



## Applicability of diatom-based indices for Water quality assessment in Iranian ecosystems: A case study of Gorganrood subbasin (NE Iran)

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| Article Info                                                              | Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
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| <b>Article type:</b><br>Research Article                                  | Water quality is a fundamental factor affecting the ecological health of rivers. However, the biological status of diatoms in relation to water quality in Iranian rivers has received limited research attention. In this study, diatom indices (TDI and GDI) alongside practical water quality indices (IRWQISC and NSFQI) were employed to assess the ecological status of four major tributaries within the Gorganrood River basin, a key river system in northeastern Iran. Water and diatom samples were collected on a monthly basis from August 2013 to May 2014. A total of 73 diatom taxa belonging to 30 genera were identified. Based on mean TDI values, the Zarringol and Chehelchay stations were classified as oligotrophic with high water quality, while the Gharachay and Oghan stations fell into class 2, indicating good quality. According to mean GDI values, all stations except Oghan (class 1, high quality) were categorized as class 2. Similarly, results from the IRWQISC and NSFQI indices placed almost all stations in the good quality class. The findings revealed a significant correlation between TDI and GDI values; however, no significant relationships were found between these diatom indices and the IRWQISC and NSFQI indices. Further research is therefore needed to clarify the applicability and reliability of diatom-based indices for water quality assessments in Iran. |
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| <b>Keywords:</b><br>Golestan<br>IRWQISC<br>NSFWQI<br>TDI<br>GDI           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

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## Introduction

Surface water pollution ranks among the most pressing global environmental challenges (Pasand et al., 2019). In response, biological assessment methods have emerged as essential complements to traditional physical and chemical analyses for water quality evaluation (Lobo & Callegaro, 2000). In recent decades, the scientific community has increasingly adopted integrated approaches that combine chemical parameters with biological indicators (Bate et al., 2004). Among the various bio-indicators employed—including benthic macro-invertebrates, fish communities, and diatoms—diatoms have proven particularly valuable due to their unique advantages as ecological indicators (Whitton & Kelly, 1995; Hill et al., 2000; Ramos et al., 2012).

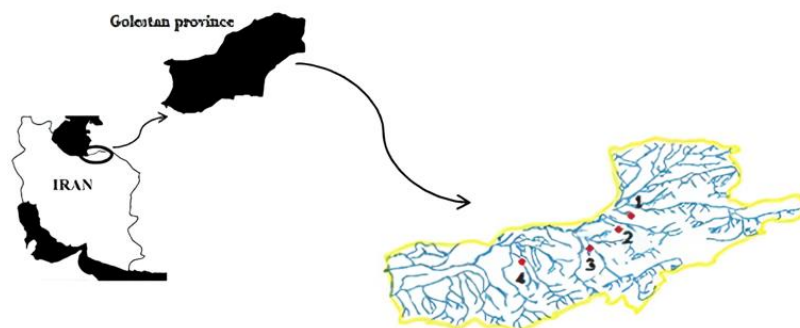
Diatoms are a large and diverse group of single-celled algae belonging to the class Bacillariophyceae. They are distributed worldwide in nearly all types of aquatic systems and are taxonomically diverse, comprising many thousands of taxa with varied ecological requirements (Stoermer and Smol, 1999). Diatoms are sensitive to stressors and respond rapidly to environmental changes through shifts in community composition and diversity (Stevenson and Pan, 2001).

In many countries, standardized methods based on diatom assemblages have been developed long ago (Descy and Coste, 1991; Kelly et al., 1998; Stevenson and Pan, 2001). In Iran, however, limited studies have utilized

diatom indicators for water quality assessment, possibly due to insufficient data on diatoms. The only notable study in this regard was conducted by Atazadeh et al. (2007), who applied the Trophic Diatom Index (TDI) in the Gharasou River (western Iran) and concluded that the TDI can serve as an effective indicator of water pollution in Iranian rivers. The Gorganrud River, one of the main rivers in northeastern Iran, is heavily influenced by land use practices (Heshmatpour et al., 2025). In this context, the water quality of this river has been evaluated using the IRWQISC and NSFQI indices in previous studies, and the results can be linked with diatom indices. Accordingly, the aim of this research was to conduct a comparative evaluation of diatom-based indices (GI and TDI) in relation to water quality indices (IRWQISC and NSFQI).

