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Trophic Status Assessment In Some Lotic Ecosystem In Turkey (Sakarya Basin)

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ABSTRACT:

- Highlights:
- Water quality
- Benthic diatoms
- Lotic ecosystems

Keywords:

- Aquatic ecosystem
- Trophic status
- Creek
- Diatom
- · Water quality

Benthic diatoms constitute a very important group of organisms in terms of providing information about the water quality of the lotic ecosystems. This research was carried out in Istanbul creek, Aliaga creek, Bostancı creek and Orta creek in the Sakarya basin. The trophic states of these creeks were evaluated according to their diatomes. In the study, the epilithic diatoms were determined to consist of 42 taxa belonging to İstanbul creek, 28 to Bostancı creek, 25 to Orta creek and 20 to Aliaga creek. A total of 51 taxa were determined in the creeks. In this study, *Cocconeis pediculus, Ulnaria ulna* and *Cymbella affinis* were found to have the highest abundant at the stations. In the creeks, water temperature, dissolved oxygen and pH were determined as respectively 9.1-14.1 °C, 8.2-9.4 mg L⁻¹, 7.1-7.5 and conductivity as 210.2 - 240.4 μ S cm⁻¹. Although different results were obtained according to the diatom indices criteria (IBD, SLA,IDSE,IDAP, TID, SID,CEE, WAT, TDI, SHE, TIT) the creeks were determined to be close to water quality class I -II according to the Surface Water Quality Regulation In general, according to the diatom index values, it has been determined that the creeks have good-medium quality water characteristics.

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INTRODUCTION

Aquatic ecosystems are ecologically and economically very important. Physicochemical parameters and biological indicators are taken into account in determining the pollution load in a river ecosystem. Since they contain indicator species in lotic aquatic systems, especially epilitic diatoms have an important place in the nutrient cycle. Diatoms indicate water quality level of aquatic environments. Diatoms have high tolerances to aquatic physiochemical parameters, flow regime, altitude, organic or inorganic toxicants and other environmental parameters (Tokatlı et al., 2020). They respond directly and rapidly to many environmental parameters such as meteorological changes, geological structure, anthropogenic activities (Stevenson, 1997; Pan et al., 2000; Chaïb and Tison-Rosebery, 2012; Tatenda et al., 2016). Because of these properties, they are used as bioindicators in aquatic systems. Determining the geographical distribution of diatoms and the factors affecting them are very important for the sustainability of water resources. Because benthic diatom species in aquatic ecosystems is crucial for the development of future water quality assessment tools (Potapova and Charles, 2003; Chaïb and Tison-Rosebery, 2012; Taylor et al., 2007). At the same time, diatoms represent an important component of phytobenthos quality parameters. Some diatom indices have been developed in different ecological regions to evaluate the ecological status of fresh waters, especially streams and rivers (Acs et al., 2004). Some of these are pollution sensitive index-IPS (Cemagref 1982), Descy and Coste Index- CEE (Descy and Coste, 1991), trophic index-TI (Rott et al. 1999), eutrophication and/or pollution index-diatom-EPI-D (Dell'Uomo 2004) is the diatom trophic index-TDI. (Kelly et al. 2008) and trophic index Turkey-TIT (Celekli et al. 2019). Water quality monitoring based on diatom indices has come to the fore as a new issue in Turkey, especially after the 2000s. In Turkey, TDI, SI and GDI indexes were first used by Gürbüz and Kıvrak (2002) to determine water quality in the Karasu River (Solak and Acs 2011). After these years, it has been widely used in the evaluation of the trophic conditions of Turkish freshwater ecosystems (Çiçek and Ertan, 2012; Tokatlı, 2012; Maraşlıoğlu et al., 2016; Becer et al., 2019).

This study was carried out to determine some water quality and epilithic diatoms of the Istanbul creek, Aliaga, Bostancı and Orta creeks. At the same time, the aim of this study was to evaluate the diatom composition of the creeks (Istanbul, Aliaga, Bostancı and Orta).

