The evaluation of ecological status in Tunca (Tundzha) River (Turkish Thrace) based on environmental conditions and bacterial features

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ABSTRACT

It is inevitable that the running waters which are used for a lot of different activities like fishing, irrigation, domestic water usage are under threat because of the settlements, industrial or agricultural activities. To provide the sustainable usage of these ecosystems we have to know their current features and their balance under changing environmental conditions. In the present study, the ecological status of Tunca (Tundzha) River which is located on Turkish Thrace was evaluated based on environmental conditions and bacterial features of the river. For this aim, the research has been carried out at 5 different stations in the river between October 2010 and September 2011 at monthly intervals. Some environmental conditions (temperature, dissolved oxygen, pH, conductivity, salinity, chloride, turbidity, hydrogen sulfide, magnesium, calcium, total hardness, NO_3-N, NO_2-N, sulfate, orthophosphate, suspended solid substances, biological oxygen demand) and bacterial features (total coliform, fecal coliform, and *Escherichia coli* bacterial abundances) were examined performing at the same time samplings. Consequently, it was determined that the water quality of Tunca River has proper physicochemical conditions allowing surviving of living things, but bacteriological findings belonging to the river was not found proper for direct use of water by human. Also, the correlations were evaluated between the obtained environmental features and the bacteria by using Spearman's index. While positive correlations were found between TMAB density and some environmental parameters (water-air temperature, EC, magnesium, nitrate nitrogen, sulfate, o-phosphate, and suspended solids); negative correlations were found between TMAB density and the other parameters (pH, hydrogen sulfide, calcium, total hardness, and BOD_5).

Keywords: Tunca River, Water quality, Coliform bacteria, Environmental condition, Physicochemical features

Introduction

Rivers are recipient areas that can be easily contaminated from environmental pollutants. The negative conditions from both continental and atmospheric environments can instantly reflected by these ecosystems (Çolakoğlu & Çakır, 2004; Bernot & Dodds, 2005). Although the studies on composition and abundance of microorganisms in biosphere have a lot of significance, in recent years it was also the investigations of bacteria in aquatic ecosystems have got an increasing significance (Jamieson et al., 2003; Hunt & Sarıhan, 2004; Agbogu et al., 2006; Niemi & Raateland, 2007; Sabae & Rabeh, 2007; Mishra & Batt, 2008; Suneela et al., 2008; Mishra et al., 2009, 2010; Saha et al., 2009; Bulut et al., 2010; Kumar et al., 2010; Venkatesharaju et al., 2010; Nguyen et al., 2016; Islam et al., 2017; Alves et al., 2018; Loucif et al., 2020). A lot of study is also carried out in our country. Çolakoğlu & Çakır (2004) attempt to investigate physicochemical and bacteriological quality of water in the Sarıçay stream. They investigated total mesophilic aerobic bacteria, total coliform, *Pseudomonas, Enterobacteriaceae, Staphylococcus, Lactobacillus, Enterococcus* and yeast-mold abundance. Physical, chemical and microbiological aspects of the aquatic ecosystem were measured in Manyas Lake by Karafistan & Colakoglu-Arik (2005). They performed total and fecal coliform bacteria, *Escherichia coli, Enterococcus, Staphylococcus, Lactobasillus* and *Pseudomonas* analysis. Toroğlu et al. (2006) were carried out total mesophilic aerobic bacteria, total coliform and fecal coliform bacteria analyzes in Aksu Stream. The physicochemical and microbiological parameters were investigated in the Karanfillicay (Bulut et al., 2010) and Egirdir Lake (Bulut et al., 2016). In these studies water samples were taken for total mesophilic aerobic bacteria, total coliform, fecal coliform and *Escherichia coli* analyzes. Koloren et al. (2011) evaluated the total coliform, fecal coliform, *Escherichia coli* and *Clostridium perfringens* levels in Gaga Lake.

Tunca River (Tundzha in Bulgarian) which is born in Bulgaria has 384 km length and it enters Turkey from Edirne province. This running water joins Meric River (Maritsa in Bulgarian; Evros in Greek) which is one of the most important tributary of Meric-Ergene River Basin and it is a boundary river between Turkey and Greece. Although there are some studies at Bulgarian and Turkish segments of Tunca River (Uzunov, 1980; Russev et al., 1984; Janeva & Russev, 1985; Uzunov & Kapustina, 1993; Kavaz, 1997; Uluçam, 1997; Öterler, 2003; Kirgiz et al., 2005; Camur-Elipek et al., 2006; Sakcali et al., 2009; Georgieva et al., 2010; Vassilev et al., 2010; Aytas et al., 2014), there is no study on correlation between environmental conditions and bacteriological features in the river. In the present study, ecological status of Tunca River was evaluated using the environmental properties and bacterial features from autochthonic and allochthonic environments.