## Material and Methods

Gorganrud River basin is located northeast of Iran in Golestan Province. The river originates from Aladagh Mountain in Bojnourd City, and after passing 250 km, flows to the Gorgan plain and ultimately reaches the Caspian Sea. Several tributaries join the Gorganrud River from east and south (Arekhi et al., 2022). The study area encompasses four tributaries of the Gorganrud River, including Zarringol in Aliabad, Gharachay in Ramian, ChehelChay in Minudasht and Oghan Rud in Galikesh (Figure 1).



**Figure 1.** Location of studied rivers (1: Oghan Rood, 2: ChehelChay, 3: Gharachay, 4: Zarringol)

Water and diatom samples were collected monthly from August 2013 to May 2014 in

four stations (Oghan, Chehelchay, Gharechay, and Zarringol) in the Gorganrud

River. According to Kelly et al. (1998), five rocks were collected from the rocky beds at each station, and epilithic diatoms were separated using a toothbrush and preserved in 3% formalin according to Ahmadi-Musaabad et al., 2019; Panahi-Mirzahasano et al., 2021, 2024. Then, organic matter was removed by oxidation with hot HNO<sub>3</sub>, and permanent diatom slides were prepared using Naphrax (three slides for each sample). Diatom species were identified using a light microscope (with 1000x magnification) based on the diatom flora (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b). To calculate the relative abundance of each species, at least 400 valves were counted. Analysis of the relationship between water quality and diatom distribution was carried out according to Kent and Cocker, 1975, using the DCA classification method and CANOCO-5 software. Shannon-Wiener indices (1998) were used to measure species diversity, and Pielou index (1966) was used to measure species evenness, and the diatom trophic index (TDI) and diatom generic index (GI) were used to express the ecological status of the river with the help of biological indices.

TDI was calculated based on weighted average equation of Zelinka and Marvan (1961) according to Kelly et al. (2001; 2014). Other index used to characterize the diatom community was generic diatom index (GI) which is based on genera (Coste and Ayphassorho 1991). The water quality classes and ecological status based on these two indices were used according to classification presented in Noga et al. (2014).

River water samples were collected, transported, stored, and then the necessary tests were performed using the standard method (APHA, 1999): Temperature, DO (Dissolve Oxygen), BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), EC (Electrical Conductivity), TDS (Total Dissolve Sediment), pH (Hydrogen potential), TH (Total Hardness), NH<sub>4</sub> (Nitrate), PO<sub>4</sub> (Phosphate), Fecal coliform and Turbidity.

In order to investigate the applicability of diatom-based indices for water quality analysis, two water quality indices (IRWQI<sub>sc</sub> as a local index and NSFQI as reference

index) were selected. Many studies in Iran have applied IRWQI<sub>sc</sub> index to assess water quality across different ecosystems (Hamed et al. 2015; Samadi 2016; Bagherpour et al. 2017; Makhloogh et al. 2017; Aghayi et al., 2019, Ebraheim et al. 2020, Heshmatpour et al. 2025). Also, NSFQI water quality index is one of the most important indices widely used for water quality assessments (Ghaziyani et al., 2020, heshmatpour et al. 2025). The parameters involved in these indices and their weights were presented by Aghayi et al., (2019) and Heshmatpour et al., (2025). The indices were calculated according to following equations:

$$NSFQI = \sum_{i=1}^n I_i W_i$$

Where the  $I_i$  is the sub-index value of each parameter,  $W_i$  is the parameter's weight score and  $n$  is the number of parameters (Oram 2007).

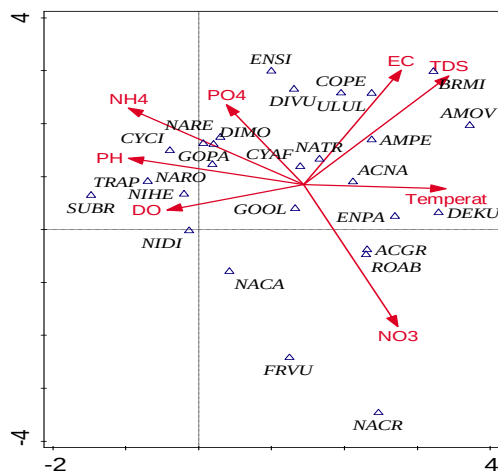
$$IRWQI = \left[ \prod_{i=1}^n I_i^{W_i} \right]^{1/\gamma}$$

Where  $\gamma = \sum_{i=1}^n W_i$ ,  $I_i$  and  $W_i$  are the sub-index value and weight scores of the parameter  $I$  respectively and  $n$  is the number of parameters (Hashemi et al., 2011; Ebraheim et al., 2020).

