EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(1): 19-26 • DOI: https://doi.org/10.26650/ASE2019555681

Research Article

# Research into the Epipelic Diatoms of the Meriç and Tunca Rivers and the Application of the Biological Diatom Index in Water Quality Assessment

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**Cite this article as:** Tokatlı, C., Solak, C. N., Yılmaz, E., Atıcı, T., Dayıoğlu, H. (2020). Research into the epipelic diatoms of the Meriç and Tunca Rivers and the application of the biological diatom index in water quality assessment. *Aquatic Sciences and Engineering, 35*(1), 19–26.

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**Submitted:**
18.04.2019

**Revision Requested:**
07.09.2019

**Last Revision Received:**
16.08.2019

**Accepted:**
10.10.2019

**Online published:**
05.12.2019

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## ABSTRACT

The Meriç River is one of the longest rivers of the Balkans and the Tunca River is the most significant tributary of the Meriç River. In the present study, the epipelic diatoms of the Meriç and Tunca Rivers were investigated and the water quality was evaluated from a physicochemical and biological perspective. Epipelic (EPP) diatoms were collected from the middlestream of the Meriç River (Edirne Province of Turkey) and from the downstream of the Tunca River (before emptying into the Meriç River) and certain physicochemical parameters including dissolved oxygen (DO), oxygen saturation (OS), pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity, nitrate ($NO_3$), nitrite ($NO_2$), ammonium ($NH_4$), phosphate ($PO_4$), sulphate ($SO_4$), chemical oxygen demand (COD) and oxidation-reduction potential (ORP) were measured during the field studies. The Biological Diatom Index (IBD) was used to determine the trophic status of the Meriç and Tunca Rivers in terms of EPP diatoms, and Cluster Analysis (CA) was applied to the detected biological data in order to classify the identified diatom taxa in terms of their dominance in the system. According to the results of the physicochemical analysis, the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, $NO_3$, $NH_4$, $SO_4$ and COD parameters; and have III. – IV. Class water quality in terms of $NO_2$ and $PO_4$ parameters. 24 diatom species were recorded in the Meriç River by counting a total of 403 valves and a total of 19 diatom species were identified by counting a total of 409 diatom valves in the Tunca River. *Cyclotella atomus* Hustedt, *Navicula gregaria* Donkin, *Nitzschia palea* (Kützing) W.Smith and *Nitzschia subacicularis* Hustedt, were determined as the most dominant species in the Meriç River and *Navicula erifuga* Lange-Bertalot, *Navicula gregaria* Donkin and *Navicula rostellata* Kützing were recorded as the most dominant taxa in the Tunca River. According to the result of the IBD, the investigated rivers were found to be in a meso-eutrophic state and according to the results of CA, three statistical clusters were formed for both rivers, and were named as "dominant taxa", "frequent taxa" and "rare taxa".

**Keywords:** Meriç River, Bentic diatoms, Biological Diatom Index, Cluster Analysis

## INTRODUCTION

Rapid world population growth, a lack of environmental awareness in society and developments in industry have been the cause of significant environmental problems - especially so in the last century. One of the biosphere components affected most by this pollution is undoubtedly freshwater ecosystems. Therefore monitoring water quality is a necessity for the sustainability and protection of water ecosystem health (Çiçek et al., 2013; Köse et al., 2014; Tokatlı et al., 2016). Using only physical and chemical water quality monitoring methods may be inadequate, and especially in recent years, biological monitoring methods and bio-indicator organisms have been widely used in the scientific community for effective research (Martin et al., 2010; Solak and Acs, 2011; Tokatlı and Dayıoğlu, 2011; Delgado et al., 2012; Atıcı and Udoh, 2016).

Diatoms, which are known to be a large part of the benthos (often 90 – 95%), can be found in all surface waters all the time. They are also one of the most important aquatic producer groups and have quick reactions to the changes in environmental variables. Therefore, diatoms, which are accepted as an important part of bio-indicator organisms, 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 Obalı, 2006; Solak et al., 2007; Kalyoncu et al., 2009; Atıcı and Obalı, 2010; Tokatlı, 2013; Aydın and Büyükışık, 2014; Tan et al., 2017). Diatom indices developed for different conditions in different habitats are one of the most widely used water quality assessment techniques and the Biological Diatom Index (IBD) is one of the most convenient indexes for evaluating the water quality by using diatom communities (Coste et al., 2009).

