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Assessment of the Microbiological Quality of Caspian Seawater and the Role of Physicochemical Factors on Microbial Load

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ABSTRACT

Introduction: Analyzing the health risk associated with the microbial contamination of seawater is necessary to ensure that there is not any threat to human or environment. The aim of this study was to evaluate the microbiological quality of Caspian sea water using indicator bacteria. Some Physicochemical parameters were studied to assed their association with the contamination level of Caspian sea water due to the important role in the growth organisms in the seawater.

Materials and Methods: In this study, 100 samples were collected from the northeastern zone of the Caspian sea, Iran, from November 2017 to December 2018. *Total coliform*, *Fecal coliform*, *E.coli*, *Fecal Streptococcus* and *Clostridium perfringens* were the indicator bacteria *and* temperature, pH, electrical conductivity, chloride and turbidity were water physicochemical factors according to standard methods.

Results: The finding showed that the mean of *Total coliform*, *Fecal coliform*, *E.coli*, *Fecal Streptococcus*, and *Clostridium perfringens* were 614.72 ± 516.13, 62.11 ± 235.30, 49.69 ± 188.24, 348.02 ± 490.01 and 3.04 ± 5.76 MPN/100 ml, respectively. Furthermore, the mean and SD of temperature, pH, electrical conductivity, chloride, and turbidity were 13.84 ^{o}C ± 5.62, 8.27 ± 0.45, 17.96 ± 6.56 µs/cm, 5776.95 ± 1996.38 mg/l and 27.48 ± 15.82 NTU, respectively. Statistical analysis showed that microbial qualities were affected significantly by physicochemical factors, but the roles of water temperature were more than others.

Conclusion: The microbiological results revealed that there was a remarkable level of contamination in some areas of Caspian sea. The results suggested that *Clostridium perfringens* provide better health risk prediction than other analyzed indicator bacteria, particularly in the warm season.

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Introduction

Coastal seas are one of the most important natural environments both in terms of biological resources and the energy resources lying on its bed and in the coastal. A variety of parameters have all been combined to threaten its environment and especially its specific microbiological characteristic due to the dumping of urban and industrial wastewaters into the sea, with their high level of pathogens such as bacteria, protozoa, viruses, helminths worms and fungi, and thus the possibility water-related illness outbreaks [1-4]. Gastroenteritis, diarrhea, typhoid, leptospirosis, primary amoebic

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meningoencephalitis, and acute respiratory infections have been identified as diseases commonly caused by these pathogens in water via both direct dermal contact and ingestion [5-6]. Hence, the evaluation of the microbiological quality of seawater aims to protect consumers from illness due to both consumption and contact [5].

The microbiological quality of seawater is evaluated by the results obtained from fecal indicator bacteria (FIB) include *total coliform*, *Fecal coliform*, *E.coli*, and *Fecal streptococci* and *Clostridium perfringens* to determine the microbiological quality of these water [7]. However, the lack of correlation between the number of some indicator bacteria and those of pathogenic microorganism has led to becoming unsuitable indicators, but the fact that indicator bacteria can be easily cultivated and is relatively inexpensive; therefore it is widely used as the indicators of fecal contamination by many countries and international organizations, including the European Union, the United States, and the World Health Organization (WHO) [8-9]. Of course, among the indicator bacteria, *Clostridium perfringens*, from spore-forming bacteria, are a better indicator for pathogens such as *salmonella*, *cryptosporidium*, *Giardia*, *adenoviruses*, and *enteroviruses* when comparing with the other indicators [10-12].

The Caspian sea is the largest inland body of water on the earth that it has a strategic and geopolitical position role for the governments of Iran, Russia, Azerbaijan, Turkmenistan, and Kazakhstan [13-14]. It has provided a suitable environment for the recreational activities of Iranian people. For this reason, pressure on this area is rising by people through ecotourism facilities, industrial factories, agriculture's drainage, etc., which in turn reduces the quality of water. Consequently, this study was designed to evaluate the microbiological quality of Caspian sea water and coastal environment according to the recommended methods. Furthermore, some physicochemical factors were studied such as temperature, pH, turbidity, electrical conductivity and chloride that might influence the microbial quality of Caspian seawater.

