Journal of Animal and Veterinary Advances 9 (6): 1077-1082, 2010
ISSN: 1680-5593

The Microbiological and Physico-Chemical Parameters with Trace Metal Pollution of Coastal Bathing Water in Dardanelles and Thracian Sea

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Abstract: The present study was undertaken to determine the microbiological (total coliforms, fecal coliforms, fecal streptococci and *Salmonella* sp.) and physico-chemical parameters (pH, conductivity, nitrate and phosphate) with trace metal concentration (arsenic, chromium) and cyanide of 74 coastal bathing water samples obtained from Dardanelles (Site 1) and Thracian Sea (Site 2). About 5 of 74 (6.75%) water samples were of unacceptable quality based on recommended criteria of microbiological (3 of 74, 4.05%) and physico-chemical with trace metal concentration (2 of 74, 2.70%) by Turkish Bathing Water, Turkish Water Pollution and European Community Bathing Water Directives. The results indicated that the value of total coliforms and fecal coliforms provided an adequate indicator of *Salmonella* sp. presence. Among the trace metals, only arsenic was detected at the below acceptable limits. Therefore, it is necessary cleaning of waste waters by purification plants, infiltration of effluent from sewage treatment plants, control industrial factors and maintaining water quality controls.

Key words: Coastal bathing water, marine pollution, microbiological quality, physico-chemical quality, trace metals

INTRODUCTION

Marine pollution is one of the most serious problems of environment that poses a major threat to the human health. Marine pollution is closely coupled to human demographics. Around the world, pollution has increased in prevalence by the growing industrialization and the population explosions (Kennish, 1997). During the past three decades, the protection and preservation of the marine environment has become a primary goal for the international community and important results have been achieved in controlling the traditional sources of marine pollution (Frank, 2007).

Marine pollution involves the destruction of the quality of water by contamination. The major source of contemporary marine pollution, accounting for >80% of all pollution is land based activities such as industrial discharges, factory effluents, agricultural wastes and sewage. National Turkish Bathing Water Directive (TBWD, 2006); Turkish Water Pollution Directive (TWPD, 2008) and European Community Bathing Water Directive (ECBWD, 2006) has contributed significantly to the improvement of the quality of sea water and considered that total coliforms, fecal coliforms, fecal streptococci and *Salmonella* sp. are the principal microbiological parameters to be evaluated. Total coliforms, fecal coliforms and fecal streptococci are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. These pathogens can cause diarrhea, cramps, nausea and headache that may pose a special health risk for infants, young children and people with severely compromised immune systems. *Salmonella* sp. is a prime example of water and shellfish transmitted human pathogen, which is frequently isolated from the marine environment, where it can remain viable for several hours. A large percentage of the gastrointestinal symptoms occurring in the general population of the developed world are attributable to infection (Efstratiou, 2001).

The determination of chemicals and trace metals concentrations in sea water may also useful tool to evaluate the quality of the marine environment and can elucidate the mechanisms of pollutants (Manfra and Accornero, 2005). Investigations of chemicals such as nitrate, phosphate and toxic heavy metals such as Arsenic (As), Chromium (Cr), Cadmium (Cd) have special importance in environmental samples. Their effects range from cancer to neuropathy to a diarrhea that is potentially harmful, especially in infants. In the past quarter century, a considerable incidence of bathing water contamination by hazardous and carcinogenic organic compounds was reported in the United States (Alley, 2007).

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The Dardanelles is a narrow strait in northwestern Turkey connecting the Aegean Sea to the Sea of Marmara. Like the Bosporus, it separates Europe and the main land of Asia. The Thracian sea is a poorly investigated area located in northeastern part of the Aegean Sea. Thracian Sea receives the fresh waters from many of the rivers draining the Balkan Peninsula, as well as large amounts of brackish water from the Black Sea and Sea of Marmara. Therefore, knowledge of the biogeochemical process occurring there is of great importance in order to understand the interactions between the Mediterranean and Black Sea ecosystems (Zeri and Voutsinou-Taliadouri, 2003).

In this study, microbiological and physico-chemical parameters with trace metal concentration were investigated coastal bathing water in Dardanelles and Thracian Sea.

MATERIALS AND METHODS

Sample collections: The total numbers of 74 bathing water samples were obtained from Dardanelles (Site 1) and Thracian Sea (Site 2) (Fig. 1). Sampling procedure was carried out according to the methods specified in TBWD (2006), TWPD (2008) and EC BWD (2006). Briefly, samples were taken 30 cm below the surface of the water. All water samples were collected in sterile brown bottle (3 L) for microbiological, physico-chemical and toxicological analysis. All samples were stored and transported in a cold box kept below 4°C. Analysis was performed within 6 h of sampling.