The Meriç and Tunca Rivers are two of the most significant lotic ecosystems for the Balkans and it is well documented that they are being exposed to intensive anthropogenic pressure - especially from agricultural and domestic applications conducted on their watersheds (Erkmen and Kolankaya, 2006; Tokatlı and Baştatlı, 2016; Tokatlı, 2017). The aim of this study was to determine the epipelic diatoms of selected stations on the Meriç and Tunca Rivers and to evaluate/compare the water quality by using certain limnologic parameters and the Biological Diatom Index (IBD).

## MATERIAL AND METHOD

### Study Area

Water and epipelic (EPP) diatom samples were collected from 2 selected stations on the Meriç and Tunca Rivers in autumn (November) 2018. A map of the study area and the selected stations is shown in Figure 1.

### Physical and Chemical Parameters

The dissolved oxygen, oxygen saturation, pH, EC, TDS, salinity and oxidation - reduction potential (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 Turbiditymeter" device during the field studies; the nitrate, nitrite, ammonium, phosphate, sulphate and chemical oxygen demand (COD) parameters were determined using a Hach Lange branded "DR3900 Spectrophotometer" device during the laboratory studies.

### Epipelic (EPP) Diatoms

A glass pipe with a diameter of 0.8 cm and 100 – 150 cm long was used for capturing EPP diatom samples. Then the diatom samples collected from the field were cleaned with acid (98% $H_2SO_4$ and 35% $HNO_3$) 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).


**Figure 1.** The Meriç River Basin and the selected stations.

### The Biological Diatom Index (IBD)

The Biological Diatom Index (IBD) values of the Meriç and Tunca Rivers were automatically calculated using the "Calculate IBD with Excel" program. The trophic statuses and quality classes of freshwater according to IBD values are given in Table 1 (Lenoir and Coste, 1996).

**Table 1.** Scale of IBD

| 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 |

### Statistical Data

Cluster Analysis (CA) according to Bray Curtis was applied to the results using the "Past" package program in order to classify the diatom species in terms of their relative abundances.

## RESULTS AND DISCUSSION

### Physical and Chemical Data

The results of the physicochemical data detected in the 2 stations selected on the Meriç and Tunca Rivers and some national–international limit values are given in Table 2. According to the criteria of the Water Pollution Control Regulations in Turkey, the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, nitrate, ammonium, sulphate and COD parameters; and have III. – IV. Class water quality in terms of nitrite and phosphate parameters (Uslu and Türkman, 1987; Turkish Regulations, 2015).

Nitrite is known as an intermediate product in the biological oxidation process reaching from ammonium to nitrate. It may reach high concentrations in low–oxygen and in especially organically contaminated water. It is also known that organic and inorganic fertilizers, municipal and industrial wastewater discharges are the most important factors in increasing the amount of ammonia and phosphate in water (Wetzel, 2001; Manahan, 2011). Özkan and Elipek (2006) investigated the water quality of the Meriç River using certain physicochemical parameters. As a result of this research, the Meriç River was reported as having II. Class water quality level in terms of phosphate, II – III. Class water quality level in terms of nitrate, and III – IV. Class water quality level in terms of nitrite. In a study performed in the same basin, and similar to the present study, the Meriç, Tunca and Ergene Rivers were reported as III. – IV. Class in terms of nitrite, ammonium and phosphate accumulations in water (Tokatlı, 2015). In another study performed in the Meriç-Ergene River Basin, the water and sediment qualities of the basin components were investigated. According to the results of this research, and similar to the present study, the Meriç River Basin was found to have I. – II. Class water quality in terms of temperature, DO, COD, pH, TDS, nitrate, ammonium and sulphate parameters; II. Class water quality in terms of nitrite parameters; and III. – IV. Class 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 Meric River (Kendirli et al., 2005). Similar to the data reported by the DSI, the nitrite and phosphate concentrations in the water from the Meriç and Tunca Rivers were detected in quite high levels and they have III. – IV. Class water quality in terms of these parameters.

