J. Black Sea/Mediterranean Environment
Vol. 16(3): 295- 310 (2010)

Distribution of members of the family *Enterobacteriaceae* in the Istanbul Strait

İstanbul Boğazı'nda *Enterobacteriaceae* familyası üyelerinin dağılımı

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Abstract

The aim of this study was to determine the distribution of the *Enterobacteriaceae* members present in the sea water samples obtained from the stations selected at the Marmara Sea entry and the Black Sea exit of the Istanbul Strait and the effect of the physical parameters on the distribution of these indicator bacteria.

A total of 126 unit isolates of the members of the *Enterobacteriaceae* were identified using API 20E (Biomereux) in the sea water samples obtained from the surface waters (0-30 cm) and various depths by monthly samplings from February 2006 to March 2007. Faecal and total coliform analyses were performed in order to state the bacterial pollution load using membrane filtration technique. Temperature, salinity, conductivity, pH and dissolved oxygen were measured by a CTD (SBE-15). The results of this study demonstrated that the highest bacterial abundance and pollution were present at the Marmara Sea

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entry of the Istanbul Strait, the number of bacteria determined below the depth of 20 m were higher than the deep discharge standards, and the deep discharge at the Marmara Sea entry of the Istanbul Strait was not attaining its purpose due to the lower layer waters were returning to the Marmara Sea instead of merging in the Black Sea, as pointed out by the bacterial levels.

Key words: Enterobacterieaceae, Istanbul Strait, total coliform, faecal coliform.

Introduction

Some members of the Family *Enterobacteriaceae,* entering to the aquatic environments through domestic and industrial wastes, are accepted as the indicators of bacteriological pollution and they are indicative of anthropogenic pollution.

There are many different kinds of active industries located near the Istanbul Strait, such as; leather, glass, battery, electric, electronics, iron casting, textiles, various metals, chemicals, cosmetics and paint. Improper domestic and industrial discharges are the main reason of the distribution and increase of the members of *Enterobacteriaceae.*

The pollution levels in the Marmara Sea have increased as a result of the effects of the Black Sea due to opposite water currents between the Black Sea and the Aegean Sea (Başsarı et al. 2000, Topcuoglu 2000, Tasdemir 2002). The Istanbul Strait connects the Sea of Marmara to the Black Sea and the Çanakkale Strait to the Aegean Sea. The Sea of Marmara separates Turkey's Asian and European regions. The less saline waters of the Black Sea reach the Mediterranean via upper currents while the concentrated saline waters of the Mediterranean reach the Black Sea via the undercurrents of the Çanakkale and Istanbul Straits (Ünlüata et al. 1990, Beşiktepe et al. 1994). Due to its peculiar oceanographic features, the Istanbul Strait offers unique opportunities for studying the bacterial role in different, poorly described conditions.

Enteric bacteria of sewage origin undergo a sudden osmotic shock when they enter the sea water and may adapt their metabolism to the new medium by means of their osmo-regulation systems. This ability of enteric bacteria aids them in gaining resistance to salt in sea environments and increases their probability of survival (Munro et al. 1989).

This study is vital to to reveal the biological contamination and determine the distribution of members of the Family *Enterobacteriaceae,* isolated from the Istanbul Strait. It was tried to demonstrate the levels of bacteriological pollution on the surface waters of the Istanbul Strait by the coliform and faecal coliform analysis and the relation between the pollution and the physical parameters, to identify the bacteria of *Enterobacteriaceae* isolated from different depths and to determine their distribution frequency.

Materials and Methods

Study area and sampling

The samplings were carried out monthly, between April 2006 and March 2007, by the Research Vessel Yunus-S. The water parameters of pH, dissolved oxygen, salinity, conductivity and temperature were measured and recorded by CTD (*SBE 19 SEACAT Profiler*) and sea water samples were taken from surface water (0–30 cm) and depths ranging from 10 to 50 meters. The Secchi depths (Kocataş 2005) were also measured in the stations shown in Figure 1. The sampling stations for the isolation of the members of the *Enterobacteriaceae* are selected at the Marmara Sea entry and the Black Sea exit of the İstanbul Strait.

