J. Black Sea/Mediterranean Environment
Vol 12: 67 – 77 (2006)

Levels of Bacterial Metabolic Activity, Indicator (Coliform, *Escherichia coli)* and Pathogen Bacteria (*Salmonella* spp.) in the Surface Water of Sapanca Lake, Turkey

Sapanca Gölü Yüzey Sularında Bakteriyel Metabolik Aktivite, İndikatör (Koliform, *Escherichia coli*) ve Patojen Bakteri (*Salmonella* spp.) Düzeyleri

Gülşen Altuğ[1*] Cumhur H. Yardimci[2] Hacer Okgerman [2] Serhan A. Tarkan[2]

[1]*Istanbul University, Faculty of Fisheries, Department of Marine Biology Istanbul, Turkey*

[2]*Istanbul University, Faculty of Fisheries, Department of Fresh Water Biology, Istanbul, Turkey*

Abstract

Surface water taken from 12 different areas in the period from July 2002 to June 2003 from Sapanca Lake, (Sakarya, Turkey) was analyzed in order to detect levels of bacterial contamination, bacterial metabolic activity, and chemical content. Level of bacteria was examined with respect to Fecal coliform, *Escherichia coli* and *Salmonella* spp. The contribution of capsule-bearing bacteria to the total number of bacterioplankton community was enumerated in order to assume the metabolically active number of bacteria. Levels of dissolved oxygen, nitrate, nitrite, orthophosphate, total hardness and calcium were calculated. Bacterial counts were made using Membrane Filtration Technique. Capsulated and non-capsulated bacteria were determined using a modified negative staining technique. As a result, the highest level of F. coliform found was $24x10^3$ MPN/ 100 ml in the surface water samples, which were taken from the western side of the Lake. While *Salmonella* spp. was found to be positive, contribution of capsulated bacteria to the total bacteria was found to be higher (19.6 %) in the same region. Results of this study have shown that level of indicator bacteria and percentage of potentially active bacteria are higher in samples of surface

[*] *Corresponding author:galtug@istanbul.edu.tr*

water collected from western part of the Sapanca Lake. Evaluation of the relations between surface fecal coliform and other environmental factors showed that the western region of the Lake was exposed to sewage wastes.
Key words: Capsulated Bacteria, Bacterial Metabolic activity, *Escherichia coli*, *Salmonella* spp. Fecal Coliform

Introduction

Bacteria have a crucial role in the bio-cycles of aquatic environments (Heissenberger 1996, Azam and Cho 1987). Most of the undissolved substrates are transformed by microorganisms in aquatic environments into available forms for other organisms. This bacterial process is important for Aquatic Microbial Ecology (Straskrabova, 1993). Under anthropogenically induced eutrophication, bacterial abundance might increase and pathogenic bacteria might be present as well (Jacob 1989), both of which influence human health (Ducklow and Carlson, 1992). It is known that there are many environmental factors which affect microorganisms' densities and activities (Refai 1979, Gaman and Sherrington 1981, Jay 1986, Jacob 1989). Since bacteria can grow easier in fresh water than in marine environments, monitoring of microbial qualities of fresh water resources are important (Straskrabova, 1993).

The Sapanca Lake, located in the northeast of Istanbul (Fig.1), is a multiplex reservoir that provides municipal water supplies for the city of Adapazari and water for wildlife and recreation. The Sapanca Lake is under the influence of chemical and biological pollution due to the fact that the inland is heavily populated with respect to dwelling, industrial activity, domestic wastes and erosion materials (Sengil, 1996, Yigit, et al, 1984). In addition to these factors, many streams discharge into the Sapanca Lake.

In aquatic environments, the number of metabolically active bacteria can be assumed by studying the contribution of capsulated bacteria to the total bacterial community. Potentially active bacteria have a well-developed capsule whereas inactive bacteria rapidly release the capsule. Active bacteria constantly renew their capsular envelope and release a significant fraction of the polysaccharide layer into the ambient water. These layers were found to be remarkably resistant to further bacterial utilization (Stoderegger and Herndl, 1998). During the course of this study visualize capsulated bacteria (the term "capsulated bacteria" is used in reference to "capsule bearing bacteria") was investigated in order to assume the level of

metabolically active bacteria in the surface water of Sapanca Lake for the first time.

