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ORIGINAL ARTICLE

Ecological status estimation of minimally disturbed rivers of the Western Mediterranean Basin (Türkiye) using diatom indices

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Abstract

The Water Framework Directive (WFD) suggests establishing reference conditions to help evaluate the ecological status of rivers in a specific ecoregion. Here we used eighteen diatom indices to estimate the ecological status of 44 candidate rivers for reference conditions in Türkiye's Western Mediterranean Basin. We collected data on epilithic diatoms and environmental variables three times (spring, summer, and fall) in 2017. Based on the redundancy analysis (RDA), seven environmental parameters significantly affected the distribution of abundant and indicator diatom species. Indicator species highly correlated with nitrate–nitrogen (NO_3–N) and total nitrogen (TN). These two environmental parameters also showed significant correlations with the Pampean Diatom Index (IDP), the Eutrophication/Pollution Index (EPI-D), the Trophic Diatom Index (TDI), and the Rott's Saprobic Index (ROTTs). Therefore, here we focused on the ecological quality status indication by these four indices. The TDI index was more consistent for estimating our streams' ecological status and revealed high ecological status for fifteen sampling sites. We conclude that areas with high ecological status will help set up a reference condition for future studies in the Western Mediterranean Basin and other basins in Türkiye.

Keywords Catchment geology · Ecological quality · Reference conditions · Running waters

Introduction

Diatoms are unicellular microscopic algae that colonize various submerged substrates in aquatic environments and are an important component of phytobenthos (Wetzel 1983; Dixit et al., 1992). Numerous studies were conducted to show the effects of environmental factors on their diversity, abundance, and distribution (Liess et al., 2009; Soininen, 2007; Solak et al., 2020; Tan et al., 2017; Tanabe et al., 2010). They are one of the most often used indicators for assessing the ecological status of rivers due to their shorter life cycles, fast growth rates, quick reactions to environmental changes, greater sensitivity to pollution and eutrophication than other organisms in the water, and ease of sampling (Çetin & Demir, 2019; Delgado et al., 2012; Karaouzas et al., 2019; Poikane et al., 2016; Vilmi et al., 2015; Waite et al., 2020).

Although diatoms have been used in the evaluation of running waters and different indices have been developed for many years, after the implementation of the Water Framework Directive (WFD) (European Union, 2000), biological quality elements (phytobenthos, benthic invertebrates, macrophytes, fish, and phytoplankton) have been favored primarily as the main assessment component, and consequently, diatom studies have gained great importance (Hering et al. 2010). As a result, countries that are part of the European Union have developed their own methods for evaluating the ecological quality of running waters using diatom communities (Žutinić et al., 2020). Diatom indices based on relative abundances of the species and weighted averages of environmental optima or autecological values for the taxa were utilized for this purpose (Smol & Stoermer, 2010). In addition to the indices developed in previous years such as Specific Pollution Sensitivity Index (IPS) (Cemagref, 1982), Trophic Diatom Index (TDI) (Kelly & Whitton, 1995), Eutrophication/Pollution Index (EPI-D) (Dell'Uomo, 2004), and the Biological Diatom Index (IBD) (Coste et al., 2009), new diatom indices have started to be created in European Union member countries (e.g., Álvarez-Blanco et al., 2013; Dell'Uomo & Torrisi, 2011). Important studies have been

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carried out in other countries in the last decades to develop new diatom indices (e.g., Lavoie et al., 2010; Ponader et al., 2007; Potapova & Charles, 2007). Trophic Index Türkiye (TIT) was developed in Türkiye in order to be used as a region-specific index in studies of ecological quality (Çelekli et al., 2017, 2019a). It has been successfully tested in numerous rivers and basins (Çelekli et al., 2018, 2019b; Sevindik et al., 2021).

The WFD's primary objective is to ensure that all surface waters in member states—including streams and rivers—achieve good ecological status (European Union, 2000). The Ecological Quality Ratio (EQR), which is the ratio between the values expected in a minimally disturbed condition and the observed value of a candidate surface water, must be established in order to assess the ecological status (Kelly et al., 2006, 2012). River types differ due to factors such as altitude, drainage area, geology, stream size, and so on. Furthermore, biological communities differ between habitats and ecoregions. Different river types and ecoregions should be considered while defining reference conditions (O'Driscoll et al., 2012). The Ministry of Agriculture and Forestry, Directorate General of Water Management (DGWM) carried out the "Establishment of Reference Monitoring Network in Türkiye, 2017–2020" project to assess reference conditions in rivers of various types in 25 basins of Türkiye. Candidate reference areas in the Western Mediterranean basin were also revealed as part of this project. TIT and several diatom indices have been used in recent years to assess the ecological quality of rivers in the Western Mediterranean Basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017). However, no studies on the bio-assessment of minimally disturbed rivers in this basin were performed. As a result, the goals of this study were to: (i) use multivariate approaches to evaluate the interactions between diatoms and environmental stressors in these rivers; (ii) assess the ecological status of minimally disturbed rivers; and (iii) identify reference sites with different types in the Western Mediterranean Basin using different diatom indices.

Materials and methods

The Western Mediterranean Basin and studied sites

The Western Mediterranean Basin is situated in Türkiye's southwest Anatolian region, between 36° 06′–37° 35′ N and 27° 13′–30° 34′ E. The Büyük Menderes, Burdur, and Antalya basins, as well as the Aegean and Mediterranean Seas, surround it. The provinces of Antalya, Burdur, Denizli, Muğla, and Aydın are all located within the limits of the Western Mediterranean Basin. The surface area is about 21,223 km^2, which constitutes 2.7% of Türkiye (DGWM 2016, 2018; Ministry of Agriculture and Forestry-MAF, 2019). The Dalaman, Eşen, Alakır, Akçay, Demre, Namnam, Kargıcak, Kocadere, and Sarıçay rivers, which release their water into the Aegean and the Mediterranean seas, are the principal running waters in the basin. The total length of the designated river water bodies is 1145 km, and the drainage area of these rivers is 14,693 km^2. The basin's altitudes range from sea level to 3086 m, with an average altitude of 877 m. The Western Taurus Mountain Range contains the basin's most important mountains, including Beşparmak, Babadağ, Gölgeli, Dumanlı, Akdağlar, Eşeler, Honaz, Bozdağ, and Sandıras (DGWM, 2016, 2018; MAF, 2019).

The typical Mediterranean climate dominates in the basin which is characterized by mild and wet winters, and dry and hot summers. The majority of precipitation and runoff events occur in the winter, although snowfall is rare. However, the characteristics of a continental climate begin to appear in the basin's interior. The basin's annual average precipitation and temperature were 840 mm and 15.8 °C, respectively (DGWM, 2016, 2018; MAF, 2019).

The sampling sites were mostly found in the Western Taurus Mountain Range, and their altitudes ranged from 16 to 1583 m (Fig. 1). A geographic information system and preliminary fieldwork were used to determine the least disturbed river sites. Attention was paid to the absence of agricultural activities and settlements near and above the selected points. Generally, the vegetation type specific to the Mediterranean flora region (maquis and coniferous forests) dominated around the sites. Samples were collected from 44 sampling sites (R1 to R44) three times (in spring, summer, and fall) during 2017. Information on the typology, latitude, longitude, altitude, and mean discharge of the sampling stations is presented in Supplementary Material Table 1. The latitude, longitude, and altitude of the sites were determined using a geographic positioning system (Magellan Sportrak Pro model GPS). River typology was determined according to the EU Water Framework Directive (European Union, 2000), and river typology information was provided by the Ministry of Agriculture and Forestry, Directorate General of Water Management (DGWM). Discharge values from the sampling stations were obtained using a dwarf flow meter (MCM-02 model) or an acoustic Doppler (Isco 4250 model area velocity, Teledyne Isco Inc.).

