40].

The lowest abundance of *E. coli* observed in the fall could be associated with high loss rate due to the wide seasonal variations in temperature and/or to predation by protozoan [16]. Indeed, rapid change in salt concentrations in marine environment might increase rates of bacterial mortality [17]. This seasonal fluctuation in bacterial counts might also be associated with water temperature, significantly correlated to *E.*

coli loss from the marine environment [41].

CONCLUSION

Increased water-associated recreational activities have created a need for sustaining safe surface waters. The current study assessed seawater quality by determining the levels of two bacterial indicators, namely total coliforms and *E. coli*, in Tripoli's coastal waters in an area impacted by sanitary sewage. Temporal and spatial variations in seawater microbial load were evident. In terms of spatial variations, highest counts of indicator bacteria exceeding international standards were detected in close proximity to a sewage outlet and in the port area. These locations are therefore contaminated hotspots and signs banning recreational activities such as swimming and fishing should be installed by authorities. Seasonal fluctuation of bacterial indicators was attributed to biotic as well as abiotic factors. In fact, the highest levels of *E. coli* measured in winter were correlated to several factors that reduced bacterial mortality such as lower temperatures and lower solar radiation doses. Microbiological pollution during the summer was associated to enhanced anthropogenic influence during this period of the year and to algal blooms that reduce the penetration of solar radiation into the water column. Finally, this study was restricted to specific areas in the coast of Tripoli, Lebanon, and represents a contribution to a large-scale monitoring program, and further studies are required for continuous monitoring of marine water of the city. Furthermore, several factors that affect the survival of bacteria in seawater such as salinity, temperature, pH, and solar radiation were not assessed in the current study, and could influence our results.

ACKNOWLEDGEMENTS

The authors would like to thank the Chamber of Commerce, Industry, and Agriculture of Tripoli and North Lebanon for assistance in water analysis.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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