MATERIALS AND METHODS

İstanbul creek (45°07'07" N; 36°26'78" E), Aliaga creek (40°38'28" N; 30°14'10" E), Bostancı creek (40°38'26"N; 30°13' 50"E), and Orta creek (40°38' 24" N; 30°13' 55" E) are located in Sakarya province, Sapanca area (Figure 1). In this study, diatom samples were collected seasonally from four creeks, among the month in January, April, July and October 2017. Randomly at least five stones were collected from each creek. The surface of stones was scraped with a toothbrush in 100 mL of distilled water as a composite sample per station (European Committee for Standardization, 2014). Diatoms were fixed in 4% formaldehyde until analysis (Anonymous, 2019). Permanent slides were prepared after boiling the diatom samples in acidic solution and the diatoms were fixed in Entellan medium (Wetzel and Likens, 1991). At least 400 valves in each slide were counted for all samples. Taxonomic identifications were made according to relevant sources (Patrick and Reimer 1966, 1975; Husted, 1985; Krammer and Lange-Bertalot 1991a, 1991b, 1999a, 1999b). The identified species were updated from the algaebase database (Guiry and Guiry, 2021) and Turkishalgae electronic publication (Maraşlıoğlu and Gönülol, 2021). In addition, the names of the authors were shortened according to Brummit and Powell (1992). In the research, IBD: Biological Diatom (Coste et al., 2009), IPS: Pollution Sensitivity (Cemagref, 1982), IDG:Generic Diatom (Cemagref, 1982; Rumeau and Coste, 1988), DESCY: Descy (Descy, 1979), SLA: Sladecek (Sladecek, 1986), IDSE: Leclercq and Maquet (Leclercq and Maquet,

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1987), IDAP: Artois-Picardie Diatom (Prygiel et al., 1996), EPI-D: Eutrophication Pollution -Diatoms (Dell'Uomo, 2004), LOBO: Lobo (Lobo et al., 2004), TID: Rott Trophic (Rott et al., 1999), SID: Rott Saprobic (Rott et al., 1997), CEE: Commission for Economical Community (Descy and Coste, 1991), WAT: Watanabe (Watanabe et al., 1990), TDI: Trophic Diatom (Kelly and Whitton, 1995), SHE: Steinberg and Schiefele (Steinberg and Schiefele, 1988) were were calculated with OMNIDIA 6.08 (Lecointe and Coste, 2017) and Trophic Turkey (TIT) was determined (Çelekli et al., 2019). In the study, Shannon – Weaver (H'), Sorensen similarity (Q), and regularity (*E*) were determined. Water temperature, conductivity, pH and dissolved oxygen were measured *in situ* with portable multiparameter instruments (IQ scientific instrument IQ150, AZ instrument 84051). Also, correlation analysis was performed to determine the relationship of diatoms and physicochemical parameters. Statistical analyzes were performed with the SPSS v.11.5 package program and p <0.05 value was evaluated as significant (Anonymous, 2004).

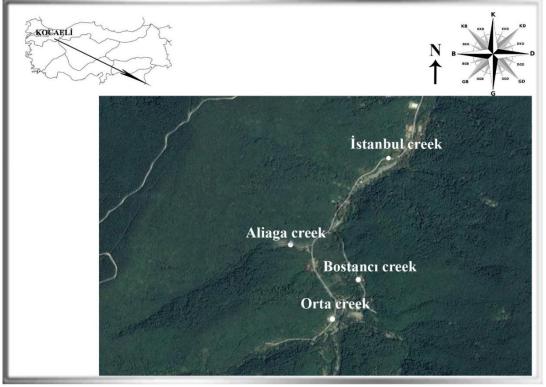


Figure 1. Research area (https://www.google.com/maps)

RESULTS AND DISCUSSION

The epilithic algal flora consisted of 51 taxa belonging to the Bacillariophyta. 42 species belonging to the İstanbul creek, 28 taxa belonging to the Bostancı creek, 25 taxa belonging to the Orta creek and 20 species belonging to the Aliaga creek, that are the important streams of the Sakarya basin, were determined. Water temperature (9.1 - 14.1 °C), dissolved oxygen (8.2-9.4 mg L⁻¹), pH (7.1-7.5) and conductivity (210.2 - 240.4 μ S cm⁻¹) were determined in the research. A list of the identified taxa was given in the Table 1.

