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Investigating Some Microbial Pollution Parameters of Seawater and Mussels (*Mytilus galloprovincialis*, Lamarck 1819) of Sinop Black Sea Coastal Zone, Turkey

(Pengkajian Parameter Pencemaran Sesetengah Mikrob Air Laut dan Kupang (*Mytilus galloprovincialis*, Lamarck 1819) di Zon Persisiran Pantai Laut Hitam Sinop, Turki)

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ABSTRACT

The presence of coliforms bacteria is one of the most prevalent problems in terms of public health in marine ecosystems over the world. In this study were investigated the physico-chemical properties of seawater and the numbers of total aerobic, total coliform, fecal coliform, E. coli *O157:H7 and fecal streptococci in seawater and mussel samples collected from Sinop environs between May and October 2011. The microbiological analysis of seawater samples showed that the difference between total coliform, fecal coliform and fecal streptococci numbers (*p*<0.05) was significant for each station. However, the difference among total aerobic bacteria numbers for each stations (*p*>0.05) were not found significant. The difference between whole counting results for mussel samples taken from different sampling sites was not significant (*p*>0.05), too. Furthermore, the results of the screening assay for the presence of* E. coli *O157:H7 showed that the strain was not detected in neither seawater nor mussel samples. In conclusion, it was determined that fecal coliform and fecal streptococci counts in the seawater and mussel samples were higher than legal (Turkish Bathing Water and Quality of Fishery Products Regulation) limit values for some stations in Sinop coastal areas.*

Keywords: E. coli *and* E. coli *O157:H7; fecal indicator bacteria; mussels; seawater*

ABSTRAK

Kehadiran bakteria koliform adalah salah satu masalah yang ketara daripada segi kesihatan awam dalam ekosistem marin dunia. Dalam kajian ini, kami mengkaji sifat fiziko-kimia air laut dan jumlah aerobik, jumlah koliform, koliform tahi, E. coli *O157:H7 dan streptokokus tahi dalam sampel air laut dan kupang yang dikumpul dari kawasan Sinop antara Mei dan Oktober 2011. Analisis mikrob ke atas sampel air laut menunjukkan bahawa perbezaan antara jumlah koliform, koliform tahi dan streptokokus tahi (*p*<0.05) adalah bererti bagi setiap stesen. Walau bagaimanapun, perbezaan antara jumlah bakteria aerobik bagi setiap stesen (*p*>0.05) didapati tidak bererti. Perbezaan antara keputusan pengiraan keseluruhan sampel kupang yang diambil dari kawasan persampelan berbeza juga tidak signifikan (*p*>0.05). Selain itu, keputusan saringan asai untuk kehadiran* E. coli *O157:H7 menunjukkan bahawa tekanan tidak dapat dikesan dalam sampel air laut mahupun kupang. Kesimpulannya, dapat ditentukan bahawa kiraan koliform tahi dan streptokokus tahi dalam sampel air laut dan kupang adalah lebih tinggi daripada had nilai yang dibenarkan (Peraturan Air Mandian serta Kualiti Produk Perikanan Turki) bagi sesetengah stesen di kawasan persisiran pantai Sinop.*

Kata kunci: Air laut; E. coli *dan* E. coli *O157:H7; kupang; petunjuk bakteria tahi*

INTRODUCTION

The presence coliforms bacteria are used as indicator of microbial pollution in saline aquatic reservoirs and the quality of shellfish harvested from the habitats. The members of the group are Gram-negative, rod-shaped and lactose fermenting microorganisms and they live in soil, freely surface water or attached to animate and inanimate surfaces. Several studies supposed that the presence coliforms in the marine environments are depend on various ecological parameters such as temperature, salinity, pH, dissolved oxygen and microbial flora (Cavallo & Stabili 2002; Kacar 2011). Global estimates demonstrated that each year more than 175 million of infection cases have been reported due to swimming, bathing in fecal contaminated waters and ingestion of contaminated seafood harvesting in the waters (Roslev & Bukh 2011). Generally, total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) are mostly prevalent indicator microorganisms using in water quality studies (Hamzah et al. 2011). Fecal coliforms include more than 60% of total coliforms and more than 90% of the fecal coliforms are members of the genus *Escherichia*. *Escherichia coli* is the most important coliform bacterium. Several types of this species are opportunistic pathogens and capable of causing disease. Furthermore, fecal streptococci group are capable of growth in high temperature (45°C), high pH (pH9.6) and

6.5% NaCl. Therefore, the members of this group have been proposed as useful to demonstrate the presence of viruses in the marine environment and bio-solids (Bitton 2005) and live longer than coliforms in seawater. They are always present in the feces of human and warm-blooded animals and unable to multiply in sewage-contaminated waters (Dionisio & Borrego 1995).