Analysis of environmental variables

Sampling for chemical analyses and measurement of physical variables were conducted at 44 sampling sites. Water temperature (*T*) (°C), dissolved oxygen concentration (DO) (mg L^{-1}), electrical conductivity (EC) (µS cm^{-1}), and pH were measured 10 cm below the water surface using a Hach HQ40 model multimeter instrument. Total organic carbon (TOC) (mg L^{-1}), chemical oxygen demand (COD) (mg L^{-1}), alkalinity (ALK) (mg L^{-1}), total phosphorus (TP) (mg L^{-1}),

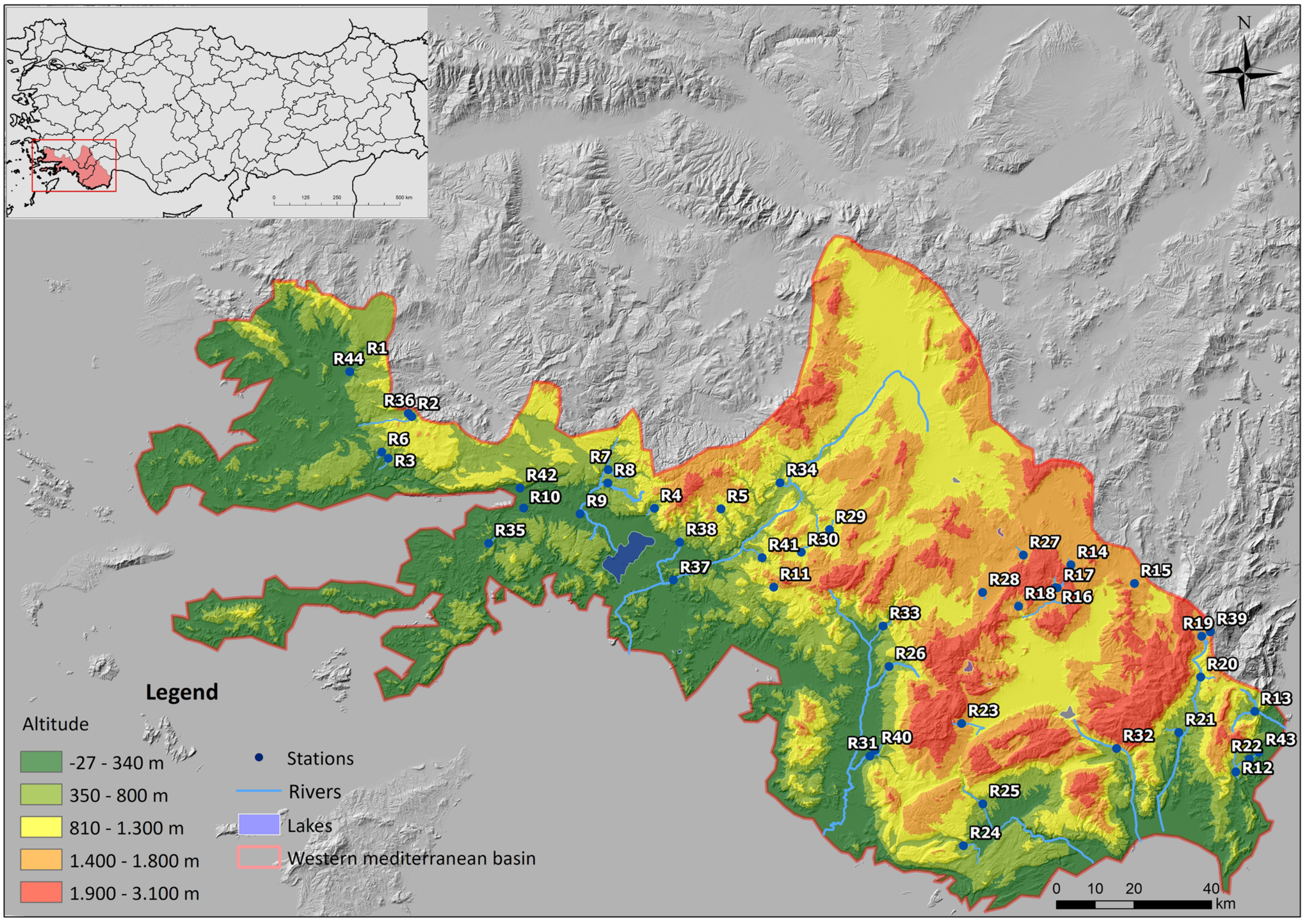

Fig. 1 Location of sampling stations in the Western Mediterranean Basin. The full names of the stations were given in Supplementary Material Table 1

nitrate–nitrogen (NO_3–N) (mg L^{-1}), organic nitrogen (ON) (mg L^{-1}), and total nitrogen (TN) (mg L^{-1}) were analyzed spectrophotometrically according to standard methods (APHA 2012) in an accredited laboratory of DGWM.

Analysis of epilithic diatoms

Sampling of epilithic diatoms was conducted at 44 sampling sites. The sampling sites (R3, R6, R10, R15, and R38) found in intermittent rivers were sampled during the flow period (Gallart et al. 2017) to ensure that the study was standardized with permanent rivers. The upper surface of at least five stones was brushed in 100 mL of distilled water, and these samples were fixed with Lugol's solution (European Committee for Standardization, 2010). Diatom samples were cleaned with hydrochloric acid and hot hydrogen peroxide, and permanent slides were mounted with Naphrax (European Committee for Standardization, 2010). Before counting, it was ensured that all species were identified by navigating randomly selected fields on the slide. More than 400 diatom valves were counted on the selected horizontal transect of the slide for each sample with an Olympus BX51 microscope using 1000 × magnification. The relative abundance of diatom species for each sample was also determined for subsequent analysis. Taxonomic books such as Krammer and Lange-Bertalot (1986, 1991a, 1991b, 1999), Lange-Bertalot (2001), Krammer (2000, 2002, 2003), Bąk et al. (2012), and Lange-Bertalot et al. (2017) were used to identify epilithic diatom species, and the current names of the species were checked according to Guiry and Guiry (2022).

Trophic Index Türkiye (TIT) was calculated according to Toudjani et al. (2017) and Çelekli et al., (2017, 2019a). 'OMNIDIA' program 5.2 (Lecointe et al., 1993) was used to calculate different diatom indices (Supplementary Material Table 2). All the indices except TIT were transformed to range from 0 to 20 in Omnidia software. The ecological status assessment of the ROTTs, EPI-D, IDP, and TDI was also done by using class boundaries according to Rott et al., (1997), Guida (2004), Gómez and Licursi (2001), Rakowska and Szczepocka (2011).