Material and Methods

The samplings were made at 5 different stations chosen from Tunca River (Figure 1) between October 2010 and September 2011 at monthly intervals. The water samples for bacterial investigation were taken from 30 cm beneath of the water surface and were put into 100 mL sterile sampling bottles under aseptic conditions and were brought to the laboratory under the condition of cold chain system. The total mesophilic aerobic bacteria (TMAB) numbers were determined by using spread plates method, while the others (total coliform, fecal coliform, and *E. coli* bacterial numbers) were determined by using Most Probable Number (MPN) method (Halkman, 2005). The culture media and incubation conditions were presented at Table 1. Nansen water sampler was used to take the water samples and the material was carried to the laboratory in lightproof bottles to analyze the physicochemical features (Egemen & Sunlu, 1999). Spearman's correlation index was used in order to determine the effects of physicochemical conditions on TMAB numbers (Krebs, 1999). Sediment and water samples were also taken at seasonally intervals to determine some heavy metal contents (iron, copper, zinc, lead, cadmium) and the concentrations were measured by using graphite-furnace atomic absorption spectroscopy (Erçal, 2007).

Results and Discussion

The data obtained from bacteriological analysis in Tunca River were presented at Table 2, and the data of physicochemical parameters were presented at Tables 3 and 4.
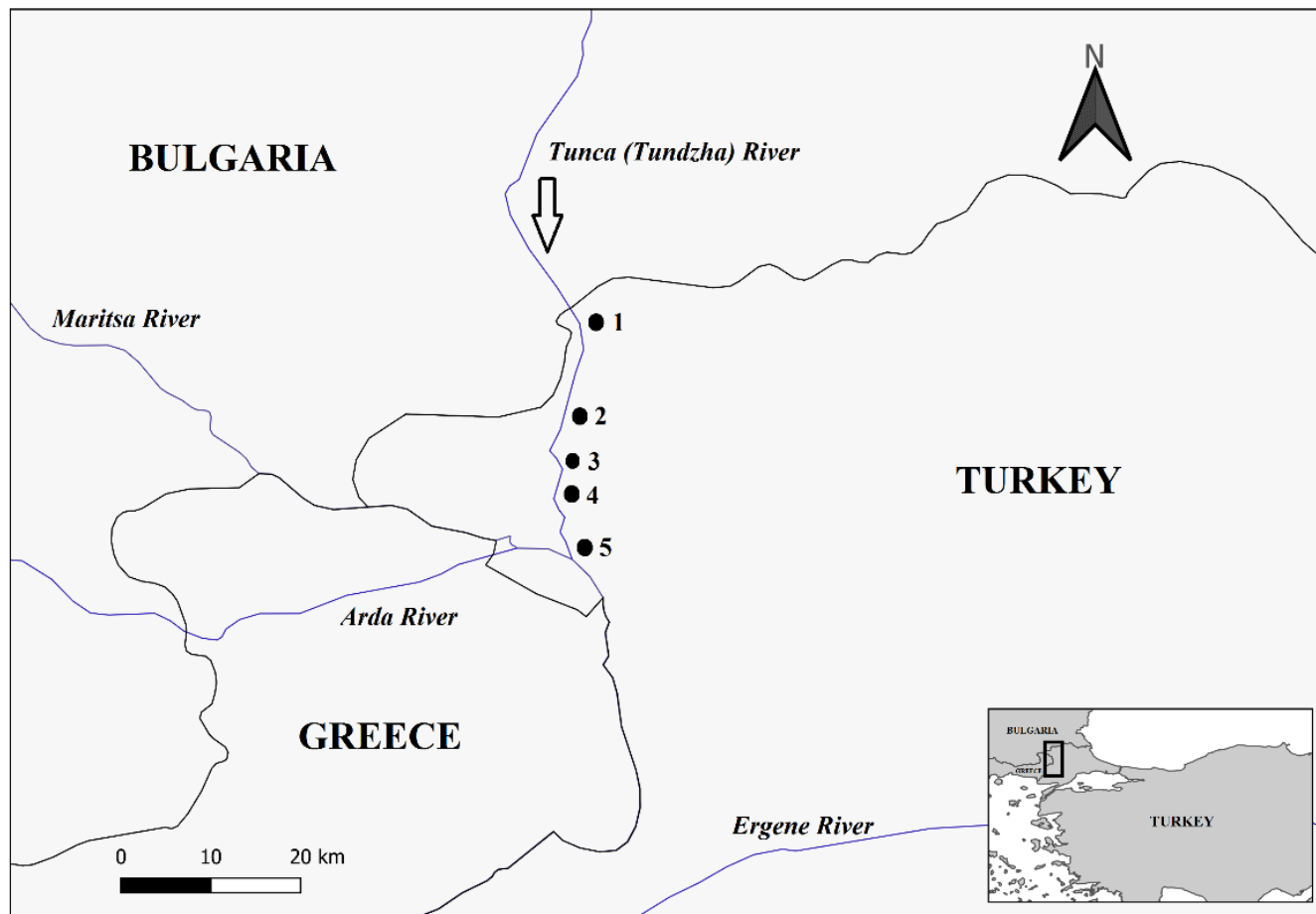
Figure 1. Location of Tunca River and the sampling stations