The sea water samples used in the bacteriological analysis were taken from the surface water and different depths by the Nansen sampling bottle and transferred into 250 ml, sterile, brown glass bottles under aseptic conditions. All the samples were taken as three replicates and transported to the laboratory on the same day, by maintaining the cold chain. The analyses were carried out according to EPA (2006) and APHA (1998).

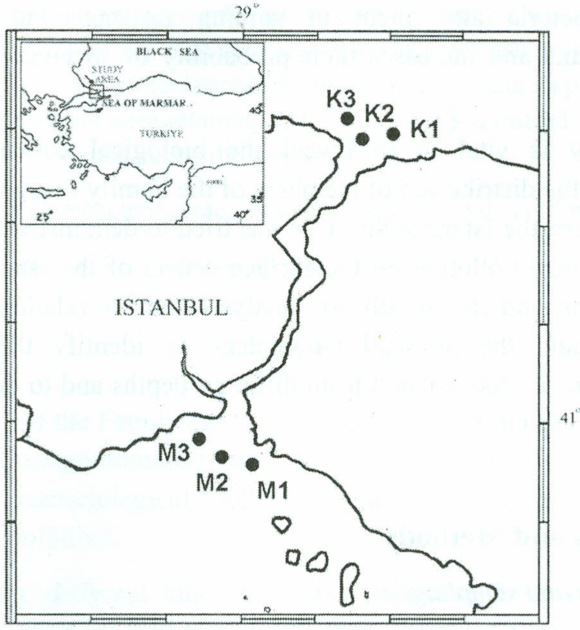


Figure 1. *The Study Area;* Stations K1-K2-K3 are located at the Black Sea exit of the Istanbul Strait and Stations M1-M2-M3 are located at the Marmara Sea entry of the Istanbul Strait.

The sampling area covers the stations at the Marmara Sea entry of the Istanbul Strait (M1: Haydarpaşa, M2: between Haydarpaşa and Ahırkapı, M3:Ahırkapı Lighthouse) and Black Sea exit (K1: Poyraz Harbour, K2: between Poyraz and Garipçe, K3: Garipçe).

Faecal coliform and total coliform tests

Water samples were filtered through a 0.45 µm membrane filter with a metal vacuum filtering set (Millipore, Germany) and then the Membran filter were placed on m-Endo for total coliform and m-FC for faecal coliform bacteria. The plates were incubated for 48 h (at 37±0.1°C and 44.5±0.1°C) and the colonies on the plates were evaluated (APHA 1999, Hitchins 1992). First, the coliform suspicious colonies were tested by cytochrome oxidase test (API Strep, BioMereux) and the oxidase negative colonies were then evaluated numerically. The colonies suspected of being fecal coliform were tested by cytochrome oxidase (API Strep, BioMereux) and indole (HIMEDIA) tests for confirmation. The numerical descriptions of bacteria were given as CFU/100 ml (colony forming units in 100 ml) by taking the average of three replicates and the results were presented with the standard deviations of the mean values (APHA 1999).

Isolation and identification of members of Enterobacteriaceae

First isolation and identification tests of isolates were applied using Indol, Metil Red, Voges–Proskauer and Citrat (IMVIC) test (FDA 1998, Bergey's Manual of Systematic Bacteriology 2007, Hitchins 1992).

Suspicious colonies were identified and confirmed by using API 20E (Bimereux, France). API 20E tests utilized included: triple sugar iron agar, motility, indole production, utilization of citrate and malonate, urea hydrolysis, decarboxylation of lysine and ornithine, deamination of arginine and phenylalanine, deoxyribonuclease, gelatinase, methyl red test, Voges- Proskauer test, and fermentation of arabinose, adonitol, inositol, sorbitol, raffinose, and rhamnose. Each isolate was identified by using the API 20E profile index after 48 h at 37°C incubation (Hitchins 1992, Madigan et al. 2009).