Data analysis

The statistical differences in environmental parameters among the sites studied in this study were determined with an analysis of variance (one-way ANOVA) test using SPSS 20.0 software. Also, the statistical differences in common environmental parameters between the sites studied in this study and disturbed sites in other studies (Çelekli & Lekesiz, 2020; Toudjani et al., 2017) were detected with one-way ANOVA. Tukey HSD was used as post hoc multiple comparison. Physical and chemical variables were logarithmically transformed for linear regression analysis. Linear regression analysis between diatom indices and environmental variables was performed with SPSS 20.0 software. To determine the suitability of canonical correspondence analysis (CCA), the gradient length was determined firstly by detrended correspondence analysis (DCA). Redundancy analysis (RDA) was performed in Canoco 5.0 (ter Braak & Smilauer, 2012) to reveal the relations between diatom species and environmental variables since the response data have a gradient 2.2 SD long. RDA was carried out on the log-normal transformed abundance data. The statistical significance of the environmental predictor variables was assessed by 999 restricted Monte Carlo permutations. Diatom species occurring in more than three samples with a relative abundance larger than 1% in at least one sample (33 taxa) were described as abundant species and were used in the RDA (Weilhoefer & Pan, 2006). Moreover, indicator species (9 taxa) were detected with Indicator Species Analysis (IndVal) and were used in the RDA. Before the IndVal, samples were clustered according to environmental variables. Clustering was performed by calculating the Euclidean distances and linking them by the Ward method according to XLSTAT software (Addinsoft, 2014). Indicator diatom species for the clusters were identified by using indicator value (IndVal) analysis (Dufrene & Legendre, 1997). Monte Carlo simulations (999 permutations) were used to assess the significance of each species as an indicator for each cluster. The IndVal analysis of indicator species in clusters was done using the labdsv package (Roberts, 2019) of R software. To analyze the relationship between diatoms and 11 environmental variables (T, EC, pH, DO, TOC, COD, ALK, TP, ON, TN, NO_3–N), we performed a RDA using relative abundance values of the 39 taxa (both indicator and abundant species). RDA was performed, initially on the whole environmental and diatom datasets. Forward selection indicated that 7 of the 11 environmental variables made a significant contribution to the variance in the diatom data. The mean values of three seasons were used to determine the relative abundance of diatoms, diatom indices, and environmental variables for RDA and linear regression analysis.

Results

Environmental variables

Table 1 and Supplementary Material Table 3 display the environmental parameters measured at 44 sampling sites. Except for TP, all environmental variables were significantly different between sampling sites ($p<0.01$). The mean T values ranged between 11.50 and 23.10 °C, with Kozağaç River (R21) with the highest mean T value ($f=5.72$, $p<0.01$). The mean pH and ON values were highest in Gürleyik River-1 (R27) ($f=8.69$, $f=8.61$, respectively, $p<0.01$), while the mean TOC and COD values were highest in Karabeyyurdu River (R35) ($f=5.59$, $f=7.57$, respectively, $p<0.01$). The highest mean EC (3090.00 μS cm^{-1}) value was found in Akyaka River (R42), while the lowest (160.03 μS cm^{-1}) was recorded in Suçıkan River (R23) ($f=516.24$, $p<0.01$). The mean NO_3–N and TN values were highest in Gürleyik River-3 (R40) ($f=4.02$, $f=5.27$, respectively, $p<0.01$). Moreover, the mean NO_3–N value was also higher in the Akyaka River (R42), while the mean TN values were also measured higher in Gürleyik River-1 (R27), Dalaman River (R37), and Akyaka River (R42). The mean DO values were measured between 3.72 and 11.19 mg L^{-1}, and the highest value was found in Av River (R24) ($f=16.61$, $p<0.01$). The mean ALK values fluctuated between 103.73 and 395.00 mg L^{-1}, and the highest value was recorded in Araplar River (R29) ($f=24.58$, $p<0.01$).

The boxplot graphs of common environmental parameters (T, EC, pH, TP, TN, NO_3–N) measured in two previous studies on disturbed rivers in the Western Mediterranean Basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017) and in this study are shown in Supplementary Material Fig. 1. Moreover, the mean and standard deviation (SD) of environmental variables measured in the disturbed rivers of Western Mediterranean Basin in two different studies (Çelekli & Lekesiz, 2020; Toudjani et al., 2017) are given in Supplementary Material Table 4. There was no statistical difference in EC values in all three studies. The highest pH ($f=6.53$, $p<0.01$) and the lowest T ($f=16.25$, $p<0.01$), TN ($f=24.31$, $p<0.01$), NO_3–N ($f=9.68$, $p<0.01$), and TP ($f=40.53$, $p<0.01$) values were recorded in our study.

Epilithic diatoms and environmental parameters

In the 44 sampling sites of the Western Mediterranean Basin, a total of 140 diatom taxa were recorded. Thirty-three diatom taxa occurring in at least three samples and accounting for more than 1% of total relative abundance

Table 1 The mean and standard deviation (SD) of environmental variables measured in the minimally disturbed stations of the Western Mediterranean Basin. (*T*: water temperature, EC: electrical conductivity, DO: dissolved oxygen, TOC: total organic carbon, COD: chemical oxygen demand, ALK: alkalinity, TP: total phosphorus, ON: organic nitrogen, TN: total nitrogen, NO_3–N: nitrate–nitrogen)

