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# **Article** Epilithic Diatom Assemblages and Indicators for the Assessment of Water Quality of Munzur Stream, Turkey

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Abstract: The epilithic diatoms of Munzur Stream, together with the chemistry and physical variables of the water, were examined in the samples taken annually from 3 stations on the creek between October 2016 and September 2017. A total of 69 diatom taxa were identified benthic habitat. Cocconeis placentula, Cymbella affinis, Cymbella excisiformis Didymosphenia geminata, Gomphonema olivaceum, and Hannaea arcus became diatoms at all that reached significant relative abundance. Shannon-diversity (H'), species evenness (J'), species richness (S) in each station varied between stations and months. Regarding diversity (H), values ranged from 1.54 (in December, at S3) to 2.99 (in October, at S3). Evenness ranged from 0.09 to 0.22 at station S3 in September (at S1, in September). Evenness ranged from 0.09 at site S3 in September, to 0.22 at S1, in September. All stations and species were close to 1 value. Canonical correspondence analysis indicated that three environmental variables (TN, TP, PO4-3) significantly affected the distribution of epilithic diatom. The studied part of stream was characterized by the species susceptible to nutrients such as Didymosphenia geminata, Cymbella excisiformis and Hannaea arcus which are found in the regions located in high lands having advanced ecological status. The results of the present study highlighted the importance of diatoms as a bio-indicator for health condition of a stream.

Keywords: Epilithic diatoms; Bioindicators, Diversity, Stream

## 1. Introduction

The importance of protecting river ecosystems globally for the well-being of humanity and nature has been increasing. These ecosystems have been destroyed in an uncontrolled way and faced rapidly with the danger of extinction. Access to usable water becomes more and more difficult as water resources have been gradually decreasing due to pollution. For the sustainability and protection of stream ecosystems, which are the leading ecosystems where environmental pollution poses a risk, current water quality conditions should be revealed and monitored in the long term. In recent years, important studies have been carried out in many countries to evaluate water quality by using not only physical and chemical parameters, but also biological elements.In accordance with the European Union Water Framework Directive (WFD),biological quality elements, including phytoplankton,phytobenthos (especially benthic diatoms), macrophytes,benthic invertebrates, and fish, should be employed asecological indicators for the assessment of surface waters (Directive, 2000; European Communities, 2009). Diatoms are organisms commonly used in bioassessments due to their short life cycle and rapid response to different stressors (Çelekli et al.2018). In fact, different diatom species have a high sensitivity to the conditions of their aquatic environment (Benhassane et al. 2020). These aquatic organisms are in constant physical, chemical, and biological interaction with their ecosystems. They are thus able to integrate environmental evolutions in the short and long terms, as well as the antagonistic or synergistic effects of different types of contaminants, impossible to demonstrate by physicochemical measurements. In order to have a better appreciation of the real biological quality in a stream, (Torrisi and Dell'uomo, 2006) recommended that instead of epiphytic diatoms, epilithic diatoms be preferentially sampled for the following reasons: Epilithic communities are more representative and more diverse than strictly epiphytic communities. In addition, stones are frequent, inert, stable and homogeneous natural substrates in streams (Benhassane et al. 2020). Diatoms are widely used in stream quality assessment due to their response to the local environment. Local environmental variables that can affect diatom communities include water chemistry, flow velocity, light, grazing, temperature, and substrate. Among the local factors, water chemistry and light amount are generally the most important variables affecting the density of diatom populations.

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The aim of the present study were to: 1. Determine the composition of benthic diatom communities in the region starting from the resource to mouth of Munzur Stream (Tunceli) which is the basic resource value of Munzur Valley National Park being one of the greatest national parks in Turkey. 2. Describe the spatial distribution of benthic diatom communities and determine the principal environmental variables influencing benthic distributions in the Munzur stream. Finally, it is to test the use of diatoms as a practical tool for estimating the overall quality of streams. To indicate possible sources of pollution and threats to the aquatic environment.

