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Evaluation of Microbial Status of Coastal Recreational Waters in the Caspian Sea – A Case Study Mazandaran Province

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ABSTRACT

Background: Mazandaran province, with the largest coastline of the Caspian Sea in the country, draws a large number of travelers and tourists every year. This study aimed to determine and interpret the microbial health quality of its coastal waters.
Methods: 19 swimming points in Sari, Miandorud, Juybar, and Babolsar counties were weekly sampled for six months. Microbial testing for collected samples was performed using the MPN method and membrane filter based on EPA method 1600. The data were analyzed using SPSS software. Descriptive, normality, one-way analysis of variance (ANOVA) and LSD tests were also conducted.
Results: The mean of enterococcus in all the stations was standard; however, fecal coliform in P4, P1, and P0 of Babolsar was high. Normality tests of geometric mean indices, normal distribution, and results of one-way analysis of variance between stations and sampling time showed a significant difference at the level of $P < 0.05$. The highest contamination was observed at P4, P1, P0, Edalat 4, and Farahabad.
Conclusion: Due to the presence of microbial contamination in some areas, determining fecal coliform indicator in addition to enterococci is necessary to accurately estimate the status of microbial contamination of coastal waters in the region.

1. Introduction

Two-thirds of the Earth is covered by water and about 99.7% of its water consists of oceans and seas. Marine ecosystems are important resources in terms of great biodiversity and various activities such as recreational and economic activities including tourism, fishing, aquaculture, shipping, and energy sources (e.g., oil and gas), etc.

Anthropogenic activities greatly affect marine environments, especially coastal areas [1]. Discharge of industrial wastewater, municipal runoff, and agricultural wastewater with the entrance of organic and mineral particles, heavy metals, and other pollutants into the sea causes damage to water quality and disrupt ecosystem function. Awareness of the growing threats and pressures on marine ecosystems has had a significant effect on developing adequate marine environmental protection [2].

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The Caspian Sea is the largest lake (an area of 436,000 km^2) in the world, and Mazandaran province has the largest coastline (more than 338 km^2) among the northern provinces of the country. Every year, a large number of tourists travel to Mazandaran province and use the beautiful beaches of the Caspian Sea and recreational waters. During summer, municipalities designate places for people to swim which serve as sanitation projects for users [3]. The health of coastal waters, especially their microbial safety is critical for swimmers and users that affects tourism and economic activities in the region.

Coastal waters are prone to pollution and can act as a means of transmitting microbial diseases [4]. Anthropogenic activities, land uses, and sources of fecal contamination are the main causes of the presence of pathogens in recreational waters. Pathogens are affected by point and non-point sources such as runoff, animal feces, and swimmers' sheds [5].

Pathogenic microbes associated with fecal contamination can pose serious risks to humans including diarrhea, vomiting, and deadly infections [6]. Tracing pathogens is very difficult and requires a lot of time and money [7]. Nowadays, fecal indicators are easy to measure, inexpensive, practical, conventional, and do not require complex equipment [8].

Significant organisms in the human and animal gastrointestinal tract are used to assess the microbial safety of drinking, recreational, and aquaculture waters. The EPA recommended fecal coliform as an indicator of fresh recreational waters and enterococci as an indicator for freshwater and saline water [9].

A microbial quality indicator of water is usually a species or a group of microorganisms that can enter the water from fecal matter which their measurement is easier compared to pathogenic microorganisms endangering human health [10]. A well-known and useful indicator should have the following characteristics: present in large quantities in human or warm-blooded animals, easily identified by simple methods and it should not grow in water, natural environments, and contaminated water, the degree to which it is eliminated by water treatment should be comparable to that of pathogenic bacteria [11]. Today, fecal indicator bacteria are used to indicate the presence of fecal contamination in the water body and the presence of intestinal pathogenic bacteria. Total coliforms, fecal coliforms, and enterococci are currently used to assess water quality pollution due to their simple and inexpensive identification compared to other pathogens [12]. These fecal indicators used in different places can change. Globally, the standards of these indicators have been announced by the World Health Organization (WHO) and the Environmental Protection Agency (EPA). The relevant standards are communicated by the relevant officials based on the existing environmental conditions in different countries. Therefore, studying the quality of water based on its use such as drinking, recreation, agriculture, aquaculture, etc. is crucial. This study, aimed to investigate the microbial quality of recreational waters of the Caspian Sea coast in Sari, Miandorud, Juybar, and Babolsar counties with the correct use of microbial indicators to determine the health of these waters for users and swimmers.