Bivalve mollusks are immobilize and filter-feeding seawater organisms, so that they accumulate several potential pathogenic bacteria and viruses in the marine environment (Popovic et al. 2010; Stabili et al. 2004). These animals may also be contaminated during processing, marketing and moving. There are two important groups of fecal indicator bacteria and they may be transmitted by consumption of raw or inadequately cooked mussels. The first of those groups is members of the family *Vibrionaceae* living in the marine environment. The other group includes coliforms that are spilled into the water from infected humans and animals (Canesi et al. 2001). It is well known that bacterial contamination of mussels is a very common problem on almost through the coast of developing countries. In recent years, several valuable papers have been published concerning microbial contamination levels of mussels harvesting in different water reservoirs in many countries (Aydın & Soyutemiz 2002; Kacar 2011; Özcakmak & Arıcı 2001; Popovic et al. 2010; Stabili et al. 2004; Yılmaz et al. 2005).

Sinop is our main research area, heavily used for recreation activities, swimming, fishing and mussels harvesting during all seasons. The coastal areas of the region also include high average population density. Sewage is often discharged directly or indirectly into the sea from various points or not-points source along the coast. The aim of the study was to determine the levels of fecal indicator bacteria in mussels (*M. galloprovincialis*) and seawater samples harvesting Sinop environs (Black Sea Region/Turkey) between May and October 2011 and physico-chemical properties of seawater samples.

MATERIALS AND METHODS

SAMPLE COLLECTION

Seawater samples were taken from 10 sampling sites in coastal areas of Sinop environs for 6 months from May to October 2011, between the same dates, mussel samples were collected from 5 sampling sites harvesting mussels through the year (Figure 1). Samples were collected to be repeated twice every month. Seawater samples were taken 10^2 mL sterile bottles directly from the undersurface. Mussels were harvested by hand and scoop in coastal reefs. All samples protecting to approximately 4°C freezers were brought to the laboratory within 3 h. Mussel samples were washed in sterile distilled water to remove epiphytes on the shell.

BACTERIOLOGICAL ANALYSIS

In the study, water and mussels samples were analyzed for total bacteria, total coliforms, fecal coliforms and fecal streptococci. The mussel samples were aseptically opened with sterile lancet; samples were homogenized in a sterile blender and were diluted with sterile dilution water. Total bacteria count for the seawater and mussel samples were diluted at the rate of 10^{-5} with sterile saline water. Then, the diluted samples were incubated to Plate Count Agar (Merck) by using pour plate technique in an incubator at a temperature of 37°C for 48 h. The plates were then checked for bacteria colony growth. The counting results were recorded as CFU/100 g or mL. The most probable method (MPN) was used for the enumeration of total and fecal coliforms in our study (AOAC 1998). Total and fecal coliforms were counted by the multiple-tube fermentation technique (triple serial dilution method). The results recorded according to table of MPN as CFU/100 g or mL. Besides, KF Streptococcus Agar (Merck) medium was used to isolate fecal streptococci and it allowed the optimal growth of all fecal streptococci strains at 24 h. In

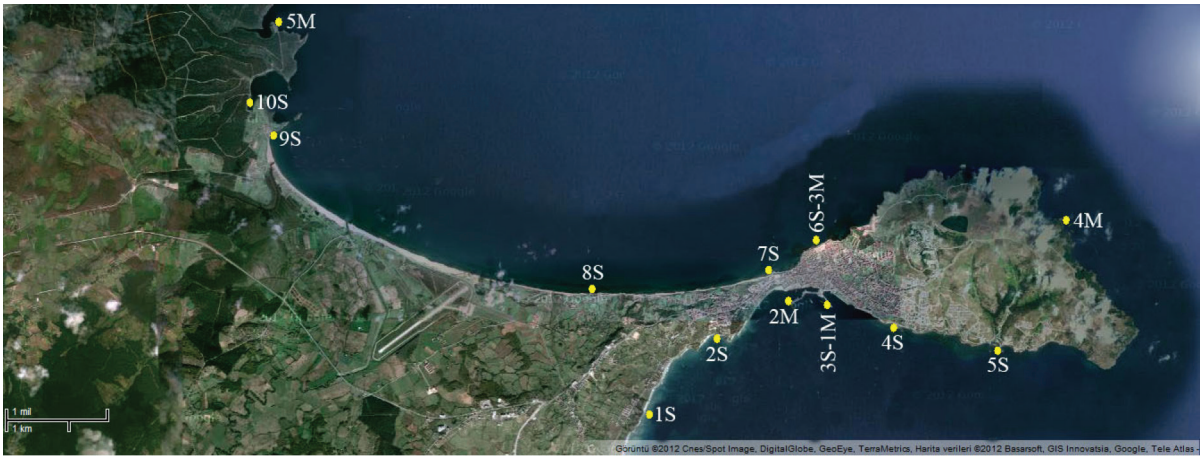

FIGURE 1. Map of sampling sites (S: Water; M: Mussel) 1S: DSI beach, 2S: Gelincik beach, 3S: Pier, 4S: Taşocağı, 5S: Karakum beach, 6S: The old market place, 7S: Outport, 8S: Bostancılı, 9S: Hotel beach, 10S: Akliman, 1M: Pier, 2M: Pier (boat stopping place), 3M: The old market place, 4M: Salesman, 5M: Hamsilos (Google Earth 2012)

addition, Gram stain and catalase test was applied to all colonies obtained in the medium. All of the experiments were repeated at least three times.

