Water Quality Assessment by Means of Bio-Indication: A Case Study of Ergene River Using Biological Diatom Index

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ABSTRACT
The Ergene River is the most significant fluvial ecosystem located in the Thrace Region of Turkey. But it is being exposed to an intensive organic – inorganic pollution by means of agricultural – industrial applications conducted around its basin. In this research, the epipelic (EPP) diatoms of the Ergene River were investigated and the water quality was evaluated using the determined physical, chemical and biological data. EPP diatom samples were collected from upstream, middlestream and downstream of the Ergene River and certain physicochemical parameters (dissolved oxygen, oxygen saturation, pH, electrical conductivity, total dissolved solids, salinity, turbidity, nitrate, nitrite, ammonium, phosphate, sulphate, fluoride, chemical oxygen demand, biological oxygen demand and oxidation-reduction potential) were measured during the field – laboratory studies. Also, the Biological Diatom Index (BDI) was used to determine the trophic status of the Ergene River in terms of EPP diatoms. According to the results of the physicochemical analysis, upstream of the Ergene River has Class I – II water quality and middle – downstream of the Ergene River have Class III – IV water quality in general. According to the results of the biological analysis, 24 diatom species were recorded in the upstream samples, 4 diatom species were recorded in the middlestream samples. and 7 diatom species were recorded in the downstream samples. Cymbopleura amphicephala (Nägeli) Krammer, Nitzschia umbonata (Ehrenberg) Lange-Bertalot and Nitzschia capitellata Hustedt were determined as the most dominant species in the up – middle – downstream of Ergene River respectively. According to the result of the BDI, the upstream of Ergene River was found to be in an oligotrophic state – has high water quality and the middle – downstream of Ergene River were found to be in a eutrophic state – have poor water quality.

Keywords: Ergene River, Bentic diatoms, Biological Diatom Index, Water quality

INTRODUCTION
Diatoms are algae that are the only organism on the planet with cell walls composed of transparent, opaline silica. Diatom cell walls are also ornamented by intricate and striking patterns of silica. Diatoms have chlorophylls a and c that are light-absorbing molecules. They collect energy from the sun and turn it into chemical energy by means of photosynthesis. Diatoms remove carbon dioxide from the atmosphere and convert it to organic carbon, and release the oxygen. Therefore, they are of vital importance for all organisms living in both aquatic and terrestrial environments. Diatoms are particular about the quality of water. For example, species have distinct ranges of pH and salinity where they will grow. They also have ranges and tolerances for other environmental factors, including nitrogenous or phosphorus concentration, flow regime, elevation, and organic or inorganic toxicants. Therefore, diatoms are also vital for assessment and monitoring of the envi-
Biological indicator organisms have been widely used in the scientific community for an effective water quality assessment research (Martin et al., 2010; Solak and Acs, 2011; Tokatlı and Dayıoğlu, 2011; Delgado et al., 2012; Atıcı and Udoh, 2016). Diatoms, which are one of the most important aquatic producer groups, can be found in all surface waters all the time. They are accepted as an important part of biological indicator organisms because of having quick reactions to environmental changes. Therefore, diatoms have been used to evaluate environmental conditions in many countries as indicators of water pollution (Ács et al., 2004; Goma et al., 2004; Atıcı and Obali, 2006; Solak et al., 2007; Kalyoncu et al., 2009; Atıcı and Obali, 2010; Tokatlı, 2013; Aydın and Büyükışık, 2014; Tan et al., 2017; Tokatlı et al., 2020). Diatom indices are one of the most widely used water quality assessment techniques and the Biological Diatom Index (BDI) is one of the most convenient indexes for evaluating water quality by using diatom communities (Coste et al., 2009).

The Ergene River is the most significant lotic ecosystem for the Thrace Region of Turkey and it is well documented that this system is being exposed to intensive anthropogenic pressure by means of agricultural and industrial applications conducted around the watersheds (Erkmen and Kolankaya, 2006; Tokatlı and Baştatlı, 2016; Tokatlı, 2017). The aim of the present research was to determine the epipelic diatoms of the Ergene River and to evaluate its water quality by using certain physicochemical parameters and the BDI.