### Biological Data

During the present study, a total of 36 diatom species were identified from the epipelic (EPP) habitats of the Meriç and Tunca rivers by counting a total of 403 valves in the Meriç and 409 valves in the Tunca. A list of identified diatom taxa with the frequency values of the investigated stations is given in Table 3 and the rel-

**Table 2.** Results of detected parameters and some national–international limit values

| Limit Values and the Results | | DO (mg/L) | OS (%) | pH | EC (mS/cm) | TDS (mg/L) | Sal (‰) | Tur (NTU) | $NO_3$ (mg/L) | $NO_2$ (mg/L) | $NH_4$ (mg/L) | *$PO_4$ (mg/L) | $SO_4$ (mg/L) | COD (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 | 25 | - |
| | II. Class | 6 | 70 | 6.5-8.5 | 1000 | 1500 | - | - | 10 | 0.01 | 1 | 0.16 | 200 | 50 | - |
| | III. Class | 3 | 40 | 6.0-9.0 | 3000 | 5000 | - | - | 20 | 0.05 | 2 | 0.65 | 400 | 70 | - |
| | IV. Class | <3 | <40 | Out of 6.0-9.0 | >3000 | >5000 | - | - | >20 | >0.05 | >2 | >0.65 | >400 | >70 | - |
| Drinking Water | TS266 (2005) | - | - | 6.5-9.5 | 2500 | - | - | 5 | 50 | 0.5 | 0.5 | - | 250 | - | - |
| | EC (2007) | - | - | 6.5-9.5 | 2500 | - | - | - | 50 | 0.5 | 0.3 | - | 250 | - | - |
| | WHO (2011) | - | - | - | - | - | - | - | 50 | 0.2 | - | - | - | - | - |
| ♦Fish Health (EC, 2006) | Cyprinid Species | 4 | - | 6-9 | - | - | - | - | - | 0.03 | 0.2 | - | - | - | - |
| | Salmonid Species | 6 | - | 6-9 | - | - | - | - | - | 0.01 | 0.04 | - | - | - | - |
| Present Study | S1 (Meriç) | 9.51<br>I. Class | 101.9<br>I. Class | 8.31<br>I. Class | 327<br>I. Class | 176<br>I. Class | 0.17 | 0.46 | 1.480<br>I. Class | **<u>0.017</u>**<br>III. Class | 0.016<br>I. Class | **0.161**<br>III. Class | 60.7<br>I. Class | 7.8<br>I. Class | 200.1 |
| | S2 (Tunca) | 8.98<br>I. Class | 96.9<br>I. Class | 8.07<br>I. Class | 777<br>II. Class | 426<br>I. Class | 0.43 | 2.46 | 2.230<br>I. Class | **<u>0.072</u>**<br>IV. Class | <u>0.249</u><br>II. Class | **1.960**<br>IV. Class | 69.6<br>I. Class | 39.1<br>II. Class | 196.4 |

*: According to another water quality classification specified by Uslu and Türkman (1987)
•: Bold data mean III. – IV. Class water quality
♦: Underlined data are not suitable for fish health
DO – Dissolved oxygen, OS – Oxygen saturation, Sal – Salinity, Tur – Turbidity
TS266 – Turkish Standards Institute, EC – European Communities, WHO – World Health Organization

