

## Investigation of Heavy Metal-Resistant Sediment Bacteria and Some Water Quality Parameters: A Case Study of Lake Bafa (Turkey)

Kacar, A.

DEU, Institute of Marine Sciences and Technology, Baku Bul. No:100, 35340, Inciralti, Izmir, Turkey

Received 3 Sep. 2014;

Revised 17 Nov. 2014;

Accepted 19 Nov. 2014

**ABSTRACT:** Surface waters such as lakes and rivers which are multipurpose fields used for drinking water supply, agricultural irrigation, fisheries and energy production are being threatened by domestic, industrial and agricultural pollution caused by rapidly growing population. Due to the insufficient numbers of studies on Lake Bafa, the largest coastal lake of Turkey, this study was conducted to investigate some physico-chemical properties of the lake's water and levels of fecal indicator bacteria in it. Additionally, bacteria species isolated from sediments that could be cultured and their heavy metal (Cu, Zn, Ni, Cr, Hg) and antibiotic resistance were determined. Levels of fecal coliforms (FC) and fecal streptococci (FS) were determined through membrane-filtration techniques. While fecal coliform levels detected in the three sampling stations were above the limit value, fecal streptococci levels were below the limit value. Seventeen isolates identified through the 16S rDNA analysis were determined to be the members of the genus *Bacillus* and *Arthrobacter*. The resistance displayed by the isolates to heavy metals was in the following order: Cu > Zn > Ni > Cr > Hg. The isolates were also resistant to ampicillin and tetracycline. The results of this preliminary study suggest that the lake which serves agriculture, fishing and recreation purposes should be regularly monitored in terms of public health.

**Key words:** Fecal indicator bacteria, Heavy metals, *Bacillus*, *Arthrobacter*, Lake Bafa

### INTRODUCTION

Surface waters like lakes and rivers which serve several purposes such as drinking water supply, agricultural irrigation, fisheries and energy production are threatened by rapidly growing population, developing technologies, and domestic and agricultural pollution (Filik-Isçen *et al.*, 2008). Aquatic ecosystems composed of many components are affected by the pollution either directly or indirectly. The majority of pollution in the water column is caused by sewage, industrial and agricultural wastes. Wastewater can lead to public health problems because it includes pathogenic bacteria and toxic components. Therefore, it is important to determine the quality of water and to maintain that quality within standards (Toroglu & Toroglu, 2009; Ouyang *et al.*, 2006).

Industrial and agricultural activities lead to the release of toxic metals which pose a danger to human health and ecosystems. In many aquatic systems, metals are the major pollutants and anthropogenic sources (Matyar *et al.*, 2010). In addition, sediment is the matrix containing organic or inorganic detrital materials and

plays the major role in storing and transporting contaminants. Heavy metals accumulate in sediment rather than in the water column and thus cause problems. Sediment is also an important habitat for many organisms and a food source for many species. The presence of heavy metals in the sediment affects the degradation of organic chemicals by microorganisms and the ecology of the environment. Bacteria in the sediment adapt to the environment in terms of survival and transport characteristics and develop resistance. In other words, they can tolerate high concentrations of heavy metals. Bacteria are able to develop various resistance mechanisms. These mechanisms are related to the formation and sequestration of heavy metal complexes. One of these mechanisms is that in which metals are converted into a less toxic form and are directly released from the cell into the water. Briefly, microorganisms play an important role in the biogeochemical cycles of metals and in the remediation of an environment contaminated with metals. Many bacterial strains carry genetic determinants conferring resistance to metals such as

\*Corresponding author E-mail: asli.kacar@deu.edu.tr

Hg<sup>+2</sup>, Ag<sup>+2</sup>, Cu<sup>+2</sup>, Ni<sup>+2</sup>, Cd<sup>+2</sup>. Most of them are present in plasmids and transposons. Soil-, water-, industrial waste- and clinical-borne bacteria have similar mechanisms too (Unaldi-Coral *et al.*, 2005). Especially, bacterial species such as *Pseudomonas* and *Bacillus* exhibit a metal absorption capacity due their high metal-binding capacity. Cell wall charges, the high surface to volume ratio, S-layer protein (surface layer protein) and metal binding proteins contribute to the high metal binding capacity of the bacteria (Chigor *et al.*, 2013; Li & Ramakrishna, 2011; Kistemann *et al.*, 2002). Due to these metal-resistant characteristics, bacteria are considered as a potential to be used to control heavy metal contamination (Altug & Balkis, 2009). Heavy metal resistance and concomitant antibiotic resistance is not an incidental phenomenon; it is due to the presence of these elements in environmental pollutants and their similar mechanisms (Kimiran-Erdem *et al.*, 2007). Ecological studies have shown that heavy metal and antibiotic resistance are important issues throughout the world. In particular, it is known that plasmids carry antibiotic and metal resistant genes. Aquatic bacteria are the most important organisms which adsorb, accumulate and transform metals in the food chain (Matyar *et al.*, 2010). In particular, antibiotic resistance is a worldwide problem, because available antibiotics are becoming unusable one by one (Toroglu & Toroglu, 2009).