MATERIAL AND METHOD

Study area
Water samples and epipelic (EPP) diatoms were collected from 3 selected stations on the Ergene River in autumn (rainy season) of 2018. A map of the Ergene River Basin and the selected stations is shown in Figure 1.

Physical and chemical parameters
The dissolved oxygen, oxygen saturation, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity and ORP parameters were determined using a Hach Lange branded “HQ40D Multiparameter” device during the field studies; the turbidity parameter was determined using a Hach Lange branded “2100Q Portable Turbidimeter” device during the field studies; the nitrate, nitrite, ammonium, phosphate, sulphate, fluoride and COD parameters were determined using a Hach Lange branded “DR3900 Spectrophotometer” device during the laboratory studies; the BOD parameter was determined using a Hach Lange branded “BOD Trak II Biological Oxygen Demand” device during the laboratory studies.

Epipelic (EPP) diatoms
A glass pipe with a diameter of 0.8 cm and a length of 100 – 150 cm was used for capturing EPP diatom samples. Then the diatom samples collected from the field were cleaned with acid (98% H₂SO₄ and 35% HNO₃) and mounted on a microscope for observation at a magnification of 1000X. Slides were prepared and approximately 400 valves were enumerated on each slide to determine the relation and abundance of each taxa (Sladecova, 1962; Round, 1993). Diatoms were identified according to Cox (1996) and Krammer and Lange-Bertalot (1986; 1988; 1991a; 1991b).

The Biological Diatom Index (BDI)
The Biological Diatom Index (IBD) values of the up – middle – downstreams of the Ergene River were automatically calculated using the “Calculate IBD with Excel” program. The trophic statuses and quality classes of freshwater according to BDI values are given in Table 1 (Lenoir and Coste, 1996).

Table 1. Scale of BDI

| Index Value | Quality Class | Trophic Status   |
|-------------|---------------|------------------|
| > 17        | High Quality  | Oligotrophic     |
| 15 – 17     | Good Quality  | Oligo – Mesotrophic |
| 12 – 15     | Moderate Quality | Mesotrophic     |
| 9 – 12      | Low Quality   | Meso – Eutrophic  |
| < 9         | Poor Quality  | Eutrophic        |

RESULTS AND DISCUSSION

Physical and chemical data
The results of the physicochemical data detected in the Ergene River and some national – international limit values are given in Table 2. According to the criteria of the Water Pollution Control Regulation in Turkey, upstream of the Ergene River has Class I – II water quality and middle – downstream of the Ergene River have Class III – IV water quality in general (Uslu and Türkman, 1987; Turkish Regulations, 2015).

It is known that the use of organic and inorganic fertilizers, and municipal and industrial wastewater discharges are the most important factors in increasing the amount of nitrogenous and phosphorus in water (Wetzel, 2001; Manahan, 2011). In a study performed in the Meriç, Tunca and Ergene Rivers, water qualities were investigated. As a result of this research and similar to the present study, water quality of the Meriç, Tunca and Ergene Rivers were reported as Class III – IV in terms of nitrite, ammonium and phosphate accumulations (Tokatlı, 2015). In another study performed in the same river basin, the Meriç – Ergene River ecosystem was found to have Class I – II water quality in terms of temperature, DO, COD, pH, TDS, nitrate, ammonium and sulphate parameters; Class II water quality in terms of nitrite parameters; and Class III – IV water quality in terms of phosphate, BOD and fecal coliform parameters in general (Tokatlı, 2019). According to the DSI observation reports, nitrogen and phosphorus are the main concerns affecting the water quality of Meriç – Ergene River Basin (Kendirli et al., 2005). Similar to the data reported by the DSI, the nitrite and phosphate concentrations in the water of middle – downstream of the Ergene River were detected in quite high levels and they have Class III – IV water quality in terms of these parameters.