Materials and Methods

The study area and collection of water samples

In this cross-sectional study, 100 water samples were collected quarterly (25 samples per each season) along the northeastern zone of the Caspian sea, Iran, from November 2017 to December 2018, which were located at the southeastern of the Caspian sea near the city of Gorgan. It has a moderate climate with an average annual temperature of 17.7°C and an average annual rainfall of 601.0 mm. The spatial characteristics of the sampling stations is presented in Figure 1. At each site, 4 to 7 seawater samples were collected in sterile glass containers (2 Litter) by holding the bottles about 30 cm below the surface in a 2-meters-deep [15]. All samples were immediately placed on ice, transported to the laboratory, and processed within two hours after collection. Moreover, quality assurance and quality control measures were used according to the Standard Methods for the Examination of Water and Wastewater [15].

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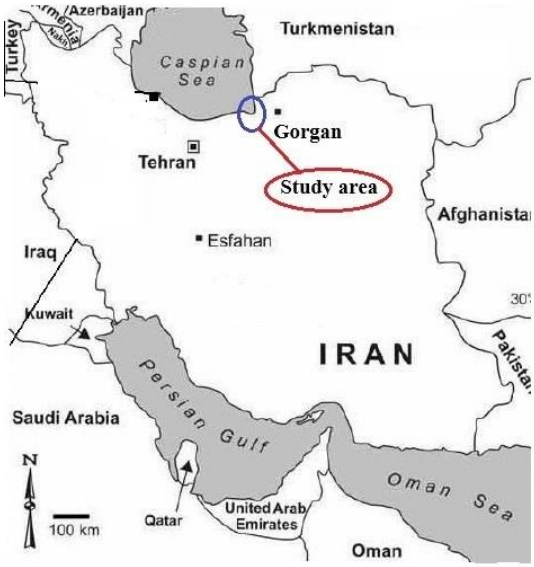

UTM coordinates of sampling stations within the study area

Sampling station	UTM coordinates	
	X	Y
S1	53°58'32.33″	37°03'59.13″
S2	53°58'19.69″	37°02'54.49″
S3	53°58'50.36″	37°01'44.43″
S4	53°58'56.51″	37°00'46.58″
S5	53°59'11.34″	36°59'35.30″
S6	53°5832.63″	36°58'17.07″
S7	53°58'54.58″	36°57'24.12″
S8	54°01'56.36″	36°55'59.41″
S9	54°02'02.54″	36°55'03.84″
S10	54°02'03.30″	36°53'50.68″
S11	54°02'14.43″	36°52'13.53″
S12	54°01'51.37″	36°49'46.74″
S13	54°01'29.94″	36°48'56.26″
S14	53°59'35.27″	36°48'13.25″
S15	53°57'19.24″	36°47'53.04″
S16	53°56'061.01″	36°47'34.51″
S17	53°54'28.82″	36°47'40.10″

Figure 1: Location of research (northeastern zone of the Caspian sea, Iran)

Determination of fecal indicator bacteria

The number of bacteria was estimated by the multiple-tube fermentation (MTF) method in a 9-tube MPN arrangement, and the result was expressed as most probable Number per 100 ml (MPN/100mL) according to the description in Standard Methods for the Examination of Water and Wastewater [15]. Detection of *total coliform* and *fecal coliform* was achieved using the selective media, Brilliant Green Lactose Bile broth, and EC medium, respectively. *Escherichia coli* was quantified by using the media of lauryl Tryptose broth containing 4-methylumbelliferyl-B-D-glucuronide (LTB-MUG) [12]. *Fecal streptococci* were also detected and enumerated using the media of Azide Dextrose broth and PSE agar as the presumptive and confirmation test, respectively [15]. For the detection of *Clostridium perfringens*, water samples were inoculated into Thioglycolate broth and then incubated in an atmosphere containing 9 - 13 % CO2 at 37 °C for 24-48 h. The positive tubes were subculture on Tryptose Sulphite Cycloserine agar, incubating at 37°C for 24 h, anaerobically. Black, grey colonies were considered as presumptive *Clostridium perfringens*. The confirmation procedure consisting of gram staining, motility, and nitrate reduction was performed on suspected colonies [12].

Analysis of physicochemical parameter

Physicochemical parameters including pH, Electrical Conductivity (EC) and Turbidity of the samples were determined in the laboratory by digital pH meter (HT-1202 Digital PH), conductivity meter (SensIon7, Hach Company, USA) and turbidity meter (2100P, Hach Company, USA) according to instrument direction and standard methods for the examination of water. Water temperature was measured in the field at the time of sample collection with an electronic digital thermometer (TP101). The Mohr method was used to determine the chloride concentration in samples, using the chromate ions as an indicator in the titration of chloride ions with a silver nitrate standard solution [15].