Another pollution problem of surface waters is that these waters are frequently contaminated with fecal matter coming from domestic and agricultural wastes. Human and warm-blooded animal feces are important hygienic parameters in natural waters. In USEPA reports, fecal bacteria have been reported to be the most significant source of contamination that poses health risk in lakes and rivers. In particular, fecal coliforms are serious indicators of the presence of sewage and farm animals' (chickens, cows, pigs, horses, etc.) waste in the environment. The presence of these bacteria in surface water is important for wildlife and agricultural activities too (Kim *et al.*, 2005; Chigbu *et al.*, 2004; Mara & Horan, 2003). Coliforms and enterococci are important indicator organisms used in monitoring water quality throughout the world. Isolation of these indicators is the sign of fecal contamination in water indicates that there may be microbial pathogens in the environment as a result of which the area can pose risks to public health (Chigor *et al.*, 2013; WHO 2008; Pruss *et al.*, 2002).

There are not enough studies conducted on the environmental characteristics and microbial diversity of Lake Bafa, the largest coastal lake of Turkey. Therefore, this study was conducted to investigate some physico-chemical properties of the lake's water

and levels of fecal indicator bacteria in it. In addition, bacteria species that could be cultured in sediments were isolated and their heavy metal and antibiotic resistance was determined.

## MATERIALS & METHODS

Lake Bafa located in the west of Turkey is a coastal dam lake which was disconnected from the Aegean Sea by the alluvial mass brought by the Büyük Menderes River. Lake Bafa located in the southeast of the Büyük Menderes River delta is of ecological importance due to the wide diversity of species living in it. The lake was declared a Nature Reserve in 1989 and a Natural Park in 1994. The maximum depth of the lake is 20 meters. Its surface area is about 7,500 hectares. Lake Bafa is fed by regular floodwaters of Büyük Menderes River, underground waters and surface waters of seasonal streams. However, unfortunately, due to supply problems, the water level in the lake is constantly dropping, and the lake is becoming contaminated. The water level has dropped about 2 meters in the last 20 years. The lake is normally brackish, but because of the drop in the water level, its salinity level has increased much. Chemical wastes of industrial facilities and household wastes of residential areas in Usak, Denizli and Aydın enter Lake Bafa through the Büyük Menderes River. On the other hand, use of fertilizers and pesticides in agricultural areas around the lake also affects the lake negatively. In some periods, elevated levels of toxic algae are observed in the lake (Yabanli *et al.*, 2013; Pazi *et al.*, 2014). Within the scope of this study, samples were collected from 17 stations for the water quality analysis of the lake and from 10 stations for the analysis of bacteria in the sediment.

Water samples were collected from the 17 stations in summer (Fig.1). The membrane filter technique was carried out for the enumeration of fecal indicator bacteria. The water samples were diluted, and each dilution was filtered through sterile nitrocellulose filters (47 mm diameter, 0.45µm pore size) (Millipore Corp., Bedford, Mass.). For FC, membrane filters were transferred to 50 mm Petri plate containing 2 ml of m-FC broth (Merck, Darmstadt, Germany) and incubated for 24 h at 44.5°C. All blue colored colonies were counted as fecal coliforms. For FS, membrane filters were transferred to the petri plates containing 2ml of Azide Dextrose Broth (Merck, Darmstadt, Germany) and incubated at 37.5°C for 24–48h and light and dark red colored colonies were counted as fecal streptococci (APHA, 1998; Isobe *et al.*, 2004).

Sediment samples were collected using Van Veen Grab from surface sediments in Lake Bafa at 10

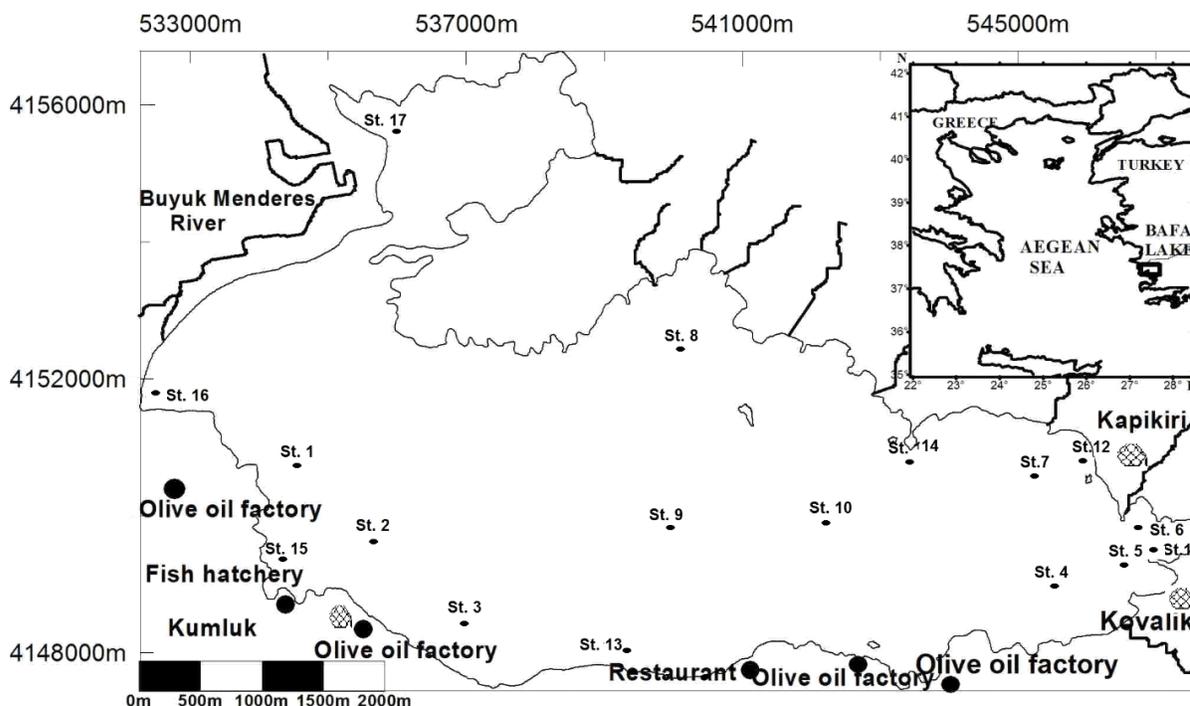


Fig. 1. Location of sampling stations in Lake Bafa ( settlement area)