Biological data
During the present investigation, a total of 31 diatom species were identified from the epipelic (EPP) habitat of the Ergene River by counting a total of 497 valves in the upstream samples, 67...
valves in the middlestream and 62 valves in the downstream. A list of identified diatom taxa with the frequency values of the investigated stations is given in Table 3. Also, the microscopy pictures of identified diatoms are given in Figure 2 and the relative abundance values of the detected EPP diatoms (higher than 1%) are given in Figure 3. Cymbopleura amphicephala (Nägeli) Krammer, Nitzschia umbonata (Ehrenberg) Lange-Bertalot and Nitzschia capitellata Hustedt, nom. inval. were determined as the most dominant species in the up – middle – downstream of the Ergene River respectively. Cymbopleura amphicephala, which was recorded as the most dominant taxon (relative abundance of 41%) for the upstream samples, is known as a cosmopolitan species found in oligo–mesotrophic waters with a low to moderate electrolyte content. Nitzschia umbonata, which was recorded as the most dominant taxon (relative abundance of 79%) for the middlestream, is a common species in eutrophic electrolyte rich waters and tolerating extremely polluted conditions. Nitzschia capitellata, which was recorded as the most dominant taxon (relative abundance of 37%) for the downstream, is a widespread species occurring in electrolyte rich and brackish waters and tolerating extremely polluted conditions (Taylor et al., 2007).

The biological diatom index (IBD)
The Biological Diatom Index (BDI), the formula of which was developed by Zelinka and Marvan (1961), is a standardized biological water quality assessment method. It is based on a total of 209 diatom taxa and provides significant information about the tro-
Table 2. Results of detected parameters and some national – international limit values

| Limit Values and the Results | Parameters | DO (mg/L) | OS (%) | pH | EC (mS/cm) | TDS (mg/L) | Sal (%O) | Tur (NTU) | NO₃ (mg/L) | NO₂ (mg/L) | NH₄ (mg/L) | *PO₄ (mg/L) | SO₄ (mg/L) | F (mg/L) | COD (mg/L) | BOD (mg/L) | ORP (mV) |
|-----------------------------|------------|-----------|--------|----|------------|------------|----------|-----------|------------|------------|------------|------------|------------|-----------|-----------|-----------|---------|
| Water Quality Classes (SKKY 2015) | | | | | | | | | | | | | | | | | | |
| I. Class                  | 8          | 90        | 6.5-8.5| 400       | 500        | -      | -      | 5          | 0.002      | 0.2        | 0.02       | 200        | 1         | 25        | 4         | -         |
| II. Class                 | 6          | 70        | 6.5-8.5| 1000       | 1500       | -      | -      | 10         | 0.01       | 1          | 0.16       | 200        | 1.5       | 50        | 8         | -         |
| III. Class                | 3          | 40        | 6.0-9.0| 3000       | 5000       | -      | -      | 20         | 0.05       | 2          | 0.65       | 400        | 2         | 70        | 20        | -         |
| IV. Class                 | <3         | <40       |       | >3000      | >5000      | -      | -      | >20        | >0.05      | >2         | >0.65      | >400       | >2        | >70       | >20       | -         |
| Drinking Water (TS266)    | -          | -         | 6.5-9.5| 2500       | -          | -      | 5      | 50         | 0.5        | 0.5        | -          | 250        | 1.5       | -         | -         | -         |
| Drinking Water (EC)       | -          | -         | 6.5-9.5| 2500       | -          | -      | -      | 50         | 0.5        | 0.3        | -          | 250        | 1.5       | -         | -         | -         |
| Drinking Water (WHO)      | -          | -         | -      | -          | -          | -      | -      | 50         | 0.2        | -          | -          | -          | 1.5       | -         | -         | -         |
| Fish Health (EC. 2006)    | | | | | | | | | | | | | | | | | |
| Cyprinid Species          | 4          | -         | 6.9    | -          | -          | -      | -      | -          | 0.03       | 0.2        | -          | -          | -         | -         | -         | -         |
| Salmonid Species          | 6          | -         | -      | -          | -          | -      | 0.01   | 0.04       | -          | -          | -          | -          | -         | -         | -         | -         |
| Present Study             | | | | | | | | | | | | | | | | | |
| Upstream                  | 9.66       | 107.6     | 7.96   | 503        | 262        | 0.26   | 0.95   | 0.92       | 0.009      | 0.012      | 0.013      | 13.1       | 0.087     | 5.4       | 0.4       | 191.6     |
| Middle Stream             | 1.86       | 20.7      | 7.54   | 2940       | 1622       | 1.65   | 32.30  | 0.725      | 0.124      | 2.210      | 1.330      | 185.0      | 0.466     | 112.0     | 21.0      | 143.3     |
| Downstream                | 3.77       | 42.1      | 7.46   | 2910       | 1607       | 1.63   | 17.70  | 0.592      | 0.144      | 2.180      | 1.320      | 158.0      | 0.583     | 83.3      | 11.0      | 130.0     |