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics version 20 with a P-value of ≤ 0.05 which is statistically significant. The normality of data was checked by the Shapiro-Wilk test before analyzing it. Descriptive statistics were applied for the presentation of *total coliform*, *fecal coliform*, *Escherichia coli*, *fecal streptococci*, *Clostridium*

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perfringens, temperature, pH, electrical conductivity, chloride concentration and turbidity levels in seawater samples. The relationship between *fecal bacteria* and physicochemical parameters was analyzed using the Pearson correlation test. Additionally, analysis of variance (ANOVA) was used to compare *fecal bacteria* concentrations among seasons and sampling locations.

Ethical issues

This research was approved at the research ethics committee of Golestan University of Medical Sciences, Iran with the Ethics Code of IR. GOUMS.REC.1395.71.

Results

According to the findings, the mean and SD of *total coliform*, *Fecal coliform*, *E.coli*, *Fecal Streptococcus*, and *Clostridium perfringens* were 614.72 ± 516.13, 62.11 ± 235.30, 49.69 ± 188.24, 348.02 ± 490.01 and 3.04 ± 5.76 MPN/100 ml, respectively. The results showed that the variation in *total coliform*, *Fecal streptococci*, *Fecal coliform*, *Escherichia coli*, *Fecal streptococci*, and *Clostridium perfringens* level were not statistically significant at different stations. The results of physicochemical analysis also showed that the mean and SD for temperature, pH, electrical conductivity, chloride, and turbidity were 13.84 ± 5.62, 8.27 ± 0.45, 17.96 ± 6.56 µs/cm, 5776.95 ± 1996.38 mg/l and 27.48 ± 15.82 NTU, respectively. For physicochemical parameters, the only significant relation was observed between electrical conductivity across places ($p = 0.45$) in accordance with the ANOVA test. The distribution average value of physicochemical parameters also showed that there were notable changes during the four seasons ($p < 0.001$) except chloride ($p = 0.158$). The mean and SD values of bacterial indicators and physicochemical parameters in seawater samples of the Caspian Sea coastal for every sampling site are presented in table 1.

Statistical analysis showed that microbial qualities were affected significantly by physicochemical factors, but the roles of temperature and electrical conductivity were more than the others. The results of correlation analysis between measured parameters and the effect of seasonal variation on physicochemical and microbiological parameters of samples are shown in table 2 and figure 2.

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Table 1: The mean ± SD Level of physicochemical parameters and FIB at the north-eastern zone of the Caspian Sea, Iran