Stations	*T* (°C)	EC (µS cm^{-1})	pH	DO (mg L^{-1})	TOC (mg L^{-1})	COD (mg L^{-1})	ALK (mg L^{-1})	TP (mg L^{-1})	ON (mg L^{-1})	TN (mg L^{-1})	NO_3–N (mg L^{-1})
R1	19.20 ± 2.86	545.00 ± 33.54	8.54 ± 0.33	8.41 ± 0.78	11.10 ± 2.85	15.80 ± 1.38	258.60 ± 54.02	0.038 ± 0.02	0.36 ± 0.21	0.47 ± 0.09	0.11 ± 0.05
R2	21.60 ± 2.99	390.67 ± 46.20	8.19 ± 0.36	7.38 ± 1.13	2.70 ± 3.82	6.90 ± 3.29	197.20 ± 24.08	0.013 ± 0.02	0.30 ± 0.22	0.30 ± 0.22	0.05 ± 0.01
R3	22.10 ± 7.35	529.50 ± 23.33	8.21 ± 0.21	8.31 ± 0.71	6.39 ± 8.32	12.80 ± 10.96	256.40 ± 16.97	0.024 ± 0.03	0.63 ± 0.23	0.69 ± 0.15	0.08 ± 0.04
R4	18.70 ± 2.54	386.00 ± 35.86	8.83 ± 0.15	8.50 ± 0.96	8.75 ± 1.47	18.60 ± 2.54	211.40 ± 23.25	0.054 ± 0.04	0.28 ± 0.15	0.41 ± 0.08	0.13 ± 0.08
R5	19.50 ± 1.19	406.00 ± 45.47	8.82 ± 0.22	7.94 ± 0.33	9.56 ± 2.48	11.40 ± 3.56	238.80 ± 15.14	0.044 ± 0.01	0.24 ± 0.21	0.24 ± 0.11	0.05 ± 0.01
R6	17.70 ± 2.25	401.00 ± 28.39	8.72 ± 0.24	8.68 ± 1.15	8.44 ± 3.55	5.00 ± 1.21	194.20 ± 47.45	0.040 ± 0.01	0.29 ± 0.24	0.49 ± 0.08	0.20 ± 0.11
R7	20.50 ± 3.44	484.00 ± 31.25	8.96 ± 0.23	8.35 ± 0.46	4.10 ± 1.54	5.00 ± 3.75	218.00 ± 25.46	0.008 ± 0.001	0.38 ± 0.26	0.38 ± 0.06	0.05 ± 0.01
R8	22.53 ± 3.04	629.00 ± 38.74	8.88 ± 0.13	8.57 ± 0.76	4.77 ± 7.40	12.70 ± 13.39	362.33 ± 76.19	0.775 ± 1.34	0.69 ± 0.23	0.69 ± 0.23	0.05 ± 0.01
R9	17.30 ± 1.36	578.00 ± 54.26	8.12 ± 0.12	3.72 ± 0.12	13.36 ± 4.45	18.10 ± 6.45	349.60 ± 32.33	0.071 ± 0.02	0.59 ± 0.24	0.59 ± 0.12	0.05 ± 0.01
R10	17.10 ± 1.18	606.00 ± 36.25	8.48 ± 0.11	7.77 ± 0.56	13.78 ± 6.58	26.35 ± 3.25	350.60 ± 35.47	0.040 ± 0.02	0.20 ± 0.17	0.20 ± 0.05	0.05 ± 0.01
R11	16.63 ± 3.36	364.00 ± 39.89	8.21 ± 0.21	7.82 ± 0.57	2.58 ± 3.61	5.00 ± 0.01	194.47 ± 6.11	0.019 ± 0.03	0.31 ± 0.22	0.35 ± 0.27	0.08 ± 0.05
R12	16.10 ± 2.14	354.00 ± 22.45	8.08 ± 0.26	8.31 ± 1.12	0.50 ± 0.01	5.00 ± 0.01	216.80 ± 24.35	0.013 ± 0.03	0.52 ± 0.16	0.52 ± 0.09	0.05 ± 0.01
R13	18.63 ± 5.65	338.67 ± 32.33	8.54 ± 0.29	9.28 ± 1.03	0.50 ± 0.01	5.00 ± 0.01	206.27 ± 54.49	0.004 ± 0.001	0.32 ± 0.24	0.36 ± 0.28	0.07 ± 0.04
R14	13.00 ± 2.13	271.00 ± 21.22	8.73 ± 0.22	8.04 ± 0.75	11.37 ± 2.47	16.60 ± 4.44	172.80 ± 12.13	0.007 ± 0.001	0.97 ± 0.23	0.97 ± 0.15	0.05 ± 0.01
R15	11.50 ± 1.11	361.00 ± 31.25	8.51 ± 0.18	8.44 ± 0.43	0.50 ± 0.01	5.00 ± 1.21	183.80 ± 15.18	0.035 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
R16	16.65 ± 4.96	344.33 ± 82.97	8.37 ± 0.45	7.76 ± 0.96	3.48 ± 5.17	5.00 ± 0.01	241.87 ± 149.02	0.031 ± 0.05	0.52 ± 0.25	0.62 ± 0.18	0.11 ± 0.05
R17	13.70 ± 1.15	433.00 ± 33.25	8.55 ± 0.25	7.89 ± 0.55	12.08 ± 3.56	23.20 ± 5.46	204.80 ± 23.45	0.003 ± 0.001	0.63 ± 0.28	0.75 ± 0.33	0.13 ± 0.05
R18	11.50 ± 0.75	281.00 ± 24.36	8.84 ± 0.16	9.05 ± 0.60	9.75 ± 4.44	5.00 ± 0.01	191.40 ± 35.78	0.003 ± 0.001	0.46 ± 0.15	0.71 ± 0.23	0.25 ± 0.15
R19	11.80 ± 1.46	386.00 ± 36.47	8.62 ± 0.18	8.81 ± 0.98	0.50 ± 0.01	5.00 ± 0.01	174.00 ± 17.14	0.066 ± 0.02	0.49 ± 0.21	0.49 ± 0.12	0.05 ± 0.01
R20	14.40 ± 2.11	290.00 ± 31.41	8.60 ± 0.15	9.16 ± 1.23	0.50 ± 0.01	5.00 ± 0.01	175.20 ± 16.84	0.009 ± 0.004	0.40 ± 0.21	0.40 ± 0.15	0.05 ± 0.01
R21	23.10 ± 2.85	333.67 ± 31.53	8.24 ± 0.06	8.57 ± 0.56	4.19 ± 6.39	9.40 ± 7.62	180.07 ± 22.45	0.003 ± 0.001	0.47 ± 0.09	0.51 ± 0.08	0.08 ± 0.05
R22	18.10 ± 1.15	419.00 ± 25.45	8.70 ± 0.15	9.29 ± 1.12	0.50 ± 0.01	5.00 ± 0.01	184.40 ± 15.43	0.054 ± 0.03	0.50 ± 0.15	0.50 ± 0.18	0.05 ± 0.01
R23	11.97 ± 3.40	160.03 ± 69.58	8.60 ± 0.29	8.88 ± 0.73	0.50 ± 0.01	5.00 ± 0.01	103.73 ± 11.09	0.003 ± 0.001	0.54 ± 0.22	0.81 ± 0.36	0.26 ± 0.18
R24	19.80 ± 2.15	504.00 ± 33.46	8.24 ± 0.23	11.19 ± 0.98	13.15 ± 2.45	21.60 ± 2.45	240.20 ± 35.45	0.014 ± 0.03	0.65 ± 0.27	0.65 ± 0.27	0.05 ± 0.01
R25	13.80 ± 1.40	369.00 ± 24.47	8.62 ± 0.24	9.90 ± 1.12	9.17 ± 1.43	15.00 ± 1.65	131.20 ± 22.74	0.070 ± 0.02	0.24 ± 0.11	0.43 ± 0.17	0.19 ± 0.05
R26	21.50 ± 3.45	274.00 ± 11.87	8.74 ± 0.15	7.45 ± 0.55	7.50 ± 3.97	13.10 ± 2.74	157.40 ± 12.16	0.052 ± 0.03	0.46 ± 0.24	0.64 ± 0.28	0.19 ± 0.05
R27	16.80 ± 1.85	263.00 ± 24.63	9.05 ± 0.16	7.97 ± 0.46	11.16 ± 3.25	18.20 ± 1.23	166.40 ± 13.36	0.003 ± 0.001	1.20 ± 0.52	1.20 ± 0.51	0.05 ± 0.01
R28	18.57 ± 4.69	400.67 ± 24.99	8.28 ± 0.19	8.64 ± 1.07	14.24 ± 12.62	20.70 ± 13.68	210.53 ± 17.26	0.006 ± 0.01	0.78 ± 0.25	0.81 ± 0.19	0.05 ± 0.01
R29	17.20 ± 1.49	782.00 ± 33.74	8.79 ± 0.21	8.35 ± 0.44	13.94 ± 5.46	19.00 ± 3.56	395.00 ± 21.85	0.037 ± 0.02	0.57 ± 0.33	0.75 ± 0.35	0.17 ± 0.06
R30	18.60 ± 2.72	441.33 ± 44.00	8.41 ± 0.38	8.23 ± 0.61	3.20 ± 4.68	5.00 ± 0.01	249.33 ± 16.86	0.005 ± 0.001	0.36 ± 0.27	0.40 ± 0.26	0.07 ± 0.03
R31	17.33 ± 3.01	357.67 ± 81.77	8.17 ± 0.27	8.83 ± 0.55	5.47 ± 5.16	15.60 ± 11.55	169.40 ± 16.67	0.077 ± 0.12	0.17 ± 0.20	0.58 ± 0.60	0.45 ± 0.39
R32	16.00 ± 2.24	298.00 ± 23.74	8.78 ± 0.19	9.30 ± 0.47	11.55 ± 4.56	16.60 ± 1.45	183.80 ± 12.11	0.098 ± 0.03	0.28 ± 0.15	0.70 ± 0.28	0.42 ± 0.21
R33	13.50 ± 1.65	341.00 ± 31.65	7.98 ± 0.11	9.18 ± 0.33	10.03 ± 3.14	16.80 ± 2.24	204.80 ± 15.78	0.003 ± 0.01	0.38 ± 0.21	0.59 ± 0.23	0.21 ± 0.11
R34	19.77 ± 1.31	590.00 ± 66.16	8.72 ± 0.23	8.33 ± 0.25	4.19 ± 6.40	9.60 ± 8.03	366.60 ± 22.47	0.031 ± 0.03	0.35 ± 0.29	0.35 ± 0.29	0.05 ± 0.01

Table 1 (continued)