## 2. Materials and Methods

Munzur Valley National Park, one of the largest national parks of Turkey, starts at 8 km from the city center of Tunceli and extends from the mountains of Munzur across the valley. Munzur stream is a stream located in Tunceli province of Turkey and originates from the mountains of Munzur. The stream, which originates from the springs in the Munzur mountains with an altitude of 3.300 m and takes its name after the Munzur Mountains, has a length of 144 km. The Munzur Stream, which is fed by numerous small streams and flows very fast in deep gorges, is quite rich in terms of both vegetation and wildlife and different natural landscapes throughout the valley, starting from the springs that give birth to the stream. Although the flow of Munzur Stream is not very regular, approximately 20 kilometers of the stream has the potential to be suitable for rafting. The Munzur Stream offers different views with its natural vegetation in the places where the bed and valley expands, interesting rock formations on steep slopes where the valley is narrow and deep, and canyons and waterfalls that can be found sporadically.

Station 1 is the source point of the Munzur stream (39° 19' 52.6" N; 39° 03' 18.5" E) its natural structure is intact. The flow rate and flow velocity of the stream increase as the number of springs where water originates increases during the spring and summer months. On the other hand, as there is the significant decrease in the number of springs during the autumn and winter months, the flow rate and the flow velocity of the stream also reduce.

Station 2 (39° 18' 02.8" N; 39° 22' 12.4" E) in the Munzur valley, its approximate distance to Tunceli is 53 km. It is the discharge area of Ovacık district's domestic wastewater to the Munzur stream. In addition, animal husbandry is also concentrated in this region. During the spring and summer months, the flow rate and flow velocity of the water are high as a result of the effect of rain and melting snow water.

Station 3 (39° 08' 03.4" N; 39° 29' 41.0" E) In the Munzur valley, its distance to Tunceli is about 8 km. During the spring and summer months, the flow rate and flow velocity of the water are higher than the first two stations due to the rain and melting snow waters and due to the fact that the stream is fed by greater number of streams and has a narrower and steeper bed.



Figure 1. Shows the sampling stations.

Dissolved oxygen, the water temperature, pH, and were measured in situ with a multi-parameter probe (Hach-Lange HQ40d). Other chemical variables such as total nitrogen (TN), nitrat (NO3-), total phosphorus (TP) and orthophosphate (PO4-3) were analyzed in the laboratory by using standard methods (APHA, 2012).

Monthly samples were taken from three stations between October 2016 and September 2017 in order to investigate the relationships between the epilithic diatoms in Munzur Stream and the environmental variable. Epilithic diatoms were collected monthly by pooling rock scrapling from 5 to 8 rocks, depending on size, into plastic containers. In each case, the upper surfaces of the rocks were scraped using a knife. To prepare permanent diatom slides, sub-samples were taken and strong acidic solution (50:50 nitric/sulphuric acid) was added to digest organic material European EN 13946 (ECS, 2014). The Naphrax was used to mount diatom slides to examine under a light microscope at the 1000x magnification and to count at least 400 valves per slide. Species identification was done according to diatom taxonomic books (Krammer, 2000, 2002, Lange-Bertalot, 2001, Lange-Bertalot et al. 2017). The abundance of all taxa was expressed as relative counts before analysis. The diatom community structural attributes of species richness (S), Shannon–Weiner index (H), and species evenness (J) were used to characterize each site. We calculated the Shannon diversity index Shannon & Weaver (1949), the Pielou evenness (Pielou 1966), species richness. These metrics are commonly used in water quality bioassessment (Stevenson et al. 2010).

Canonical Correapondence Analysis (CCA) was used to display relationship among 49 spesies and 7 environmental variables. Before CCA, Detrended Correspondence Analysis (DCA) was used to see compadibility of our data for CCA. During CCA, rare species were removed from the data to eliminate the influence of multi-colinearity and arceffect. Besides, both species and ecological data were long-transformed and tested with the Monte Carlo test (499 permutations). CANOCO 4.5 Ter Braak & Smilauer (2002) and SPSS 22.0 statistical packages were applied for multivariate statistical and comparative analyses, respectively.