*: According to another water quality classification specified by Uslu and Türkman (1987)
- Bold data means III. – IV. Class water quality
- Underlined data is not suitable for fish health
DO – Dissolved oxygen, OS – Oxygen saturation, Sal – Salinity, Tur – Turbidity; F: Fluoride; ORP: Oxidation – Reduction Potential
TS266 – Turkish Standards Institute, EC – European Communities, WHO – World Health Organization
In this research, a total of 31 diatom taxa were identified and they were used to calculate the Biological Diatom Index (BDI) scores of the up – middle – downstreams of the Ergene River in order to determine the trophic status. The BDI values of the investigated locations are given in Figure 4. According to the calculated BDI values for the EPP habitats, upstream of the Ergene River was found to be in an oligotrophic state – has high water quality (score range of >17) and middle – downstream of the Ergene River were found to be in a eutrophic state – have poor water quality (score range of <9).

The physical and chemical parameters used to assess water quality may only indicate the current status of the investigated aquatic ecosystem. However, the bioindicator organisms like diatoms may indicate the long-term changes in water ecosystems. Therefore, they have been widely used for the bio-assessment of aquatic habitats in almost all the countries of Europe due to their broad distribution and their quick reaction to environmental changes in water (Acs et al., 2004; Torissi and Dell’Uomo, 2006; Solak and Acs, 2011). Many diatom indices have been developed and they are widely used to determine the quality and trophic levels of water ecosystems. In a study performed in Poland, the Biological Diatom Index (BDI) was used for the assessment of water quality in the Pilica River. As a result of this study, the ecological state of the Pilica River changed from good (oligo – mesotrophic) to moderate (mesotrophic) (Szulc and Szulc, 2013). In another research performed in Vietnam, the BDI was used to evaluate the water quality of the Dong Nai River. As a result of this study, the water quality of the investigated river varied from good (oligo – mesotrophic), moderate (mesotrophic), to low (meso – eutrophic) levels (Pham, 2017). Several studies have also been carried out in different aquatic habitats of Turkey. Gürbüz and Kıvrak (2002) applied Saprobity Index (SI) and Trophic Diatom Index (TDI) in order to assess the water quality of Karasu River. According to the results of this investigation, the Karasu River was found to be in a eutrophicated state and organically polluted. Kalyoncu et al. (2009) investigated the Darıören Stream by ecological methodologies to evaluate the impact of pollution on epilithic diatom assemblages. Solak (2011) also used the SLA, EPI-D, TDI and DESCY indices to evaluate the water quality of the Upper Porsuk River (Kütahya). In two studies performed in the Gürleyik and Seydisuyu Streams, the BDI was used to assess the water qualities and the results of these studies showed that in line with the investigated physicochemical data, the Gürleyik and Seydisuyu Streams were found to be in a mesotrophic state (Tokatlı, 2012, Atıcı et al., 2018).