After testing for data normality, Spearman's correlation coefficients were used to analyze the relationships between water quality indices and diatom-based indices. In addition, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (SPSS 16) was used to examine differences in species diversity, evenness, and richness among stations.

## Results

In total, 73 diatom taxa belonging to 30 genera were identified. In 4 sampling stations, the highest abundance belonged to the species *Achnanthes minutissimum*. The results of the DCA classification, used to examine the relationship between epilithic diatom assemblages and physicochemical water quality parameters, are presented in Figure 2.



**Figure 2.** DCA classification diagram using epilithic species abundance data and water quality parameters at the study stations during the sampling period

As shown in Figure 2, the most influential water quality parameters were  $\text{NO}_3$ , TDS, EC, and  $\text{NH}_4$ . *Achnanthes gracillimum*

(ACGR) and *Rhoicosphenia abbreviata* (RHAB) showed positive correlations with  $\text{NO}_3$ ; *Brachysira microcephala* (BRMI) correlated positively with TDS; and *Cocconeis pediculus* (COPE), *Navicula tripunctata* (NATR), and *Ulnaria ulna* (ULUL) were positively correlated with EC. Additionally, *Diatoma moniliformis* (DIMO) showed a positive correlation with  $\text{PO}_4$ , while *Gomphonema parvulum* (GOPA) and *Navicula reichardtiana* (NARE) were positively correlated with  $\text{NH}_4$ . Furthermore, *Navicula rostellata* (NARO) and *Nitzschia incospicua* (NIIN) exhibited positive relationships with pH and DO, respectively.

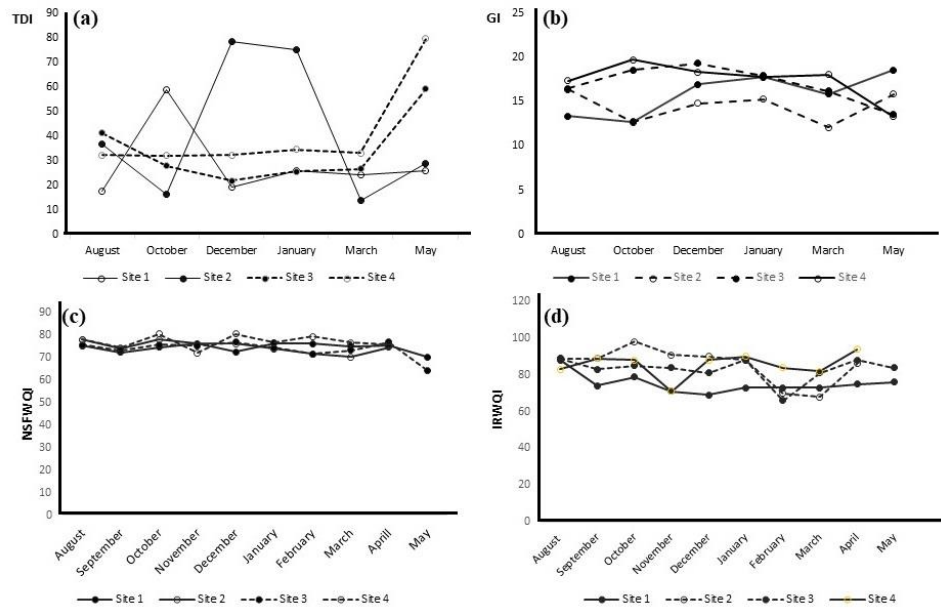
The values for the TDI, GI, Shannon diversity, Simpson diversity, Pielou evenness, NSFQI, and IRWQISC indices were calculated according to the formulas provided in the references, and their mean values are presented in Table 1.

**Table 1:** Mean value and standard deviation for measured indices

| Index             | Zarringol    | Gharachay   | Chehelchay  | Oghan       |
|-------------------|--------------|-------------|-------------|-------------|
| TDI               | 28.71± 15.15 | 41.56±28.44 | 33.78±14.05 | 40.65±19.03 |
| GI                | 15.87±2.37   | 14.52±1.73  | 17±2.08     | 17.42±2.17  |
| Shannon diversity | 1.3±0.46     | 1.66±0.47   | 1.55±0.33   | 1.48±0.33   |
| Simpson diversity | 0.56±0.15    | 0.71±0.15   | 0.64±0.21   | 0.67±0.08   |
| Pielou Evenness   | 0.4±0.43     | 0.5±0.33    | 0.57±0.56   | 0.31±0.05   |
| NSFWQI            | 73.4±29.41   | 73.5±42.19  | 72.5±95.36  | 73.3±30.59  |
| IRWQISC           | 73.5±58.17   | 84.8±91.67  | 82.6±46.13  | 85.5±83.78  |