locations (Sta.1-Sta.10) which are shown in Fig. 1. Samples were diluted ( $10^{-7}$ ) and inoculated on Nutrient Agar Medium by the spread plate method. The agar plates were incubated at  $26^{\circ}\text{C}$  during 2-3 days. Seventeen bacterial colonies were selected and purified on Nutrient Agar plates (Nithya & Pandian, 2009; Nithya *et al.*, 2011; Kacar & Kocyigit, 2013). Genomic DNA's of isolates were extracted using a Bacterial Genomic DNA Isolation Kit (Zymo Research, USA) after incubation in Nutrient Agar for 1 day at  $26^{\circ}\text{C}$ . The universal bacterial primers (27F and 1522R) were used for polymerase chain reaction (PCR) amplification of 16S rDNA. FastStart Taq DNA Polymerase dNTPack Kit (Roche, Germany) and 0.2 M of primers, 10 ng of template DNA were used for PCR. PCR was performed for 30 cycles (5 min denaturing step at  $95^{\circ}\text{C}$  in the first cycle; 55 s denaturing at  $95^{\circ}\text{C}$ , 40 s annealing at  $52^{\circ}\text{C}$ , and 1.5 min extension at  $72^{\circ}\text{C}$ , with a final extension step at  $72^{\circ}\text{C}$  for 7 min (Kacar & Kocyigit, 2013). The DNA sequence analyses were performed using an automatic sequence analyzer system (ABI Prism 3100) (REFGEN Biyoteknoloji, Turkey). The chimera check tool of the Ribosomal Database Project (<http://rdp.cme.msu.edu>) was used to check possible chimeric sequences. The sequenced results were compared with 16S rDNA sequences in NCBI database (<http://www.ncbi.nlm.nih.gov>) by BLAST searching. A phylogenetic tree was constructed by the

neighbor-joining method using the MEGA 6 program (Thompson *et al.*, 1994; Altschul *et al.*, 1997).

The Minimum Inhibitory Concentration (MIC) for each bacterial isolate for five heavy metals was determined using Mueller–Hinton Agar (Difco, USA) containing  $\text{HgCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{CuCl}_2$ ,  $\text{ZnCl}_2$  and  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ . Metal stock solutions were sterilized at  $0.2\ \mu\text{m}$  membrane filter and concentrations of heavy metals were ranged from 0.01 to 10.0 (mM). Agar plates were incubated at  $26^{\circ}\text{C}$  for 24-48 h. (Matyar *et al.*, 2008; Kacar & Kocyigit, 2013).

The antibiotic susceptibility of the bacterial isolates was determined by an agar disk diffusion test using Mueller-Hinton Agar (Difco, USA) and six antibiotics: Ampicillin (AM,  $10\ \mu\text{g}$ ), Chloramphenicol (C,  $30\ \mu\text{g}$ ), Tetracycline (TE,  $30\ \mu\text{g}$ ), Gentamicin (CN,  $10\ \mu\text{g}$ ), Tobramycin (TM,  $10\ \mu\text{g}$ ), and Vancomycin (VA,  $30\ \mu\text{g}$ ) (Bioanalyse, Turkey). The bacterial strains were grown overnight in LB liquid medium and spread on Mueller Hinton agar using sterile swabs. Antibiotic discs were placed on the agar plates and incubated at  $30^{\circ}\text{C}$ . The diameter of the inhibition zones was measured after 24-48 h. The bacterium was classified as resistant (R), intermediate (I) or susceptible (S), according to the information supplied by the manufacturer (BBL, MD, USA) (Matyar *et al.*, 2008; Kacar & Kocyigit, 2013).

Physico-chemical parameters such as dissolved oxygen (DO) (Winkler methods), pH, temperature and salinity of the water were measured by a WTW pH/Cond 304i/Set.

## RESULTS & DISCUSSION

A summary of water quality data from 17 stations on the water temperature, salinity, pH and dissolved oxygen (DO) is presented in Table 1. Lake Bafa is generally brackish, and salinity values at the stations did not show notable differences except at stations 16 and 17. The pH of the aquatic environment is an important indicator of the water quality and the extent of the pollution in the watershed areas. A pH range of 6.5–8.5 is consistent with the guidelines suggested by WHO (2003). The pH values assessed for Lake Bafa ranged between 7.65 and 9.08, which indicate the moderately alkaline nature of the lake water. The range of temperature values varied between 26.5 and 32°C, and 27.3 and 29.1°C in the surface and bottom water, respectively. The concentrations of DO varied between 3.28 and 11.29 mg/L in the all stations. High DO values were recorded at coastal stations 12, 13 and 15. In these stations were thought to be high primary production because of summer sampling period. On the other hand, low DO values were found at St16 and bottom water at St3 and St8. In lakes, deep water becomes depleted of oxygen depending on several factors. For instance, if microbial activity is very high, the excessive organic matter leads to oxygen depletion. In addition, the water mass becomes stratified during the summer through temperate climates. After stratification, bottom layers become anoxic (Madigan *et al.*, 2003; Kamala-Kannan & Krishnamoorthy, 2006).