2. Materials and Methods

In this study, the coastal waters in Sari, Miandorud, Juybar, and Babolsar counties were weekly sampled in May, June, July, August, September, December, January, and February in 2019 (Figure 1).

Sampling was done in 1-liter sterile bottles, approximately 30 cm below the water surface. The samples were transferred to the laboratory in an icebox at 4°C within 8 h. The tests were performed by the MPN method and membrane filter according to recommended Standard Methods (9222D and EPA 1600) [13, 14]. To determine enterococci by the membrane method, 100 mL of the sample was passed through a 0.45 μm membrane filter. The samples were then concentrated, cultured in Streptococcus agar KF culture medium and incubated at a temperature of 37±1 for 24-48 h. Confirmation tests were performed if red, pink, or distinguished colonies were observed. The esculin test and culture in Bile Aesculin Azide Agar were performed and placed in a greenhouse with a temperature of 44 ± 0.5 for 24-48 h. The formation of brown to black colonies with the spread of these two colors in the medium of streptococci was esculin-positive. The catalase test was then performed by dissolving a colony from the previous medium in a drop of hydrogen peroxide. Intestinal streptococci are catalase negative. M-Endo culture medium was used to determine total coliformsand M-Fc culture medium was used to determine coliform feces. Alongside the sampling, environmental parameters including temperature, salinity, dissolved oxygen, and pH were also measured with the portable multi-parameter water quality measurement Hack model 156.

Figure 1: Sampling stations in the coastal waters of the Caspian Sea Mazandaran province

The results were compared with international standards, national standards, the Ministry of Health, and the standards of several other countries, and the microbial quality of the sampling sites was determined. Statistical analyzes were performed using SPSS software (version 19). Descriptive statistics including mean, SD, and geometric mean were obtained. The samples were distributed by Kolmogorov-Smirnov, Shapiro–Wilk test, and Skewness normality tests. After determining the normality of the data, parametric statistical methods were used. Further, one-way ANOVA and LSD tests were used to compare the sampling locations and times.

3. Results and Discussion

The means and geometric means of microbial indices including Total coliform, Fecal coliform, and enterococci in the study area were obtained to be (346.98 + 23.8000, 247.07), (124.22 + 4.94802, 85.29), and (29.26 + 1.20742, 23.24) CFU/100mL, respectively. The minimum fecal coliform and enterococci were 3, and their maximum values were 1100 CFU/100 mL. Normality coefficients, Kurtosis, and Skewness of Total coliform, fecal coliform, and enterococci were obtained to be (8.274, 2.462), (4.277, 1.867), and (1.774, 23.24), respectively.

Environmental parameters such as pH, temperature, salinity, and dissolved oxygen of the solution were measured. The mean value and range of environmental parameters were obtained to be as follows: pH (8.2150± 0.01, 7.63-8.81), salinity (10.27 ± 0.07, 7.51-12.91ppt) (part per thousand), dissolved oxygen (8.3179±0.03, 7.12- 9.77 mg/L), water temperature (26.1348±0.15, 20.20- 29.80 °C), and air temperature (28.9300±0.15, 23.11-33.00 °C). 7.63-8.81), salinity (10.27 ± 0.07, 7.51-12.91ppt) (part per thousand), dissolved oxygen (8.3179 ± 0.03, 7.12- 9.77 mg/L), water temperature (26.1348 ± 0.15, 20.20- 29.80 °C), and air temperature (28.9300 ± 0.15, 23.11-33.00 °C).

The normality test for the distribution of samples of all three indices was performed by Shapiro-Wilk and Kolmogorov-Smirnov tests which showed that the geometric mean of the three microbial indices has a normal distribution at the level of $P < 0.05$.

The amount of total coliform was in the standard level and most of it belonged to P1 (953 MPN/100 mL) and P0 (823 CFU/100mL). In different months of sampling, the range of mean changes of total coliform was from 208 MPN/100ml in July to 474 MPN/100ml in January (Figure 2).

Due to the normal distribution of microbial indicators, One-Way Variance Analysis was performed which showed a significant difference between microbial indices in sampling stations at the level of $P < 0.05$ (Table 1).

The mean and geometric means obtained for fecal coliform and enterococci indicate the difference between these two means in the measured microbial indicator in sampling sites which were shown in Figures 3.