Microbiological analysis: A total of 74 samples were examined by using the Membrane Filter (MF) Technique (Sartorius, 3 branch manifolds) for total coliforms (100 mL), fecal coliforms (100 mL), fecal streptococci (100 mL) and *Salmonella* sp. analyzed in 1000 mL marine water according to American Public Health Association (APHA, 1998), Slanetz and Bartley (1957) and United States Pharmacopeia (USP) (1995) (Table 1).

Physico-chemical analysis: Marine water analyzed for pH, conductivity, nitrate, phosphate, As, Cd, Cr and CN elements. The pH and conductivity were measured

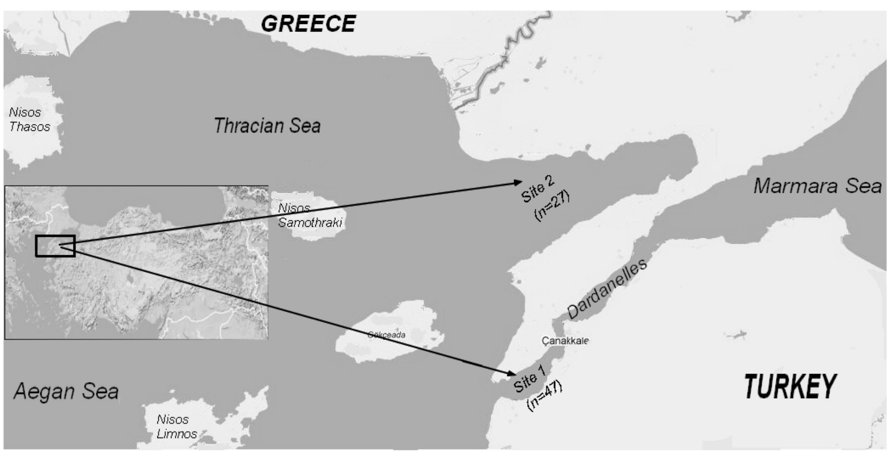

Fig. 1: Location of water sampling points in Dardanelles and Thracian Sea

Table 1: The incubation conditions and microbiological medias that are used in microbiological analysis

Analysis	Culture media	Incubation conditions	Interpretation	References
Total coliforms	ENDO Agar (Sarto No:14053)	35-37°C; 24 h	Bacteria develop sharply contoured, dark red, mucoid or nucleated colonies	APHA (1998)
Fecal coliforms	ENDO Agar (Sarto No:14053)	44.5°C; 24 h	Bacteria develop sharply contoured, dark red colonies	APHA (1998)
Fecal streptococci	Azide Agar (Sarto No: 14051) BHI (Brain Heart Infusion) Broth	37°C; 24-48 h	Bacteria form small reddish brown colonies (approx. 1 mm ⌀) with smooth peripheries, BHI with ose, finally catalase, gram strain	Slanetz and Bartley (1957)
Salmonella sp.	Bismut Sulfit Agar (According to Wilson and Blair) (Sarto No: 14057) Selenite Cystine Broth	37°C; 18-48 h	Selenite cystine broth (enrichment) to plate (streak with an inoculating loop) the sample on MF, light-colored colonies with brown to black centers	United States Pharmacopeia (1995)

with a pH meter and ion-selective meter (Sartorius PP 50, Germany). As (Merck, Merckoquant 1.17927) concentration was determined by using visual test strips. Nitrate (Merck, Spectroquant 1.14942), phosphate (Merck, Spectroquant 1.14543), Cr (Merck, Spectroquant 1.14758) and CN (Merck, Spectroquant 1.14800) concentration was measured photometrically at 520 nm wavelength (Thermospectronic Aquamate 2000E UV visible spectrophotometer, USA) by using test kits. According to the manual descriptions, the minimum detection limits for As, nitrate, phosphate, Cr and CN were 0.005, 0.2, 0.05, 0.01 and 0.002 mg L^{-1}, respectively.

Statistical analysis: To compare the physico-chemical analysis and heavy metal concentrations of Site 1 and 2, independent samples t-test was performed using SPSS 8.0 program package (Anonymous, 1999).

RESULTS AND DISCUSSION

About 5 of 74 (6.75%) marine water samples were of unacceptable quality based on recommended criteria of microbiological (3 of 74, 4.05%) and physico-chemical with trace metal concentration (2 of 74, 2.70%) by TBWD (2006), TWPD (2008) and EC BWD (2006). The dispersions of microbiological analysis of marine water samples are shown in Table 2.