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Table 1. Epilithic diatoms in the research stations

Species/Stations	Istanbul Creek	Bostancı Creek	Aliaga Creek	Orta Creek
Achnanthidium affine (Grunow) Czarnecki	-	+	+	-
Achnanthidium minutissimum (Kütz.) Czarnecki	-	+	+	-
Amphora ovalis (Kütz.) Kütz.	+	+	-	-
Caloneis silicula (Ehrenb.) Cleve	+	+	-	-
Campylodiscus clypeus (Ehrenb.) Ehrenb. ex Kütz.	-	+	-	-
Cocconeis pediculus Ehrenb.	+	+	+	+
Cocconeis placentula Ehrenb.	+	+	+	+
Cymbella affinis Kütz.	+	+	-	+
Cymbella neocistula Krammer	-	+	+	-
Cymbella tumida (Bréb.) Van Heurck	+	+	+	-
<i>Cymbella</i> sp.	+	+	-	+
Cymbopleura reinhardtii (Grunow) Krammer	+	-	+	+
Denticula tenuis Kütz.	+	+	-	+
Diatoma ehrenbergii Kütz.	+	-	+	+
Diatoma vulgaris Bory	+	+	+	+
Didymosphenia geminata (Lyngbye) Mart. Schmidt	+	-	+	+
Encyonema leibleinii (C.Agardh) W.J.Silva, R.Jahn,	+	+	-	-
T.A.V.Ludwig, and M.Menezes				
Encyonema minutum (Hilse) D.G.Mann	+	-	-	+
Encyonema silesiacum (Bleisch) D.G.Mann	+	-	_	-
Encyonema ventricosum (C.Agardh) Grunow	+	+	+	+
Encyonopsis microcephala (Grunow) Krammer	+	-	_	+
<i>Europia bilunaris</i> (Ehrenb.) Schaarschmidt	+	-	_	-
Fragilaria crotonensis Kitton	+	-	+	_
Gomphonella olivacea (Hornem.) Rabenhorst	+	+	Τ	-
Gomphonema angustum C.Agardh	+	Т	-	-
Gomphonema lateripunctatum E.Reichardt and Lange-Bert	+	-	-	-
		-	-	-
Gomphonema parvulum (Kütz.) Kütz.	+	+	+	-
Gomphonema truncatum Ehrenb.	+	+	-	-
Meridion circulare (Greville) C.Agardh	+	-	-	+
Melosira lineata (Dillwyn) C.Agardh	+	-	-	-
Melosira varians C.Agardh	+	-	+	+
Navicula cryptocephala Kütz.	-	+	+	+
Navicula lenzii Krasske	+	-	-	+
Navicula menisculus Schumann	+	-	-	-
Navicula radiosa Kütz.	+	+	-	-
Navicula rhynchocephala Kütz.	-	+	+	+
Nitzschia dissipata (Kütz.) Rabenh.	+	-	-	-
Nitzschia graciliformis Lange-Bertalot and Simonsen	+	-	-	-
Nitzschia palea (Kütz.) W.Smith	-	-	-	+
Nitzschia recta Hantz. ex Rabenh.	+	+	-	+
Nitzschia sp.	+	+	-	+
Odontidium hyemale (Roth) Kütz.	+	-	+	-
Odontidium mesodon (Kütz.) Kütz.	+	-	-	-
Planothidium lanceolatum (Bréb. ex Kütz.) Lange-Bertalot	-	-	+	-
Reimeria sinuata (W.Gregory) Kociolek and Stoermer	-	+	-	+
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	+	+	+	+
Surirella brebissonii Krammer and Lange-Bertalot	+	-	_	+
Surirella minuta Bréb. ex Kütz.	+	+	_	+
<i>Surrella</i> sp.	+	т _	-	–
<i>Ulnaria acus</i> (Kütz.) Aboal		-	-	-
	+	+	-	+
Ulnaria ulna (Nitzsch) Compère	+	+	+	-

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Diatom Assemblages

In the study, *Cocconeis pediculus, Ulnaria ulna,* and *Cymbella affinis* were the most abundant species in epilitic composition. Diatom indices (IBD: Biological Diatom, IPS: Pollution Sensitivity, IDG:Generic Diatom, DESCY: Descy, SLA: Sladecek, IDSE: Leclercq and Maquet, IDAP: Artois-Picardie Diatom, EPI-D: Eutrophication Pollution -Diatoms, LOBO: Lobo, TID: Rott Trophic, SID: Rott Saprobic, CEE: Commission for Economical Community, WAT: Watanabe, TDI: Trophic Diatom, SHE: Steinberg and Schiefele, TIT: Trophic Turkey) were evaluated in the research. Eutrophication/organic load or water quality rates was evaluated. According to diatom indices values were given in the Table 2.