The mean and geometric mean of the fecal coliform microbial index of P0, P1, and P4 were higher than the standard which indicates more pollution in the swimming points of Babolsar city compared to Miandorud, Juybar, and Babolsar.

The obtained geometric mean of enterococci microbial indicator for sampling sites was within the standard. Moreover, enterococci mean was higher than the geometric mean and the standard in Edalat 4 and P4 sampling sites (Figure 3).

Test for microbial contamination index showed significant differences between sampling sites (Table 2).

The mean microbial indicator of fecal coliform was higher than the standard in June, July, August, and January, while its geometric mean exhibited higher values compared to the standard in June and August. Moreover, the means of enterococcus in August and June with 50.23 and 43.17 CFU/100 ml and in August, with a geometric average of 40.14 CFU / 100ml was above the standard (Figure3).

One-way variance analysis showed the difference between the indices in different months of sampling at the level of $P < 0.05$ (Table 2).

LSD test between microbial indices showed significant differences between May and August (0.065), May and December (0.015), May and January (0.002), June and January (0.046), July and August (0.051), July and December (0.12), July and January (0.002), August and May (0.065), August and July (0.051), September and December (0.038), and September and January (0.006).

According to Iranian standards, the sampling sites of Negin, Toska, Edalat 1 and 2, Larim, Chapkroud, Miroud, parking lots No. 2, 3, 5, 6, 7, 8, and 9 in Babolsar did not show microbial contamination. Parking stations No. 4, 1, and zero Babolsar, Edalat 4, and Farahabad had the highest pollution among the sampling stations, respectively.

The correlation coefficient of total coliform and enterococci index (r = 0.318, Sig = 0.000), total coliform and fecal coliform (r = 0.521, Sig = 0.000), and fecal coliform and enterococci (r = 0.598, Sig = 0.000) had a positive correlation between the indicators at a significance level of $P < 0.01$.

Indicators showed a negative correlation with environmental parameters of temperature, salinity, dissolved oxygen, and pH at a significance level of $P < 0.01$.

Seawater pollution is recognized as a global problem, and it should be continuously monitored and compared with national and international standards (that vary from country to country) to report the status of pollution for determining the general health and quality of coastal water [15, 16]. Microbial indices are used for microbial monitoring of recreational waters. Enterococci and Escherichia coli (*E.coli*) are considered as indicator organisms determining the microbial quality of seawater [17]. In 2012, the WHO recommended *E. coli* and Enterococcus for freshwater and Enterococcus for saline water (18). These two indicators act differently in different environments [19].