Station name	Temperature (^{O}C)	pH	E.C (µS/cm)	Chloride (mg/L)	Turbidity (NTU)	*Total coliform* (MPN/100ml)	F. Coliform (MPN/100ml)	*E. coli* (MPN/100ml)	*Fecal streptococci* (MPN/100ml)	*Clostridium perfringens* (MPN/100ml)
S1	11.50 ± 7.23	8.31 ± 0.27	15.69 ± 6.21	6483.19 ± 345.34	33.35 ± 17.29	603.50 ± 579.44	0.75 ± 1.50	0.50 ± 0.75	587.50 ± 594.94	4.03 ± 8.05
S2	11.75 ± 6.99	8.34 ± 0.40	16.88 ± 5.49	5205.19 ± 697.14	33.18 ± 16.10	877.50 ± 445.00	0.75 ± 1.50	0.50 ± 0.75	551.75 ± 633.06	4.30 ± 7.88
S3	12.00 ± 6.78	8.40 ± 0.35	15.78 ± 6.16	5604.56 ± 1681.67	33.55 ± 12.49	832.00 ± 536.00	0.00 ± 0.00	0.00 ± 0.00	568.75 ± 614.20	2.00 ± 3.31
S4	14.17 ± 6.62	8.52 ± 0.45	17.41 ± 5.12	4954.03 ± 662.26	33.41 ± 15.39	453.67 ± 506.37	0.00 ± 0.00	0.00 ± 0.00	368.67 ± 566.50	5.35 ± 9.03
S5	13.25 ± 6.90	8.62 ± 0.89	10.00 ± 11.49	4317.69 ± 254.93	33.18 ± 17.92	556.50 ± 627.63	115.00 ± 230.00	85.00 ± 110.00	283.00 ± 544.75	4.58 ± 7.70
S6	13.25 ± 6.90	8.21 ± 0.54	9.77 ± 11.76	5169.69 ± 1913.30	29.17 ± 16.42	825.75 ± 548.50	115.00 ± 230.00	115.00 ± 230.00	551.00 ± 633.93	6.03 ± 11.33
S7	14.57 ± 5.68	8.41 ± 0.40	18.56 ± 6.32	5777.88 ± 2501.55	18.55 ± 13.17	551.71 ± 534.95	17.00 ± 33.94	11.25 ± 18.55	472.43 ± 587.04	7.20 ±10.80
S8	14.92 ± 6.17	8.21 ± 0.29	17.75 ±5.43	5936.78 ± 1229.48	16.95 ± 12.49	417.17 ± 533.99	0.50 ± 1.22	0.00 ± 0.00	369.00 ± 566.24	2.67 ± 3.32
S9	14.74 ± 5.55	8.20 ± 0.59	18.47± 5.92	5975.66 ± 3009.27	22.81 ± 10.92	525.14 ± 542.57	161.29 ± 414.02	115.00 ± 230.00	393.71 ± 509.11	2.46 ± 3.90
S10	14.89 ± 5.36	8.16 ± 0.48	18.71 ± 6.05	7202.95 ± 2133.84	36.36 ± 25.26	647.57 ± 545.55	163.71 ± 413.01	163.71 ± 413.01	227.00 ± 420.57	0.83 ± 1.33
S11	14.50 ± 4.12	8.16 ± 0.53	20.40 ± 5.54	7105.33 ± 2495.19	29.33 ± 15.85	827.25 ± 545.50	280.75 ± 546.27	131.29 ± 214.02	11.25 ± 18.55	1.10 ± 0.90
S12	14.50 ± 4.72	8.19 ± 0.38	22.13 ± 4.99	5569.95 ± 1010.10	17.69 ± 13.54	401.83 ± 543.34	4.33 ± 9.22	4.33 ± 9.22	367.17 ± 567.65	4.58 ± 6.64
S13	13.25 ± 5.32	8.28 ± 0.53	27.00 ± 2.08	5889.45 ± 2073.23	34.43 ± 32.13	826.00 ± 548.00	275.75 ± 549.50	227.00 ± 420.57	17.00 ± 21.95	0.83 ± 1.05
S14	13.13 ± 6.25	7.97 ± 0.49	17.83 ± 4.80	6216.94 ± 1601.13	21.93 ± 9.35	608.25 ± 572.93	0.00 ± 0.00	0.00 ± 0.00	390.75 ± 519.89	2.00 ± 3.31
S15	13.93 ± 6.80	8.16 ± 0.44	16.69 ± 6.68	6207.94 ± 2537.86	26.77 ± 10.19	551.57 ± 535.52	0.00 ± 0.00	0.00 ± 0.00	212.86 ± 402.69	0.99 ± 1.28
S16	13.88 ± 6.61	8.37 ± 0.59	22.92 ± 5.24	5356.06 ± 3013.35	29.43 ± 17.71	581.00 ± 601.16	0.75 ± 1.50	0.75 ± 1.50	312.50 ± 529.74	1.55 ± 2.42
S17	13.88 ± 6.61	8.16 ± 0.49	17.58 ± 4.95	4956.69 ± 401.59	25.73 ± 6.78	827.25 ± 545.50	0.75 ± 1.50	0.75 ± 1.50	301.00 ± 534.26	2.30 ± 4.60

Table 2: Pearson correlation coefficient between indicator bacteria and physicochemical parameters

Indicator Bacteria	physicochemical parameters				
	Temperature (^{o}C)	pH	Cl (mg/L)	EC (µS/cm)	Turbidity (NTU)
Total coliform	-0.371**	-0.560**	0.193*	0.178*	0.285**
Fecal coliform	-0.068	-0.129	0.055	0.286**	-0.061
Fecal streptococci	-0.659**	-0.023	0.197*	0.433**	0.001
Clostridium perfringens	0.605**	0.506**	-0.111	-0.337**	-0.251**