The study herein has been planned and carried out for the purpose of determining the level of bacterial contamination, bacteriological metabolic activity and water quality in the samples of the surface water collected from the Sapanca Lake in the period between July 2002 to June 2003.

Materials and Methods

Study area and sampling:

Sapanca Lake is located in the Northeast of the Marmara region in Turkey. Its surface area is 46.8 km^2 with a maximum depth of 55 meters. Water samples were taken from 12 stations from different parts of the Sapanca Lake. Ten stations were located at the discharge points of the streams. One station was at the middle point of the Sapanca Lake. The twelvth station was at the point where the network for tapping drinking water to Adapazari is located. (Figure1).

The samples of the surface water were collected and transported daily to the laboratory in the period between July 2002 to June 2003.

Bacterial Methods:

Bacterial counts were made using Membran filtration (Millipore) and Most Probable Number (MPN) Technique with different media (Harrigan 1998).

Fecal Coliform: The analysis is based on the principle of numerical identification of incubation test of LSTB and BGLB positive tubes at 45±0.2°C according to the Most Probable Number Method (FAO 1992, FDA 1998, Harrigan 1998).

Escherichia coli: The analysis depends on the identification test of strains, which were derived from possitive EC Broth tubes with IMVIC (FDA 1998).

***Salmonella* spp**: The analysis is based on the identification, using biochemical and serologic tests, of suspicious colonies from selective solid medium after selective enrichment and unselective prior enrichment at 37 ^oC in liquid medium (ICMSF 1978, Borcaklı et al 1992, Harrigan 1998).

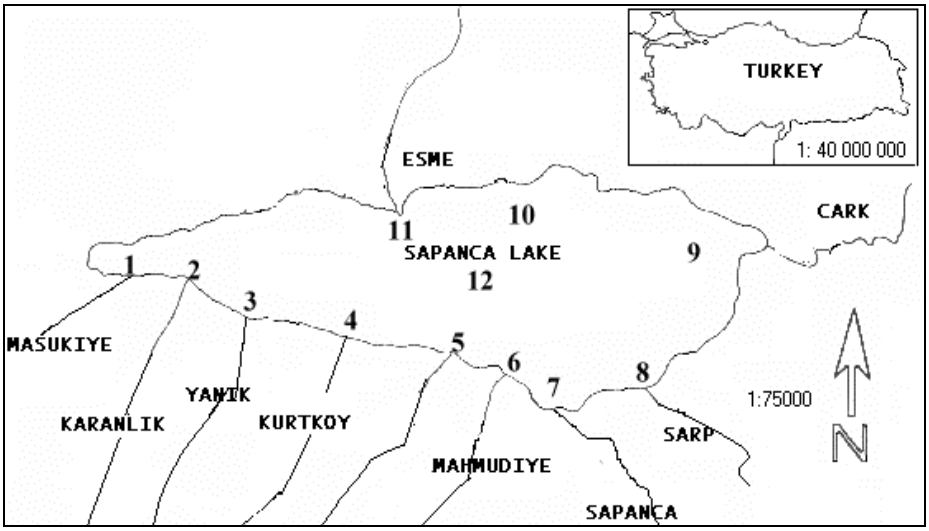

Figure 1. Study Area. 1. Point of the discharge of Masukiye Stream, 2. Point of the discharge of Karanlık Stream, 3. Point of the discharge of Yanık Stream, 4.Point of the discharge of Kurtkoy Stream, 5. Front area of the Aquaculture of Carp, 6. Point of the discharge of Mahmudiye Stream, 7. Point of the discharge of Sapanca Stream, 8. Point of the discharge of Sarp Stream, 9.Cark Stream, 10. Network of Adapazari Drinking Water, 11. Point of the discharge of Esme Stream, 12.Middle point of the Sapanca Lake

***Discrimination of capsulated and non-capsulated bacteria*:** Principally the same method as Stoderegger and Herndl (2001), which is a modification of Plante & Shriver (1998) staining method, was used to discriminate capsulated from non-capsulated bacteria.