Table 1. Culture media and incubation conditions on growth of Microorganisms

Microorganisms	Culture Media	Temperature	Times
TMAB	PCA	35 ±0.5°C	24 ±2 hours
Total Coliforms	LST Broth	35 ±0.5°C	24 ±2 hours
Fecal Coliforms	LST Broth	35 ±0.5°C	24 ±2 hours
	EC Broth	44.5 ±0.2°C	24 ±2 hours
Esherichia coli	LST Broth	35 ±0.5°C	24 ±2 hours
	EC Broth	44.5 ±0.2°C	24 ±2 hours
	EMB Agar	35 ±0.5°C	24 ±2 hours
	Tryptone Broth	35 ±0.5°C	24 ±2 hours
	MR-VP Broth	35 ±0.5°C	24-48hours
	Simmon's Citrate Agar	35 ±0.5°C	24 ±2 hours

Table 2. The numbers of bacteria determined from Tunca River

	ST. 1	ST. 2	ST. 3	ST. 4	ST. 5
Total Mesophilic Aerobic Becteria (cfu/100mL)					
October	1.25×10^5	1.68×10^5	1.97×10^5	1.91×10^5	1.63×10^5
November	6.2×10^4	1.24×10^5	2.1×10^5	3.04×10^5	2.95×10^5
December	3.6×10^4	9.1×10^4	7.6×10^4	1.02×10^5	1.66×10^5
January	6.9×10^4	8.6×10^4	8.2×10^4	6.7×10^4	8.1×10^4
February	5.4×10^4	6.6×10^4	4.8×10^4	6.5×10^4	7.6×10^4
March	4.8×10^4	5.9×10^4	4.6×10^4	8.7×10^4	1.07×10^5
April	6.7×10^4	6.4×10^4	9.6×10^4	3.44×10^5	2.01×10^5
May	5.3×10^4	1.48×10^5	1.65×10^5	2.34×10^5	1.5×10^5
June	2.9×10^5	5.5×10^5	7.3×10^5	5.2×10^5	4.8×10^5
July	4.5×10^5	1.0×10^6	4.0×10^5	6.0×10^5	5.9×10^5
August	1.7×10^5	2.0×10^5	3.1×10^5	4.8×10^5	7.7×10^5
September	1.6×10^5	3.4×10^5	3.8×10^5	5.2×10^5	4.2×10^5
Total Coliform (MNP/100mL)					
October	2.1×10^3	2.3×10^3	1.5×10^3	9.2×10^2	9.3×10^3
November	$<3.0 \times 10^2$	2.3×10^3	1.5×10^3	1.5×10^4	1.1×10^5
December	9.2×10^2	4.3×10^3	9.2×10^2	9.3×10^3	2.4×10^4
January	2.3×10^3	7.5×10^3	4.3×10^3	1.1×10^5	2.1×10^4
February	4.3×10^3	4.3×10^3	2.8×10^3	1.1×10^5	7.5×10^3
March	$<3.0 \times 10^2$	9.3×10^3	9.2×10^2	4.6×10^4	2.3×10^3
April	3.6×10^2	2.3×10^3	3.6×10^2	9.3×10^3	1.1×10^5
May	$<3.0 \times 10^2$	7.4×10^2	9.2×10^2	9.3×10^3	1.1×10^5
June	7.4×10^2	9.3×10^3	7.4×10^2	4.6×10^4	2.4×10^4
July	2.3×10^3	4.6×10^4	2.8×10^3	$>1.1 \times 10^7$	4.6×10^4
August	$<3.0 \times 10^2$	9.3×10^3	1.5×10^3	1.5×10^4	1.5×10^4
September	$<3.0 \times 10^2$	9.2×10^2	4.6×10^4	2.4×10^4	7.5×10^3
Fecal Coliform (MPN/100mL)					
October	9.2×10^2	2.3×10^3	1.5×10^3	9.2×10^2	9.3×10^3
November	$<3.0 \times 10^2$	2.3×10^3	9.2×10^2	1.5×10^4	1.1×10^5
December	9.2×10^2	4.3×10^3	3.6×10^2	9.3×10^3	2.4×10^4
January	2.3×10^3	4.3×10^3	4.3×10^3	4.6×10^4	2.1×10^4
February	1.5×10^3	4.3×10^3	2.1×10^3	1.1×10^5	7.5×10^3
March	$<3.0 \times 10^2$	2.1×10^3	9.2×10^2	1.5×10^4	2.3×10^3
April	3.6×10^2	2.3×10^3	3.6×10^2	9.3×10^3	1.1×10^5
May	$<3.0 \times 10^2$	7.4×10^2	9.2×10^2	9.3×10^3	1.1×10^5
June	7.4×10^2	9.3×10^3	3.6×10^2	4.6×10^4	2.4×10^4
July	2.3×10^3	9.3×10^3	2.0×10^3	1.5×10^4	1.5×10^4
August	$<3.0 \times 10^2$	9.3×10^3	9.2×10^2	1.5×10^4	9.3×10^3
September	$<3.0 \times 10^2$	9.2×10^2	2.4×10^4	2.4×10^4	7.5×10^3
***E. coli* (MPN/100mL)**					
October	9.2×10^2	2.3×10^3	7.4×10^2	9.2×10^2	9.3×10^3
November	$<3.0 \times 10^2$	2.3×10^3	9.2×10^2	1.5×10^4	1.1×10^5
December	9.2×10^2	4.3×10^3	3.6×10^2	9.3×10^3	2.4×10^4
January	2.3×10^3	4.3×10^3	4.3×10^3	4.6×10^4	2.1×10^4
February	1.5×10^3	4.3×10^3	2.1×10^3	1.1×10^5	7.5×10^3
March	$<3.0 \times 10^2$	2.1×10^3	9.2×10^2	1.5×10^4	2.3×10^3
April	3.6×10^2	2.3×10^3	3.6×10^2	9.3×10^3	1.1×10^5
May	$<3.0 \times 10^2$	7.4×10^2	9.2×10^2	2.1×10^3	1.1×10^5
June	7.4×10^2	9.3×10^3	3.6×10^2	4.6×10^4	2.4×10^4
July	2.3×10^3	9.3×10^3	2.0×10^3	1.5×10^4	1.5×10^4
August	$<3.0 \times 10^2$	9.3×10^3	9.2×10^2	7.5×10^3	4.3×10^3
September	$<3.0 \times 10^2$	9.2×10^2	9.3×10^3	2.4×10^4	7.5×10^3