ISOLATION OF *E. COLI* AND SCREENING OF *E. COLI* O157:H7

Sample taken from Lactose Broth (Merck) positive tubes were inoculated to EC broth (Merck) and was incubated for 24 h at 44.5°C. After incubation period, gas positive tubes were selected as presumptive isolates of *E. coli*. Presumptive *E. coli* samples were grown again onto Eosine Methylene Blue (EMB) agar plates to observe for green metallic sheen production as confirmatory test. The characterization of a total of 41 strains of *E. coli* including 40 native isolates and 1 reference (*E. coli* ATTC 25922) strain were also carried out according to cultural, microscopic and biochemical tests. Biochemical tests were performed according to the methods described by Brenner and Farmer (2005) and Welch (2006). Fluorocult® LMX Broth Modified (Merck) broth were used for the identification of *E. coli* and coliforms from samples. The sample inoculated LMX tubes were incubated 37°C for 24 h and development of a blue-green color was evaluated as the presence of total coliforms in the tubes. Then, all tubes were exposed to a 366 nm long-wave UV light and fluorescence in the tube was assessed as the presence of *E. coli*. All tested isolates were also screened for the presence of *E. coli* O157:H7 strains, onto Fluorocult® E. coli O157:H7 Agar (Merck) as mentioned to the above method (Anonymous 2006a).

PHYSICO-CHEMICAL PARAMETERS

Temperature, pH, dissolved oxygen and conductivity values of the seawater samples were measured using by HACH portable HQD model multi-function measuring device at the time of sampling. All the tests were run in triplicate and expressed as the meant ± standard deviation.

STATISTICAL ANALYSIS

The differences between the results of microbiological counts of seawater and mussel samples taken from different stations was evaluated by one-way analysis of variance (ANOVA) using the PASW statistics 18 (IBM SPSS Inc.) program. The data that important in result analysis of variance was tested to level $p<0.005$ according to Post-Hoc Tukey HSD test. In addition, graphics were made using Microsoft Excel 2007.

RESULTS

In this study, the pH values were found to range from 7.68 to 9.74 for sea water. The pH values exceed the determined limits value (7.5 to 8.5) at all stations in October. Furthermore, the seawater temperature was determined to be the seasonal normal standards. The conductivity values usually increase from May to October. Dissolved oxygen (DO) values of station 6 (3.09 to 6.32) were lower than the other stations (4.64 to 17.35). The data of physico-chemical parameters of sampling stations are given in Table 1.

All bacterial counting values of seawater and mussel samples are shown in Figures 2 and 3. According to the results of microbiological enumeration, total aerobic bacteria counts gathering water samples were the highest at Station 6 with 1.07×10^8 CFU/100 mL value in August, while the lowest numbers were with 7.0×10^4 CFU/100 mL value at Station 8 in September. According to the statement of MPN, the highest numeric values of total coliforms bacteria in seawater samples at some stations were determined as >1100 CFU/100 mL, while the minimum values were found at Station 1 in June. It was shown that fecal coliform counts of the harvesting water samples were the highest at the Station 7 (1100 CFU/100 mL) in July, while the highest (except for October) fecal streptococci counts were found at the Station 6 with 1.68×10^6 CFU/100 mL value. Our findings showed that there was an inverse relationship between numbers of FC and FS, except for Station 6 (Figure 2).

In addition, the results of total aerobic bacteria counts in mussel samples showed that the highest values were shown at Station 5 with 8.4×10^7 CFU/100 g value in August, but the lowest values were at Station 4 (2.0×10^4 CFU/100 g) in June. According to total coliform bacteria counts of the harvesting mussel samples, the highest numerical values were found at Stations 2, 3, 4 and 5 with >1100 CFU/100 g value in September and at Stations 2, 3 and 4 in August, whereas the lowest values were shown at the Station 1 with 4 CFU/100 g value in September. The highest value for fecal coliform was found >1100 CFU/100 g according to MPN. Furthermore, the highest values numbers of fecal streptococci for mussel samples were determined at Station 4 (4.0×10^4 CFU/100 g) in August and at Station 3 (3.0×10^4 CFU/100 g) in August (Figure 3).

These results showed that the numbers of FC and FS for the seawater and mussel samples were over of recommended values for some stations. Furthermore, the microbiological analyses of seawater samples obtained from different stations showed that the difference between TC, FC and FS numbers ($p<0.05$) was significant for all stations, but the difference was not significant for total aerobe bacteria numbers ($p>0.05$), this situation was confirmed using Post-Hoc Tukey HSD test.

In addition, the difference among counting results of mussel samples harvesting different stations was not significant ($p>0.05$).

During the sampling period a total of 40 *E. coli* strains were isolated on EMB agar; particularly 85% of them were isolated from the seawater and 15% from mussels. All isolates presumptive *E. coli* were confirmed according to morphological and biochemical characteristics.

The strains were also utilized to X-Gal substance and emitted fluorescence under UV lamp onto Fluorocult® Modified LMX Broth. On the other hand, the results of the screening tests onto Fluorocult® *E. coli* O157:H7 Agar showed that this strain wasn't found among all tested strains; however, six strains were given false positive.