Microbiological results obtained from the analysis of 17 water samples taken from Lake Bafa are given in Table 2. In Turkey, according to the Water Pollution Control Regulations (The Official Gazette, 2004) the standard number of fecal streptococci and fecal coliform colony per 100 ml water sample should not exceed 100 and 200, respectively. High concentrations of fecal coliforms were found at the coastal stations St11 ( $4 \times 10^2$  cfu 100/ml), St12 ( $2.1 \times 10^2$  cfu 100/ml) and St8 surface and bottom water ( $1 \times 10^3$  cfu 100/ml and  $3 \times 10^2$  cfu 100/ml, respectively). On the other hand, results of fecal streptococci were determined below the boundary value in all the sampling stations. Lake Bafa receives anthropogenic inputs coming from hotels, restaurants, fish farms, agriculture areas, olive oil factories around it and from the river flowing into it. The results indicate that there is no significant microbial pollution caused by untreated human and animal waste except

three stations (St 8, St11 and St12). Although confined to a few stations, the high number of indicator bacteria poses a risk of fecal contaminated water-borne diarrhea, dysentery, typhoid and infections of skin, ear, nose etc. for those who make use of the lake for swimming, fishing and other recreational purposes. As a result, it is essential to determine sources like St8, St11 and St12 with high fecal coliform levels likely to cause non-point pollution and inputs affecting the lake, and to frequently monitor the entire region.

Fecal indicator bacteria concentrations were compared to those of other studies carried out in lakes of Turkey. In their study (2008), Filik-Isken *et al.* found high summer fecal coliform levels at some stations in Lake Ulubat receiving run-off from a river. They thought that this increase in fecal coliform bacteria concentrations in Lake Ulubat might be due to the seasonal differences in human activities which increased in the summer. Working with the same indicator bacteria, Toroglu & Toroglu (2009) found that bacterial contamination level was high at all sampling stations ( $4.6 \times 10^2$ – $1.1 \times 10^3$  cfu 100/ml) in Lake Gölbaşı, which was exposed to human, industrial and agricultural wastes.

Phylogenetic analysis using 16S rDNA indicated that the 17 sediment strains belong to genus *Bacillus* and *Arthrobacter* (*B. cereus* BF1, *B. thuringiensis* BF2, *B. atrophaeus* BF3, *B. aguilmaris* BF4, *B. thuringiensis* BF5, *B. aguilmaris* BF6, *B. cereus* BF7, *B. subtilis* BF8, *B. simplex* BF9, *B. amyloliquefaciens* BF10, *B. drementensis* BF11, *B. aguilmaris* BF12, *B. fusiformis* BF13, *B. subtilis* BF14, *B. cereus* BF15, *A. nitroguajacolicus* BF16 and *B. pumilus* BF17) reported in this article were submitted to GenBank and assigned the accession numbers KJ524499, KJ524500, KJ524501, KJ524502, KJ524503, KJ524504, KJ524505, KJ524506, KJ524507, KJ524508, KJ524509, KJ524510, KJ524511, KJ524512, KJ524513, KJ524514 and KJ524515, respectively (Fig. 2).

Minimum inhibitory concentrations of heavy metals are shown in Table 3. Sediment isolates showed higher tolerance to Cu, Zn and Ni. In the case of copper and zinc *B. cereus* BF1, *B. thuringiensis* BF2, *B. thuringiensis* BF5, *B. aguilmaris* BF 12, *B. subtilis* BF14, *Arthrobacter nitroguajacolicus* BF16 exhibited high resistance to both copper and zinc compared to other isolates and they showed no growth in 6mM. Besides, nine strains (*B. cereus* BF1, *B. thuringiensis* BF2, *B. atrophaeus* BF3, *B. thuringiensis* BF5, *B. aguilmaris* BF6, *B. cereus* BF7, *B. subtilis* BF8, *B. aguilmaris* BF 12, *B. subtilis* BF14,