(ST:station; cfu:colony forming unit; MPN:most probable number)

Table 3. Annual average values of physicochemical properties of the water samples in Tunca River

PARAMETERS	ST. 1	ST. 2	ST. 3	ST. 4	ST. 5	Mean
Water temp (ºC)	14.5	14.5	14.6	14.7	15.4	14.7
Air temp (ºC)	14.5	15	15.3	15.1	16.2	15.2
pH	8.92	8.52	8.41	8.41	8.31	8.51
EC (µS/cm)	549	556	568	574	574	564
DO (mg/L)	5.83	5.80	5.50	5.64	5.45	5.64
Turbidity (cm)	60	61	62	63	64	62
Salinity (‰)	0.088	0.086	0.081	0.087	0.088	0.086
Chlorides (mg/L)	37.82	40.23	40.90	41.32	41.65	40.38
H_2S (mg/L)	0.284	0.461	0.497	0.337	0.550	0.425
Ca^{+2} (mg/L)	61.05	62.65	60.78	58.55	56.51	59.90
Mg^{+2} (mg/L)	15.44	17.78	18.15	18.76	18.67	17.76
Total hardness (FSº)	17.0	18.3	17.6	17.1	17.1	17.4
NO_2-N (mg/L)	0.023	0.021	0.023	0.024	0.026	0.023
NO_3-N (mg/L)	5.648	5.859	5.389	6.708	6.621	6.045
Phosphate (mg/L)	0.056	0.055	0.057	0.060	0.057	0.057
Sulphate (mg/L)	2.013	2.104	2.212	2.204	2.203	2.147
SSS (mg/L)	302	295	320	345	394	331

(ST:station; EC:conductivity, DO:dissolved oxygen, SSS:suspended solid substances)

The highest average values for the TMAB numbers in Tunca River was recorded as 6.08×10^5 cfu/100 mL at month July. Also, it was found that the increasing of TMAB numbers in the summer and the autumn was remarkable (Table 2). Çolakoğlu & Çakır (2004) reported that, in summer and autumn, most diversification of bacteria was observed. This situation can be explained by rising of water temperature in summer season and decreasing of flow, and increasing of organic substances which has been joined the water because of rain fall in autumn (Elmacı et al., 2008; Mishra et al., 2009; Saha et al., 2009; Bulut et al., 2010). Koloren et al. (2011) reported that the number of indicator bacteria and rain fall is directly proportional. Bulut et al. (2016) reported that TMAB ranged between with 0 to 172 cfu/100 mL in Egirdir Lake. TMAB in the Golbasi Lake was determined as 20×10^3 cfu/mL (Toroglu & Toroglu, 2009). In our study, TMAB ranged between 3.6×10^4-1.0×10^6 cfu/100 mL. When the total coliform, fecal coliform, and *E. coli* bacterial numbers were evaluated considering the sampling stations, the highest values were recorded in the 4th and 5th stations which are located near to city center of Edirne Province (Table 2), but the 1st station which is the most away from city center has been observed to be has the least bacteriological numbers. These high values can be explained by the sewages enter to the river from the 4th and 5th stations. Although, Karafistan & Colakoglu-Arik

(2005) reported that Lake Manyas is threatened by anthropogenic pollution. Our findings demonstrated similar results. Bulut et al. (2016) reported that *E. coli* was not detected at all stations in Egirdir Lake. But our findings, *E. coli* were found at all times and stations in Tunca River.