TABLE 1. Physico-chemical properties of sampling stations

	May				June				July			
	pH	T	DO	Cn	pH	T	DO	Cn	pH	T	DO	Cn
Station 1	8.34±0.21	14.7±0.25	8.74±0.12	27.4±0.3	8.91±0.74	22.6±0.26	8.05±0.1	27.4±0.2	8.43±0.14	26±0.2	7.33±0.11	28.3±0.14
Station 2	8.59±0.52	12.4±0.26	12.47±0.08	27. 5±0.2	8.09±0.08	20.9±0.18	9.61±0.3	26.9±0.15	8.24±0.11	24.9±0.17	8.91±0.02	28.2±0.07
Station 3	8.43±0.16	10.2±0.11	10.06±0.03	27±0.2	8.95±0.6	20.6±0.11	8.51±0.22	27.2±0.3	8.65±0.07	25.4±0.15	8.89±0.02	28.2±0.05
Station 4	8.76±0.10	12.8±0.12	14.41±0.02	27.1±0.2	8.88±0.0	21.5±0.72	10.64±0.03	26.9±0.2	9.05±0.08	27.3±0.3	17.35±0.05	27.7±0.11
Station 5	8.61±0.09	12.3±0.20	12.9±00	27.1±0.3	8.95±0.62	23.1±0.5	8.49±0.11	27.1±0.07	9.18±0.03	27±0.21	10.51±0.13	28.2±0.03
Station 6	8.48±0.00	13.8±0.16	10.06±0.03	26.6±0.2	9.3±0.09	21±0.12	8.09±0.03	26.4±0.2	9.6±0.11	31.1±0.26	11.32±0.11	28.4±0.12
Station 7	8.48±0.00	13±0.05	9.4±0.09	25±0.15	9.11±0.03	22.8±0.11	6.99±0.22	27±0.2	8.61±0.09	26.8±0.2	6.93±0.3	28.1±0.06
Station 8	8.49±0.11	13.3±0.26	9.72±0.00	26.3±0.2	9.3±0.08	22.3±0.11	8.01±0.14	27.6±0.05	8.86±0.02	26.4±0.2	7.63±0.12	28.5±0.07
Station 9	8.51±0.11	13.6±0.25	9.59±0.05	27.1±0.2	9.2±0.03	22±0.11	11.42±0.09	27.8±0.16	9.07±0.06	28.9±0.6	10.56±0.11	28.7±0.03
Station 10	8.49±0.10	13.1±0.05	10.57±0.02	27.4±0.26	9.19±0.11	22.4±0.1	8.73±0.25	27.6±0.32	8.93±0.07	28±0.2	8.77±0.24	28.6±0.02

	August				September				October			
	pH	T	DO	Cn	pH	T	DO	Cn	pH	T	DO	Cn
Station 1	8.43±0.13	27.5±0.24	6.99±0.26	28.9±0.5	8.46±0.16	26.2±0.21	6.99±0.21	28.6±0.04	9.62±0.02	18.2±0.18	8.36±0.00	29.6±0.12
Station 2	7.68±0.11	28.8±0.3	7.3±0.12	28.3±0.3	8.53±0.72	25.6±0.16	8.16±0.11	29.1±0.08	9.71±0.01	18.2±0.14	8.11±0.05	29.5±0.1
Station 3	8.29±0.17	27.8±0.2	7.05±0.06	28.8±0.12	8.56±0.11	25.4±0.11	7.12±0.05	28±0.05	9.68±0.00	18.8±0.11	8.24±0.05	29.7±0.21
Station 4	8.28±0.11	28.3±0.2	7.58±0.1	29.1±0.14	8.64±0.12	26±0.14	11.08±0.08	27.7±0.1	9.74±0.03	18.1±0.2	8.99±0.03	26.4±0.5
Station 5	8.49±0.2	28.2±0.00	8.46±0.11	28.6±0.2	8.39±0.2	26.3±0.2	10.48±0.06	28.3±0.12	9.64±0.09	18.5±0.23	9.2±0.1	29.4±0.11
Station 6	9.73±0.3	30.7±0.21	21.44±0.3	27.7±0.2	9.76±0.0	29.3±0.21	21.71±0.1	28.5±0.16	9.66±0.05	18.7±0.21	8.01±0.06	29.5±0.03
Station 7	8.73±0.16	28.6±0.18	7.09±0.02	28.1±0.21	8.71±0.09	26.1±0.15	7.34±0.15	26.4±0.06	9.69±0.00	17±0.24	4.64±0.04	29±0.8
Station 8	8.91±0.1	29.8±0.22	9.21±0.09	29±0.1	8.63±0.11	23.2±0.13	7.91±0.2	28.6±0.14	9.03±0.21	17.1±0.16	9.34±0.06	29.8±0.04
Station 9	9.26±0.05	29.4±0.18	8.26±0.05	28.4±0.2	9.23±0.08	25.4±0.11	11.03±0.07	29.2±0.11	9.08±0.24	17.5±0.2	8.95±0.00	29.9±0.01
Station 10	9.18±0.11	28.7±0.2	8.27±0.02	29.1±0.23	9.01±0.10	24.4±0.09	8.15±0.13	28.8±0.00	9.09±0.06	17.4±0.2	8.96±0.02	29.8±0.01