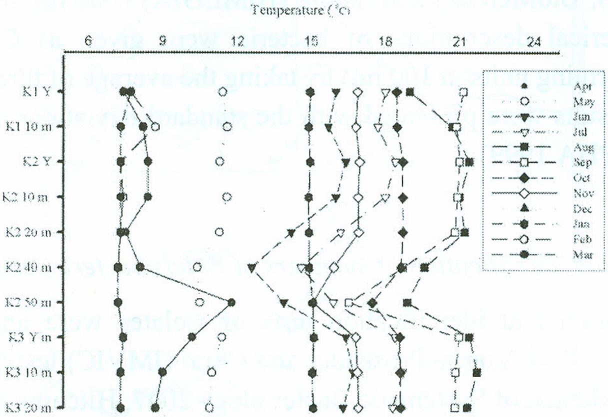
Statistical analysis

The bacterial concentrations were log-transformed (base 10) and statistical analysis was performed by using the Instat GraphPad Software, San Diago, CA, USA.

Results

Abiotic parameters

The main physico-chemical characteristics of the sampling points such as temperature, salinity, pH, and dissolved oxygen are reported in Figure 2.



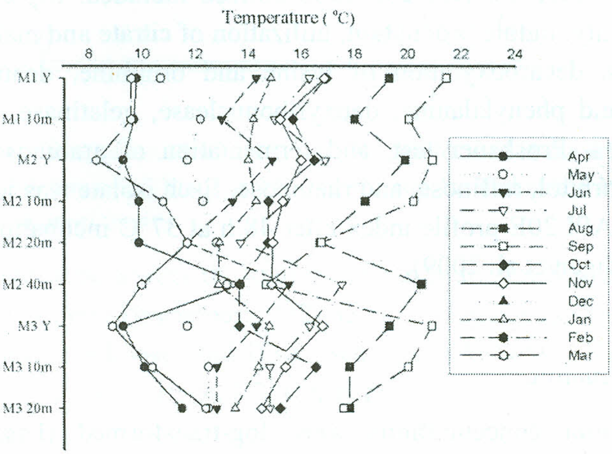


Figure 2. Yearly values of: Temperatures (T, °C), Salinity (S, ‰), dissolved oxygen (O_2, mg/L), measured at the various sampling sites.