Based on the mean TDI values, Zarringol and Chehelchay stations were classified as oligotrophic with high water quality, while Gharachay and Oghan stations fell into the oligo-mesotrophic category with good quality. According to the mean GI values, all stations except Oghan, which was classified as high quality, were placed in the good

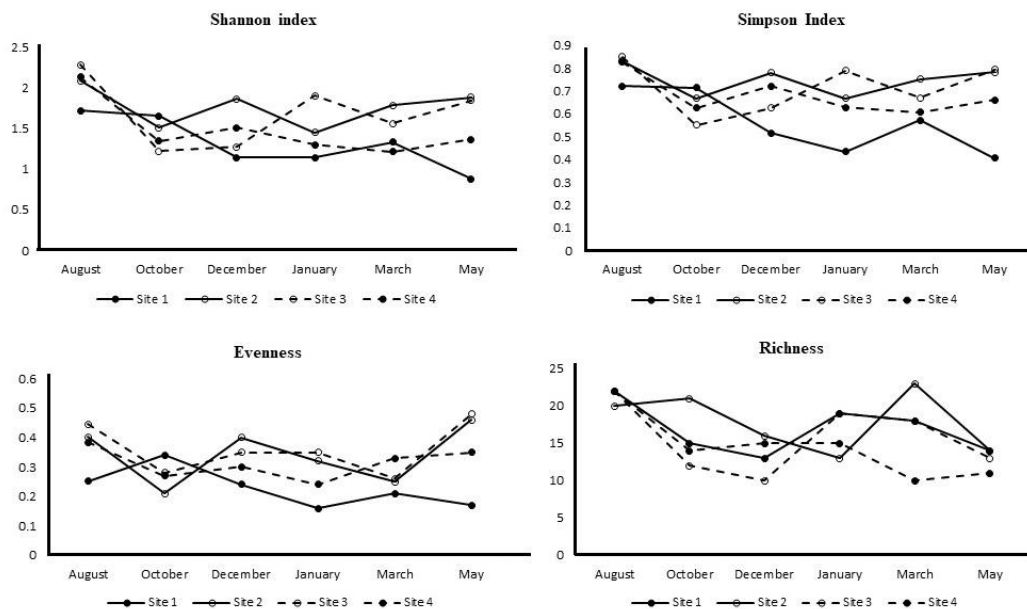
quality class. Monthly variations in TDI and GI values are presented in Figure 2a and 2b, respectively. Furthermore, the NSFQI and IRWQISC values for all four stations (Table 1) fell within the good quality class, and their monthly fluctuations are shown in Figure 2c and 2d.



**Figure 2.** Monthly variation of TDI values (a), GI values (b), IRWQI<sub>SC</sub> (c) and NSFQI (d) at the four sampling sites

Shannon diversity index ranged between 0.88 and 2.28 and Simpson index 0.4-0.84. Both indices showed highest values in Chehelchay station in August and the lowest value in Zarringol station in May. The highest evenness was observed in Chehelchay station

in May (0.68) and the lowest value in Zarringol station in January (0.16). The highest number of taxa was determined in the Gharachay station (23 taxa), while, the lowest number was observed in Chehelchay station (10 taxa) (Figure 3).



**Figure 3.** Values of the diversity indices during sampling period

Spearman's correlation coefficients showed that TDI and GI values are significantly correlated; however, there was no significant correlation between TDI, GI, Shannon

diversity, Simpson index, Pielou evenness index and richness index with IRWQI<sub>SC</sub> and NSFQI (Table 2).

**Table 2.** Spearman's Correlation coefficients between measured indices

| Indicator species | TDI     | GI      | NSFWQI  | IRWQI   | Shannon | Simpson | Evenness | Richness |
|-------------------|---------|---------|---------|---------|---------|---------|----------|----------|
| TDI               | 1.000   | 0.426*  | 0.185   | 0.210   | 0.263   | 0.341   | 0.610**  | -0.310   |
| GI                | 0.426*  | 1.000   | 0.094   | -0.164  | -0.430* | -0.396  | -0.106   | -0.362   |
| NSFWQI            | 0.185   | 0.094   | 1.000   | 0.551** | -0.075  | -0.107  | -0.107   | -0.016   |
| IRWQI             | 0.170   | -0.185  | 0.551** | 1.000   | 0.250   | 0.250   | 0.250    | 0.197    |
| Shannon           | 0.263   | -0.430* | -0.075  | 0.250   | 1.000   | 0.978** | 0.695**  | 0.584**  |
| Simpson           | 0.341   | -0.396  | -0.107  | 0.250   | 0.978** | 1.000   | 0.762**  | 0.493*   |
| Evenness          | 0.610** | -0.106  | -0.080  | 0.120   | 0.695** | 0.762** | 1.000    | -0.128   |
| Richness          | -0.310  | -0.362  | -0.016  | 0.197   | 0.584** | 0.493*  | -0.128   | 1.000    |

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

In addition, the ANOVA followed by Tukey's multiple comparison test of diversity, evenness, and richness among stations indices showed no significant differences between sites ( $p > 0.05$ ).