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

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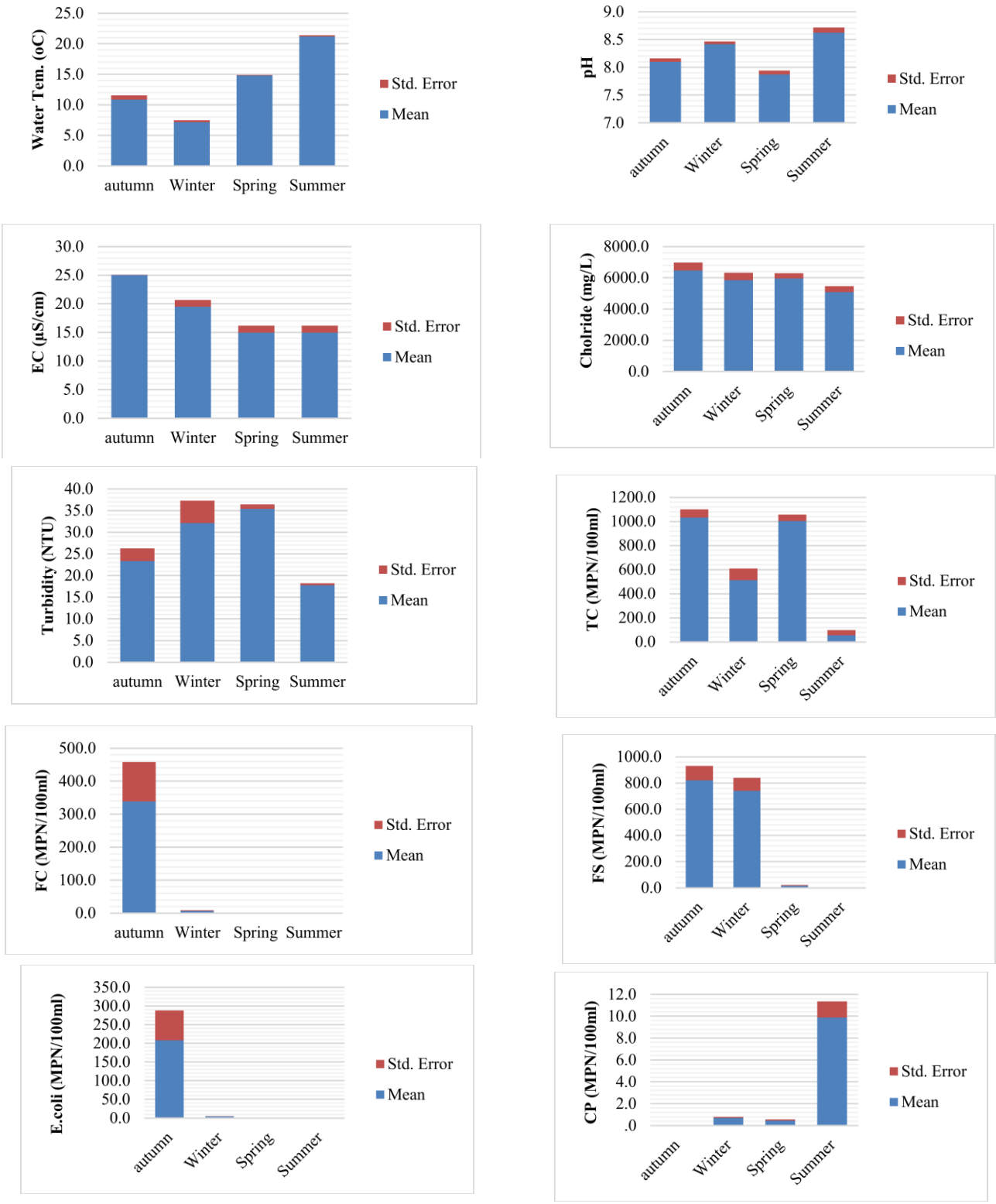

Figure 2: Seasonal variation of physicochemical parameters and indicator of bacteria