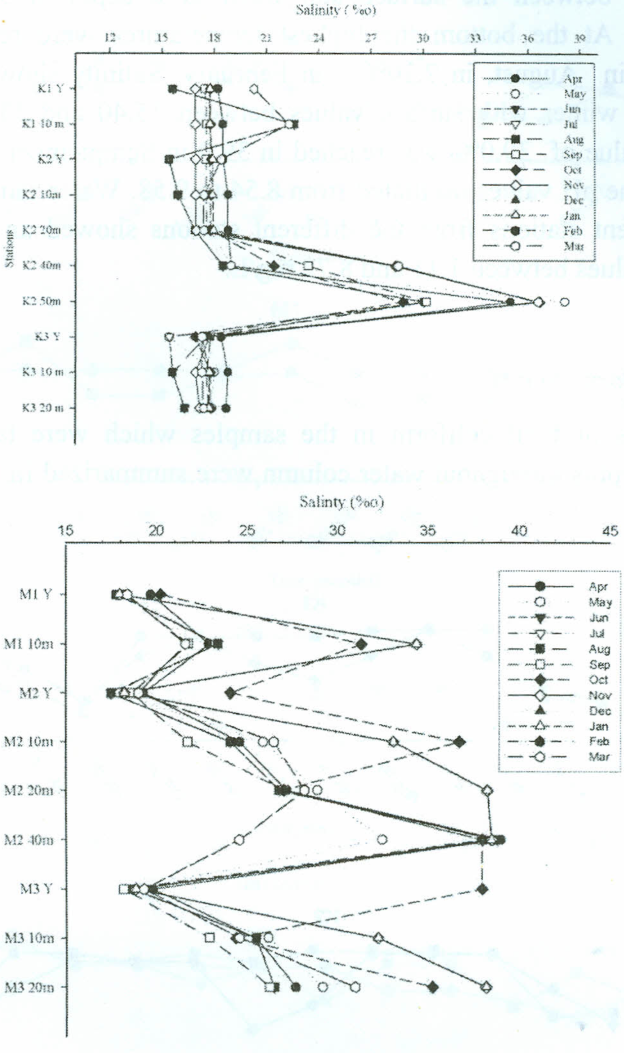


Figure 2 continued. Yearly values of: Temperatures (T, °C), Salinity (S, ‰), dissolved oxygen (O_2, mg/L), measured at the various sampling sites.

The lowest seasonal temperatures were reached in 7.00- 26.09 °C both at the surface water and the bottom. Summer warming up started in 16.00 with temperatures never above 21.09°C and the highest temperature values were observed in August at the surface, when the greatest thermal excursion between the surface and water at a depth of 50 m was observed. At the bottom the highest temperatures were recorded in 22.70°C, in August, in 7.20°C , in February. Salinity showed lowest values in winter with surface values between 15.40 and 33.0‰. The highest value of 39.0‰ was reached in 39.0 in Semptember with deep waters. The pH values oscillated from 8.54 to 9.58. Water samples from the different stations from the different stations showed an dissolved oxygen values between 1.11 and 8.72 mg/L.

Total coliform counts

The levels of total coliform in the samples which were taken from various depths throughout water column were summarized in the Figure 3.