DO: Dissolved oxygen (mg/l); Cn: Conductivit; T: Temperature (C°)

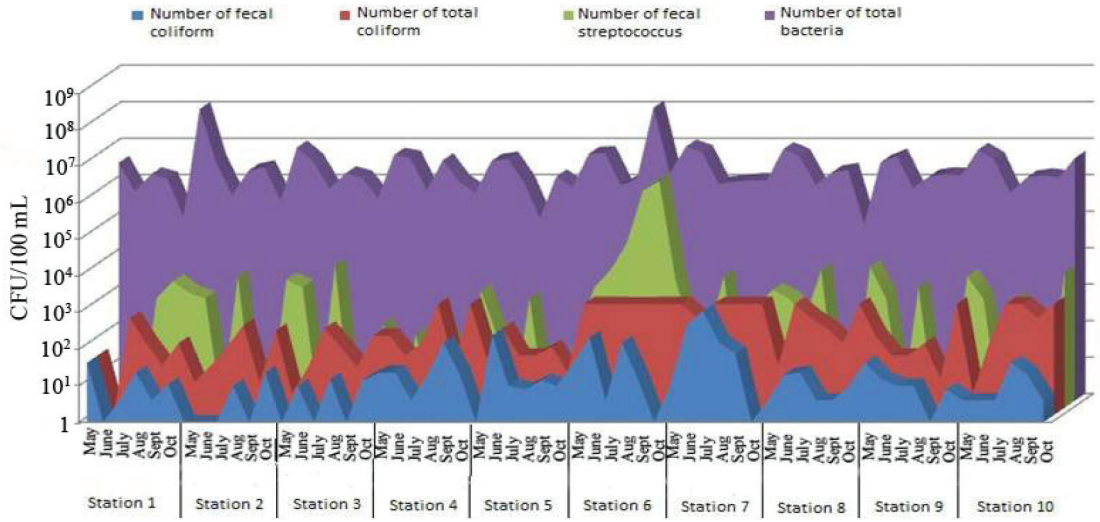

FIGURE 2. The chart of all bacterial counting results in seawater samples

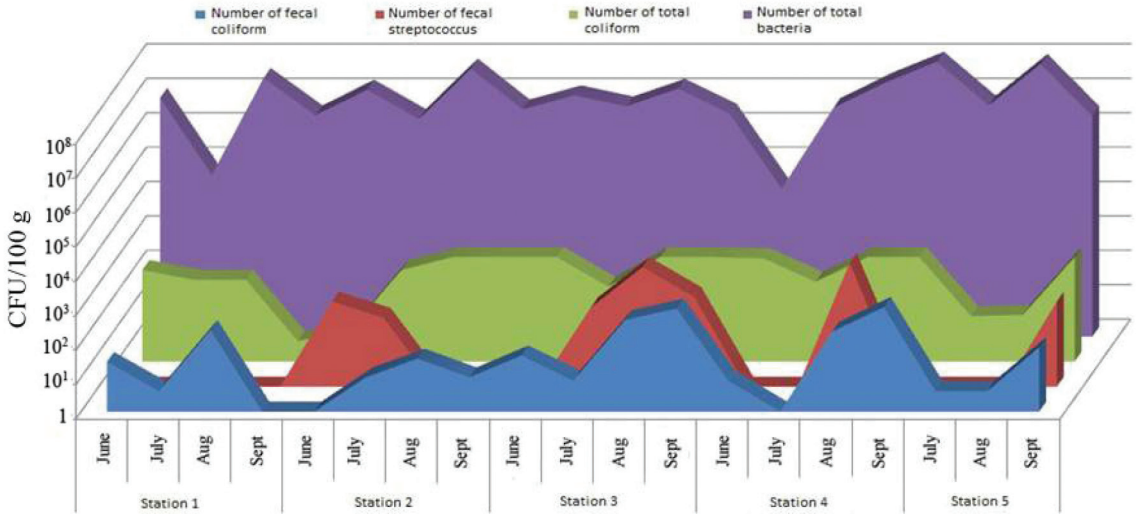

FIGURE 3. The chart of all bacterial counting results in mussel samples

DISCUSSION

Microorganisms in water and seafood including several pathogenic bacteria cause serious diseases threatening human health, such as diarrhea, dysentery, typhoid and cholera. The pathogens contaminate the coastal waters and other aquatic habitats as a result of human and animal activities (Robin et al. 2012). In this study, we investigate the aspect fecal indicator bacteria of seawater and mussel samples harvesting Sinop environs intensively using for recreational and other human activities, as well as physico-chemical properties of seawater samples between May and October 2011.

Hernandez-Delgado and Toranzos (1995) reported that the optimum pH value was 6 to 7 for growth of coliforms. Hamzah et al. (2011) also determined that the optimum pH value was between pH 7.57 to 7.79 for growth of coliform bacteria in a conducted study on the coastal water. Based on our findings, optimum pH value was observed as 7.68 to 9.74 and it was found suitable for coliforms growth. Values of pH obtained in this study were similar to previous studies by Aydın et al. (2010) and Perez et al. (2008). In addition, Mayo (1995) notified that dissolved oxygen concentration had no impact on survival or decreases in numbers of fecal coliforms. Our results were parallel with this study.