The water and air temperature values of Tunca River were observed at seasonal expectancy that the values range between minimum 2°C and maximum 28°C (Figure 2). Also, Spearman's index showed that a positive correlation between the TMAB numbers and temperature values (for water temperature r= 0.576, p<0.05 in the autumn and r= 0.571, p<0.05 in the spring; for air temperature r= 0.894, p<0.05 in the April, r= 0.975, p<0.01 in the August and r= 0.533, p<0.05 in the autumn).

While the average values of dissolved oxygen (DO) in the river was observed as 5.64 mg/L, the lowest DO levels were measured at sampling stations which are the nearest to city center. Speraman's index showed that a negative correlation between the DO values and TMAB numbers in Tunca River (r = -0.894, p<0.05 in October; r = -0.894, p<0.05 in May; r= -0.949, p<0.05 in November). This situation can be explained by the organic materials found in water were decomposed by activities of aerobic microorganisms and thus oxygen consumed. Also, it was observed that the water temperature and DO have an inversely correlation in the study (Figure 2).
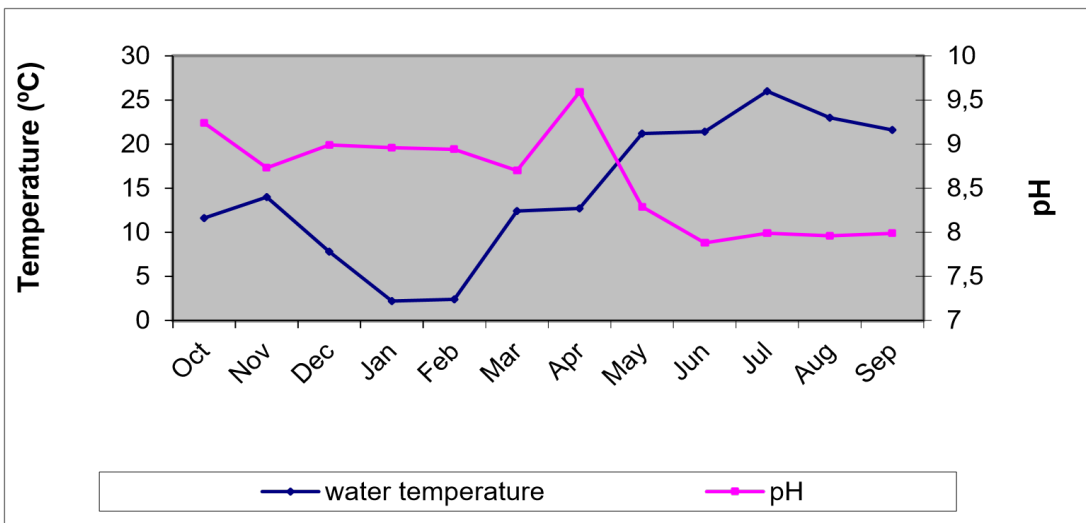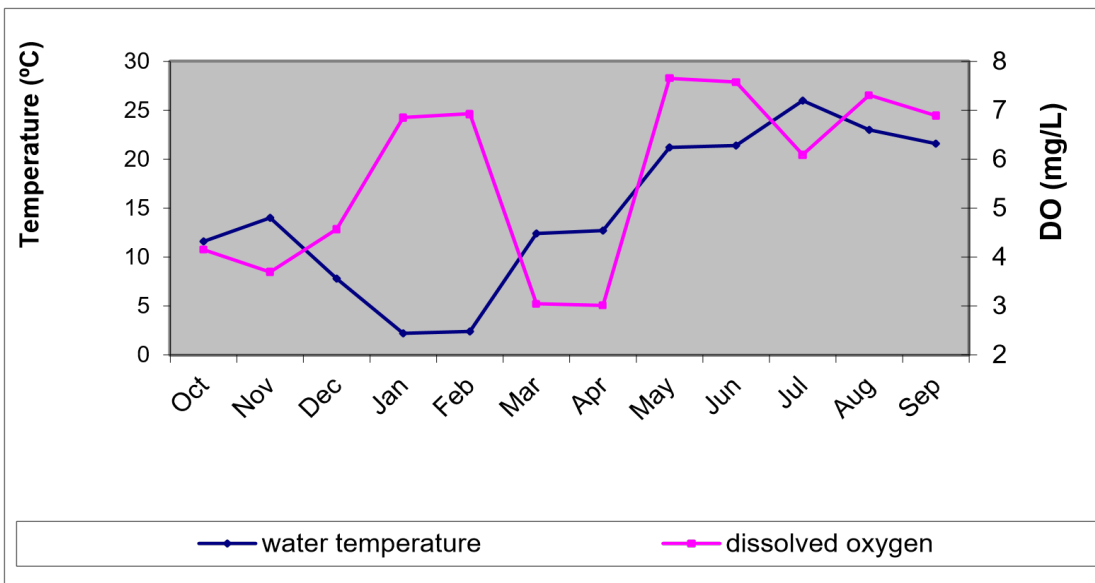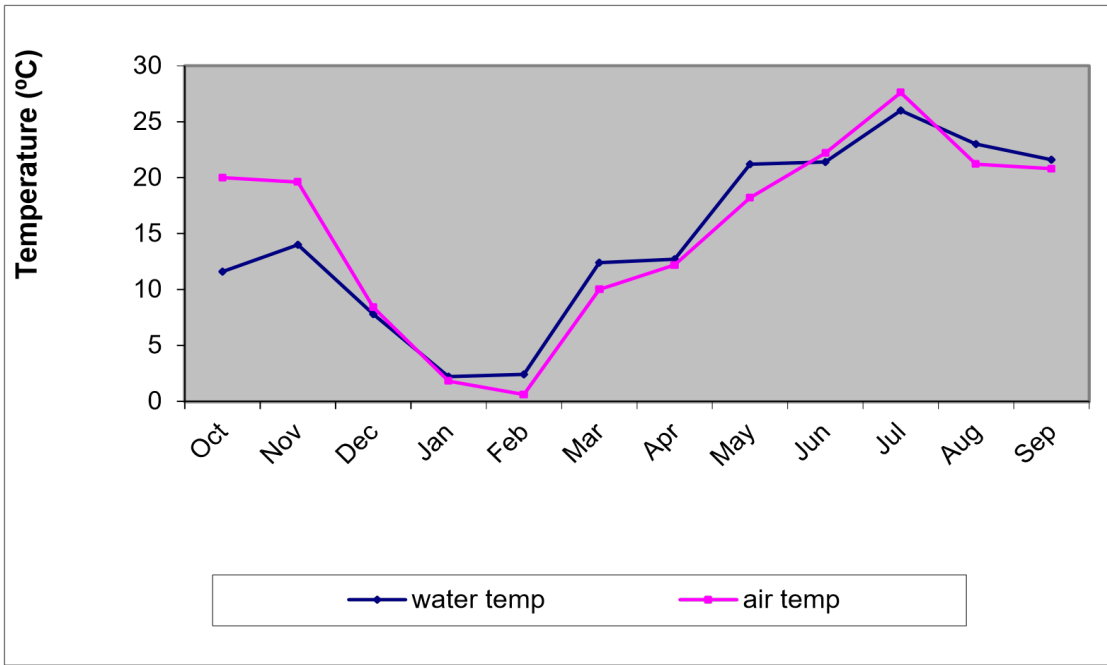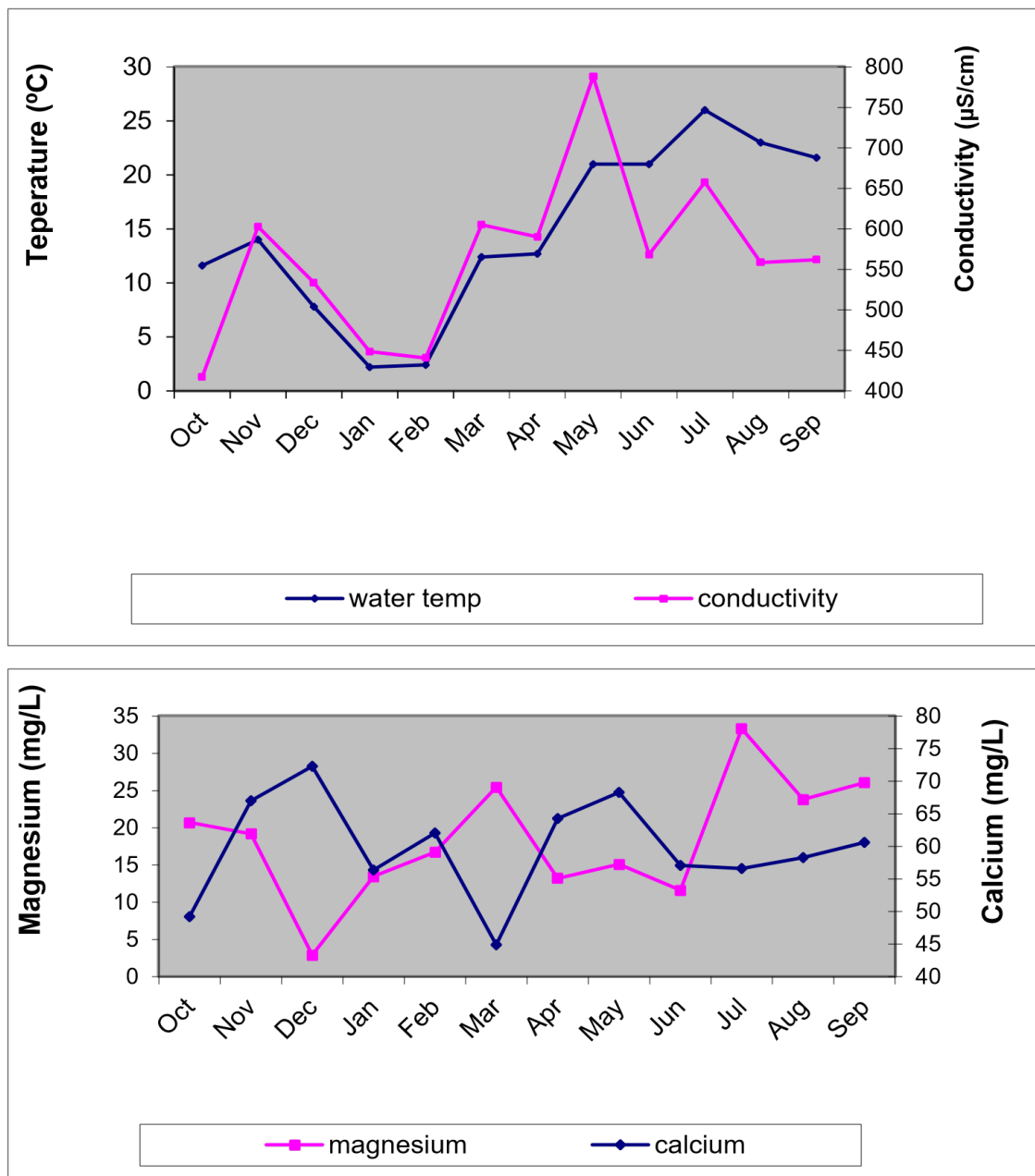
Figure 2. Monthly averages of some physicochemical parameters of Tunca River