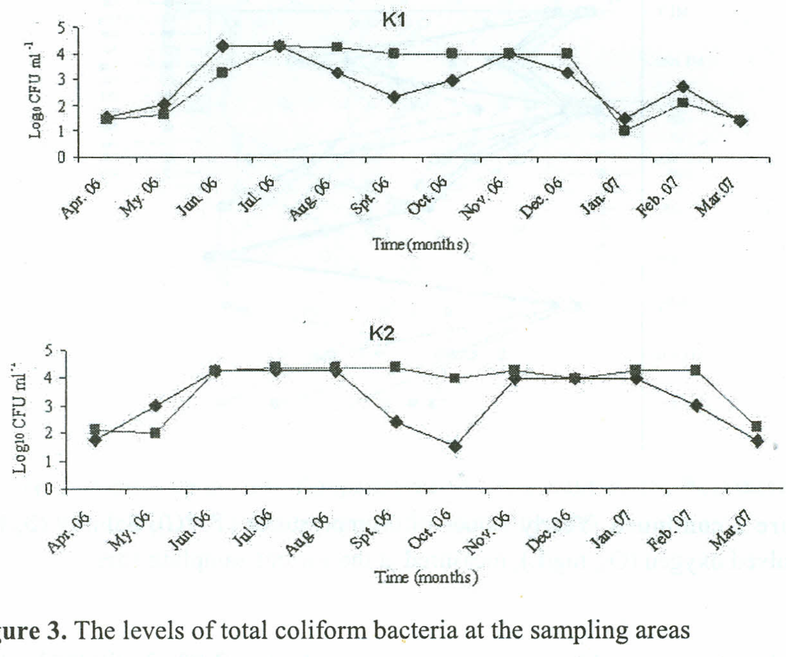


Figure 3. The levels of total coliform bacteria at the sampling areas

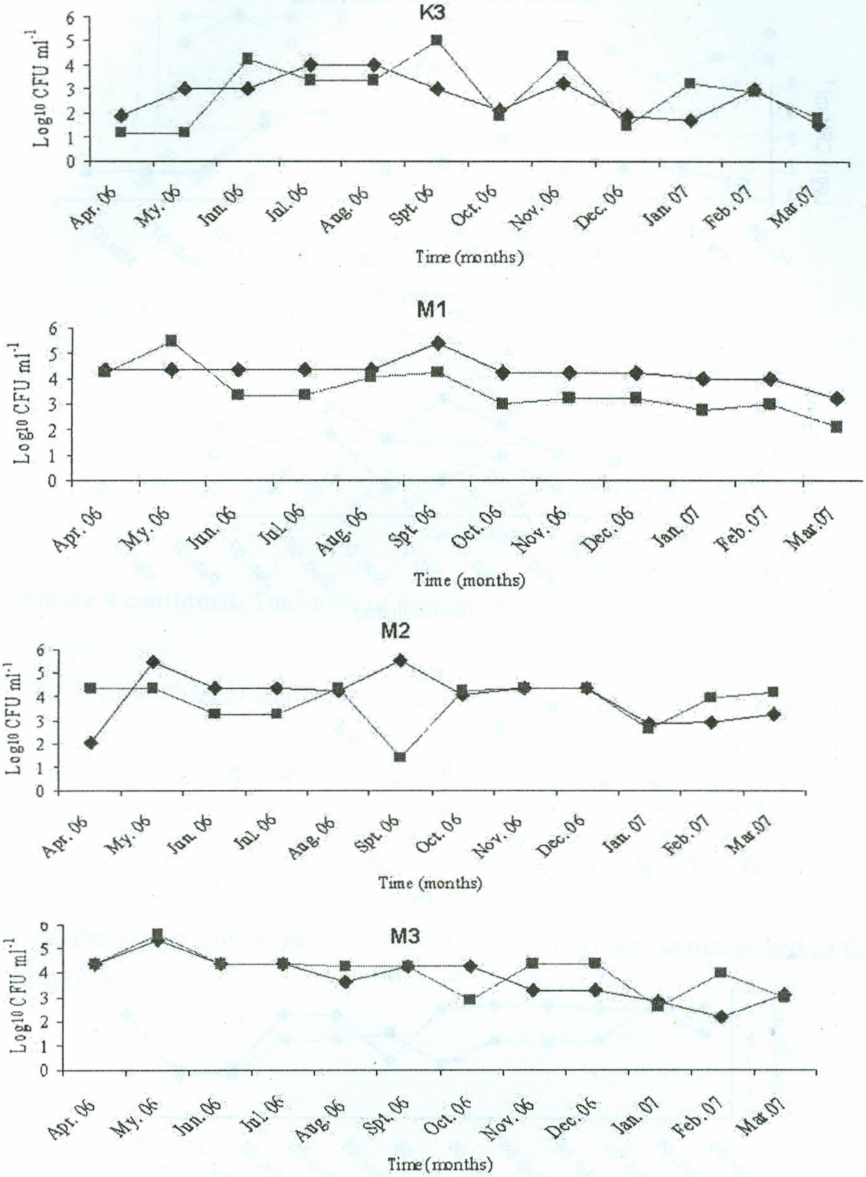


Figure 3 continued. The levels of total coliform bacteria at the sampling areas

Fecal Coliform Bacteria

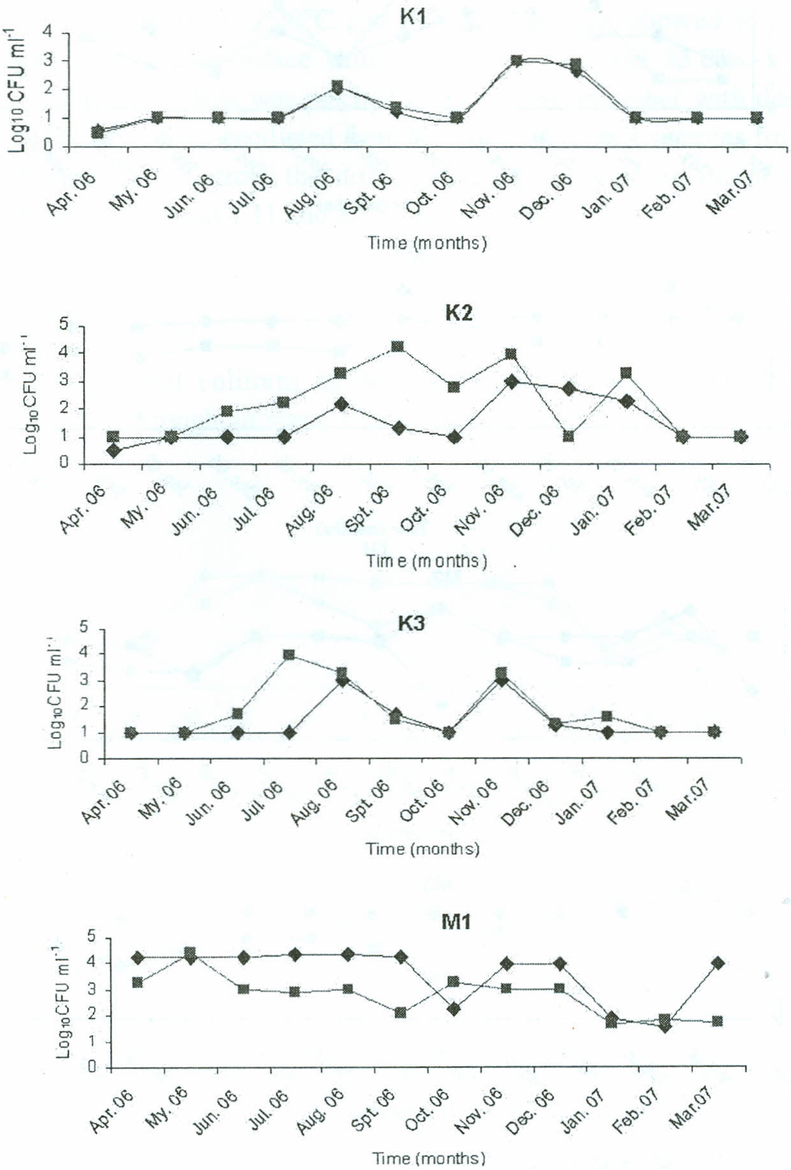


Figure 4. The levels of fecal coliform bacteria at the sampling areas.