| Table 3. Identified diatom taxa |
|------------------------------|
| Species Code | Diatom Taxa | Upstream | Middlestream | Downstream |
| 1 | Amphora pediculus (Kützing) Grunow | + | - | - |
| 2 | Cocconeis pediculus Ehrenberg | + | - | - |
| 3 | Cricula subminuscus (Manguin) C.E.Wetzel & Ector | - | + | - |
| 4 | Cyclotella meneghiniana Kützing | + | + | - |
| 5 | Cymbella excisa Kützing | + | - | - |
| 6 | Cymbopleura amphi cephalia (Nägeli) Krammer | + | - | - |
| 7 | Diatoma vulgaris Bory | + | - | - |
| 8 | Diplooneis separanda Lange-Bertalot | + | - | - |
| 9 | Enyonema ventricosum (C.Agardh) Grunow | + | - | - |
| 10 | Geissleria decussis (Östrup) Lange-Bertalot & Metzeltin | + | - | - |
| 11 | Gymnotheca italicum Kützing | + | - | - |
| 12 | Grunowia sinuata (Thwaites) Rabenhorst | + | - | - |
| 13 | Melosira varians C.Agardh | + | - | + |
| 14 | Navicula amphicerosis Lange-Bertalot & U.Rumrich | - | - | + |
| 15 | Navicula antonii Lange-Bertalot | + | - | - |
| 16 | Navicula capillatoradiata H.Germain ex Gasse | + | - | - |
| 17 | Navicula cryptococephala Kützing | + | - | - |
| 18 | Navicula cryptotenella Lange-Bertalot | + | - | - |
| 19 | Navicula gregaria Donkin | - | - | + |
| 20 | Navicula radiosa Kützing | + | - | - |
| 21 | Navicula reichardtiana Lange-Bertalot | + | - | - |
| 22 | Nitzschia amphibia Grunow | - | - | + |
| 23 | Nitzschia capitellata Hustedt, nom. inval. | - | + | + |
| 24 | Nitzschia dissipata (Kützing) Rabenhorst | + | - | - |
| 25 | Nitzschia linearis W.Smith | + | - | - |
| 26 | Nitzschia sublinearis Hustedt | + | - | - |
| 27 | Nitzschia subtilis (Kützing) Grunow | + | - | - |
| 28 | Nitzschia umbonata (Ehrenberg) Lange-Bertalot | - | + | + |
| 29 | Tryblionella hungarica (Grunow) Frenguelli | - | - | + |
| 30 | Ulnaria acus (Kützing) Aboal | + | - | - |
| 31 | Ulnaria ulna (Nitzsch) Compère | + | - | - |
In the present investigation, the BDI was used to assess the water quality of the Ergene River and the detected data was compared with the results of limnological data. Similar to the resulting limnological data, upstream of the Ergene River was found to be in an oligotrophic state and has high water quality according to the result of the BDI and has Class I – II water quality according to the results of physicochemical parameters. Middle – downstream of the Ergene River were found to be in a eutrophic state and have poor water quality according to the result of the BDI and have Class III – IV water quality according to the results of physicochemical parameters. The detected similarities in water quality status between the results of the BDI scores and the physicochemical parameters indicate that the BDI may be used to reflect changes in ecological conditions of the Ergene River.

Figure 2. Microscopy pictures of Ergene River diatoms.
CONCLUSION

It is clearly known that biological water quality assessment is much more effective than investigated physicochemical data in terms of reflecting any environmental effects on aquatic ecosystems. Therefore, in order to make a better aquatic ecosystem quality assessment research, physicochemical data should be supported by biological data. In the present study, the epipelic diatoms of the Ergene River were investigated and the water quality of this significant river was evaluated using the Biological Diatom Index (BDI).

As a result of this research, 24 diatom species were recorded in the upstream samples, 4 diatom species were recorded in the middlestream samples and 7 diatom species were recorded in the downstream samples. *Cymbopleura amphicephala* which has a narrow ecological valence and low tolerance, *Nitzschia umbonata* and *Nitzschia capitellata* which have a wide ecological

Figure 3. Relative abundance of diatoms in the Ergene River.
The results of this study also revealed the benefits of using biotic and abiotic factors together in water quality assessment studies and showed that minor changes in environmental conditions may cause major effects in the diatom communities. While the sampling frequency is perhaps not sufficient and more research is needed for the assessment of quality status of the investigated water ecosystem, the results of the present research do have the characteristics of a preliminary research with the aim of providing resources for any future bioindication investigation in the region.

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