Table 2. Diatom indices in the streams

					Eutrophication/organic load or water
İndices/Creek	İstanbul	Aliağa	Bostancı	Orta	quality rates
IBD/7	4.59	4.96	5.24	5.50	1 (worse)-7 (best)
IPS/5	3.30	4.01	4.10	3.95	1 (worse)-5 (best)
IDG/5	4.03	3.75	4.15	4.14	1 (worse-5 (best)
DESCY/5	3.85	3.90	3.93	3.86	1 (worse)-5 (best)
SLA/4	1.16	1.73	1.39	1.55	0 (best)-4 (worse)
IDSE/5	3.59	3.43	3.49	3.48	1 (worse)-5 (best)
IDAP/5	3.43	4.17	4.00	3.36	1 (worse)- 5(best)
EPI-D/4	1.09	1.32	1.10	1.36	0 (best)-4 (worse)
LOBO/4	2.59	2.62	2.19	2.68	1 (best)- 4 (worse)
TID/4	2.14	2.12	2.05	2.57	0.3 (best)-3.9 (worse)
SID/4	1.38	1.82	1.59	1.68	1 (best)-3.8 (worse)
CEE/10	5.58	7.79	7.62	6.65	0 (worse)-10 (best)
WAT/100	73.28	74.44	74.22	67.29	0 (worse)-100 (best)
TDI/100	33.36	52.69	49.26	33.39	0 (best)-100 (worse)
SHE/7	5.51	5.64	5.71	5.78	1 (worse)-7 (best)
TIT/4	2.52	2.03	2.02	2.42	0 (best)-4 (worse)

In general, according to the diatom indices values, it has been determined that the creeks have good-medium quality water characteristics. The results were determined significant Pearson correlations between diatom indices value and some water physical and chemical parameters. The high negative correlation in terms of temperature value was TIT (r = -0.955 *, p < 0.05) and the high positive correlations were determined between CEE (r = 0.962 *, p < 0.05) and TDI (r = 0.961 *, p < 0.05).

Table 3. Pearson correlation coefficients between physico-chemical parameters and c	Table 3. Pearson c	erricients between physico-chemical param	ers and diatom indices
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	Temperature	pН	Dissolved Oxygen	Conductivity
IBD	0.228	0.809	0.848	0.617
IPS	0.783	0.996**	-0.136	0.925
IDG	-0.565	0.067	0.480	-0,370
SLA	0.768	0.697	-0.109	0.940
IDSE	-0.862	-0.847	0.141	-0.996**
IDAP	0.946	0.556	-0.843	0.668
EPID	0.298	0.414	0.496	0.661
LOBO	-0.306	-0.411	0.581	-0.069
TID	-0.404	0.095	0.967*	0.089
SID	0.807	0.752	-064	-0.966**
CEE	0.962*	0.890	-0.488	0.921
WAT	0.477	-0.088	-0.981 *	-0.013
TDI	0.961*	0.621	-0.808	0.712
SHE	-0.186	-0.139	0.688	0.157
TIT	-0.955*	-0.761	0.716	-0.773

* Corellation is significant at the 0.05 level

** Corellation is significant at the 0.01

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The highest positive correlation in terms of pH value was determined as IPS (r = 0.996 **, p < 0.01). In terms of dissolved oxygen value, the high negative correlation was found between WATT (r = -0.981 *, p < 0.05) and the high positive correlations were found between TID (r = 0.967 *, p < 0.05). The highest negative correlation in terms of conductivity value was determined as IDSE (r = -0.996 *, p < 0.01) and SID (r = -0.966 **, p < 0.01) indices. The Pearson correlation coefficients of the mentioned parameters are given in Table 3. The correlations of IPS, IDG, DESCY, EPID and LOBO indices with other indices are low so they are not included in the table 4. It was determined that the highest positive correlation between the diatom indices (according to p < 0.01 status) between IBD and SHE(0.997**), SLA and SID (0.996 **), IDAP and TDI (0.997 **). The negative high correlation (according to the p < 0.05 status) with SLA and IDSE (-0.966^*), IDSE with SID (-0.985 *), IDAP with TIT (-0.957 *), TID with WAT (-0.988 *), CEE with TIT (-0.956 *) and between TDI and TIT (-0.976 *) were determined. This situation is thought to be due to the fact that low values in some as indicate poor quality and in others very good quality. Pearson's correlation values between the biotic indices were given in Table 4.