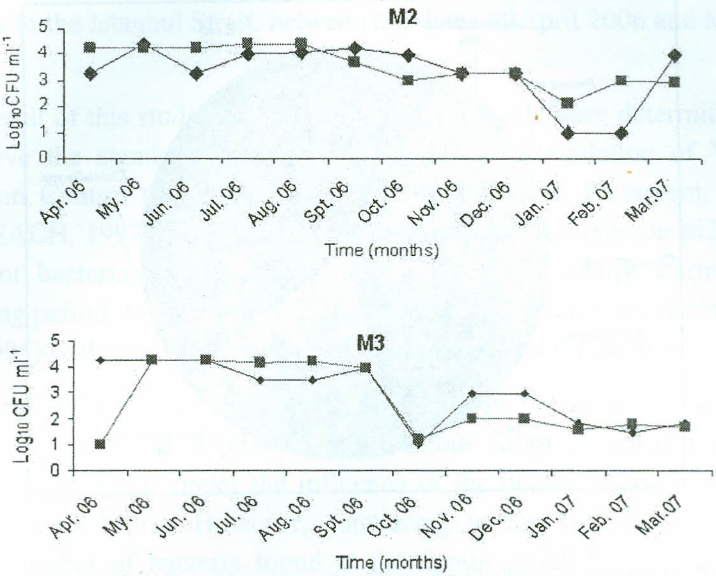


Figure 4 continued. The levels of fecal coliform bacteria at the sampling areas.

The levels of fecal coliform bacteria in the samples which were taken from various depths throughout water column were summarized in the Figure 4.

Distribution of Enterobacterieaceae members

Distribution of Enterobacterieaceae members (%) was summarized in the Figure 5.