1. Gelatin coated slides (0.1% gelatin solution and 0.01% $CrK(SO_4)_2$ x $12H_2O$) were prepared and stored frozen until use.
2. Each sample was fixed with 2% glutaraldehyde (final conc.) and filtered onto 0.2 µm polycarbonate filter.
3. The filter was transferred on the gelatin-coated slide and frozen in a horizontal position. It was stored at -20 ºC until analysis.
4. Before enumeration, the filter was thawed. The filter area was coated firstly with 0.25 % Congo red (3 to 5 drops), thereafter with Maneval's stain (three to four drops about 1 min.).
5. The slide was examined under a phase contrast microscope (Stoderegger and Herndl 2001).

***Enumeration of total bacteria*:** In order to carry out enumeration of total bacterial abundance, 5 ml samples were stained with acridine orange (Hobbie *et al.,* 1977.) and filtered onto black polycarbonate filters (0.2µm pore size, Millipore) and examined with an

epifluorescence microscope at 1250 x magnification. At least 300 bacteria per filter were counted.

***Chemical Methods*:** Chemical analyses (Nitrate, Nitrite, Orthophosphate, Dissolved Oxygen, Total Hardness, and Calsium) were made according to Boyd & Tucker (1992), APHA (1987), Bianucci & Bianucci, (1987).

Results

Contribution of capsulated bacteria to the total number of bacteria in surface water, which were taken from 12 stations, has been summarized in Table 1.

Table 1. The yearly average of the percentage of capsulated bacteria from different stations (2002-2003)

Stations	***Capsulated Bacteria %***
1.Masukiye	17.3±1.8
2.Karanlik	**19.6**±2.1
3.Yanik	16.4±2.4
4.Kurtkoy	13.3±1.9
5 Front of the basin of Carp	17.4 ±2.2
6.Mahmudiye	17.1±2.2
7. Sapanca	15.0±2.4
8. Sarp	13.8±1.9
9. Cark	13.0±2.5
10.Regional water supply	11.5±2.3
11. Esme	15.2±2.1
12. Middle point of the Lake	10.4±1.9

The percentage of capsulated bacteria was found to be higher (19.6±2.1) in samples of surface water, which were taken from the western region of the Sapanca Lake (Karanlık Stream) as compared to other stations. Samples, which were taken from the middle point of the lake, yielded lower values than the other stations.

The average minimum and maximum levels of F. Coliform, *E. coli* MPN values of surface water samples from Sapanca Lake (2002-2003) were summarized in Table 2.

Table 2. Level of Fecal Coliform, *E. coli* and *Salmonella* spp. in the surface water from Sapanca Lake, Turkey in the period between July

2002 and May 2003 (Minimum-Maximum Most Probable Number MPN/100 ml)

Stations	**F. Coliform**	**E. coli**
1.Masukiye	$12x10^2$ - **$\geq 24x10^3$**	*92x10 - 12x10²*
2.Karanlik	$12x10^2$ - **$\geq 24x10^3$**	*92x10 – 35x10²*
3.Yanik	92x10 – $16x10^2$	*45x10 – 92x10*
4.Kurtkoy	24x10 – 92x10	*95x10 – 24x10*
5 Front of the basin of Carp	95x10-$24x10^2$	*2.3x10 - 9.5x10*
6.Mahmudiye	5.4x10 – $12x10^2$	*9.5x10 – 9.5x10²*
7. Sapanca	$12x10^2$ - $95x10^2$	*24x10 – 92x10*
8. Sarp	92x10 - $15x10^2$	*5.4x10 – 12x10²*
9. Cark	9.5x10 - 24x10	*23x10 - 9.5x10*
10.Regional water supply	**<10 - 2.3x10**	*<10 – 2.3x10*
11. Esme	$12x10^2$ - $95x10^2$	*92x10 - 15x10²*
12. Middle point of the Lake	***<10 - 2.3x10***	*<10 – 2.3x10*

Results of analyses on water samples were found higher in September than other months. Fecal coliform reached the highest level at the first and second stations: max $\geq 24x10^3$ MPN / 100 ml. The second station showed the yearly average highest bacterial value. In addition, *Salmonella* spp. was found positive in September in only this station. Levels of Nitrate, Nitrite, and Orthophosphate are shown in Figure 2.