**Table 1. Values of physico-chemical parameters in Lake Bafa**

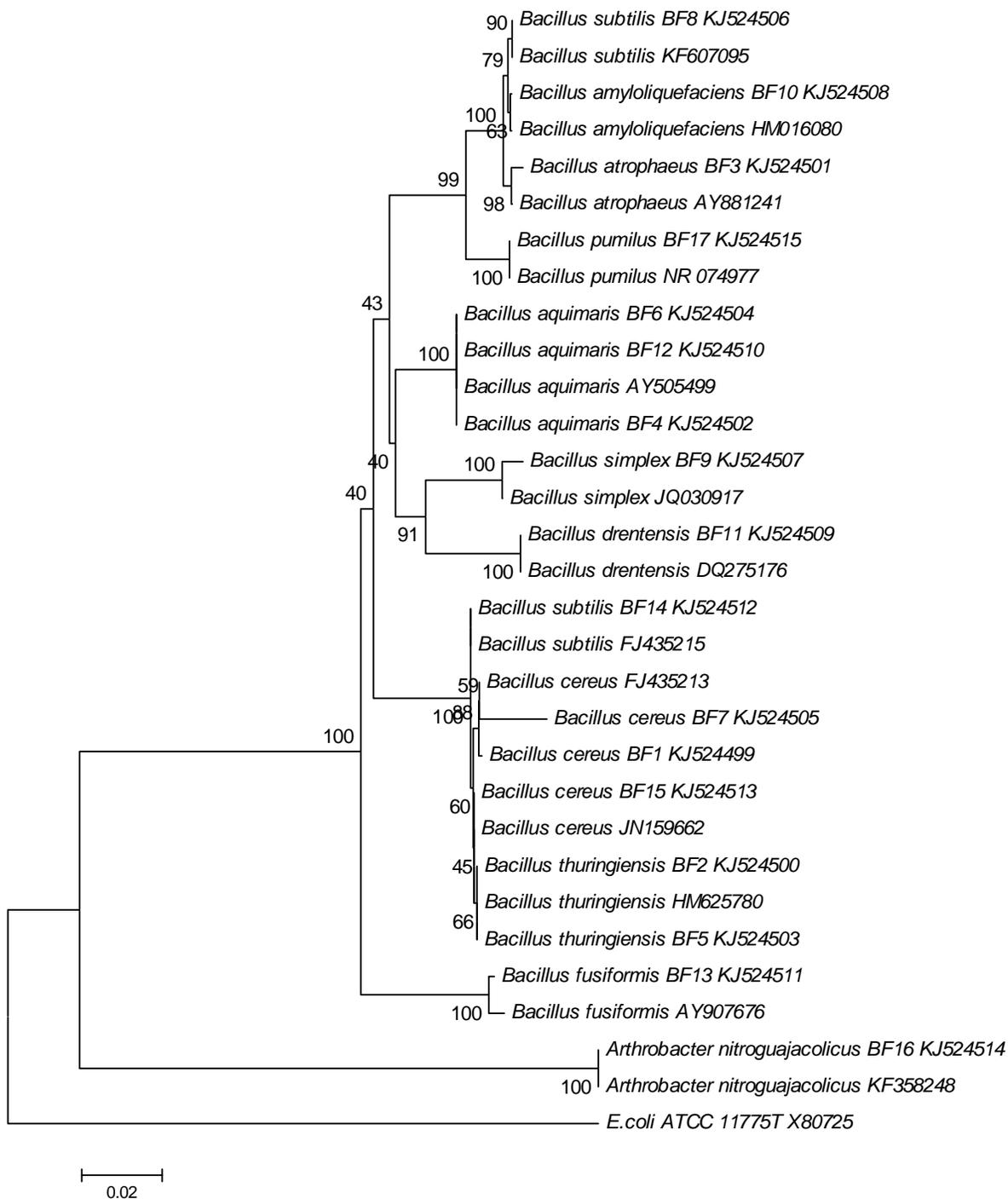
Station No.	Physico-chemical parameters				
	Surface	Temperature	Salinity	pH	DO
St 1		28.1	14.2	8.30	6,68
St 2		27.9	14.2	8.25	5,15
St 3		28.7	14.1	8.32	7,05
St 4		29.2	14.1	8.39	7,55
St 5		29.3	14.2	8.44	7,2
St 6		28.6	14.3	8.22	6,05
St 7		29.6	14.3	8.37	7,46
St 8		28.5	14.1	8.35	7,59
St 9		28.5	14.0	8.32	7,17
St 10		28.7	14.1	8.36	7,4
St 11		32.0	14.2	8.40	6,49
St 12		28.5	14.7	8.55	9,33
St 13		28.6	14.1	8.72	11,2
St 14		28.7	14.0	8.38	8,81
St 15		26.5	14.3	8.21	11,29
St 16		26.8	16.3	7.99	3,65
St 17		29.7	17.4	9.08	6,89
<b>Bottom</b>					
St 1 (3.5m)		27.9	14.2	8.28	6,84
St 2 (4m)		27.6	14.2	8.21	5,3
St 3 (8m)		27.6	14.0	8.13	3,9
St 4 (3m)		29.1	14.1	8.38	6,96
St 7 (10m)		29.0	14.2	8.27	6,04
St 8 (14m)		27.3	13.9	8.13	3,28
St 9 (15m)		27.4	13.1	7.65	5,8
St 10 (12m)		28.4	14.1	8.33	6,43

Note: Samples were collected at only one depth at the shore stations and the stations 5, 6, and 11-17, because they were at shallow waters

**Table 2. Concentrations of fecal indicator bacteria from Lake Bafa**

Station No.	Fecal Coliforms (cfu/100ml)		Fecal Streptococci (cfu/100ml)	
	Surface	Bottom	Surface	Bottom
St 1	8.5x10 <sup>1</sup>	5x10 <sup>1</sup>	3	<1
St 2	<1	<1	<1	<1
St 3	2x10 <sup>1</sup>	<1	<1	<1
St 4	<1	<1	<1	<1
St 5	<1	-	<1	-
St 6	<1	-	<1	-
St 7	1x10 <sup>1</sup>	<1	5	<1
St 8	1x10 <sup>3</sup>	3x10 <sup>2</sup>	<1	<1
St 9	<1	<1	<1	<1
St 10	<1	<1	<1	<1
St 11	4x10 <sup>2</sup>	-	5	-
St 12	2.1x10 <sup>2</sup>	-	5x10 <sup>1</sup>	-
St 13	1.5x10 <sup>2</sup>	-	<1	-
St 14	9x10 <sup>1</sup>	-	1.2x10 <sup>1</sup>	-
St 15	7.5x10 <sup>1</sup>	-	<1	-
St 16	3.5x10 <sup>1</sup>	-	<1	-
St 17	1x10 <sup>2</sup>	-	1x10 <sup>1</sup>	-

Note: Samples were collected at only one depth at the shore stations and the stations 5, 6, and 11-17, because they were at shallow waters



**Fig. 2.** The phylogenetic dendrogram of 17 isolates based on partial 16S rDNA gene sequence (approx. 1400bp)

*B. cereus* BF15, *A. nitroguajacolicus* BF16) were resistant to nickel. The susceptibility of isolates to chromium ranges from 2 mM to 3 mM. On the other hand, mercury was the highest toxic metal for all of the isolates. When all the metals were considered, the following six strains were found to be the most resistant strains: *B. cereus* BF1, *B. thuringiensis* BF2,

*B. thuringiensis* BF5, *B. aquimaris* BF 12, *B. subtilis* BF14, *A. nitroguajacolicus* BF16.