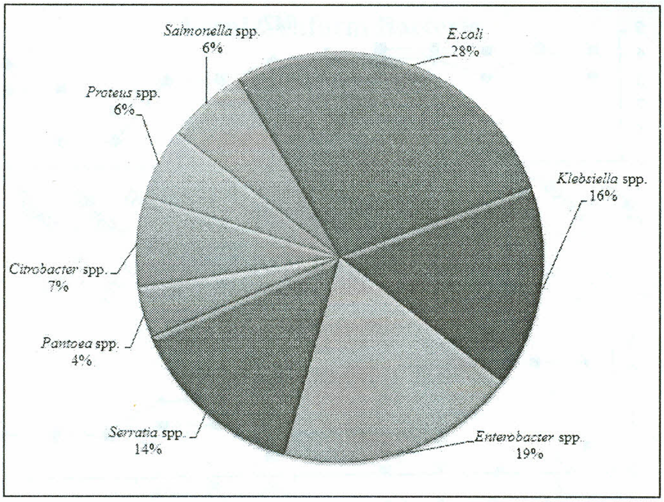


Figure 5. Distribution of *Enterobacterieaceae* members (%) during the study period.

E. coli was more abundant isolate among the 126 strains to be 27.28%. *E. gergoviae* and *E. aerogenes* was reported as a less common (1.59%) species.

Domestic and industrial input showed their effects at stations (M1-M2 and M3) which were placed between Ahırkapı and Üsküdar. The mean level of fecal coliform was detected to be $12x10^3$ ml CFU/100 ml in the surface water samples which were taken from entrance of the Yenikapı-Üsküdar. However, the levels of bacteria were increasing to be $24x10^3$ CFU/100 ml in the samples which were taken from deeper points, depending on the deep discharge. There was no correlation between indicator bacteria and nutrients.

Discussion

The relation between the levels of coliform and fecal coliform bacteria and physical parameters was determined by the analysis of the sea water samples obtained from the sea surface and different depth levels at the stations in the Istanbul Strait, between the dates of April 2006 and March 2007.

As a result of this study, the indicator bacteria levels were determined to be above the standard values published by the Regulation of Water Pollution Control (Official Gazette, 1988), European Parlament, 2006 and BEACH, 1997.In the entrance of the Marmara Sea (station M2), the indicator bacteria levels were always above the standards during the sampling period. In all stations, there was a significant linear relationship ($p < 0.005$) with the numbers of fecal coliform and total coliform.

The fecal coliform and total coliform levels in the seawater samples taken from different depths of the Istanbul Strait revealed that the researched area was under the influence of the domestic and industrial sources of pollution. However, comparing to the surface water, the higher number of bacteria found at the depths of 20-50 m is a more serious problem than the presence of the pollution. An obvious increase was observed by comparison of the values of the fecal coliforms measured from the mid and lower layer waters of the Istanbul Strait between 1997 and 2004 (İSKİ, 1988, İSKİ, 2005). This situation was also confirmed by the findings of our study.

This information explains the reason of the high levels of bacteria we found below the depth of 20 m. It can be understood from this case that the deep discharge in the Marmara Sea entrance of the Istanbul Strait is not properly functional according to its purpose and the bacteria carried by domestic waste water are returning back to the Marmara Sea instead of mixing to the Black Sea by the lower layer waters.

Moreover, even if these waters reached to the Black Sea, they carry much above the level of bacteria compared the coliform (1000 / 100 ml) and fecal coliform (200/100 ml) values of the deep sea discharge water must meet according to 25 687 No. Directive of Water Pollution and Control (2004). So it can be understood here that the water treatment of the deep-sea discharge is not functioning properly. From the Black Sea exit of the Istanbul Strait where the coast interaction is at a minimum level, that is to say from north to south, in addition to the surface pollution, a sudden increase in the surface water salinity was determined in some periods.An increase in the nitrite levels was also observed in the bottom waters during the periods that salinity and fecal coliform values increased.

In this period, the rapid increase in fecal coliform levels was associated with the vertical mixing of the waste water discharging to the lower layer, due to the geomorphology of the southern part with the influence of meteorological conditions.

Among the isolates of *Enterobacteriaceae* members, *E. coli* was the most frequent with a frequency of %28 (Figure 4:40). This situation confirms the presence of the appropriate abundance of the nutrients to *E. coli* adaptation in the research area.