Total coliforms and fecal coliforms are used universally as microbiological indicators of water quality and are commonly used for determining the quality of bathing waters. The fecal streptococci, members of the genus *Enterococcus* are considered as to be a good indicator because they are more resistant than coliforms and environmental stress (Gleeson and Gray, 2004). In this study, total coliforms were isolated from 9 (12.16%), fecal coliforms from 7 (9.45%) and fecal streptococci from 7 (9.45%) samples. Similar prevalence rates of total coliforms and fecal coliforms were reported in Belgium, France and Italy (Figueras *et al*., 1997). In a study that was conducted by Arvanitidou *et al*. (2002) in Northern Greece, 68.5, 45.2 and 58.4% of 197 bathing water samples were positive for total coliforms, fecal coliforms and fecal streptococci, respectively. In another study, the incidence of total coliforms, fecal c oliforms and fecal streptococci was isolated from 87.1, 80.0 and 97.5% of samples (Efstratiou *et al*., 2009). The present study also demonstrated that the mean load of indicators in Dardanelles (2.1×10^2, $<10^1$, $<10^1$ cfu 100 mL^{-1}) were lower than Thracian Sea (5.6×10^2, 1.5×10^1, 2.5×10^1 cfu 100 mL^{-1}). These may be due to the large population producing sewage and strong tourism sector.

Salmonella sp. is not routinely examined in water samples. The efficiency of indicators to predict the presence of *Salmonella* sp. has been examined in several countries. In Greece, Papadakis *et al*. (1997) correlated *Salmonella* sp. detection percentages to coliform groups. In Spain, Morinigo *et al*. (1990) recorded better correlation of fecal coliforms with *Salmonella* sp. isolation. In UK, Public Health Laboratory Service (PHLS), 1959 demonstrated significant correlation between the occurrences of total coliforms and *Salmonella* sp. Efstratiou *et al*. (2009) assessed, the value of total coliforms and fecal coliforms in predicting the presence of *Salmonella* sp. The findings showed approximately similarity with these results. In this study, *Salmonella* sp. was detected in samples which had high counts of total coliforms and fecal coliforms. Two of the total samples examined (2.70%) were found positive for *Salmonella* sp. The study which has higher result (13.8%) than ours was done by Efstratiou *et al*. (2009). Contrary, according to the annual report of EU in 1991 and 1994, *Salmonella* sp. was not detected in the seawater of Greece, Denmark and Luxembourg (Figueras *et al*., 1997).

The mean concentration of physico-chemical parameters and heavy metals measured in Site 1 and 2 during summer season are presented in Table 3.

Coastal zones are important ecological systems that are facing many problems with sudden increase of population and rapid economic development (Bald *et al*., 2000). In order to solve these problems, physico-chemical parameters such as pH, conductivity, nitrate and phosphate concentration are used for an indicator of quality loss. pH may fluctuate rapidly over the day depending on biological activities in the surrounding environment (Beer *et al*., 2006). In this study, all samples had pH levels below the permitted limits. The highest pH levels were 8.62, 8.33 and the lowest were 7.19, 7.42 in Site 1 and 2, respectively. Similar (Perez *et al*., 2008) and

Table 2: The results of microbiological analysis of marine water samples (n = 74)

			Dispersion (Site 1/2)					
Parameters	EU BWD[1]	TBWD[2] TWPD[3]	ND	ND-9	10-99	100-999	1000-9999	10000-99999
Total coliforms (cfu/100 mL)	10000 (100 mL)	10000 (100 mL)	65 (44/21)	-	2 (2/0)	5 (0/5)	-	2 (1/1)
Fecal coliforms (cfu/100 mL)	2000 (100 mL)	2000 (100 mL)	67 (46/21)	-	1 (0/1)	4 (0/4)	2 (1/1)	-
Fecal streptococci (cfu/100 mL)	Not mentioned*	1000 (100 mL)	67 (45/22)	-	4 (1/3)	2 (1/1)	1 (0/1)	-
Salmonella sp.	0 (1000 mL)	0 (1000 mL)	72 (46/26)	2 (1/1)	-	-	-	-

[1]EU Bathing Water Directive 2003; [2]Turkish Bathing Water Directive (TBWD), 2006; [3]Turkish Water Pollution Directive (TWPD), 2008; *Mandatory level is not specified

Table 3: The results of physico-chemical and toxicological analysis of marine water samples (n = 74)

Parameters	EU BWD[1] (MAC[2])	TBWD[3] TWPD[4] (MAC)	Site 1** (Dardanelles) (n:47)	Site 2** (Thracian Sea) (n:27)	Samples higher than permitted level (Total) Total (%)	Site 1 (%)	Site 2 (%)
pH	6-9	6-9	8.246±0.246[a]	8.001±0.247[b]	-	-	-
Conductivity* ($\mu S\ cm^{-1}$)	Not mentioned	Not mentioned	8963.830±243.744	8722.960±188.658	-	-	-
Nitrate * (NO_3) ($mg\ L^{-1}$)	Not mentioned	10	8.018±11.361	5.585±7.803	-	-	-
Phosphate* (PO_4) ($mg\ L^{-1}$)	Not mentioned	Not mentioned	0.076±0.244	0.054±0.039	-	-	-
Arsenic (As) ($mg\ L^{-1}$)	0.05	0.05	0.010±0.025	0.009±0.013	2 (2.70)	1 (2.12)	1 (3.70)
Chromium* (Cr) ($mg\ L^{-1}$)	Not mentioned	0.05	0.034±0.023	0.029±0.023	-	-	-
Cyanide* (CN) ($mg\ L^{-1}$)	Not mentioned	0.05	0.002±0.002	0.002±0.003	-	-	-