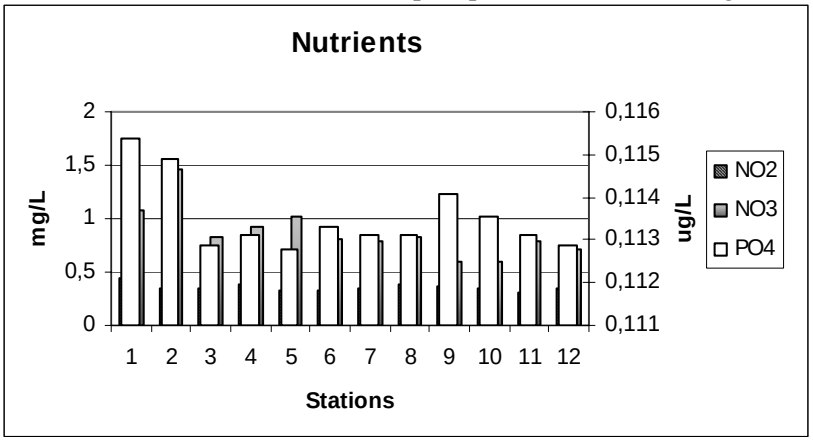

Figure 2. Nitrate, Nitrite, and Orthophosphate values of surface water samples for a period of one year (2002-2003).

Levels of Dissolved Oxygen, Total Hardness and Calcium are shown in Figure 3.

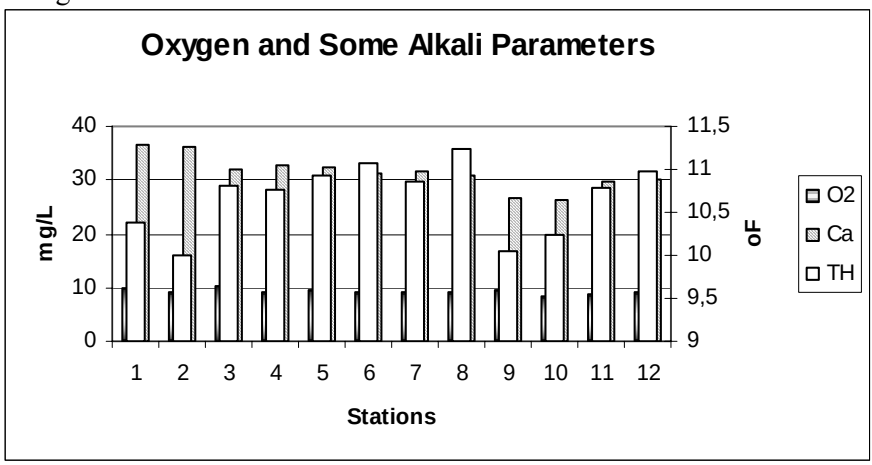

Figure 3. Dissolved Oxygen, Total Hardness and Calcium values of surface water samples for a period of one year (2002-2003).

Discussion

In our study we investigated the contribution of capsule-bearing bacteria to the total bacterioplankton community to assume the level of metabolically active bacteria in the surface water of the Sapanca Lake. Production of the capsular envelope is related to specific environmental conditions. Although some specific reactions for the biosynthesis of specific exopolymers are well known and frequently used in biotechnology, the knowledge on the factors regulating capsule exopolysaccharide formation is limited (Heissenberger *et al*., 1996). The bacterial capsule has many functions like adsorption of nutrients or protection against predators. Furthermore, it plays an important role in the formation of biofilm on surfaces (Costerton *et al*., 1987; Decho, 1990). Intact or metabolically active bacterioplankton is enveloped by a polysaccharide capsule fixed to the constantly renewed outer cell wall of the bacteria (Heissenberger *et al*., 1996; Stoderegger and Herndl, 1998). It was observed that there was no statistically significant difference ($P>0.05$) between the date of sampling with respect to the contribution of capsulated bacteria to the bacterioplankton community. However, in the surface water of the western portion of the Lake, percentage of capsulated bacteria was significantly higher ($P<0.001$) than the other parts of the Lake. This situation can be explained as different effects of environmental

factors. It is known that there are many environmental factors which affect the densities and activities of microorganisms (Gaman and Sherrington, 1981; Jay, 1986; Jacob, 1989). In this study, despite the higher level of capsulated bacteria in samples from the western part of the Sapanca Lake, nutrient concentrations were detected to be under the tolerable limits. Stoderegger and Herndl (2001) reported that a higher percentage of capsulated bacteria were found in areas with generally higher nutrient concentrations. This situation led to do hypothesis that nutrients may be used by macrophytes which were abundant in the western part. There were no significant differences ($P>0.05$) in contribution of capsulated bacteria to the bacterioplankton community among the various sampling periods.