Similarly, in the present study, we found a relationship between heavy metal and antibiotic tolerance. In antibiotic assay, the isolates exhibited a maximum resistance to Ampicillin and Tetracycline. Especially, the isolates *B. cereus* BF1, *B. thuringiensis* BF2, *B.*

Table 3. MIC values of sediment isolates against heavy metals

Isolates	Heavy metals (mM)				
	Hg	Cr	Cu	Zn	Ni
B. cereus BF1	0.01	3.0	6.0	6.0	4.0
B. thuringiensis BF2	0.01	3.0	6.0	6.0	4.0
B. atrophaeus BF3	0.01	3.0	4.0	2.0	4.0
B. aguilmaris BF4	0.01	2.0	4.0	2.0	2.0
B. thuringiensis BF5	0.01	3.0	6.0	6.0	4.0
B. aguilmaris BF6	0.01	2.0	4.0	6.0	4.0
B. cereus BF7	0.01	3.0	4.0	6.0	4.0
B. subtilis BF8	0.01	3.0	6.0	1.0	4.0
B. simplex BF9	0.01	2.0	6.0	0.6	2.0
B. amyloliquefaciens BF10	0.01	2.0	2.0	1.0	2.0
B. drentensis BF11	0.01	2.0	4.0	2.0	2.0
B. aguilmaris BF12	0.01	3.0	6.0	6.0	4.0
B. fusiformis BF13	0.01	2.0	4.0	2.0	2.0
B. subtilis BF14	0.01	3.0	6.0	6.0	4.0
B. cereus BF15	0.01	3.0	6.0	4.0	4.0
Arthrobacter nitroguajacolicus BF16	0.01	3.0	6.0	6.0	4.0
B. pumilus BF17	0.01	3.0	4.0	4.0	2.0

thuringiensis BF5, B. aguilmaris BF 12, B. subtilis BF14, A. nitroguajacolicus BF16 showed high resistance compared to the other isolates (Table 4). In a study by Yilgor *et al.*, (2012) in Bafa Lake, heavy metal contents of sediments were determined and the mean metal concentrations in sediments obtained decreased in the following order; Fe > Mn > Ni > Cr > Zn > Cu > Pb > Hg. According to their results, the mean concentrations of metal were lower than the background levels in earth crust except Cr, Ni and Hg. The highest Cr and Ni content in the lake were derived from the mafic and ultramafic rocks cropping out in the drainage area of Büyük Menderes Rivers. The source of Hg could be attributed to the pesticides which are used in the agricultural area around the lake and the other source of this metal was anthropogenic inputs via the Büyük Menderes River. These results showed that the identified bacteria are able to survive in sediments polluted by heavy metals and they could develop resistance to heavy metals. Bacillus species have been studied extensively, because of their taxonomic and ecological significance and their applicability for biotechnological and industrial purposes (Harper *et al.*, 2003; Borsodi *et al.*, 2003).

In various studies, the presence of Bacillus species was reported in lake sediments. Borsodi *et al.*, (2003) characterized that B. pseudofirmus, B. halmapalus, B. cohnii, B. marinus which were isolated from Lake Valencei (Hungary). They suggested that sediment bacteria present in the upper sediment layer

of shallow lakes could be influenced by strong wind-wave action and that oxygen could be the main electron acceptor; therefore, mainly aerobic respiratory microbes (e.g. Bacillus species) are widely present in such environments or are responsible for the degradation of organic carbon. In another study, Kamala-Kannan & Krishnamoorthy (2006) investigated Publicat Lake (India) contaminated with mercury and other metals. In the study, they analyzed heavy metal tolerance of B. cereus strains isolated from the lake's sediments, and determined that they exhibited high metal tolerance. In the same study, chromium and nickel were identified as the least toxic metals for all the isolates. They also analyzed the physico-chemical parameters and determined that oxygen, salinity and pH values of the lake were suitable for the growth, reproduction and similar metabolic activities of organisms, and then reported that solubility, toxicity and bioavailability of toxic metals to bacteria was associated with this too.