### 3. Results

The physical and chemical data obtained from the station are shown in Table 1. In this study, water temperature and pH did not show significant changes between stations, while dissolved oxygen levels were slightly different. Orthophosphate showed significant changes both between stations and between seasons. Total nitrogen and nitrate values are minimum in winter and maximum in spring and early summer. While decreasing in summer, it started to rise again in the last months of autumn. A total of 69 taxa were identified in the stream (Table 2).

Cocconeis placentula, Cymbella affinis Cymbella exiformis, Diatoma vulgaris, Didymosphenia geminata, Gomphonema olivaceum, Hannaea arcus and Ulnaria ulna were important in both relative abundance and frequency of occurrence at all stations. Cymbella minuta was an important species observed in all stations in Munzur Stream, especially at stations 2 and 3 in terms of relative abundance and frequency of occurrence, it was a species found with a significant relative abundance at station 2 in September and mostly at station 3. Diatoma vulgaris, Cymbella affinis and Cocconeis placentula are among the most dominant species in the study area and they were found continuously in all stations. Hannaea arcus was recorded with different frequencies and relative abundance in all stations in the study area. While it was a species constantly found at station I, it had a significant relative abundance especially in February at stations 2 and 3. Didymosphenia geminata was recorded at different frequencies and relative abundance at all stations. While it was mostly observed species at stations 1 and 3, it was constantly found at station 2. Gomphonema olivaceum reached a significant relative density at all stations and was significant in terms of frequency of occurrence. Gyrosigma acuminatum was recorded with low relative densities in Munzur Stream; it was found generally at Station 1 and rarely at Stations 2 and 3. Nitzschia palea was found rarely and with low relative density at the station 3. Nitzschia amphibia was found at the station 2 which is under the influence of domestic waste. Ulnaria ulna was a species found generally with low relative abundance and constantly at all stations. Regarding diversity (H), values ranged from 1.54 (in December, at S3) to 2.99 (in October, at S3). It is difficult to obtain a clear seasonal or temporal pattern. Evenness ranged from 0.09 to 0.22 at station S3 in September (at S1, in September). Large fluctuations did not allow establishing reasonable spatial or temporal trends (Fig. 2). Evenness ranged from 0.09 at site S3 in September, to 0.22 at S1, in September (Fig. 3).

All sites and species were close to 1 value. CCA analysis was performed to explain the relationships between diatom species and environmental factors during the study period. 16% of the cumulative variance in the diatom species was explained by the CCA first two axies with 73.1 % the species–environment correlation.In the present study, samples could not be taken for 2 months because of the adverse weather conditions and the species identified for 3 times and more were used; Denticula elegans, Didyosphenia geminata and Cymatoplora solea were negatively correlated with temperature which indicated that they found at low temperatures (autumn months). Diatoma ehrenbergi, Ulnaria ulna and Navicula viridula var. linear is were affected by orthophosphate, while Navicula micropupula, Navicula proctata, and Gyrosigma acuminatum were negative correlated with orthophosphate (Fig. 4).

The results of the CCA showed that the abundance of Cymbella lanceolata and Nitzschia amphibia were associated with TP while Cymbella affinis and Gomphonema olivaceum were negatively correlated with this factor. TN and NO3 were effective in spring and summer, TP, and DO were effective in autumm and winter months (Fig. 4). TP and PO4 were more effective at stations 2 and 3. In this study, 7 environmental parameters, 3 sampling stations, CCA were plotted between dominant species and within each month.