Sapanca Lake is under the influence of biological pollution due to the fact that the inland is heavily populated with respect to industrial and recreational activity. Measuring concentrations of indicator bacteria is more cost effective than testing for specific pathogens and provides information relevant to health risks associated with water-contact activities. High concentrations of fecal indicator bacteria in waters where humans have recreational contact may present a risk of infection from other pathogenic microorganisms. Particularly, fecal coliform reached the highest level in the western part of the Sapanca Lake (Stations 1 and 2) as $\geq 24x10^3$MPN / 100 ml. At the beginning, this situation seemed to be a local fecal pollution. However, analyses show that this local pollution is moves to other stations due to climatic condition like wind, rain and waves. Moreover, *Salmonella* spp. was found positive in surface water samples taken from second station. This stiation led to the hypothesis that there is potential health risk in this area.

The highest F. *coliform* and *E. coli* values were found in the western part of the lake in August and September. These high values may be positively correlated with the increase of discharge from the streams into the Lake due to increased seasonal human activity and recreation on the coastline. Minimum bacteria numeration was detected in samples taken from the point of the regional water supply and the middle part of the Lake. Similarly percentage of capsulated bacteria was detected to be lower in samples taken from the middle point of the Lake.

Primary data for Sapanca Lake indicate that it is an oligotrophic lake. Our study showed that high concentrations of fecal coliform particularly related with the western part of the Lake will affect the trophic level negatively in time. Despite normal concentrations of dissolved oxygen, total hardness, calcium and nutrients, fecal pollution entering the Lake comes mainly from point-sources. The Lake is

exposed to sewage and wastewater from the streams (particularly from the western part). Sewage outlets, urban wastewater, and agricultural runoff contribute to the current condition of these sources which contamine the lake. Monitoring microbial quality of these areas is important in terms of saving natural resources and correct usage of the products which have economical importance.

Özet

Sapanca Gölü'nde belirlenen 12 istasyondan Haziran 2002 - Temmuz 2003 tarihleri arasında aylık örneklemelerle, yüzey sularında bakteriyolojik kirlilik, bakteriyel metabolik aktivasyon düzeyi ve su kalitesi analizleri yapılmıştır. Fekal koliform, *Escherichia coli* ve *Salmonella* spp.ölçümleri membran filtrasyon tekniği kullanarak, yapılmış, toplam bakteri sayımına kapsüllü bakteri oranı modifiye organik boyama tekniği ile belirlenerek metabolik olarak aktif bakteri sayısı bulunmuştur. Sapanca Gölü'nün batı tarafında en yüksek toplam koliform miktarı $24x10^3$ MPN/ 100 ml olarak kaydedilirken, aynı istasyonda *Salmonella* spp. pozitif bulunmuştur. Kapsüllü bakterinin toplam bakteriye oranı yine aynı istasyonda % 19.6 olarak en yüksek düzeyde kaydedilmiştir. Sonuçlar indikator bakteri ve bakteriyel metabolik aktivasyon oranının gölün batı tarafında daha yüksek olduğunu gösterirken, çevresel şartların fekal koliform düzeyi ile ilişkilendirilmesi ile Sapanca Gölü'nün batı tarafından sürekli kanalizasyon kaynaklı girdi aldığı görülmüştür.

Acknowledgment

This work was supported by the Research Fund of the University of Istanbul. Project number: 1760/21122001.

References

APHA (1987). Standart Methods for Examination of Water and Wastewater. American Public Health Association, Washington.