Removal of metals from aquatic environment via biological methods has some potential advantages. In particular, many of gram-positive bacteria have high metal biosorption capacity. Especially, Bacillus species are the most studied organisms in terms of metal removal. These species are of importance because they can easily be cultured and display high tolerance to the heavy metal toxicity (Ince-Yilmaz, 2003; Samanta *et al.*, 2012). In a similar study conducted by Ince-Yilmaz (2003) on the Tigris River

Table 4. Antibiotic resistance of sediment isolates

Isolates	Antibiotic Resistance (mm)					
	Ampicillin	Tetracycline	Chloramphenicol	Vancomycin	Gentamicin	Tobramycin
<i>B. cereus</i> BF1	6	17	22	16	26	18
<i>B. thuringiensis</i> BF2	6	16	16	15	24	16
<i>B. atrophaeus</i> BF3	14	28	18	18	30	29
<i>B. aguimaris</i> BF4	18	32	28	22	30	26
<i>B. thuringiensis</i> BF5	6	8	19	14	24	23
<i>B. aguimaris</i> BF6	22	30	30	26	30	26
<i>B. cereus</i> BF7	10	8	29	19	28	18
<i>B. subtilis</i> BF8	14	22	28	24	34	32
<i>B. simplex</i> BF9	8	32	18	22	29	28
<i>B. amyloliquefaciens</i> BF10	16	30	21	23	31	30
<i>B. drentensis</i> BF11	35	38	38	35	36	36
<i>B. aguimaris</i> BF12	6	15	21	16	23	17
<i>B. fusiformis</i> BF13	10	18	9	20	28	25
<i>B. subtilis</i> BF14	6	12	14	16	22	17
<i>B. cereus</i> BF15	14	26	13	23	23	14
<i>Arthrobacter nitroguajacolicus</i> BF16	6	10	21	16	26	23
<i>B. pumilus</i> BF17	18	15	12	20	29	27

in the eastern region of Turkey, *Bacillus* sp. strain EB1 isolated from the metal-contaminated sediment was investigated in terms of its heavy metal accumulation capacity. When tested for the minimum inhibitory concentration in terms of its resistance to heavy metals, the toxicity order was determined as follows: Cd = Co > Cu > Ni > Zn > Mn. Also, a similar relationship was observed between the heavy metal resistance and metal tolerance. It was found to be resistant to all of the tried  $\beta$ -lactams such as Ampicillin, Mezlocillin and Piperacillin but susceptible to amino glycosides; Gentamicin, Amikacin and Tobramycin. At the end of the study, it was reported that *Bacillus* isolate would exhibit high metal biosorption capacity under aerobic conditions, and that it might be used as an in situ bioremediation medium in metal contaminated in aquatic systems. In a similar study, (Li & Ramakrishna, 2011; Samanta *et al.*, 2012) reported that the important step in the development of bioremediation systems was the identification of microorganisms capable of surviving in areas contaminated with heavy metals, and that in the next steps, biochemical and molecular

mechanisms should be investigated in detail in order to ensure and increase the resistivity.

## CONCLUSIONS

It is concluded that the results confirmed that the lake, except for some locations, was not currently facing any serious microbial contamination-related environmental pollution risk. However, although it is not possible to reach a definitive conclusion since the study was a preliminary study, it is considered that the fecal indicator bacteria analysis should be regularly conducted and that contamination sources should be carefully monitored because they are likely to pose serious health risks to beneficiaries of this large lake mainly used for recreational purposes. In addition, the bacteria isolated from the sediment found to be resistant to some heavy metals. The MICs of heavy metals were different for each strain but the general order of resistance to the metals was found to be as Cu > Zn > Ni > Cr > Hg. As stated in similar studies, it is thought that isolation and identification of the isolates able to survive in environments susceptible to heavy metal pollution likely to be

caused by industrial wastes is of importance for the elimination of this pollution and, that these isolates can be used in bioremediation studies to be conducted in the future.

#### ACKNOWLEDGEMENTS

Financial support by the Turkish Scientific and Technical Research Council (TUBITAK) (Grant No:110Y002) for this work is gratefully appreciated and I would like to extend my sincerest thanks to Prof. Dr. Erdeniz Özel.