**Table 3.** Identified diatom taxa

| Diatom Taxa | Tunca River | Meriç River |
|---|---|---|
| *Amphora pediculus* (Kützing) Grunow | + | + |
| *Bacillaria paxillifera* (O.F.Müller) T.Marsson | + | - |
| *Cocconeis pediculus* Ehrenberg | + | - |
| *Craticula subminuscula* (Manguin) Wetzel & Ector | - | + |
| *Cyclotella atomus* Hustedt | - | + |
| *Cyclotella meneghiniana* Kützing | - | + |
| *Cymbella tumida* (Brébisson) Van Heurck | + | - |
| *Diatoma vulgaris* Bory | - | + |
| *Encyonema minutum* (Hilse) D.G.Mann | - | + |
| *Frustulia vulgaris* (Thwaites) De Toni | + | + |
| *Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin | - | + |
| *Gyrosigma kuetzingii* (Grunow) Cleve | + | - |
| *Melosira varians* C.Agardh | + | + |
| *Navicula amphiceropsis* Lange-Bertalot & U.Rumrich | - | + |
| *Navicula capitatoradiata* H.Germain ex Gasse | - | + |
| *Navicula erifuga* Lange-Bertalot | + | + |
| *Navicula germainii* J.H.Wallace | + | - |
| *Navicula gregaria* Donkin | + | + |
| *Navicula perminuta* Grunow | - | + |
| *Navicula radiosa* Kützing | - | + |
| *Navicula recens* (Lange-Bertalot) Lange-Bertalot | + | - |
| *Navicula rostellata* Kützing | + | + |
| *Navicula simulata* Manguin | + | |
| *Nitzschia amphibia* Grunow | - | + |
| *Nitzschia clausii* Hantzsch | + | - |
| *Nitzschia dissipata* (Kützing) Rabenhorst | - | + |
| *Nitzschia dubia* W.Smith | + | - |
| *Nitzschia inconspicua* Grunow | + | - |
| *Nitzschia linearis* W.Smith | - | + |
| *Nitzschia palea* (Kützing) W.Smith | + | + |
| *Nitzschia sociabilis* Hustedt | - | + |
| *Nitzschia subacicularis* Hustedt. nom. inval. | - | + |
| *Pantocsekiella ocellata* (Pantocsek) K.T.Kiss & Ács | - | + |
| *Reimeria sinuata* (W.Gregory) Kociolek & Stoermer | - | + |
| *Stephanodiscus hantzschii* Grunow | + | - |
| *Tryblionella calida* (Grunow) D.G.Mann | + | - |

ative abundance values of the detected EPP diatom species, which have relative abundance values higher than 1% for the Meriç and Tunca Rivers, are given in Figure 2. *Cyclotella atomus* Hustedt, *Navicula gregaria* Donkin, *Nitzschia palea* (Kützing) W. Smith and *Nitzschia subacicularis* Hustedt, nom. inval. were found as the most dominant species in the Meriç River and *Navicula erifuga* Lange-Bertalot, *Navicula gregaria* Donkin and *Navicula rostellata* Kützing were found as the most dominant taxa in the Tunca River.

*Navicula rostellata*, which is found as the most dominant taxon (relative abundance of 50%) for the Tunca River, is a cosmopolitan eutrophic species. *Navicula erifuga*, which is found as the second most dominant taxon (relative abundance of 25%) for the Tunca River, is a cosmopolitan species found in eutrophic, brackish waters or those with very high electrolyte content. Both species with very high abundance values in the Tunca River are tolerant to critical levels of pollution (Taylor et al., 2007). *Navicula gregaria*, which is found as the most dominant taxon (relative abundance of 52%) in the Meriç River, is a cosmopolitan species. It is very common in eutrophic to hypertrophic freshwaters with a moderate to high electrolyte content. *N. gregaria* tolerant of strongly polluted conditions may also be found in brackish waters. It is also known as a good indicator species for these conditions. *Cyclotella atomus*, which is found as the second most dominant taxon (relative abundance of 12%) in the Meriç River, occurs in the electrolyte rich waters (Taylor et al., 2007).