Stations	T (°C)	EC (µS cm^{-1})	pH	DO (mg L^{-1})	TOC (mg L^{-1})	COD (mg L^{-1})	ALK (mg L^{-1})	TP (mg L^{-1})	ON (mg L^{-1})	TN (mg L^{-1})	NO_3–N (mg L^{-1})
R35	19.60 ± 2.12	882.00 ± 51.45	8.53 ± 0.21	7.68 ± 0.45	19.37 ± 7.45	36.50 ± 4.43	552.60 ± 33.45	0.067 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
R36	21.20 ± 3.14	334.00 ± 21.65	8.67 ± 0.17	7.59 ± 0.14	6.94 ± 3.23	10.20 ± 2.75	161.40 ± 12.81	0.048 ± 0.03	0.17 ± 0.11	0.17 ± 0.11	0.05 ± 0.01
R37	17.30 ± 1.45	496.00 ± 45.43	8.79 ± 0.16	9.26 ± 0.56	11.70 ± 5.14	15.00 ± 3.74	263.00 ± 11.49	0.142 ± 0.09	0.65 ± 0.32	1.31 ± 0.55	0.66 ± 0.35
R38	15.20 ± 1.07	331.00 ± 32.14	8.18 ± 0.11	8.60 ± 0.21	0.50 ± 0.01	5.00 ± 0.01	170.00 ± 15.64	0.014 ± 0.01	0.21 ± 0.12	0.47 ± 0.31	0.25 ± 0.15
R39	16.17 ± 5.37	490.00 ± 92.80	8.34 ± 0.30	8.47 ± 1.12	2.74 ± 3.88	9.20 ± 7.33	198.30 ± 33.04	0.006 ± 0.01	0.34 ± 0.25	0.37 ± 0.28	0.07 ± 0.03
R40	20.87 ± 1.00	584.33 ± 49.32	8.31 ± 0.19	8.51 ± 0.17	5.19 ± 8.12	9.30 ± 7.45	261.07 ± 18.12	0.013 ± 0.01	0.67 ± 0.22	2.15 ± 1.47	1.40 ± 1.40
R41	18.30 ± 3.47	413.33 ± 24.42	8.53 ± 0.34	8.32 ± 0.06	3.36 ± 4.95	5.00 ± 0.01	236.20 ± 11.11	0.012 ± 0.02	0.23 ± 0.16	0.23 ± 0.16	0.05 ± 0.01
R42	18.00 ± 1.65	3090.00 ± 21.45	7.86 ± 0.17	6.68 ± 0.23	7.43 ± 1.23	10.70 ± 2.41	213.00 ± 16.44	0.044 ± 0.03	0.37 ± 0.21	1.30 ± 0.21	0.93 ± 0.33
R43	16.60 ± 1.52	211.40 ± 20.10	8.66 ± 0.19	9.54 ± 0.48	0.50 ± 0.01	5.00 ± 0.01	120.80 ± 11.23	0.008 ± 0.005	0.69 ± 0.33	0.69 ± 0.35	0.05 ± 0.01
R44	21.77 ± 2.94	545.33 ± 42.83	8.30 ± 0.24	8.50 ± 0.41	3.68 ± 5.51	10.20 ± 9.01	255.67 ± 22.80	0.016 ± 0.01	0.32 ± 0.24	0.54 ± 0.43	0.24 ± 0.17

are given in Supplementary Material Table 5. Nine diatom taxa were determined as indicator species (Table 2). *Amphora ovalis* (Kützing) Kützing, *Cocconeis lineata* Ehrenberg, *Cymbella affinis* Kützing, *Cymbella neocistula* Krammer, *Fragilaria capucina* Desmazières, *Gomphonema affine* Kützing, *Gomphonema angustum* Agardh, *Gomphonema minutum* (Agardh) Agardh, *Navicula angusta* Grunow, *Navicula radiosa* Kützing, and *Ulnaria ulna* (Nitzsch) Compère were commonly recorded in the sites of the basin.

The results of RDA are given in Fig. 2. The diatom–environmental correlations of RDA axes 1 and 2 are high, and the first two axes account for 73.0% of the variance in the diatom–environmental relationships. Most of the indicator species such as *A. ovalis, Campylodiscus hibernicus* Ehrenberg, *Gyrosigma acuminatum* (Kützing) Rabenhorst, *N. angusta, Nitzschia dissipata* (Kützing) Rabenhorst, *Nitzschia sigmoidea* (Nitzsch) Smith, *Surirella amphioxys* Smith were strongly correlated with NO_3–N, TN and site R40. ALK, COD, and EC were correlated mainly with sites such as R10, R29, R34, R35, R42, and species such as *U. ulna, Delicatophycus delicatulus* (Kützing) Wynne, *Gomphonema commutatum* Grunow, and *Ulnaria acus* (Kützing) Aboal. In the opposite side of the first axis, pH was separated from other parameters with sites such as R14, R17, R19, R23, R25, R27, and species such as *C. affinis, G. angustum, G. minutum, Gomphonema parvulum* (Kützing) Kützing, *Meridion circulare* (Greville) Agardh, and *N. radiosa*.

Diatom indices

Linear regression analysis was performed on the index scores and environmental parameters (Table 3). All the environmental parameters except T, pH, and TP showed significant correlations with 13 index scores ($p < 0.01$ or $p < 0.05$). EC, COD, NO_3–N, and ALK had 6, 4, 3, and 3 significant correlations with the diatom indices, respectively. The average scores of 18 diatom indices are given in Table 4. Due to the high correlation of indicator species with NO_3–N and TN in the RDA, the ecological status of four indices (EPI-D, TDI, IDP, ROTTs) that show high correlation with NO_3–N and TN is also given in Table 4. Average percentage information about the species pool which is used by diatom indices is given in Fig. 3. The EPI-D, TDI, IDP, and ROTTs indices used 88%, 99%, 65%, and 77% of the species, respectively.

Discussion

Due to the mountainous geography of the Western Mediterranean Basin, few studies on its rivers have been conducted, and the water quality of lower altitude (lowland) rivers has

Table 2 Indicator species and indicator values (IndVal) for the most important species ($p < 0.05$) in clustered groups

Indicator species	Code	Cluster	IndVal	*p* value
Amphora ovalis (Kützing) Kützing	AOVA	6	0.8435503	0.003
Campylodiscus hibernicus Ehrenberg	CHIB	6	0.8429119	0.018
Gomphonema commutatum Grunow	GCOM	3	0.9756098	0.018
Gyrosigma acuminatum (Kützing) Rabenhorst	GYAC	6	0.9305605	0.025
Navicula angusta Grunow	NAAN	6	0.9274227	0.015
Nitzschia dissipata (Kützing) Rabenhorst	NDIS	6	0.9267803	0.042
Nitzschia sigmoidea (Nitzsch) W.Smith	NSIO	6	0.9385793	0.022
Surirella amphioxys Smith	SAPH	6	0.9836066	0.033
Ulnaria acus (Kützing) Aboal	UACU	3	0.8640074	0.024