There was not a continuous, significant correlation between the dissolved oxygen concentration and the levels of indicator bacteria. The highest dissolved oxygen concentration was recorded as 9.21mg / l at the surface and a decrease in the dissolved oxygen (0.36 mg / l) value was recorded due to the increasing depth. This has been linked to the decrease in the primary production and increase in the oxygen consumption due to the biochemical reactions related to bacterial activity.

In the sampling stations, the surface water temperatures ranged between 7-26°C during the year. A significant relation was detected between the sea water temperatures and the presence of indicator bacteria in the periods of high bacterial pollution.

Enteric bacteria of sewage origin can adapt their metabolism to the new environment by osmoregulation systems after the sudden osmotic shock when they enter into the sea water. This ability of enteric bacteria increases the resistance of these bacteria to the salinity in the marine environment and their chances of survival (Munro et al. 1989). The salinity was measured as the minimum (‰15-18) at the surface, increased with depth and reached to the maximum (‰39) at the depths of 40-50 m. However, even in the highest salinity levels, the indicator bacteria could grow at the level of $24x10^3$. In the light of the above information, this can be accepted as the adaptability of the indicator bacteria to this salinity level. At all stations, the PH levels were ranged between 8:46 and 9:59 during the year and although there was not a significant difference between the stations and depth levels, the samples of the surface water were found to be slightly higher. This was associated with the increasing H_2CO_3 (O_2 + $H_2O \leftrightarrow H_2CO_3$) amount during the increasing phytoplankton production in the surface water. The lowest secchi disc measurements were recorded at the station M2, located in the south of the Istanbul Strait. The indicator bacteria levels were also high in this station, as this was associated with the survival trends of bacteria attaching to the particles rather than free-living.

The results showed that there was no decrease in the bacteria level throughout study period. This situation in turn indicated that anthropological pollution inputs were continual. As the ecosystem health, public health and the economic use of the natural resources are concerned, immediate measures should be taken to control the sources of pollution in the Istanbul Strait.

Özet

Bu çalışma sonunda elde edilen bulgular, İstanbul Boğazı'nda indikatör bakteri oranı ile değerlendirdiğimiz bakteriyolojik kirliliğin en yüksek Marmara Denizi girişinde olduğunu göstermiştir. 20 metrenin altında tespit ettiğimiz yüksek bakteri sayısı İstanbul Boğazı Marmara Denizi girişi bölgesinde derin deşarjın amacının gerçekleşmediğini evsel atıklar yoluyla taşınan bakterilerin alt tabaka suları ile Karadeniz'e karışmak yerine Marmara Denizine döndüğünü göstermiştir. Derin deşarj akıntılardan etkilenmeden Karadeniz'e ulaşsa bile bile dip tabakaların taşıdığı bakteri sayısının sağlaması gereken değerlerin üzerinde olduğu yani derin deşarja verilen atık suların sağlıklı olarak arıtılmadığı tespit edilmiştir. İstanbul Boğazı'nda, *Enterobacteriacea* üyelerinin tür dağılımı ayrıntılı bir şekilde bu çalışma ile ortaya konmuştur. Ulaşılan bulgular, İstanbul Boğazı'nda endüstriyel, evsel ve tarım kaynaklı atıkların herhangi bir arıtma işlemine sokulmadan veya sağlıklı çalışmayan arıtım işlemlerinden geçerek doğrudan doğaya verilmesi ortamda bakteriyel dirençliliğin gelişi güzel dağılımı gibi mikro düzeyde önemli değişikliklere yol açmaktadır. Kirlilik kaynaklarının etkisi altında olan İstanbul Boğazı'nda ekosistem sağlığı, halk sağlığı ve doğal kaynakların ekonomik kullanımı düşünüldüğünde, bölgede kirlilik girdilerinin sağlıklı bir şekilde kontrolünü sağlayacak önlemlerin alınması kaçınılmazdır

Acknowledgments

The authors thank to Istanbul University Scientific Researches Projects Unit (BAP588/14082006) for the financial supports. The authors also thank the crew of the research vessel Yunus-S for their help in sampling.

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Received: 13.12.2010
Accepted: 27.12.2010