So far, some studies including seawater quality, benthic organisms and heavy metal contents of harvesting mussel in Sinop coastal area have been conducted (Karayucel et al. 2003; Koloren et al. 2012; Kurt & Ozkoc 2004). To the best of our knowledge, there were no detailed studies focusing over the microbiological pollution of Sinop costal water, except for one done by Gökkurt (2007). In this study, it was found that the number of total coliform, fecal coliform and fecal streptococci bacteria were 8-660, 0-28 and 0-60 CFU/mL, respectively, at the sampling sites

in Sinop coasts during the summer season. The results in our study showed that the numbers of total coliforms, fecal coliforms and fecal streptococci bacteria were 0->1100, 0-1100 and 0-9×10^5 CFU/mL, respectively, in the same period. Our findings concerning bacterial counts were over the values determined by Gökkurt. This case clearly showed that microbial pollution level has increased over time.

According to the Turkey Bathing Water Quality Standard, the acceptable fecal coliform count is 2×10^2 MPN/100 mL. In addition, according to the European Union (EU)-Blue Flag, the acceptable fecal coliform and fecal streptococci counts are 2×10^3 and 1×10^3 MPN/100 mL, respectively (Anonymous 2006b). Çotuk and Kimiran (2004) stated that the number of total and fecal coliform was at 10^3 to 10^4 100/mL at Bosphorus; both values exceed the legal limits. The other study showed that total coliform values were between 5±2 and 26±55 and fecal coliform values were between 4±2 and 24±50 in the monitoring at eight stations around the Prince Islands in Marmara Sea (Türkdoğan-Aydınol et al. 2012). In this study, it was also statistically confirmed that there was no significant difference between total and fecal coliform counts for the whole sampling stations, except for one. Aydın et al. (2010) determined that TC, FC, FS and *Salmonella* sp. counts were over of standard value for about 5 of 74 (6.75%) coastal bathing water samples obtained from Dardanelles and Thracian sea. In a study done by Gurun and Erdem (2013), they took samples in seven points from discharge regions of the Ayamama stream to Marmara sea and examined the bacteria such as TC, FC, FS, *Salmonella* spp. and total mezophilic aerobic heterotrophic. They found that in all of seven areas, bacteria counts were higher than the seawater quality standards. Our findings showed that fecal coliform numbers were higher than the recommended standard value at sampling Stations 5, 6 and 7 (Figure 2). In our study, it also was found that the numbers of fecal streptococci were higher than the acceptable limit value especially at Station 6 and this case was almost the same at the other stations (Figure 2). These results clearly showed that Sinop coastal waters were faced with pollution as a result of swimming and recreational activities during the summer periods. Our findings were in good agreement with Gurun and Erdem (2013). On the other hand, Tuğrul-İçemer et al. (2007) reported that the annual average values of TC, FC and FS 102, 28, 8 and 110, 17, 8 CFU/mL for Lara and Konyaaltı beaches of Antalya, respectively and these values do not exceed the legal limit values for EU and Turkey Bathing Water Quality Directive.

Live bivalve mollusks specified upper limit values for fecal coliform were determined as 300 MPN/100 g according to Quality of Fishery Products Regulation (Regulation of Fisheries 1995). Many studies have been conducted concerning the bacterial pollution levels of mussels growing in many countries. Kaşgar (1992) determined that fecal coliforms counts in mussels collected from Bosporus (Rumeli Kavağı) were higher than the standard value. In the study done by Yılmaz et al. (2005) on mussel harvesting in the Marmara Sea (Gelibolu Region), total aerobic mesophilic bacteria numbers as 2.1×10^4 - 1.9×10^6 CFU/g, coliform group bacteria counts as 2.9×10^2 - 8.2×10^3 CFU/g and the numbers of *E. coli* as 78-2.5×10^2 CFU/g were determined. Furthermore, few studies also stated that the content of fecal coliform bacteria of mussel was much higher than seawater (Akar 2009; Stabili et al. 2004). In another study, it showed that levels of fecal coliforms were higher than the legal limit value (>300 MPN/100 g) at all sampling stations throughout the study (Kaçar 2011). The results of our findings indicated that levels of fecal coliform bacteria in some months at Stations 3 and 4 were higher than the standard value. Our results were in good agreement with other studies.

Enzyme tests were used for identification of *E. coli* strains in another step of our study. These tests have been used for the identification of *E. coli* and coliforms isolated from both human and environmental specimens. On the other hand, phenotypic characteristics and enzymes tests were completely consistent with the phenotypes of the isolates. In some studies on this subject, Rice et al. (1990) reported that *E. coli* strains isolated from a total of 460 human, 105 cows and 55 horses were screened and 95.5% β-glucuronidase-positive were isolates in 24 h and 99.5% positive after 28 h of incubation. Doğan and Halkman (1998) isolated 190 *E. coli* strains in the studies and they reported that all colonies gave the fluorescent positive in LST + MUG media. In our study, it was also confirmed that a total of 40 isolates were consisted of *E. coli* species according to biochemical characteristics and β-glucuronidase activities. Our findings were in agreement with the previous studies.