### Discussion

The primary objective of this study was to evaluate the applicability of several diatom-based biotic indices for water quality assessment in the Gorganrood River. To this end, the TDI, GI, Shannon index, Pielou evenness index, richness index, IRWQISC, and NSFWQI were calculated for the study stations over the sampling period.

IRWQISC is a national index recognized as a reliable tool for water quality assessment (Pourshahabi et al., 2018; Heshmatpour et al., 2025). NSFWQI is also among the most widely used indices for water quality analysis. Aghai et al. (2019), Ebraheim et al. (2020), and Heshmatpour et al. (2025) have stated that both indices are necessary for a more comprehensive assessment of surface waters in Iran. Our results show a significant correlation between IRWQISC and NSFWQI. According to both indices, all stations were classified as having good water quality.

Kelly and Whitton's (1995) TDI was applied to monitor the trophic status of the Gorganrood River. TDI values exhibited temporal variation across all study sites, a pattern also confirmed by Kelly et al. (2008), who recommended repeated sampling—specifically three times per year over two years—to provide a robust basis for assessment. They noted that more frequent sampling might risk pseudo-replication; accordingly, we excluded additional data in our study. Atazadeh et al. (2007) applied the

TDI in the Gharasou River in western Iran and found it useful for monitoring ecological conditions in streams. Although our TDI results placed the study stations in high and good quality classes, we found no correlations between TDI and either IRWQISC or NSFWQI. In comparison with TDI, GI values were more stable across all stations throughout the study period; however, similar to TDI, GI showed no significant correlation with IRWQISC or NSFWQI. A positive correlation was observed between GI and TDI in our study, consistent with findings by Maraşlıoğlu et al. (2020). An advantage of the GI index is that it requires identification only to the genus level.

Diversity indices are commonly used in diatom studies (Ndiritu et al., 2006; Kupe et al., 2008; Jüttner et al., 2012). It has been demonstrated that heavily polluted waters typically support low diversity and species richness in diatom assemblages (Lobo and Kobayasi, 1990); however, factors other than pollution may also affect species diversity (Lobo et al., 1995). Assemblages with low diversity are often dominated by only a few species (low evenness). Shannon index values range from 0 to 5, usually falling between 1.5 and 3.5, and rarely exceeding 4.5 (Magalef, 1972). In our study, values mainly ranged between 1 and 2 during the sampling period. The Simpson index ranges from 0 (low diversity) to 1 (Magurran, 2004). Our Simpson index results were mostly between 0.5 and 0.8, indicating higher diversity than the Shannon index. This discrepancy arises because the Shannon index is strongly influenced by species richness and rare species, whereas the Simpson index gives greater weight to common species and is

more sensitive to abundant taxa (Magurran, 2004). Strong correlations were observed among the diversity indices, which is unsurprising as they represent aspects of the same phenomenon. According to ANOVA test results, there were no significant differences in diversity indices between sites ( $p > 0.05$ ). Similar findings were reported by Jüttner et al. (2012). Heino et al. (2009) attributed such results to insufficient environmental gradients and weak differences in water chemistry, which in turn are reflected by the absence of clear differences in species diversity components.

### Conclusions

Many authors have examined the applicability of diatom indices for water quality assessment and confirmed their usefulness in their respective regions, owing to the widespread distribution of diatom taxa with similar environmental tolerances (Bere and Tundisi, 2011; Bere, 2016). Conversely, other researchers have argued that diatom indices developed for a particular region may not be suitable for evaluating water quality elsewhere (Pipp, 2002). In our study, although the results of diatom indices and

water quality indices were generally similar—with all measured rivers classified as having good quality, with few exceptions—we found no significant correlations between the indices. This lack of correlation occurred despite the fact that most dominant taxa are cosmopolitan. Further research and additional data from other regions appear necessary to clarify the usefulness of diatom indices for water quality assessments in Iran.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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