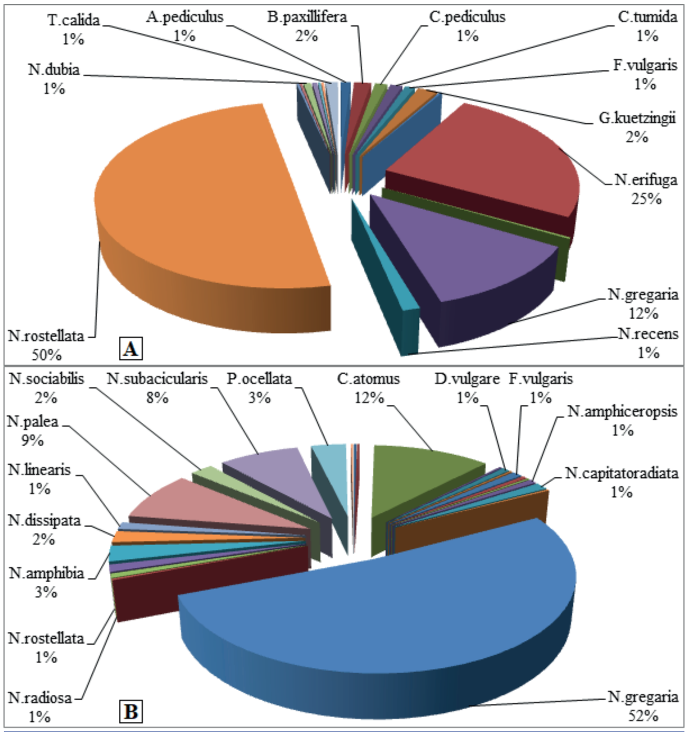


**Figure 2.** Relative abundance of diatoms in Tunca (A) and Meriç (B) rivers.

## Cluster Analysis (CA)

Cluster Analysis (CA), which classifies the objects, is one of widely used multivariate statistical techniques and hierarchical agglomerative clustering is the most common approach in CA applications (Shrestha and Kazama, 2007; Kazi et al., 2009; Tokatlı et al., 2014). In the present study, CA was applied to the results in order to classify the EPP diatoms of the Meriç and Tunca Rivers according to their relative abundance values. According to the results of CA, three statistically significant clusters were formed both for the diatoms of the Meriç and Tunca Rivers and these detected clusters were named as "dominant taxa", "frequent taxa" and "rare taxa" (Figure 3).

It is known that the Meriç River, which is one of the largest rivers flowing on Europa territory, is a transboundary fluvial ecosystem. Therefore the quality of its waters is of substantial importance for irrigation, industrial, recreation and domestic use for Bulgaria, Greece and Turkey and the control-management of the Meriç catchment is of mutual interest for the neighboring countries. In a study performed in the upstream of the Meriç River Basin, Cluster Analysis (CA) was applied for a model assessment of the water quality of the Meriç River on Bulgarian territory using long-term monitoring data from 21 sampling sites characterized by 8 surface water quality indicators. The application of CA to the indicators resulted in 3 significant clusters showing the impact of biological, anthropogenic and eutrophication sources and the results identified the dominant role of the industrial wastes and agricultural activities in water pollution (Papazova and Simeonova, 2013).