Table 4. Pearson's correlation values between the biotic indices used

	IBD	SLA	IDSE	IDAP	TID	SID	CEE	WAT	TDI	SHE	TIT
IBD	1										
SLA	0.498	1									
IDSE	-0.584	-0.966*	1								
IDAP	-0.037	0.532	-0.650	1							
TID	0.618	0.235	-0.116	-0.679	1						
SID	0.528	0.996**	-0.985*	0.579	0.193	1					
CEE	0.462	0.761	-0.894	-0.867	-0.267	0.813	1				
WAT	-0.640	-0.123	0.029	0.734	-0.988*	0.088	0.314	1			
TDI	0.042	0.563	-0.691	0.997**	0.636	0.613	0.902	0.687	1		
SHE	0.997**	° 0.561	-0.648	0.038	0.581	0.591	0.529	-0.595	0.116	1	
TIT	-0.234	-0.577	0.737	-0.957*	0.536	-0.637	-0.956*	-0.568	-0.976*	-0.303	1

* Corellation is significant at the 0.05 level

** Corellation is significant at the 0.01

Biological examination and monitoring aquatic systems enables us to understand ecological integrity of environment and the reactions of living things to it. Diatoms, which are among the elements of biodiversity, react quickly and sensitively to environmental quality factors. Therefore, Bacillariophyta members are generally considered to be important ecological indicators of aquatic systems (Cox, 1996; Potapova and Charles, 2003; Kelly et al., 2008; Celekli et al., 2019). In the study, the epilithic diatoms were determined to consist of 42 taxa belonging to İstanbul creek, 28 to Bostancı creek, 25 to Orta creek and 20 to Aliaga creek. A total of 51 taxa were determined in the lotic ecosystems. The total number of species determined in the study, 37% were determined in the Istanbul creek, 25% in the Bostancı creek, 22% in the Orta creek and 16% in the Aliaga creek. The highest rate found in Istanbul creek may have been caused by the flow of other streams into this stream and the transportation of species. In the study, Achnanthidium minutissimum species, which is widely found in different water qualities and survives from clean to wastewater and α - and β - mesosaprobic conditions, was detected only in Aliaga and Bostanci creek. In this study, C. pediculus, Ulnaria ulna and Cymbella affinis were found to have the highest rate. Patrick and Reimer (1966) stated that Lange-Bertalot classified C. pediculus as pollutionintolerant, while C. pediculus was sometimes considered a pollution-tolerant species. According to generally assessment shows that C. pediculus can be determined in a relatively wide range of environmental conditions (Brown and Olive, 1995). Clean and slightly dirty water indicator specified as C. affinis, in Turkey in the work done in Akcay, has been identified as predominant in areas where the

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water is clean (Kelly and Whitton, 1995; Pabuçcu et al., 2007). This species has been identified in İstanbul Stream, Bostancı and Orta creeks. *Cymbella affinis*, which is an alkaliphilic species (Gómez and Licursi, 2001) was dominat in Orta Creek. In particular, it has been reported that *Cymbella affinis* frequently develops as benthic especially in rivers and is dominant in clean waters (Varol and Şen, 2014). The Orta creek has been determined to be cleaner because it is far from allochthonous factors and is located in the forest. *Ulnaria ulna* was reported to be the most common species indicator organic pollution tolerant, widespread and anthropogenic pollution in rivers from oligotrophic to eutrophic (Van Dam et al,1994; Cox, 1996; Iliopoulou-Georgudakig et al., 2003). In the study, *C. pediculus* and *Ulnaria ulna* were determined at the highest rate in Aliaga creek. The abundance of these species may be the result of anthropogenic activities around the stream. Similar results have been reported in many research carried out in order to classify the water quality by using diatom indices and lotic systems in Turkey (Gün, 2011; Solak, 2011; Tokatlı, 2012; Çiçek and Ertan, 2012; Maraşlıoğlu et al., 2016; Çetin and Demir, 2019).