The pH values were measured to have the lowest values in summer period and the highest in winter (Figure 2). The reason of the decrease in the summer might be the decomposition of organic materials by increasing activities of microorganisms. While the pH values change between 7.77 and 9.98, it was observed to have a negative correlation between the TMAB numbers and pH values ($r = -0.900$, $p<0.05$ in June; $r = -1.000$, $p<0.01$ in October; $r = -0.699$, $p<0.01$ in autumn; $r = -0.651$, $p <0.01$ in spring).

The decreasing of conductivity (EC) values of the river at winter season can be explained by decreasing of temperature in cold months (Figure 2). The average EC values were recorded as 564 µS/cm and it was observed to have a positive correlation between the EC and the TMAB numbers ($r= 1.000$, $p<0.01$ in August).

Although, the highest value was observed at month June, salinity levels in Tunca River did not change during the sampling periods. Excessive evaporation from surface of river and the lowest water level at month June can explain this situation.

The secchi disc depth was found to be very high in the summer because of the highest light permeability. But the secchi disc depth was measured very low at autumn season because of erosion towards to the river by the effect of rain.

The measured minimum H_2S rate was recorded in the winter season as 0.028 mg/L. Activities of microorganisms decrease in winter season because of falling of water temperature, and thus H_2S values decrease, too. Furthermore, a negative correlation was observed between H_2S values and TMAB numbers in the river ($r = -0.975$, $p<0.01$ in October).

While it was recorded that the calcium values at minimum 36.07 and maximum 76.95 mg/L; the magnesium values were observed minimum 0.48 and maximum 42.13 mg/L. Furthermore, an inverse correlation was determined between Ca^{+2} and Mg^{+2} levels in the river (Figure 2). Also, a negative correlation was observed between TMAB numbers and Ca^{+2} values ($r = -1.000$, $p<0.01$ in December); and a positive correlation between TMAB numbers and Mg^{+2} values ($r = 0.533$, $p<0.05$ in autumn).

Annual mean total hardness (TH) in Tunca River was measured as 17.4 FH°. A negative correlation was determined between TH value and TMAB numbers (in June $r= -0.894$, $p<0.05$; in winter $r= -0.740$, $p<0.01$; in spring $r= -0.651$, $p<0.01$).

The average nitrite nitrogen values were recorded as 0.023 mg/L in Tunca River. This value showed that the river has beta-mezosaprobic level towards to alfamezosaphrobic (Kazancı & Dugel, 2009). The maximum values belonging nitrite

nitrogen were measured as 0.143 mg/L in November. The reason of this increase in November might be explained because of decrease bacterial activities transforming nitrite to nitrate by the intermediate product of nitrification with the temperature falling. In this study, the average nitrate nitrogen values were recorded as 13.969 mg/L with the highest ratio in May. This value showed that the river has beta-mezosaprobic level (Kazancı & Dugel, 2009). Furthermore, a positive correlation was found between NO_3-N values and TMAB numbers ($r = 0.575$, $p<0.05$ in winter). Our results on the increasing of nitrate levels in spring season are similar the results the study performed by Hunt & Sarıhan (2004) in Saricam Stream.