[1]EU Bathing Water Directive 2003; [2]MAC: Maximum Acceptable Concentration; [3]Turkish Bathing Water Directive (TBWD), 2006; [4]Turkish Water Pollution Directive (TWPD), 2008; [a, b]Means with different letters in a same line are significantly different from one another ($p<0.05$); *These parameters must be checked by the competent authorities when there is a tendency towards the eutrophication of the water; **The values which were determined under minumum detection limit, calculated as 0

lower (Ohline *et al*., 2007; Ayon *et al*., 1999) results were detected in previous studies. These differences may be originated from detection methods, sampling procedures, geographical conditions and climatic factors. On the other hand, significant differences in pH were found between the Site 1 and 2 ($p<0.05$). The results were consistent with the conductivity measurements. Conductivity, the ability of water to conduct on electrical current, is directly related to the amount of inorganic substances (salts and minerals). The concentration of Nitrate (NO_3) and Phosphate (PO_4) increase the conductivity of the water. It is also useful measurement to determine the salinity which is the main variable regulating the concentrations of dissolved materials (Gianguzza *et al*., 2002). In the present study, the conductivity of samples were found with the range of 8410-9310 $\mu S\ cm^{-1}$ in Site 1 and 8000-9250 $\mu S\ cm^{-1}$ in Site 2 ($p>0.05$). Likewise, Site 1 has higher nitrate and phosphate concentrations than Site 2 ($p>0.05$).

In coastal waters, trace metal concentrations tend not the correlate well with nutrients. Due to the biogeochemical and sedimentological process, coastal zone may introduce amounts of pollutants including trace metals (Zeri and Voutsinou-Taliadouri, 2003). Investigations of As, Cr and CN have special importance in environmental samples. In the present study, As concentration in Site 1 and 2 was detected 0.010 and 0.009 $mg\ L^{-1}$, respectively. As and its compounds have been used as agricultural pesticides. As coming from pesticide application is quickly absorbed onto soil particles in treated areas and lingers for years after treatments has ceased. Due to the high volatility of As and its compounds, they can be easily delivered to the coastal waters. On the other hand, lower (Byrd, 1988) and higher (De Boer *et al*., 2001; Tornes *et al*., 2002) results were detected in previous studies. The difference between the results may be due to the industrial facilities ranging from bronzing of material, hardening of shots to laser material and nature of the pesticides applied in agricultural areas.

Cr is a widespread aquatic environmental pollutant which is used in various industries such as in the galvanization, steel, leather and paint industries (Hirata *et al*., 2000). In this study, all samples were in acceptable levels and ranges belong to Site 1 was found less high ($p>0.05$). This range can be explained that there are seven large leather industrial establishments along the coast in Canakkale city. Moreover, a study performed in the five stations in Iskenderun Bay in Turkey showed that significant correlation was found between the metal concentration and temperature, pH, salinity parameters (Turkmen *et al*., 2004). In previous studies, higher (Hirata *et al*., 2000), lower (Dahab and Al-Madfa, 1997) and in similar (Yaymtas *et al*., 2007) concentrations were reported. The differences may be due to the settlement areas, deep of sampling point and determination method.

CN, natural compound found in plants and animals, most commonly inputs the marine waters via the commercial fishing (McClintock and Baker, 2001). The findings were slightly similar to the study obtained by Guven *et al*. (2001) and Dzombak *et al*. (2006).

CONCLUSION

During next 25 years, world population expected to exceed 7 billion people by the turn off the century and the coastal population anticipated doubling. Therefore, marine pollution problems must be effectively addressed at local, national and international levels in order to avert further habitat and ecological destruction. In conclusion, it is necessary cleaning of waste waters by infiltration of effluent from sewage treatment plants, controlling industrial factors and maintaining water quality controls because aquatic organisms (especially fish and mussels) accumulate heavy metals in their tissues or organs in higher quantities. This affects all ecosystems and human health by directly and indirectly as in food chain (Yaymtas *et al*., 2007). This study is a first in Dardanelles and Thracian Sea that the results supply valuable information about the microbiological and physico-chemical of coastal bathing water quality. Also the results may help as background reference concentrations in evaluating the water quality for next studies.

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