In the creeks were determined as: water temperature, dissolved oxygen, pH varied from 9.1-14.1 °C, 8.2-9.4 mg Γ^{-1} , 7.1-7.5, and conductivity as 210.2- 240.4 μ S cm⁻¹. Water temperature is very important on species and in many physicochemical conditions. It varies depending on the climatic and atmospheric conditions of the aquatic environment, as well as the flow rate characteristics. It has been determined that the water temperature values in the streams evaluated in the study vary seasonally within normal limits. It was determined that the research stations are alkaline according to their pH classification. Lotic systems were identified as the class I water quality according to the Surface Water Quality By-Law (YSKY, 2016). Water quality assessment made with physicochemical data in rivers expresses instantaneous pollution, but evaluations made according to algae give us information about pollution in the medium term (Kalyoncu, 2004). There were plenty of pebbles and stones in the streams and creek beds where the research was made, and the waters were very clear. It was determined that this situation supports the hydromorphological properties and water quality of the research stations. These indicators, as stated by WDF, hydromorphology is an important element in assessing the water quality of the river ecosystem. (Meier et al., 2013).

In correlation analysis, that the highest positive correlation between the diatom indices between IBD and SHE, SLA and SID, IDAP and TDI were determined. This positive correlation shows that the indices are consistent with each other and can be used together. SLA and IDSE, IDSE with SID, IDAP with TIT, TID with WAT, CEE with TIT and between TDI and TIT were determined that negative high correlation this research. This situation may be due to the difference in type and quality scale included in the es, as well as being used for different geographical regions. In the evaluation made according to biotic es in Istanbul creek; according to IBD, SHE, SLA, TDI were determined as water quality Class I. According to SID water quality Class I-II (oligosaprop) were determined. Also, the IDAP indices was determined as water quality Class II in the Istanbul creek. Water quality in Aliağa stream was determined as Class I according to IBD, SHE, SLA, IDAP values. While water quality class was determined as I-II according to SID in diatom indices and according to TDI quality as II (start of organic pollution). The water quality of Bostanci Stream was determined as Class I according to the values of IBD, SHE, SLA, IDAP diatom indices and as Class I-II according to the SID indice value. This creek according to TDI was determined as water quality class II (start of organic pollution). Although different results were obtained according to the diatom indices criteria, the streams were determined to be close to water quality class I-II according to the Surface Water Quality Regulation. These aquatic ecosystems were determined as good-medium quality when evaluated according to both water quality and diatom indices. Shannon-Weaver diversity (H') is used to determine the diversity of the communities in the ecosystem

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and the trophic level of the environment. Shannon-Weaver diversity (H') is used to determine the diversity of the communities in the ecosystem and the trophic level of the environment. A Shannon-Weaver diversity indice (H') value above 3 indicates that the water is clean and the taxa numbers are balanced, while low H' values below 3 indicate low diversity and more polluted ecosystems in the community (Clarke and Warwick, 2001). In the study, it was determined that the diversity indice values ranged from 3.41 to 5.35. According to these values, it has been determined that the streams have good quality water characteristics and balanced taxa number. In the research, similar results were found between the other determined parameters and Shannon – Weaver diversity (H') values. The Sorensen similarity indice (Q) has been determined from 0.47 to 0.63. According to these values, the closest similarity was determined between Istanbul creek and Orta creek (0.63), while the farthest similarity was determined between Aliaga creek and Orta creek. In addition to, the regularity indice (E) was determined from 0.90 to 0.95 in this study. According to these values, it has been determined that the species distributions of the researched water resources are regular and close to each other. In a study conducted on Liman creek, Findik creek, Karaçay creek, Kuruçay creek, Kurtköy creek, Mahmudiye creek, Istanbul creek and Sarp creek in the Sakarya basin, the Shannon-Weaver diversity index (H') value ranged between 0 and 2.94. This shows that the streams are under the pressure of pollution (Sevindik et al., 2023). The regularity index values of the same streams were determined between 0.01 and 1 (Sevindik et al., 2021). Istanbul creek, Orta creek, Aliaga creek and Orta creek, located in the same basin, were determined as good quality water. It is thought that the different situation detected in the streams in the same basin may be due to the distance of the streams to the settlements, the flow rate and the meteorological conditions that change throughout the year.

CONCLUSION

The ecological status of the water resources in this study was evaluated according to diatoms, which are indicators of biological quality. The water sources (Istanbul, Aliağa, Bostancı and Orta) where the research was conducted flow into Sapanca lake, which is used as drinking and irrigation water source. Therefore, it is very important to develop biological evaluation criteria in order to know and classify the quality of potable water resources. Due to its geographical location Turkey is a very rich country in terms of biodiversity. This diversity also affects the biological implementations. However, as prepared according to ecological and environmental conditions can be used in many different countries might give different results in Turkey's water resources. In this respect, it is recommended to determine biological water quality determination systems and eco-regions for monitoring aquatic organisms.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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