		Min.	Max.	Mean
1st Station 2 <sup>nd</sup> Station	Water Temperature (°C)	5.10	12.18	8.10
	Dissolved Oxygen (mg/L)	8.63	11.71	10.27
	рH	7.82	8.48	8.33
	$NO3$ (mg $I-1$ )	0.40	1.30	0.99
	$PO4$ (mg $I-1$ )	0.12	0.30	0.22
	$TP (mg I-1)$	0.06	0.62	0.26
	TN (mg $I^{-1}$ )	0.07	1.30	0.46
	Water Temperature (°C)	6.00	15.60	10.40
	Dissolved Oxygen (mg l-1)	8.21	11.23	9.65
	рH	8.00	8.72	8.29
	$NO3$ (mg $I-1$ )	0.58	1.30	1.06
	$PO4$ (mg $I-1$ )	0.09	0.56	0.30
	$TP (mg I-1)$	0.05	0.80	0.30
	TN (mg $I^{-1}$ )	0.08	1.60	1.16

Table 1. Mean values of physico-chemical parameters during study period of Munzur Stream.



Table 2. Epilithic diatoms of Munzur Stream and yearly mean relative abundance at the stations.







Table 3. Summary of CCA results. First two axes explained 73.1% of relationships between 49 species and 7 environmental

variables.					
Axes	1	$\overline{2}$	3	4	Total inertia
Eigenvalues	0.146	0.127	0.048	0.035	1,709
Species-environment correlations	0.879	0.795	0.658	0.705	
Cumulative percentage variance of species data	8.6	16	18.8	20,8	
of species environment relation	39.2	73.1	86,1	95.4	
Sum of all eigenvalues				1,709	
Sum of all canonical eigenvalues				0.373	



Figure 2. Diversities (H') of epilithic diatom communites.



Figure 3. Evennes (J') of epilithic diatom communites.



Figure 4. CCA diagrams of all data and species with variables in (a) Sampling site, environmental variable(s) and sampling months (b) distribution of species, occurred three or more times, with sampling sites.

#### 4. Discussion

Benthic diatoms function as an important component to maintain the health of aquatic ecosystems. Several studies have reported that composition and structure of the benthic diatom is influenced by various chemical and physical environmental variables (Mirzahasanlou et al., 2020). Diatom community composition is often described as the relative abundances of species in a site. In Munzur Stream, Diatoma vulgaris, Cymbella affinis and Cocconeis placentula have been the species that are constantly found in all months and at all stations and have reached a significant relative density. The relative abundance of species in the community defines both species tolerance and preference for prevailing conditions, and the competitiveness of species as a species is the highest under the most appropriate conditions. Hoagland et al. (1982) stated that community composition can change even if species richness or productivity remains unchanged, so it is perhaps the most relevant measure of the biological response to changes in the environment.

These species were likely to be more successful in their tolerance and competition with other species. Navicula, Nitzschia and Gomphonema were the genera represented with the highest number of species in the present study. Bere (2011) stated that Navicula, Nitzschia and Gomphonema are cosmopolitan genera and they are genus with a wide tolerance range against various water quality variables. Cocconeis placentula is considered a cosmopolitan species as it is a diatom commonly found in different freshwater bodies with different electrolyte contents. Çelekli et al. (2019) reported that Cocconeis placentula is a species tolerant to pollution associated with different water qualities from good ecological to poor conditions. Omar (2010) state that the species Cymbella affinis and Cocconeis placentula are the indicators of barely polluted or clean waters (Klee, 1991; Lange-Bertalot, 1978) state that Diatoma vulgaris are the indicators of barely polluted or clean waters. It has been found that Cymbella affinis was mostly encountered in alkaline and calcareous habitats (Round, 1984). In epiphytic and epilithic flora where this species is littoral; it has been stated that it can be seen in streams and still waters (Adrian, 2011) and it was determined to be one of the indicator species for waters that are in good condition in terms of biological water quality class (Rimet et al., 2005). Cymbella affinis was one of the species constantly found at all stations and one of the most dominant species in the study area in Munzur Stream. Most of the taxa belonging to the genus Cymbella are indicator organisms of waters with rich oxygen concentration and poor organic nitrogen content (Van Dam et al., 1994). The species Cymbella minuta is usually found in alkaline waters (Edward et al., 2000). Also in the present study, Cymbella minuta, in all months of sampling, was one of the important species in terms of relative abundance and frequency of occurrence especially at the stations 2 and 3. At the station 2 which is under the influence of domestic waste it reached a significant relative density in September. Kelly et al. (1995) stated that Cymbella minuta is a species tolerant to organic pollution and is commonly found in waters where organic pollution occurs. Štefková (2006) noted that Cymbellam minuta is an alkaliphilus species.