In many studies, Fluorocult *E. coli* O157:H7 Agar medium has been the successful used for screening the presence of *E. coli* O157:H7 strain. Belloso (2000) examined a total of 493 water samples from different regions in Santa Fe Province and this strain was not found in any of the tested samples. In addition, *E. coli* O157:H7 strain was not determined in the other research done by Öztelli (2004) in central Bayburt Province. Our findings are in line with these studies.

Conclusion

Our findings confirmed that the counts of fecal coliforms and fecal streptococci in the seawater and mussel samples were higher than the legal limits for a few sampling site in Sinop. However, the level of microbial pollution remained at the acceptable values at the others sampling stations. As a result, it is possible to say that microbial infections can be prevented by adequate freezing and cooking, proper storage and processing after harvesting and avoidance of cross-contamination during mussel handling. In addition, the coastal contamination due to enteric bacteria leads to quality decline of seawater resources that pose a public health risk and economic loss. At the same time, this study may help as reference in evaluating the seawater and seafood quality for next studies.

REFERENCES

Akar, S. 2009. İzmir İç Körfezi'nde, Kıyı Sularında ve Kara Midyelerde (*Mytilus galloprovincialis* Lamarck, 1819) Fekal Koliform Bakterilerin İzlenmesi. MSc, University of Dokuz Eylül, İzmir, p. 97 (unpublished).

Anonymous. 2006a. *Merck Microbiology Manual*. 12th ed. Merck KGaA, Darmstadt, Germany. pp. 287-288.

Anonymous. 2006b. Yüzme suyu kalitesi yönetmeliği. Resmî gazete, (26048).

AOAC (Association of Official Analytical Chemist). 1998. *Official Method of Analysis of AOAC International*. 16th ed., 4th Revision.

Aydın, A. & Soyutemiz, E. 2002. Bazı Balık Türlerinden ve Kum Midyelerinden (Venus gallina) *Vibrio parahaemolyticus* İzolasyonu ve İdentifikasyonu. *Turk. J. Vet. Anim. Sci*. 26: 1249-1253.

Aydın, A., Kahraman, T. & Aydın, H. 2010. The microbiological and physico-chemical parameters with trace metal pollution of coastal bathing water in Dardanelles and Thracian Sea. *J. Anim. Vet. Advan*. 9: 1077-1082.

Belloso, C.O. 2000. Absence of *Escherichia coli* 0157:H7 in waters interdisciplinary perspectives on drinking water risk assessment and management. *IAHS Pub*. 260: 33-35.

Bitton, G. 2005. Microbial indicators of fecal contamination. PhD, Universty of Florida, Gainesvilla, FL. p. 71 (unpublished).

Brenner, D.J. & Farmer, J.J. 2005. Order XIII. "Enterobacteriales". In *Bergey's Manual of Systematic Bacteriology*, 2nd ed. Vol. 2 (The Proteobacteria), part B, edited by Garrity, G.M., Brenner, D.J., Krieg, N.R. & Staley, J.T. New York: Springer. pp. 587-607.

Canesi, L., Pruzzo, C., Tarsi, R. & Gallo, G. 2001. Surface interactions between *Escherichia coli* and Hemocytes of the Mediterranean mussel *Mytilus galloprovincialis* Lam. leading to efficient bacterial clearance. *Appl. Environ. Microbiol*. 67(1): 464-468.

Cavallo, R.A. & Stabili, L. 2002. Presence of vibrios in seawater and *Mytilus galloprovincialis* (Lam.) from the Mar Piccolo of Taranto (Ionian Sea). *Water Res*. 36: 3719-3726.

Çotuk, A. & Kimiran, A. 2004. İstanbul Boğazı Deniz Suyu Örneklerinde Indikatör Bakterilerin Dağılımı. *Orlab-online Mikro. Derg*. 2(5): 22.

Dionisio, L.P.C. & Borrego, J.J. 1995. Evaluation of media for the enumeration of faecal streptococci from natural water samples. *J. Microbiol. Met*. 23: 183-203.

Doğan, H.B. & Halkman, A.K. 1998. *E. coli* Sayımında EC Broth+Triptofan ile LST Broth+MUG Besiyerlerinin Kullanılması. *Gıda*. 23(6): 425-429.

EU (European Union). 2006. Directive 2006/7/EC of the European Parliament and the Council Concerning the Management of Bathing Water Quality. *Offic. J. Eur. Commun*. 64: 37-61.

Gökkurt, O. 2007. Sinop Kıyılarında Potansiyel Deşarj Noktalarının Suyun Kalitesine ve Canlılara Etkileri. MSc, University of On Dokuz Mayıs, Samsun, p. 202.

Gurun, S. & Erdem, A.K. 2013. Examination of the level of bacteriological pollution in the discharge area of the Ayamama Stream to the Marmara Sea. *Ekoloji* 22(86): 48-57.