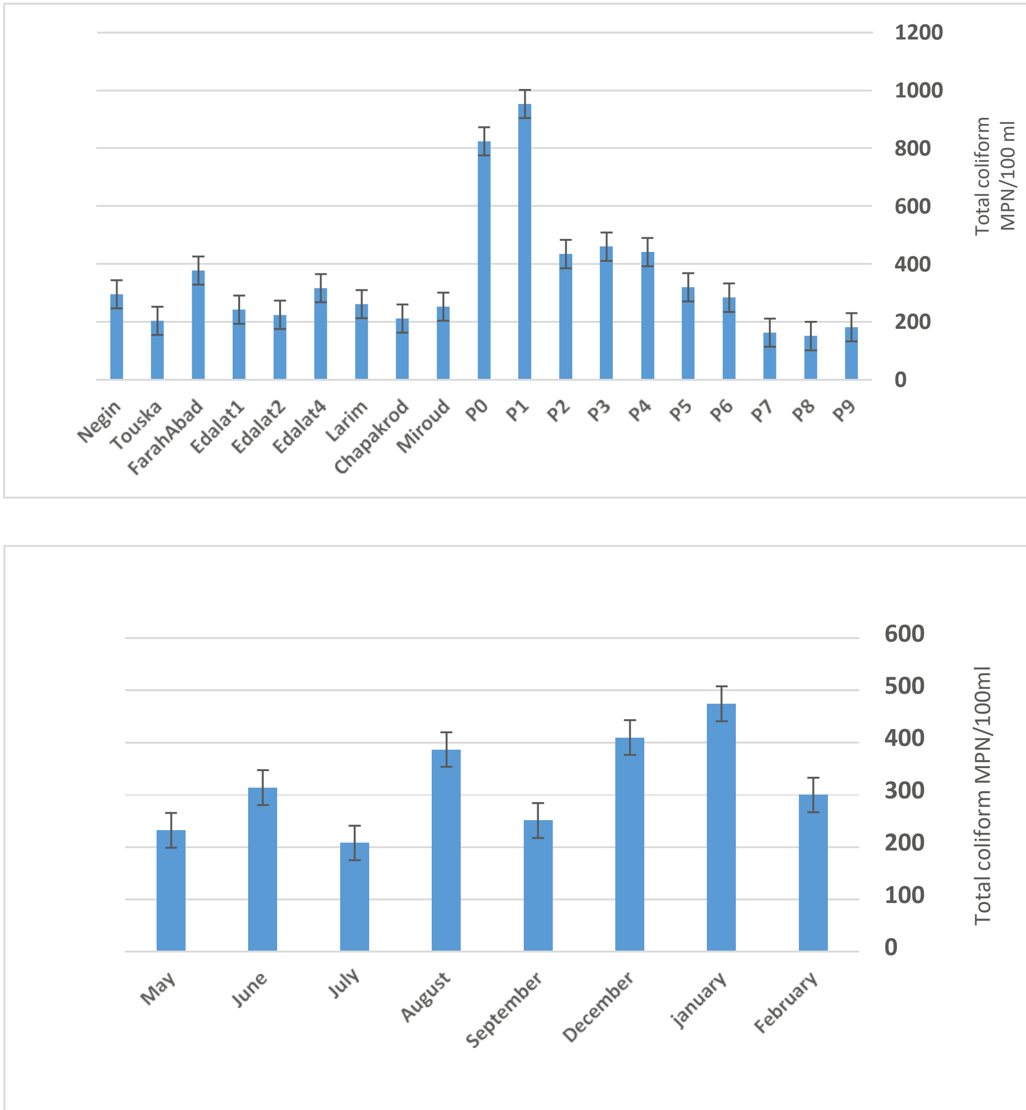


Figure 2: Change in the mean total coliform (Total coliform) at sampling sites and months

In many parts of the world, coastal recreational waters are monitored; some of the studies conducted for monitoring coastal waters are listed as below: Southern California waters by Cordero *et al.* (2012) , Doreen *et al.* (2005), Hawaii waters by Fujioka *et al.* (2015), Lake Erie by King *et al.* (2016), etc. [20-23]. In this study, the Caspian Sea recreational coastal waters in Sari, Miandorud, Juybar, and Babolsar counties were monitored, and microbial quality was studied using microbial indicators, and its compliance with standards was assessed. The Caspian Sea has less salinity than the waters of the oceans and seas and is considered brackish water. In this study, both microbial indices of fecal coliform (*E. coli)* and enterococci were sampled and measured.

Due to the exponential bacteria growth, in the global health guidelines from which the Iranian standard has been adopted, the standards for Geometric Mean (GM) and Single Sample are stated separately and are different from each other with specific standards; the Single Sample standard is higher than the geometric mean and indicates the microbial quality of the water at the time of sampling [18]. However, in studies conducted in our country, it was seen that the mean of microbial indices was compared with the standard, which causes errors in announcing the status of contamination. In the present study, compared to previous studies and according to world guidelines, the geometric mean of the microbial index was calculated and measured with the geometric mean mentioned in the standard [4, 9, 11, 13].

Table 1: Results of one-way variance analysis of microbial indicators in sampling stations