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Discussion

The results of bacteriological analysis showed that there was microbial contamination above the permissible limit for recreational activity in some sampling locations. The presence of indicators of bacteria in seawater is dangerous to human health due to the potential presence of pathogens. Therefore, it is essential to routinely check the microbial contaminants threating seawater safety and to ensure compliance with quality standards and public health [16]. The correlation analyses revealed that temperature as one of the most important physical characteristics of seawater had a negative significant effect on the *fecal coliform* and *fecal streptococci* population. A similar result was found in four seasons of a year, so far the lowest occurrence of indicator bacteria were observed in the summer, presumably due to high temperature and solar radiation. Moreover, a higher level of *total coliform*, *Fecal coliform*, *Escherichia coli*, and *fecal streptococci* were observed in autumn, it may be attributed to intense rainfall that occurs in this period. Previous studies have also shown that intense rainfall highly influenced the occurrence of fecal indicator bacteria [17-18]. The results of the study showed that there was a significant association between autumn and spring seasons with summer ($p < 0.001$). However, the statistical analysis showed no significant difference between summer and winter. In addition, the average value of *fecal coliform* in autumn was significantly higher than in the winter, spring, and summer. Unlike to coliform bacteria, the highest and lowest *Clostridium perfringens* levels were detected in the summer, probably due to the greater resistance of the spores to environmental stresses such as osmotic, sunlight, and temperature. Based on the results, the bacteriological examination based on culturing *Clostridium perfringens* can be effective for assessing the public health safety of recreational water, especially in summer when the highest number of people are present for recreational activities. Skanavis and Yankoà reported that classic indicator bacteria had a little value in predicting the presence of pathogens and *Clostridium perfringens* are better indicators, due to their higher resistance to sunlight and longer survival under stressed environment condition [19].

Another important factor, which affected the occurrence of bacteria, was electrical conductivity. Correlation analyses showed that there was a significant positive association between electrical conductivity value and the number of *Total coliforms*, *Fecal coliform*, *Escherichia coli* and *Fecal streptococci* of samples ($p < 0.001$), while this significant association was negative between electrical conductivity and *Clostridium perfringens* level ($p < 0.001$). The data showed that with increasing electrical conductivity, a decrease was observed in the value of indicator bacteria in seawater. This may be due to the fact that specific characteristics of seawater, such as osmotic pressure and the toxicity of *inorganic salts* have a negative effect on the indicator bacteria population. Similarly, the result of Karbasdehi et al. study also showed that there was a negative association between the average number of *fecal bacteria* and *inorganic salt* [20].

The results of this study showed that chloride concentration was in a high degree of variability ranged from 2836.45 to 11533.95 mg/l. Statistical analysis showed that there was a significant association between Chloride level and an average number of *total coliform* and *Fecal streptococci*, but it had no effect on the growth of *Fecal coliform* and *Clostridium perfringens*. Shehane et al. reported that a negative correlation was found between salinity and F. Coliform and enterococci, while this effect was not significant for *Clostridium perfringens* [21-22].

In respect to pH as a key variable of the aquatic environment, the findings showed that the average values of pH were in the range between 7.35 and 9.93. The locations with the largest pH were found in the stations 5, 14, 4 and 3, respectively. The present study revealed that there was a reverse correlation between pH and the level of *total coliform* and *Clostridium perfringens.* Seawater with a pH range of 7.5-8.5 influenced the E. coli mortality, while in alkaline environments; *fecal*

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coliform decreased about 30% with the increase in one unit of pH [23].

Turbidity as a significant physical parameter does not have a health-based recommendation for seawater, but turbidity higher than 5 NTU may promote water contamination [15]. The findings revealed that in 90.8%, 32.3%, 6.1%, 30.8% and 75.4% of samples were positive for *total coliform*, *Fecal coliform*, *Escherichia coli*, *F. streptococci*, and *Clostridium perfringens*, respectively, turbidity was above than 5 NTU. However, the finding showed there was no statistically significant association between turbidity and *Fecal coliform* and *fecal streptococci* level, but *Clostridium perfringens* and *total coliform* considerably affected by turbidity (Table 2).

In order to determine the sources of fecal contamination, *fecal coliform* to *fecal streptococci* ratio was used as an index to distinguish human fecal pollution from animal fecal pollution [15] in each station (data do not show). According to the findings of this study, only stations 11 and 13 had a *fecal coliform* to *fecal streptococci* ratio greater than 4, highlighting that human waste was the main source contamination. The result is in accordance with other studies that reported anthropogenic activities and land used had a high effect on the microbiological contamination of coastal environmental [20,24]. While, *Fecal coliform* to the *fecal streptococci* ratio was ≤ 0.7 in other stations, indicating that non-human, animals and agricultural runoff were the dominant sources of pollution. However, the risk presented by fecal contamination from nonhuman sources was potentially less than human sources, but some documents indicate that some nonhuman fecal sources may pose risks, compared to those risks from human sources [19, 25].

Conclusions

The microbiological results revealed that there was a remarkable level of contamination in some areas; consequently, this area could be unsafe for recreational activities. The results suggested that *Clostridium perfringens* provide better health risk prediction than other analyzed indicator bacteria, particularly in the warm season.

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Conflict of interest

The authors have declared no conflict of interest for the publication of this article.

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