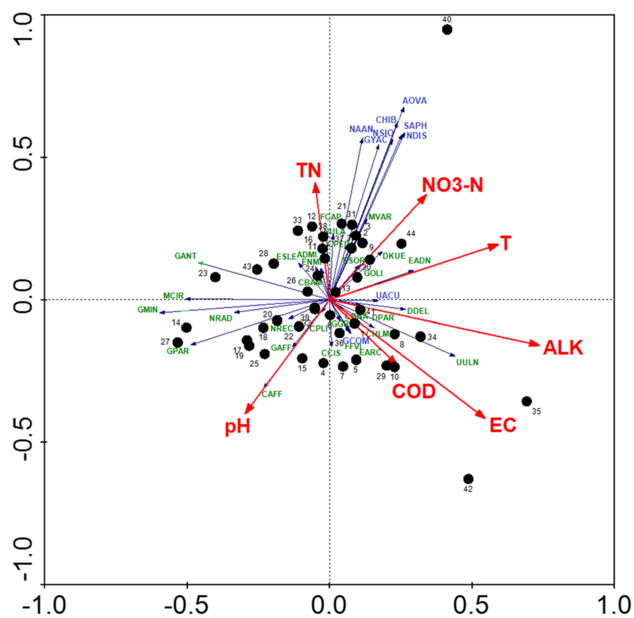

Fig. 2 Ordination diagram of the redundancy analysis (RDA) with the scores of diatoms relative abundance and environmental variables in different sampling sites. Environmental variables: *T*: water temperature, EC: electrical conductivity, TN: total nitrogen, NO_3–N: nitrate–nitrogen, ALK: alkalinity, COD: chemical oxygen demand. Full names and abbreviations of species are given in Table 2 and Supplementary Material Table 5. Indicator species were colored with blue

been investigated in these studies (Çelekli & Lekesiz, 2020; Kazancı & Dügel, 2000; Toudjani et al., 2017). The measured environmental variables, including biological oxygen demand, EC, TOC, TP, TN, and NO_3–N, showed that some rivers were seriously nutrient and organically polluted. However, in some areas with less severe anthropogenic activity, good or high water quality was found. Although industrial activity-related pollution has been seen in some rivers near cities like Antalya, Denizli, Aydın, and Muğla, domestic waste and agricultural activity-related pollution are the predominant pollutants in the basin (Directorate General of Environmental Management-DGEM, 2016; Toudjani et al., 2017). Furthermore, intensive tourism and aquaculture activities have impacted the water quality of the basin's rivers (DGEM, 2016). In terms of environmental parameters (TP, TN, NO_3–N), it was seen that the values measured in our study are lower than the values measured in two previous studies on disturbed rivers in the Western Mediterranean Basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017). The most important reason for this is that these 44 sites selected as candidates for minimally disturbed rivers are chosen from the high-altitude points away from human influence as much as possible.

Based on the RDA results, NO_3–N, TN, and site R40 were correlated with most of the indicator species such as *A. ovalis, C. hibernicus, G. acuminatum, N. angusta, N. dissipata, N. sigmoidea,* and *S. amphioxys*. With the exception of *N. angusta,* all of these species were classified as pollution-tolerant taxa in European rivers (Kelly et al., 2008; Van Dam et al., 1994). However, *N. angusta* was described as pollution-sensitive taxa (Carayon et al., 2019; Van Dam et al., 1994). Studies have not been found that give information about the trophic status and ecological preferences of this species in the rivers of Türkiye. It is interesting that this species, which was defined as oligotrophic in rivers of Europe, showed a high correlation with NO_3–N in our study. *A. ovalis, N. angusta,* and *N. dissipata* were also reported in the disturbed rivers of the Western Mediterranean Basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017). Site R40 was described as moderate or pool ecological status based on IDP and TDI indices. Similar to the findings of Toudjani et al. (2017), NO_3–N was found as the influential environmental variable in the distribution of diatom species in our study. Although the selected sites were chosen from places where human impact is minimal, untreated domestic wastes from small-scale settlements may affect the NO_3–N concentration, especially in site R40.

Species such as *D. delicatulus*, *G. commutatum*, *U. acus,* and *U. ulna* and sites such as R8, R10, R29, R34, R35, and R42 were correlated mainly with ALK, COD, and EC. These species except *D. delicatulus* were classified as intermediate-pollution-tolerant or pollution-tolerant taxa in different studies (Kelly et al., 2008; Salinas-Camarillo

Table 3 Linear regression analysis values (R^2) between diatom indices and environmental variables (*T*: water temperature, EC: electrical conductivity, DO: dissolved oxygen, TOC: total organic carbon, COD: chemical oxygen demand, ALK: alkalinity, TP: total phosphorus, ON: organic nitrogen, TN: total nitrogen, NO_3–N: nitrate–nitrogen) (*$p<0.05$; **$p<0.01$)

	IDAP	EPI-D	IBD	SHE	DICH	WAT	IPS	SLA	DES	IDSE	IDG	CEE	TDI	LOBO	IDP	ROTTt	ROTTs	TIT
log (*T*)	0.050	0.007	0.001	0.014	0.001	0.012	0.019	0.028	0.051	0.022	0.031	0.005	0.005	0.034	0.004	0.041	0.001	0.015
log (pH)	0.002	0.009	0.019	0.031	0.001	0.007	0.009	0.070	0.039	0.001	0.037	0.018	0.078	0.001	0.001	0.018	0.007	0.010
log (EC)	0.064	0.072	0.007	**0.090***	0.031	**0.186****	0.078	0.075	**0.267****	**0.310****	0.033	**0.180****	0.023	0.047	0.067	**0.089***	0.018	0.018
log (DO)	0.014	0.017	0.002	0.058	**0.089***	0.001	0.051	0.001	**0.113***	0.025	0.001	0.008	0.007	0.016	0.058	0.046	0.037	0.023
log (COD)	**0.180****	0.069	0.035	0.075	**0.103***	0.007	**0.088***	0.035	0.044	0.047	0.013	0.060	0.010	0.001	0.048	**0.099***	0.079	0.020
log (TOC)	**0.116***	0.080	0.011	0.031	0.036	0.030	0.067	0.003	0.030	0.066	0.001	0.044	0.004	0.010	0.023	0.035	0.016	0.001
log (TN)	0.012	0.001	0.055	0.003	0.010	0.001	0.028	0.015	0.032	0.001	0.030	0.006	0.049	0.055	0.001	0.008	**0.111***	0.001
log (NO_3–N)	0.001	**0.130***	0.015	0.077	0.038	0.002	0.009	0.057	0.009	0.076	0.054	0.025	**0.089***	0.044	**0.119***	0.018	0.023	0.033
log (ON)	0.008	0.040	0.046	0.010	0.069	0.001	0.067	0.001	0.084	0.028	0.004	0.024	0.008	0.013	0.034	0.042	**0.089***	0.026
log (TP)	0.045	0.005	0.001	0.009	0.036	0.004	0.005	0.012	0.012	0.061	0.004	0.008	0.002	0.004	0.024	0.072	0.009	0.001
log (ALK)	0.017	0.032	0.001	0.053	0.039	**0.111***	0.023	0.035	0.084	**0.152****	0.024	**0.105***	0.004	0.018	0.046	0.084	0.044	0.002

Bold ones describe the statistically significant regression values

et al., 2021; Van Dam et al., 1994). In particular, *U. ulna* was highly correlated with EC and COD in the RDA. Bere and Tundisi (2011) have reported this species as tolerant to organic pollution, and it has broad tolerance to EC ranges (Bere & Tundisi, 2009; Venkatachalapathy & Karthikeyan, 2012). On the other hand, *D. delicatulus* is the indicator species of oligotrophic to mesotrophic waters with rich calcium bicarbonate ($CaCO_3$) content (Lange-Bertalot et al., 2017). Carbonate-rich sedimentary rocks such as calcite ($CaCO_3$) have been described as the most common geogenic source of ALK in stream water (Wedepohl, 1978). As a matter of fact, this species showed higher correlations with ALK and sites R8 (74.2% mean abundance) and R34 (59.1% mean abundance) in the RDA. All these sites (R8, R10, R29, R34, R35, R42) except R35 were high or good ecological status based on EPI-D, TDI, and ROTTs indices. The R35 site indicated medium ecological quality according to the ROTTs, while good ecological quality according to the EPI-D and TDI. In this station, EC and COD values were high and *U. ulna* was the dominant species with a mean abundance of 75.6%.