One of the dominant diatoms frequently observed in the study area is Hannaea arcus. This species is not very tolerant to organic pollution and distributed in relatively uncontaminated, neutral or alkaline habitats (Bahls, 1993). Species belonging to the genus Hannaea are observed in fast flowing, oligotrophic, neutral and low nitrate concentration water environments (Krammer and Lange-Bertalot, 1991a; Rott et al., 1999). Hannaea arcus have been reported to have higher abundance in cool and fast flowing streams. Çelekli et al. (2018) determined in their study that there were species sensitive to nutrients such as Hannaea arcus in areas with good ecological status.

Didymosphenia geminata was observed at different frequencies and relative abundance at all stations in our study area, especially at Station 1, where the stream had cooler and cleaner water and oxygen levels were generally higher than other stations. It has been noted that this species is generally seen in low nutrient and high oxygen conditions; in northern regions, higher altitudes and mostly oligotrophic habitats, but sometimes it can also exist in waters with high nutrient content (Whitton et al., 2009). Çelekli et al. (2018) stated that Didymosphenia geminata and Hannaea arcus are the species that are susceptible to nutrients and are found in areas of high ecological quality. The composition of dominant species of the Munzur Stream has characteristics of numerous montane rivers and streams with lime bottom in different physical-geographical regions Stenina and Steryagova (2019). Species such as Hannaea arcus and Didymosphenia geminata prefer fast-flow rivers with cold weakly mineralized water.

In Munzur Stream Gomphonema olivaceum reached significant relative density at all stations and was also important in terms of occurrence frequency. Butcher (1946) stated that Gomphonema olivaceum is an indicator species

of clean and high calcareous waters. It has been noted that the genus Nitzschia is the most commonly observed genus in waters with a rich concentration of nutrients, poor in dissolved oxygen, and under the influence of organic pollution (Palmer, 1969; Van Dam et al., 1994). Nitzschia palea has been recorded as the most tolerant species to organic pollution worldwide (Gómez, 1998; Gómez and Licursi, 2001; Soininen, 2002; Dere et al., 2006; Szczepocka and Szulc, 2009). This species can be found even in eutrophic and heavily polluted waters. Again, several researchers have stated that this species is an indicator of organic pollution in rivers (Khan, 1990; Kelly, 1998; Soininen, 2002). In the present study, Nitzschia palea was found with low relative density and rarely at the station 3. It has been indicated that Nitzschia amphibia Grunow is tolerant to organic pollution (Van Dam et al., 1994). This species was also found with low relative density and rarely at the station 2 in the study area. The fact that a small number of these species were encountered at the stations 2 and 3 in our study area indicates that the pollution level of the stream in our study area is not yet at significant levels.

The station 2 is the discharge point of the district's domestic wastewater, therefore organic pollution indicator species were found with low relative density at these stations. Ulnaria ulna was a species found continuously with low relative densities in general at all stations in Munzur Stream. In the CCA results, it was also found on the orthophosphate side in autumn and winter. Sienkiewicz (2013) stated that Ulnaria ulna has wide trophy tolerance and can live in oligo eutrophic water. The species recorded in Munzur Stream were Gyrosigma acuminatum, Gyrosigma attenatum. Gyrosigma acuminatum was the species generally found at station 1 and rarely at the stations 2 and 3. The genus Gyrosigma was reported to be observed very commonly in calcerous waters (Round, 1984).