Azam, F., Cho. B.C. (1987). Bacterial utilization of organic matter in the sea. In: Fletcher M. (ed) Ecology of microbial communities. Cambridge University Press, Cambridge, pp. 261-268.

Bianucci, G., Bianucci, E.R. (1987). L' analisi Chimica Delle Acque Naturali ed Inquinate. pp. 320. Ulrico Hoepli Editore.Milano.

Borcaklı. M., Kalafatoglu, H., Aran. N., Topal, S. (1994). Gıdalarda Temel Mikrobiyolojik Analiz Yontemleri. TUBİTAK-MAM, Gıda ve Sogutma Teknolojisi Bolumu Yayınları, Yayın No: 128, Gebze-Kocaeli 53.

Boyd, C.E., Tucker, C.S. (1992). Water Quality and Pond Soil Analyses for Aquaculture. Alabama Agricultural Experiment Station. Aubum University Press. Ed. Lowell T. Frobish.183 Alabama

Costerton, J.W., Cheng, J., Geesey, T.I., Nickel, J.C., Dasgupta, M., Marrie, T.J. (1987). Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.* 41: 435-464.

Decho, A.W., (1990). Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.* 28: 73-153.

Ducklow, H.W., Carlson, C.A., (1992). Oceanic bacterial production. *Adv. Microb. Ecol.* 12: 113-181.

FAO (1992). Manual of Food Quality Control 4. Rev.1 Microbiological Analysis. Food and Agricultural Organization of the United Nations, Rome.

FDA (1998). "Bacterial Analytical Manual" 8th ed., Revision A. AOAC International, Washington D.C

Gaman, P.M., Sherrington, M., (1981) Food Poisoning And It's Prevention The Science of Food Pergamon Press pp 199-218.
Harrigan, W. F (1998). " Laboratory Methods in Food Microbiology" Academic Press, San Diego.

Heissenberger, A., Leppard, G.G., Herndl, J.G. (1996). Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. *Appl. Environ. Microbiol.* 62: 4521-4528.

Heissenberger, A., Leppard, G.G., Herndl, J.G. (1996). Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. *Appl. Environ. Microbiol.* 62: 4521-4528.

Hobbie, J.E, Daley, R.J, Jasper, S. (1977). Use of Nucleopore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225-1228.

ICMSF (1978). Microorganism in Foods. Vol.1. University of Toronto Press, Toronto.

Jacob, J.M. (1989). Safe Food Handling World Health Organization Genava 142.

Jay, J.M. (1986). The Microbial Spoilage of Foods Perspectives. In Biotechnology and Applied Microbiology In: D.I Alani and M. Mooyoung (eds) New York pp 325-342.

Plante, C.J., Shriwer, A.G., (1998). Differential lysis of sedimentary bacteria by Arenicola marina L., examination of cell wall structure and exopolymeric capsules as correlates. *J. Exp. Mar. Biol. Ecol.* 229: 35-52.

Refai, M.F., (1979). Manuel of Food Quality Control 4. Microbiologycal Analyses Food and Agriculture Organization of the United Nations Rome.

Sengil, I.A., (1996). Sapanca Golunde Derinlige Baglı Plankton Hareketinin Arastırılması ve Su Alma Yapısı Hakkında On Rapor. SAU Çevre Muh. Bol. Sakarya.

Stoderegger, K., Herndl, G.J., (1998). Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnol. Oceanogr. 43: 877-884.

Stoderegger, K.E., Herndl, G.J. (2001). Visualization of the exopolysaccharide bacterial capsule and its distribution in oceanic environments. *Aquat. Microb. Ecol.* 26: 195-199.

Straskrabova, V. (1993). Function of Bacteria and Bacterial Activity in Lakes ILEC / UNEP Internatıonal Training Course Limnologycal Bases of Lake Management Tihany Hungary pp 80-83.

Yigit, V., Muftugil, N., Ozalp, N., Ergen, C., Arvas, H., Yolcular, H. (1984). Sapanca Golunun Su Kirliligi ve Besin Durumu Uzerinde Bir Arastırma TUBITAK Marmara Bilimsel ve Endustriyel Arastırma Enstitusu Beslenme ve Gıda Teknolojisi Bölümü Yayın no:78

Received 13.05.2005
Accepted: 15.09. 2005