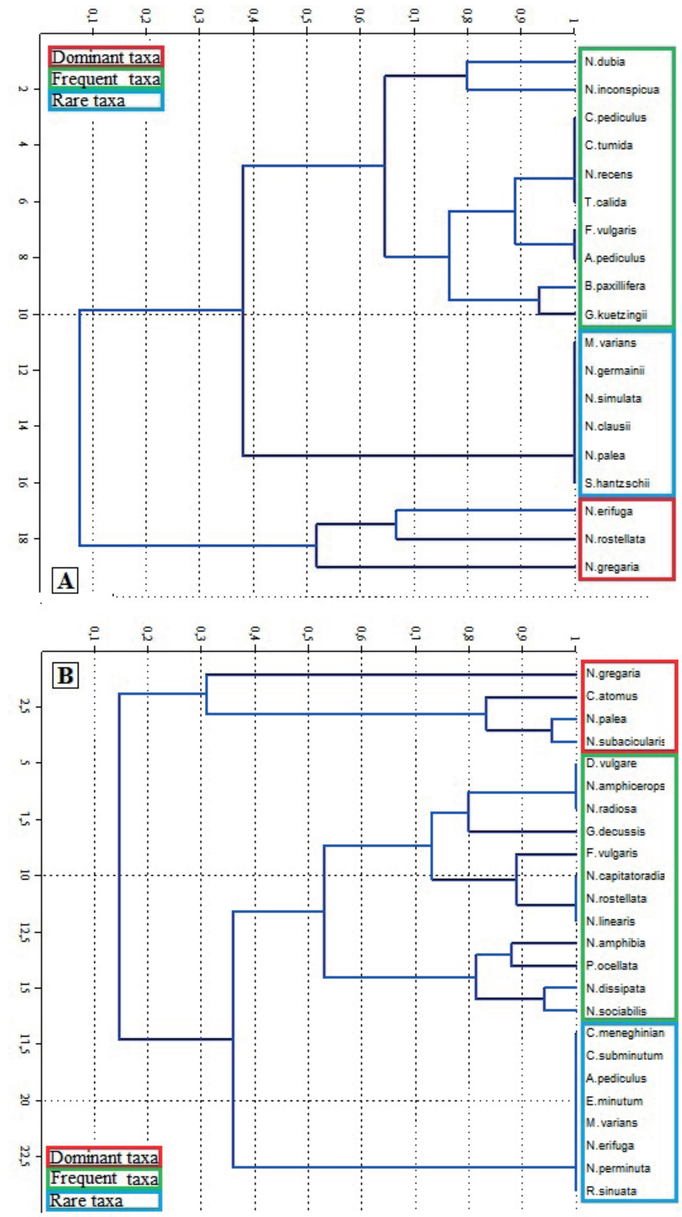


**Figure 3.** Tree dendrograms of CA for Tunca (A) and Meriç (B) Rivers.

## The Biological Diatom Index (IBD)

The Biological Diatom Index (BDI) is a standardized biologically water quality assessment method. The BDI, the formula of which was developed by Zelinka and Marvan (1961), is based on a total of 209 diatom taxa and provides information about trophic levels of the investigated aquatic ecosystems (Coste et al., 2009). In the present study, a total of 36 diatom taxa were identified and 34 of them were used to calculate the Biological Diatom Index (BDI) scores of the Meriç and Tunca Rivers in order to determine the trophic statuses. The BDI index values of the investigated rivers are given in Figure 4. According to the calculated BDI values for the EPP habitats of the investigated aquatic ecosystems, the Meriç and Tunca Rivers were in "meso – eutrophic state" and had "low water quality" (score range of 9 – 12) in general.

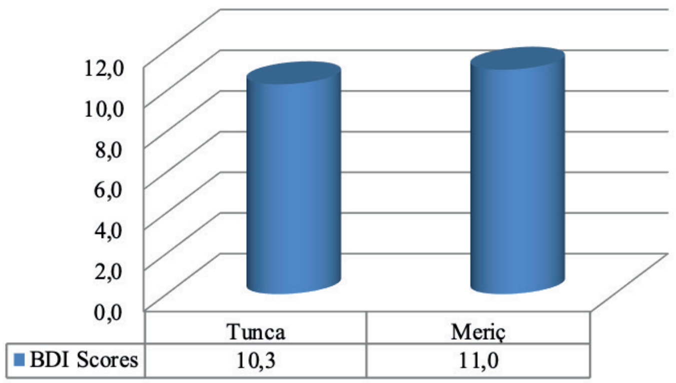


**Figure 4.** BDI scores of Tunca and Meriç Rivers.

The physicochemical parameters used to assess the water quality may only indicate the current status of aquatic habitat. But the diatoms, which should be used in monitoring programs of rivers ecological assessment according to the Water Framework Directive (WFD), may indicate the long term changes in aquatic ecosystems. Therefore they have been widely used for the bio-assessment of lotic and lentic ecosystems for a long time in almost all the countries of Europe due to their broad distribution and their ability to integrate changes occurring in water composition (Acs et al., 2004; Torissi and Dell'Uomo, 2006; Solak and Acs, 2011).