#### REFERENCES

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. (1997). Gapped BLAST and PSIBLAST: a new generation of protein database search programs, *Nucleic. Acids. Res.*, **25**, 3389-3402.
- Altug, G. and Balkis, N. (2009). Levels of some toxic elements and frequency of bacterial heavy metal resistance in sediment and sea water. *Environ. Monit. Assess.*, **149**, 61-69.
- APHA (American Public Health Association). (1998). Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association. Washington, DC.
- Borsodi, A. K. Vldar, P., Cech, G., Gedeon, G., Beszteri, B., Micsinai, A., Reskone, M. N. and Marialigeti, K. (2003). Bacterial activities in the sediment of Lake Velencei, Hungary. *Hydrobiologia*, 506-509, 721-728.
- Chigbu, P., Gordon, S. and Strange, T. (2004). Influence of inter-annual variations in climatic factors on fecal coliform levels in Mississippi sound. *Water Res.*, **38**, 4341-4352.
- Chigor, V. N., Sibanda, T. and Okoh, A. I. (2013). Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa. *Environ. Sci. Pollut. Res.*, **20**, 4125-4136.
- Filik-Iscen, C., Emiroglu, Ö., Ilhan S., Arslan, N., Yilmaz, V. and Ahiska, S. (2008). Application of multivariate statistical techniques in the assessment of surface water quality in Uluabat Lake, Turkey. *Environ. Monit. Assess.*, **144**, 269-276.
- Harper, D. M., Childress, R. B., Harper, M. M., Boar, R. R., Hickley, P., Mills, S. C., Otieno, N., Drane, T., Vareschi, E., Nasirwa, O., Mwatha, W. E., Darlington, J. P. E. C., Escuté- Gasulla, X. (2003). Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia*, 506-509.
- Ince-Yilmaz, E. (2003). Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. *Res. Microbiol.*, **154**, 409-415.
- Isobe, K.O., Tarao, M., Chiem, N.H., Minh, L.Y. and Takada, H. (2004). Effect of environmental factors on the relationship between concentrations of coprostanol and fecal indicator bacteria in tropical (Mekong Delta) and temperate (Tokyo) fresh- waters. *Appl. Environ. Microb.*, **70** (2), 814-821.
- Kacar, A. and Kocyigit, A. (2013). Characterization of Heavy Metal and Antibiotic Resistant Bacteria Isolated from Aliaga Ship Dismantling Zone, Eastern Aegean Sea, Turkey. *Int. J. Environ. Res.*, **7**(4), 895-902.
- Kamala-Kannan, S. and Krishnamoorthy, R. (2006). Isolation of mercury resistant bacteria and influence of abiotic factors on bioavailability of mercury—A case study in Pulicat Lake North of Chennai, South East India. *Sci. Total Environ*, **367**, 341-353.
- Kim, G., Choib, E. and Lee, D. (2005). Diffuse and point pollution impacts on the pathogen indicator organism level in the Geum River, Korea. *Sci. Total Environ.*, **350**, 94-105.
- Kimiran-Erdem, A., Arslan, E. O., Sanli-Yurudu, N. O., Zeybek, Z., Dogruoz, N. and Cotuk, A. (2007). Isolation and Identification of Enterococci from Seawater Samples: Assessment of Their Resistance to Antibiotics and Heavy Metals. *Environ. Monit. Assess.*, **125**, 219-228.
- Kistemann, T., Claßen, T., Koch, C., Dagendorf, F., Fischeider, R., Gebel, J., Vacata, V. and Exner, M. (2002). Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. *Appl. Environ. Microbiol.*, **68**, 2188-2187.
- Li, K. and Ramakrishna, W. (2011). Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth. *J. Hazard. Mater.* **189**, 1-2, 531-539.
- Madigan, M. T., Martinko, J. M. and Parker, J. (2003). *Brock Biology of Microorganisms* (10th ed.). New Jersey: Pearson Education.
- Mara, D. and Horan, N. (2003). *The Handbook of Water and Wastewater Microbiology*. Academic Press, London, UK, p. 819.
- Matyar, F., Akkan, T., Uçak, Y. and Eraslan, B. (2010). *Aeromonas* and *Pseudomonas*: antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (northeast Mediterranean Sea). *Environ. Monit. Assess.*, **167**, 309-320.
- Matyar, F., Kaya, A. and Dinçer, S. (2008). Antibacterial agents and heavy metal resistance in gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey, *Sci. Total Environ.*, **407**, 279-285.
- Nithya, C., Gnanalakshmi, B. and Pandian, S. K. (2011). Assessment and characterization of heavy metal resistance bacteria in Palk Bay sediment. *Mar. Environ. Res.*, **7**(1), 283-294.
- Nithya, C. and Pandian, S. K. (2009). Isolation of heterotrophic bacteria from Palk Bay sediments showing heavy metal tolerance and antibiotic production. *Microbiol. Res.*, **165**, 578-593.
- Ouyang, Y., Nkedi-Kizza, P., Wu, Q. T., Shinde, D. and Huang, C. H. (2006). Assessment of seasonal variations in surface water quality. *Water Res.*, **40**, 3800-3810.

- Pazi, I., Gonul L.T., and Kucuksezgin F. (2014). Pesticide and PCB residues in biotic and abiotic environment in Lake Bafa (in Turkish). *Ege J. Fish. Aqu. Sci.* **30**(4), 175-182.
- Pruss, A., Kay, D., Fewtrell, L. and Bartram, J. (2002). Estimating the burden of disease from water sanitation and hygiene at a global level. *Environ. Heal. Perspect.*, **110**, 537–542.
- Samanta, A., Bera, P., Khatun, M., Sinha, C., Pal, P., Lalee, A. and Mandal, A. (2012). An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain *Bacillus* sp. isolated from municipal waste. *J. Microbiol. Biotech. Res.*, **2** (1), 178-189.
- The Official Gazette. (2004). Water Pollution Control Regulations (in Turkish). No. 25687, 31 December, Ankara.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting position specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673-4680.
- Toroglu, E. and Toroglu, S. (2009). Microbial pollution of water in Golbasi lake in Adiyaman, Turkey, *J. Environ. Biol.* **30**(1), 33-38.
- Unaldi-Coral, M. N., Korkmaz, H., Arıkan, B. and Coral, G. (2005). Plasmid mediated heavy metal resistances in *Enterobacter* spp. isolated from Sofulu landfill, in Adana, Turkey. *Ann. Microbiol.*, **55**(3), 175-179.
- WHO (World Health Organization). (2008). Guidelines to drinking water quality (3rd Edn). Vol. 1 World Health Organization. Geneva, Switzerland.
- WHO (World Health Organization). (2003). Guidelines for safe recreational-water environments, vol.1, coastal and fresh waters, World Health Organization, Geneva, Switzerland.
- Yabanlı, M., Coşkun, Y., Öz, B., Yozukmaz, A., Sel, F. and Öndeş, S. (2013). Determination Of The Content Of Heavy Metal In The Lake Water And Sea Bass (*Dicentrarchus Labrax*) Obtained From Bafa Lake And Evaluation In Terms Of Public Health (in Turkish). *Bornova Vet. Bil. Derg.*, **35** (49), 15-23.
- Yılgor, S., Kucuksezgin, F. and Erdeniz Ozel, E. (2012). Assessment of Metal Concentrations in Sediments from Lake Bafa. (Western Anatolia): An Index Analysis Approach. *Bull Environ Contam Toxicol.*, **89**, 512–518.