The average sulfide values were measured as 2.14 mg/L in the river. Also, a positive correlation was determined between SO_4^{-2} values and TMAB numbers ($r = 0.975$, $p<0.01$ in August; $r = 0.900$, $p<0.05$ in September; $r = 0.515$, $p<0.05$ in summer).

It was measured the o-PO_4^{-3} (ortho-phosphate) values between minimum 0.020 and maximum 0.146 mg/L, and a positive correlation was found between o-PO_4^{-3} and TMAB numbers ($r= 0.975$, $p<0.01$ in November; $r= 0.900$, $p<0.05$ in December; $r= 0.975$, $p<0. 01$ in July; $r= 0.751$, $p<0.01$ in winter). The values indicated that the river has beta-mesotrophic conditions according to the o-PO_4^{-3} levels.

The values of suspended solid substances (SSS) were measured at very high levels in November with 546 mg/L. Erosion material might have entered to the river by influence of rainfall, and thus the SSS values have reached to the high levels. It is seen that the data on SSS we obtained in the present study show similarity with the results of the study performed by Öterler (2003) in Tunca River. However, our findings on SSS were found to be higher than the findings from another study performed by Uluçam (1997) in the river. Furthermore, a positive correlation was determined between SSS values and TMAB numbers ($r= 0.900$, $p<0.05$ in April; in September $r = 0.900$, $p<0.05$).

As it was compared with studies carried out in Tunca River in previous, it was observed that biological oxygen demand (BOD_5) values increased at time. The entering of pollutant material to the river might lead to this situation. Also, a negative correlation was found between BOD_5 values and TMAB numbers ($r = -0.975$, $p<0.01$ in November; $r= -0.900$, $p<0.05$ in May; $r = -0.900$, $p<0.05$ in September).

Summarized, according to Spearman's Correlation Index, positive correlations were found between TMAB density and some environmental parameters (water-air temperature, EC, magnesium, nitrate nitrogen, sulphate, o-phosphate, and suspended solids); negative correlations were found between

TMAB density and the other parameters (pH, hydrogen sulphide, calcium, total hardness, and BOD₅).

The obtained data for heavy metals were shown at Table 4. According to the observed heavy metal values, the lead concentrations were found at high level.

When the obtained data on the physicochemical variables were evaluated according to Surface Water Quality Control Regulation of Turkey (Anonymous, 2016), the water quality of Tunca River was found at first quality level in terms of some parameters (temperature, pH, chloride, nitrate nitrogen, sulfate, phosphate, and cadmium). However, some observed parameter values (DO, nitrite nitrogen, iron, copper, and zinc) have signed that the river has second quality level. And the river was found at fourth quality level according to the findings of total coliform, fecalcoliform, BOD₅, and lead.

Conclusions

According to the some physicochemical findings observed in this study, it was determined that the water quality of Tunca River has proper conditions allowing surviving of living things. However, bacteriological findings belonging to the river was not found proper for direct use of water by human. Especially, the existence of *E.coli* in the river has shown that a serious contamination with fecal matter. Furthermore, the high levels belonging coliform, fecal coliform and *E. coli* indicate that the possibility the presence of other pathogenic microorganisms. Consequently, using the water of river will has been restricted by emergence of some resistant bacteria (Toroglu et al., 2006).

Consequently, it is suggested that pollution sources reaching to the river should be determined in order to remove present pollution of Tunca River or to prevent it to be more polluted. Therefore, it is also required to repeat similar analysis frequently and to follow the changes to appear in the water quality.

Table 4. Annual average values of heavy metals of the water and sediment samples in Tunca River

Station ↓ Heavy Metals →	Fe (mg/L)	Cu (mg/L)	Zn (mg/L)	Pb (mg/L)	Cd (mg/L)
ST. 1 **Water**	1.82	0.08	0.39	4.21	ND
Sediment	78.25	1.01	1.19	57.43	ND
ST. 2 **Water**	1.36	0.11	0.31	4.91	ND
Sediment	91.62	1.82	2.47	61.49	ND
ST. 3 **Water**	0.75	0.08	0.16	4.69	ND
Sediment	348.20	1.51	9.23	74.05	ND
ST. 4 **Water**	1.19	0.03	2.69	5.42	ND
Sediment	191.41	1.98	2.56	66.64	ND
ST. 5 **Water**	2.08	0.09	0.48	5.31	ND
Sediment	266.96	1.85	3.56	66.64	ND

(ND:not determined)

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