In Danish streams, Baattrup-Pedersen et al. (2022) have found that pollution-sensitive and pollution-tolerant taxa coexisted in highly alkaline rivers, and it became difficult to explain the causal relationship between ALK and pollution indicator environmental variables. Kelly et al. (2020) have also stated that ALK stands out as the most important abiotic predictor variable in phytobenthos reference sites, and it became difficult to detect least disturbed conditions in high ALK rivers due to geological conditions which often resulted in situations well-suited to settlement and agriculture. Similarly in our study, high ALK, EC, COD, and pollution-sensitive and pollution-tolerant taxa were observed in streams with different trophic conditions (high, good, medium). Therefore, a new approach should be developed by taking into account the ALK status of the least disturbed rivers during the revision of the ecological quality indices.

Species such as *C. affinis, G. angustum, G. minutum, G. parvulum, M. circulare,* and *N. radiosa* were located near sites such as R14, R17, R19, R23, R25, R27 and were associated with high pH in the RDA. All these sites were high or good ecological status based on EPI-D, TDI, and ROTTs indices. These aforementioned species, except *C. affinis* and *N. radiosa*, were also found in other studies of this basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017). *Cymbella affinis, G. angustum, M. circulare*, and *N. radiosa* were described as oligo-saprobic species (Bere & Tundisi, 2011; Carayon et al., 2019; Çelekli et al., 2019a, 2019b; Jüttner et al., 1996; Kelly et al., 2008; Van Dam et al., 1994), while *G. minutum* and *G. parvulum* were proposed as intermediate-pollution tolerant taxa in different watercourses (Çelekli et al., 2019a; Hausmann et al., 2016; Kelly et al., 2008; Sevindik & Kucuk, 2016).

Table 4 The mean diatom index values at 44 sampling sites of the Western Mediterranean Basin and corresponding ecological status of most significantly correlated indices with environmental parameters (blue: high, green: good, yellow: moderate, orange: poor, red: bad)

Stations	IDAP	EPI-D	IBD	SHE	DICH	WAT	IPS	SLA	DES	IDSE	IDG	CEE	TDI	LOBO	IDP	ROTTt	ROTTs	TIT
R1	12.2	14.9	17.9	16.9	17.1	12.0	15.9	13.3	17.6	14.6	17.7	16.0	16.2	9.1	12.7	13.0	17.8	1.68
R2	11.0	15.2	14.8	15.6	17.2	11.8	15.8	13.6	14.7	14.2	16.9	14.5	16.8	12.8	12.4	10.8	17.5	2.36
R3	12.8	15.6	18.7	16.9	18.2	13.3	18.0	12.4	15.8	14.2	14.1	16.3	11.0	8.0	13.3	13.8	17.8	2.08
R4	10.0	15.2	17.1	14.6	9.8	19.7	15.2	13.3	13.7	15.1	13.0	17.5	10.5	1.4	10.6	9.4	15.0	3.51
R5	10.8	14.0	15.2	14.8	12.9	13.8	13.5	12.6	9.1	12.1	15.1	15.4	13.1	11.1	10.9	8.0	14.2	3.18
R6	12.2	15.5	17.2	18.5	18.3	14.4	16.4	14.2	16.3	15.3	15.2	17.3	15.5	9.9	14.0	15.2	17.2	1.98
R7	10.6	14.7	17.0	16.4	16.0	11.9	14.4	13.5	11.3	11.8	17.4	16.6	16.9	12.0	11.6	12.4	17.0	1.54
R8	10.5	18.8	20.0	19.1	18.1	11.1	18.7	13.0	14.5	13.8	18.9	17.3	19.2	12.5	13.2	10.2	17.6	1.49
R9	11.3	15.5	17.1	14.9	10.6	18.5	15.3	13.2	10.9	14.4	13.4	16.6	10.9	2.6	10.6	8.7	13.5	3.50
R10	9.9	16.4	17.2	17.4	18.2	15.3	17.6	13.3	16.0	16.2	15.1	17.2	12.9	11.8	14.1	14.2	16.3	2.08
R11	11.7	17.3	16.3	19.3	19.3	18.3	19.0	13.4	15.5	16.6	13.8	18.1	11.0	13.6	14.9	15.8	16.0	2.12
R12	13.2	17.8	17.2	19.4	19.6	19.0	19.2	12.5	15.6	16.6	14.7	19.1	10.0	5.9	14.8	15.9	17.1	2.46
R13	16.7	17.5	16.8	19.2	19.3	19.4	19.6	13.9	15.3	16.8	14.3	17.7	11.3	10.1	15.1	16.0	16.0	2.01
R14	10.1	15.6	16.9	17.6	16.6	13.5	15.3	13.6	15.9	15.3	14.3	16.0	14.0	10.3	12.9	14.9	16.1	1.90
R15	15.3	15.3	20.0	19.7	17.2	10.5	15.2	14.7	15.1	13.7	18.2	14.3	19.7	10.5	11.7	17.6	18.6	1.44
R16	13.6	17.1	16.7	19.6	19.1	18.7	18.5	13.5	15.3	16.4	13.9	18.7	12.0	7.5	14.4	15.7	16.2	2.41
R17	14.7	16.1	17.6	19.0	18.4	15.5	16.3	14.1	15.2	16.0	15.7	17.7	15.2	10.4	13.6	16.2	16.9	1.87
R18	17.0	15.5	19.2	18.9	16.8	13.8	16.4	14.0	15.9	15.0	15.7	17.2	15.6	8.5	11.9	15.9	17.9	2.65
R19	12.8	15.3	17.1	16.7	16.1	12.3	15.5	13.8	15.3	15.0	15.4	15.3	13.1	10.4	12.6	15.1	15.6	1.74
R20	17.4	16.5	17.1	19.0	17.3	18.2	17.0	13.4	15.4	16.2	13.8	19.1	12.0	4.9	12.7	14.0	16.3	3.10
R21	12.2	15.2	15.1	16.1	15.3	14.7	16.1	12.2	13.3	14.7	14.1	16.1	12.4	15.6	13.7	10.4	13.9	1.78
R22	10.6	16.2	14.8	16.1	16.8	15.0	16.2	11.4	12.8	14.8	14.4	16.6	12.1	17.4	13.0	10.2	14.9	2.20
R23	13.5	16.4	17.5	17.2	15.9	17.2	16.9	13.5	15.3	15.9	15.1	18.3	12.2	10.4	12.9	13.7	16.6	2.73
R24	10.5	17.2	16.2	19.5	19.6	18.8	18.7	14.1	15.3	16.5	14.0	17.5	11.7	10.2	14.9	16.0	16.1	2.09
R25	10.2	17.2	15.8	18.6	19.0	18.3	18.5	12.4	14.8	16.2	13.8	16.4	11.1	13.2	14.6	14.9	15.6	2.13
R26	-	16.8	17.1	19.6	19.2	17.4	18.0	14.6	15.3	16.7	14.8	17.7	14.7	10.3	14.4	16.6	16.5	1.95
R27	9.3	15.7	18.0	18.4	17.3	14.1	16.0	14.0	14.9	15.4	16.6	17.0	16.2	10.3	12.7	16.3	17.1	1.95
R28	11.7	16.3	17.8	18.3	17.2	15.1	17.0	13.4	15.4	16.0	15.6	16.2	13.1	11.4	13.6	15.5	17.4	2.22
R29	11.0	14.5	16.7	16.8	14.4	15.8	14.3	13.5	16.9	14.4	12.9	17.2	12.4	6.6	11.3	11.0	17.3	3.21
R30	15.1	16.1	19.2	18.3	16.8	14.2	16.6	14.4	15.5	16.3	17.2	17.5	17.2	13.1	13.3	15.8	17.4	2.26
R31	13.3	15.7	17.1	18.1	13.9	18.4	15.8	13.6	14.3	15.3	14.2	17.8	11.9	4.8	11.7	11.9	17.0	2.98
R32	10.5	15.6	17.3	16.8	12.3	19.2	15.6	13.3	15.5	15.1	13.3	19.4	11.1	1.8	10.9	10.1	17.6	3.47
R33	10.9	13.4	14.9	14.0	13.0	12.6	14.3	11.6	9.3	12.3	13.8	13.9	11.5	14.2	10.8	6.1	12.7	3.04
R34	11.0	18.0	20.0	19.4	17.3	10.6	17.8	14.5	13.0	13.7	19.0	14.7	19.4	10.5	12.0	16.3	18.4	2.15
R35	10.5	12.2	12.2	13.7	9.8	12.5	11.9	11.5	6.2	11.3	13.8	14.5	10.6	16.0	10.6	4.1	6.8	3.46
R36	11.6	16.7	17.0	19.4	18.5	18.0	17.5	13.7	15.0	16.3	14.4	18.7	13.5	8.2	13.5	15.1	16.5	2.74
R37	14.3	14.6	16.9	15.9	14.8	12.8	15.4	12.4	15.5	11.4	15.4	14.3	11.0	12.1	11.8	12.5	15.1	2.19
R38	11.9	15.0	17.2	14.6	11.4	18.6	15.8	13.2	14.5	15.0	13.6	17.3	10.8	3.5	10.9	9.2	15.0	3.46
R39	5.8	15.5	18.5	18.5	17.2	15.2	16.5	12.3	15.5	14.8	14.8	17.6	13.3	5.4	12.7	14.4	17.4	2.63
R40	9.5	12.3	20.0	15.1	18.4	11.0	18.2	11.2	13.4	12.9	11.9	14.8	5.8	4.0	10.8	13.3	19.0	2.94
R41	13.9	17.3	16.7	18.9	18.9	19.2	19.0	13.6	15.2	16.6	14.2	18.7	11.9	11.3	14.7	15.1	15.8	2.38
R42	10.5	14.3	15.5	15.3	13.9	10.9	13.4	12.6	6.0	10.7	16.1	14.9	16.6	13.1	11.0	9.3	15.1	1.93
R43	10.5	16.2	16.5	17.3	15.5	18.4	16.2	13.2	14.6	15.6	13.2	18.3	10.1	2.6	11.8	11.4	14.7	3.38
R44	9.8	17.6	20.0	17.2	17.6	15.9	18.6	13.3	13.7	16.0	18.0	13.9	11.0	10.8	11.9	16.9	19.5	1.73