Diatom indices are widely used worldwide to determine the quality and trophic levels of aquatic systems. In a study performed in Portugal, the water qualities of the Caiman and Mau River Basins were evaluated using the Specific Pollution Index (SPI) and the Biological Diatom Index (BDI). According to the results of this research, the water and diatom data showed a medium to good water quality. However, samples collected near the mining areas (Coval da Mó) revealed a low abundance and diversity of diatom taxa indicating a stressed environment (Nunes et al., 2003). In another study performed in Poland, the Biological Diatom Index (BDI) was used for the estimation of water quality in the central section of the Pilica River, located in Łódź province. As a result of this study, the ecological state of the Pilica River changed from good (oligo – mesotrophic) to moderate (mesotrophic), which corresponded with the physicochemical analysis of the water (Szulc and Szulc, 2013). Pham (2017) used the Biological Diatom Index (BDI) to evaluate the water quality of the Dong Nai River (Vietnam). As a result of this study, similar to the present study, the water quality of the investigated river varied from good (oligo – mesotrophic), moderate (mesotrophic), to low (meso – eutrophic) levels, based on the BDI values. Although water quality assessment using diatom indices has been used worldwide for many years, several studies have been also been carried out in Turkey especially in the last 15 – 20 years. Gürbüz and Kıvrak (2002) were applied saprobity index (SI), trophic diatom index (TDI) and the percentage pollution tolerant valves in order to assess the water quality of Karasu River and they found that the Karasu River were eutrophicated and organically polluted. Kalyoncu et al. (2009) investigated the Darıören Stream by ecological methodologies to evaluate the impact of the pollution on epilithic diatom assemblages. SLA, EPI-D, TDI and DESCY indices were used by Solak (2011) in Upper Porsuk River (Kütahya) and according to the results of this study, the water quality levels of the investigated stations were found in different trophic conditions.

In this study, the Biological Diatom Index (IBD) was used to assess the water quality of the Meriç and Tunca Rivers and this data was compared with the limnological parameters data. Similar to the resulting limnological data, the Meriç and Tunca Rivers were found to be in a "meso–eutrophic state" and had "low water quality" in terms of the calculated IBD values. In a number of studies performed in the Gürleyik, Ankara and Seydisuyu Streams (Central Anatolian Region of Turkey), the IBD was used to evaluate the water quality of these lotic ecosystems. According to the results of these studies, and in line with the investigated physicochemical data, the Gürleyik, Ankara and Seydisuyu Streams were found to be in a mesotrophic state (Tokatlı, 2012, Atici ve Ahiska, 2005; Atıcı et al., 2018). In the present study, the detected similarities in water quality status between the results of the IBD scores and the limnological parameters indicate that the IBD may be used to reflect changes in ecological conditions of the basin – potentially after making some revisions in the index.

## CONCLUSION

Biological water quality assessment is much more effective (especially in the long term) in reflecting any effects on water ecosystems than investigated psychochemical data. Therefore any limnological data has to be supported by biological data to make a much better assessment. In this study, the epipelic diatoms of the Meriç and Tunca Rivers were investigated and the water quality of these fluvial ecosystems were evaluated using the Biological Diatom Index. As a result of this study, it was determined that the Meriç and Tunca Rivers have I. – II. Class water quality in terms of dissolved oxygen, oxygen saturation, pH, EC, TDS, $NO_3$, $NH_4$, $SO_4$ and COD parameters; and have III. – IV. Class water quality in terms of $NO_2$ and $PO_4$ parameters and the water of these rivers is in a meso–eutrophic state according to the results of the IBD. It was also determined that the biologically determined water qualities of the rivers showed a high similarity with the physico–chemically determined water qualities - especially in terms of nitrogen and phosphorous compounds. The results of this study also showed the benefits of using biotic and abiotic factors together in water quality assessment studies and show 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 ecosystems, the results of the present study do have the characteristics of a preliminary research with the aim of providing resources for any future bio-indication studies in the region.

**Financial Disclosure:** The present study was funded by the Trakya University, Commission of Scientific Research Projects (Project No. 2018/154).

**Ethics committee approval:** Ethics committee approval is not required.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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