Hamzah, A., Kipli, S.H., Ismail, S.R., Una, R. & Sarmani, S. 2011. Microbiological study in coastal water of Port Dickson. *Sains Malaysiana* 40(2): 93-99.

Hernandez-Delgado, E.A. & Toranzos, G.A. 1995. *In situ* replication studies of somatic and male-specific coliphages in a tropical pristine river. *Water Sci. Technol*. 31: 247-250.

Kacar, A. 2011. Some microbial characteristics of mussels (*Mytilus galloprovincialis*) in coastal city area. *Environ. Sci. Pollut. Res*. 18: 1384-1389.

Karayucel, S., Kaya, Y. & Karayucel, İ. 2003. Sinop Bölgesinde Akdeniz Midyesinin (*Mytilus galloprovincialis* Lamarck, 1819) Kondisyon Faktörü ve Biyokimyasal Kompozisyonu Üzerine Çevresel Faktörlerin Etkisi. *Turk. J. Vet. Anim. Sci*. 27: 1391-1396.

Kaşgar, H.S. 1992. İstanbul Boğazının Deniz Suyu ve Midyelerinin Fekal Koliform Bakteriler Bakımından İncelenmesi, MSc, University of İstanbul, İstanbul, p. 46 (unpublished).

Koloren, Z., Avsar, C. & Kaya, D. 2012. Monitoring of *Cryptosporidium* species in water supplies of Sinop, Black Sea, Turkey by acid-fast staining method. *J. App. Bio. Sci*. 6(3): 41-43.

Kurt, P.B. & Ozkoc, H.B. 2004. A survey to determine levels of chlorinated pesticides and PCBs in mussels and seawater from the Mid-Black Sea Coast of Turkey. *Mar. Pollut. Bull*. 48(11-12): 1076-1083.

Mayo, A.W. 1995. Modeling coliform mortality in waste stabilization ponds. *J. Environ. Enginer.* 121(2): 140-152.

Özcakmak, S. & Arıcı, M. 2001. Samsun'da Satılan Midyelerde *Vibrio parahaemolyticus* Varlığı Üzerine Bir Araştırma. Hotel Rest. Food Derg., pp. 68-77.

Öztelli, Y. 2004. Bayburt İli Merkez İlçede İçme Sularında Enterohemorajik *Escherichia coli* (O157:H7)'nin Araştırılması. MSc, University of Süleyman Demirel, Isparta, p. 49 (unpublished).

Perez, M.C.G., Anfusu, E., Acevedo, A. & Machuca, J.A.P.V. 2008. Microbiological indicators of faecal contamination in waters and sediments of beach bathing zones. *Int. J. Hyg. Environ. Health* 211: 510-517.

Popovic, N.T., Skukan, A.B., Dzidara, P., Rakovac, R., Perovic, I., Kozacinski, L., Jadan, M. & Gorski, D. 2010. Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic Coast of Croatia. *Vet. Med*. 55(5): 233-241.

Rice, E.W., Allen, M.J. & Edberg, S.C. 1990. Efficacy of β-glucuronidase assay for identification of *Escherichia coli* by the defined-substrate technology. *Appl. Environ. Microbiol*. 56: 1203-1205.

Robin, R.S., Vardhan, K.V., Muduli, P.R., Srinivasan, M. & Balasubramanian, T. 2012. Preponderance of enteric pathogens along the coastal waters of Southern Kerala, Southwest Coast of India. *Marine Sci*. 2(1): 6-11.

Roslev, P. & Bukh, A.S. 2011. State of the art molecular markers for fecal pollution source tracking in water. *Appl. Microbiol. Biotechnol*. 89: 1341-1355.

Stabili, L., Immacolata-Acquaviva, M.I. & Cavallo, R.A. 2004. *Mytilus galloprovincialis* filter feeding on the bacterial community in a Mediterranean coastal area (Northern Ionian Sea, Italy). *Water Res*. 39(2-3): 469-477.

Regulatio of Fisheries (Su Ürünleri Yönetmeliği). 1995. Resmi Gazete 22223, Ankara, p. 43.

Tuğrul-İçemer, G., Keleş, C. & Karaca, H. 2007. Antalya Plajlarında Mikrobiyolojik Deniz Suyu Analizi. *Türk Sucul Yaşam Derg*. 3(5): 553-560.

Türkdoğan-Aydınol, F.I., Kanat, G. & Bayhan, H. 2012. Sea water quality assessment of Prince Islands' Beaches in İstanbul. *Environ. Monit. Assess*. 184(1): 149-160.

Welch, R.A. 2006. The Genus *Escherichia*. In *The Prokaryotes: A Handbook on the Biology of Bacteria*. 3rd ed, Vol. 6, edited by Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. & Stackebrandt, E. New York: Springer. pp. 60-71.

Yılmaz, İ., Bilgin, B. & Öktem, B. 2005. Occurrence of *Vibrio* and other pathogenic bacteria in *Mytilus galloprovincialis* and *Venus gallina* harvested from the Marmara Sea. *Türk J. Vet. Anim. Sci.* 29: 409-415.

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