Four indices (EPI-D, TDI, IDP, and ROTTs) were significantly correlated with NO_3–N and TN values. Among these indices, EPI-D and ROTTs indicated high ecological quality at 35 and 37 sites, while they indicated good ecological quality at 9 and 6 sites, respectively. On the other hand, IDP defined the sites as good or moderate ecological quality. TDI determined 15 sites as high, and 26 sites as good ecological status. Site 40 which was highly correlated with TN, NO_3–N, and pollution-tolerant species, was determined as poor ecological quality according to TDI. Besides, sites which contain intermediate-pollution-tolerant and pollution-tolerant species such as *U. ulna, U. acus, G. parvulum, G. minutum, G. commutatum* were described as good ecological status with TDI. For this reason, considering the diatom species and environmental parameters, it can be concluded that the TDI gives better results in determining the ecological quality of the sites. This index was used in studies such as Karasu River (Gürbüz and Kivrak 2002), Upper Porsuk Creek (Solak, 2011), and Acarlar Floodplain (Sevindik & Kucuk, 2016) and found compatible with indicator diatom species and environmental parameters. EPI-D and

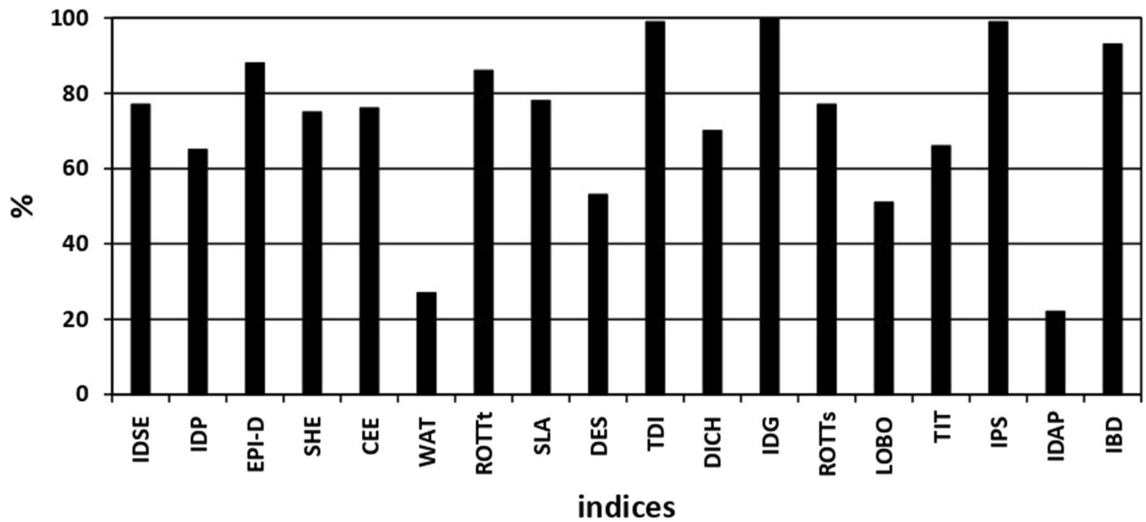

Fig. 3 Percentage of species used in indices

TIT indices were tested in previous studies of the rivers in the Western Mediterranean Basin (Çelekli & Lekesiz, 2020; Toudjani et al., 2017). TIT index, which was highly correlated with TP, was found effective in evaluating the ecological quality of these rivers. However, in our study, TIT did not give convenient results, since it did not show a significant correlation with TP or other environmental parameters. This index, which gives consistent results in more disturbed rivers of the same basin, did not work in pristine rivers; consequently, it should be revised considering these kinds of pristine rivers.

In conclusion, it was found that 44 pristine rivers which were selected to determine their potential as reference sites in the Western Mediterranean Basin, had better water quality than the disturbed rivers in terms of environmental variables (TP, TN, NO_3–N) measured in the basin. According to the RDA results, the distribution of abundant and indicator diatom species in the rivers was highly influenced by the catchment geology and nutrients, as evidenced by the NO_3–N, TN, ALK, EC, pH, and COD values. Due to the high correlation between NO_3–N, TN, and indicator species, the ecological quality status of 4 indices (EPI-D, TDI, IDP, and ROTTs), which showed high correlations with NO_3–N and TN, was determined. However, TDI gave more consistent results with environmental parameters and diatom species. The 15 high-ecological quality sites according to TDI will help set up reference conditions for future studies in the Western Mediterranean Basin and other basins in Türkiye.

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Author contributions TOS designed the experiments, conducted the field sampling, identified and counted the diatoms, analyzed the data, and wrote the text. TÇ participated in project management, designed the experiments, and analyzed the data. UG, HT, and AGT conducted the field sampling and data curation. All authors read and agreed to the published version of the manuscript.

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