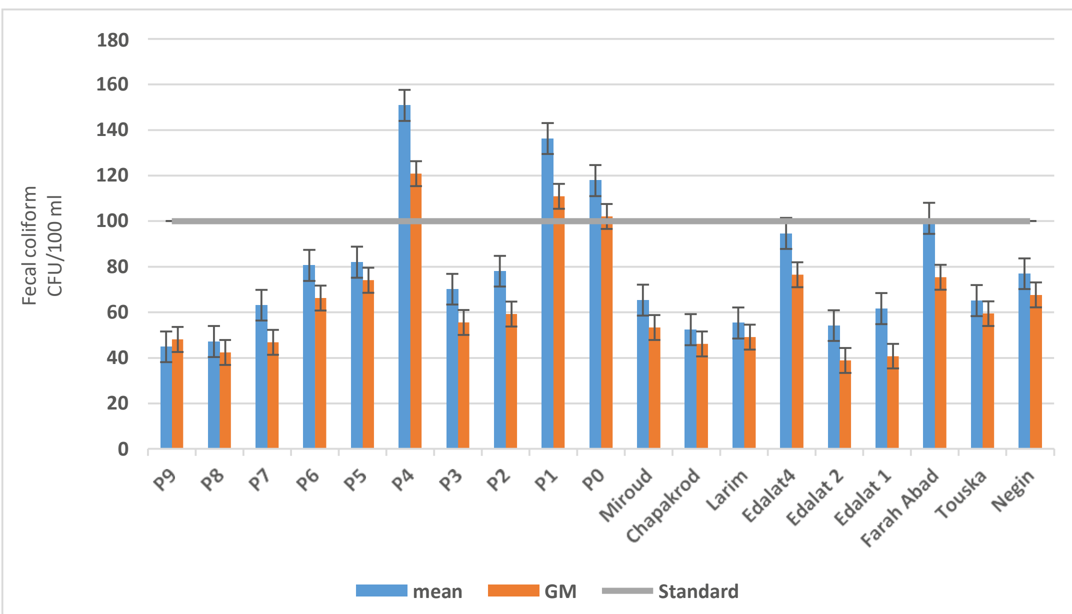
		Sum of Squares	df	Mean Square	F	Sig.
FC	Between Groups	126392.329	18	7021.796	2.144	.007
	Within Groups	435539.750	133	3274.735		
	Total	561932.079	151			
TC	Between Groups	6500229.186	18	361123.844	7.388	.000
	Within Groups	6500696.649	133	48877.418		
	Total	1.300E7	151			
Enterococci	Between Groups	9000.513	18	500.029	2.719	.001
	Within Groups	24460.375	133	183.913		
	Total	33460.888	151			

Also, in the recent instructions, the Single Sample standard should be measured for instantaneous investigation of water pollution during swimming. According to the current standard of the country (mean enterococci for saline water microbial index ≤ 40), the geometric mean of enterococci microbial index was within the standard levels and did not show microbial contamination. Before the 2017 guidelines, fecal coliform was also measured as a microbial indicator which was also measured and compared to the standard in this study; for parking lots 4, 1, and zero, the obtained geometric mean was observed to be higher than the standard and indicated contamination. Therefore, this study also confirmed that monitoring the microbial contamination index is important to determine the microbial health of coastal recreational waters. Choosing the right indicator, calculating and matching it correctly with the standard, and correct awareness are important steps in managing these water resources and predicting their quality status [18].

Sampling sites located in Babolsar city showed more contamination. The presence of a large number of buildings, motels, hotels, residential, welfare and entertainment complexes of the coast, lack of sanitary disposal of sewage, easy access to the beach, high density of tourists in the recreation season, the release of sewage, and the proximity of the river estuary are effective factors in increasing contamination in the region, which confirms the study by Ho LC *et al.* (2011) [24].

In the case of sampling months, the highest contamination was observed in June, July, and August which is due to the increase in the number of travelers and tourists. In January, an increase in microbial pollution was observed due to increased rainfall and runoff and their entry into the sea; this is confirmed by Allen *et al.* (2003) [25].

In this study, microbial contamination showed a negative correlation with environmental parameters such as salinity, dissolved oxygen, temperature, and pH. Anderson (2005) found that increased salinity killed fecal bacteria and a combination of biological, chemical, and physical factors in saline water affected the presence of indicator bacteria [26].