There was no extremely dramatic differences between stations in terms of distribution of diatoms; however, species such as Cocconeis placentula, Cymbella affinis, Gomphonema olivaceum and Hannaea arcus were generally recorded in higher relative abundance and more frequently. In the present study, the first station in the stream is the headwater source and apparently includes clean water where there was relatively low amount pollution although the flow rate was relatively low. The station receives a sufficient amount of light due to its open surface area in which four species (Cymbella affinis, Denticula tennuis, Diatoma vulgaris and Gomphonema olivaceum) exhibited dominancy over others. Gomez and Licuris (2001) stated in their study that these species were dominant in the regions which are closer to the resource and where the water is clean. In the present study, TN and TP play an important role in structuring benthic diatom communities, followed by WT pH, DO, and NO3-. Major nutrients [i.e., nitrogen (N) and phosphorus (P)] concentration of surface waters was a primary factor contributing to variation in benthic-algal assemblages (Leland and Porter, 2000). These results were similar to the studies by (Christie and Smol 1993; Schönfelder et al., 2002), and they demonstrated that TN and TP were two of the important ecological variables. Results of the CCA indicated the benthic diatom assemblage was significantly correlated with nutrient supply during the sampling period (Biggs and Smith, 2002) provided evidence that flood frequency and nutrient supply were the primary constraints on benthic-algal biomass accrual in New Zealand Rivers. Species diversity is frequently used in ecological status assessment for explaining spatial and temporal patterns of biotic communities, some studies have found that species biodiversity may increase or decrease with the increase of pollution and it mainly depends on the degree and type of pollution (Chena et al., 2016). The diversity index in the present study ranged between 1.54 and 2.99. Seasonally diversity index was recorded to be maximum in summer and minimum in winter. The Shannon-Weiner diversity index showed the maximum value (2.99) at site 3.

According to Patrick (1973), algal species richness and diversity increase generally with temperature, although this is modulated by the climatic characteristics of the basins. Evenness Index (J) was used according to (Pielou, 1966) to measure species distribution and it ranges from 0 to 1. Evenness ranged from 0.09 at site S3 in September, to 0.22 at S1, in September (Fig. 3). All sites and species were close to 1 value, which means that the diatoms are distributed equally (Mahmoud et al., 2018). The result of the present study showed that the diversity indices (H' J') did not show any significant difference between stations. Moreover, the spatial variation in communities may also reflect the effects of unmeasured environmental variables Virtanen and Soininen (2012).

In this study, the stream is located in a mountainous area, forest is the main landscape type, the water quality is relatively better than in the urban streams, and the diatoms are oligotrophic species. Evaluating ecological condition of water masses via physical and chemical variables is one of the related approaches for water quality; chemistry at any time of sampling is the instant view of water quality Bere and Tundisi (2011). As water quality indicators, diatoms have been proved to be accurate and cost-effective and they can provide a comprehensive picture of stream conditions Virtanen and Soininen (2012). Munzur stream may be classified as oligotrophic with the mean value of nitrate and phosphate. The occurrence of members of various epilithic diatoms is supported with the oligotrophic characteristic of the stream. Areas of high and good ecological status are characterized by nutrient-sensitive species such Cymbella affinis Cymbella excisiformis, Didymosphenia geminata, Hannaea arcus and Gomphonema olivaceum. In conclusion, in this study, physico-chemical factors affecting this distribution with existing taxa were determined in Munzur Stream, whose benthic diatoms have not been studied before. The taxa determined are new records for the study area since this is the first study conducted and will make a significant contribution in terms of determining the biodiversity of the region.

## Conflicts of Interests

Authors declare that there is no conflict of interests

#### Financial Disclosure

Author declare no financial support.

## Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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