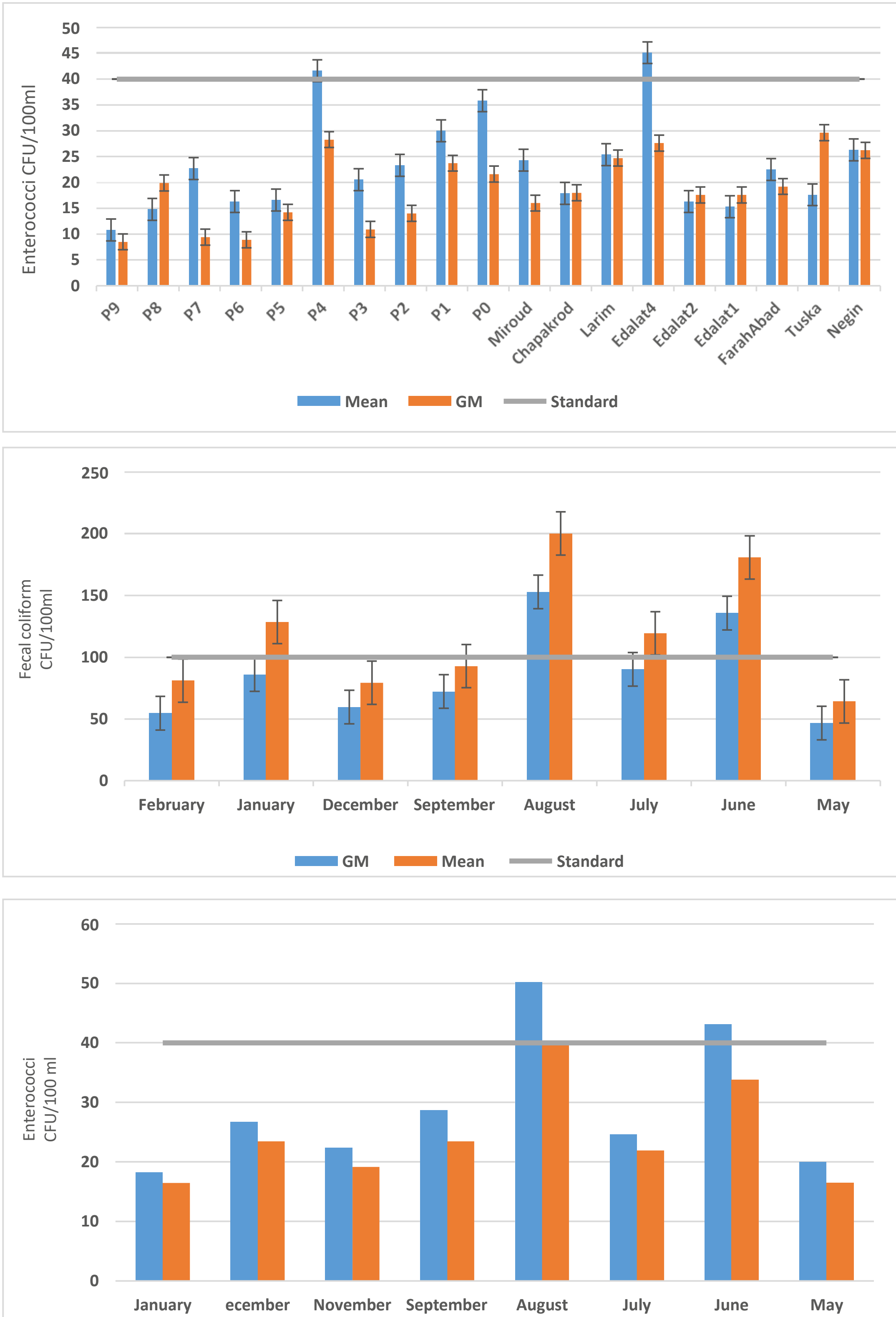


Figure 3: Mean and geometric mean changes of coliform fecal and enterococci microbial index at sampling sites and sampling months

Table 2: Results of one-way analysis variance of microbial indices in sampling months

		Sum of Squares	df	Mean Square	F	Sig.
Total coliform	Between Groups	1531151.166	7	218735.881	2.746	.010
	Within Groups	1.147E7	144	79670.345		
	Total	1.300E7	151			
Fecal coliform	Between Groups	234316.184	7	33473.741	14.713	.000
	Within Groups	327615.895	144	2275.110		
	Total	561932.079	151			
Enterococci	Between Groups	7997.730	7	1142.533	6.461	.000
	Within Groups	25463.158	144	176.827		
	Total	33460.888	151			

4. Conclusion

To announce the microbial status of coastal waters, comparing the geometric mean with the standard is correct due to the exponential growth of microbes. According to the environmental conditions of the region, in addition to counting enterococci as the only microbial index of seawater, the study of the fecal coliform microbial index in the coastal waters of the Caspian Sea is also essential. Also, to determine the daily microbial quality status, the Single Sample standard of the two mentioned indicators to determine and announce the microbial contamination status at the time of sampling is inevitable.

Authors' Contributions

Seyed Mohsen Kazemitabar: Writing-original draft; Writing-review and Editing. **Reza Amirnezhad:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review and Editing. **Aptin Rahnavard:** Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization. **Farid Gholamreza Fahimi:** Investigation; Methodology; Project administration; Resources; Software.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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