IRANIAN JOURNAL OF BOTANY 30(2), 2024
DOI: 10.22092/ijb.2024.364664.1451

WATER QUALITY ASSESSMENT USING A MULTI-DESCRIPTOR APPROACH: INTEGRATING CHEMISTRY, DIATOM ASSEMBLAGES, AND NON-TAXONOMICAL DIATOM METRICS IN THE KASHKAN RIVER, LORESTAN PROVINCE, IRAN

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Received 2024.01.12; accepted for publication 2024.08.20

Safiallah, S., Saadatmand, S., Kheiri, S. & Iranbakhsh, A. 2024.12.30: Water quality assessment using a multi-descriptor approach: integrating chemistry, diatom assemblages, and non-taxonomical diatom metrics in the Kashkan River, Lorestan province, Iran. *-Iran. J. Bot. 30(2): 234-253.* Tehran.

The Kashkan River, situated in western Iran, is a significant waterway, primarily utilized for drinking water supply and agricultural purposes. Consequently, rigorous monitoring of the river's water quality is imperative. Diatom's teratological forms, and ecological stress indicators, play a crucial role in water quality assessment programs. Understanding the correlation between environmental factors and these teratological forms is crucial for accurate water quality indices. The primary objective of this study was to investigate the diatom teratological communities in the Kashkan River and explore their correlation with heavy metals, aiming to elucidate water quality dynamics. In spring 2019, 48 epipelic and epilithic samples were collected along the Kashkan River, considering the impact of anthropogenic activities on the river. Three distinct teratological forms were identified comprising type 1) deformities in valve outlines, type 2) deformities in striations, and type 3) mixed deformities. Notably, valve deformities in *Achnanthidium minutissimum* and *Diatoma moniliformis* were the most prevalent. Furthermore, the concentrations of Cd, Fe, and Cr in the sediments emerged as significant environmental variables associated with elevated heavy metal levels in the Kashkan River. Principal component analysis (PCA) highlighted that sedimentary Fe, Cu, and Cr concentrations were key factors influencing the composition of diatom teratological forms. Consequently, implementing a water quality management program is vital to enhance the overall water quality in the Kashkan River.

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Keywords: Anthropogenic activities; freshwater diatoms; heavy metals; Kashkan River; teratology; water monitoring

ارزیابی کیفیت آب رودخانه با استفاده از رویکرد چند توصیف‌گر: شیمی، جوامع دیاتومه‌ها و شاخص‌های غیر تاکسونومیک در رودخانه کشکان، استان لرستان، ایران

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رودخانه کشکان یکی از رودخانه‌های بزرگ در غرب ایران است و به‌عنوان یک مسیر آبی که عمدتا بیشتر برای کشاورزی و شرب استفاده می‌شود مورد توجه قرار می‌گیرد. بنابراین پایش دقیق بر کیفیت آب رودخانه ضروری است. اشکال تراتولوژیکی دیاتومه‌ها، همراه با دیگر شاخص‌های

استرس‌زای اکولوژیکی، نقش مهمی در برنامه‌های پایش کیفیت آب در نظر گرفته می‌شوند. شناخت ارتباط بین عوامل محیطی و اشکال تراتولوژیک برای شاخص‌های دقیق کیفی آب حائز اهمیت است. مطالعه حاضر به‌منظور بررسی جوامع اشکال تراتولوژیکی دیاتومه‌ها در رودخانه کشکان و بررسی همبستگی آنها با فلزات سنگین با هدف آشکار کردن کیفیت آب رودخانه انجام شد. در این مطالعه ۴۸ نمونه اپی‌پل و اپی‌لیت با در نظر گرفتن فعالیت‌های انسانی در طول رودخانه کشکان در فصل بهار سال ۱۳۹۸جمع‌آوری شد. سه تیپ از اشکال تراتولوژیکی دیاتومه ها شناسایی شد: تیپ اول براساس تغییرات والو، تیپ دوم بر اساس تغییرات استریا و تیپ سوم بر اساس ترکیب تغییرات. شایع‌ترین شکل تراتولوژیک در تغییر والو دیاتومه‌های Achnanthidium minutissimum و Diatoma moniliformis مشاهده شد. علاوه بر این، غلظت کادمیوم، آهن و کروم در رسوبات به‌عنوان متغیرهای زیست محیطی مهم مرتبط با افزایش غلظت بالای فلزات سنگین در رودخانه کشکان مشاهده شد. همچنین، تجزیه و تحلیل مولفه‌های اصلی (PCA) نشان داد که غلظت آهن، غلظت مس و غلظت کروم در رسوبات از عوامل کلیدی مؤثر بر ترکیب اشکال تراتولوژی دیاتومه‌ها است. بنابراین، اجرای برنامه مدیریت کیفیت آب برای ارتقای کیفیت آب رودخانه کشکان ضروری به نظر می‌رسد.

INTRODUCTION

Water, covering approximately 75% of Earth's surface, is vital for all living organisms. Water has, a key role in human life (Lozan & al. 2007). To advance our current understanding of water quality, it is imperative to investigate the factors influencing it. Surface water monitoring studies consistently examine key parameters, including nutrient concentrations (e.g., nitrate, phosphate), heavy metal levels, turbidity, and salinity (Adeosun & al. 2016; Ameen 2019; Blanco & Bécares 2010; Mateo-Sasgasata & al. 2018; Saghali & al. 2014; WHO 2011). However, relying solely on physico-chemical assessments of water quality poses challenges in comprehensively assessing ecological stress. Aquatic biota, on the other hand, offer valuable insights into environmental conditions and contribute significantly to the overall health of the aquatic ecosystems.

Diatoms, among the key indicator organisms, exhibit remarkable sensitivity to environmental factors (Solak & al. 2020). These microorganisms, capable of thriving without laboratory culture, can be directly sampled from natural field environments (Martin & Reyes Fernández 2012). Their adaptability to low light conditions and survival in polluted water underscores their ecological resilience. Researchers have extensively investigated diatom responses to heavy metal stress, revealing significant teratological forms characterized by alterations not only in size and population but also in striae patterns and valve structures (Falasco & al. 2009a; Falasco & al. 2009b). Notably, the frequency of deformed diatom structures holds promise as a parameter for biomonitoring heavy metal contamination in water (Pandey & al. 2014), and consequently, predicting changes in aquatic ecosystem functioning necessitates an understanding of the processes that define the diverse patterns of biological communities (Donato-R & al. 2022).

Given their wide pH tolerance, diatom species are valuable indicators for monitoring environmental changes (Hervé & al. 2012). The study of diatoms has a rich historical context, with contributions from researchers such as Foged (1959), Hirano (1973), Kogan (1973), Krammer & Lange-Bertalot (1986, 1988), Patrick & Reimer (1966), and Williams (1985). In recent years, the taxonomy of freshwater diatoms in Iran has garnered interest, with studies conducted by Ahmadi Musaabad & al. (2019), Atazadeh & al. (2007), Cheraghpour & al. (2013), Jamalo & al. (2006), Kheiri & al. (2013, 2018a, 2018b, 2019, 2022, 2023, 2024), Mohebbi & al. (2016), Nejadsattari (2005), Pourgholam & al. (2014), Witkowski & al. (2007), Zarei-Darki (2011a, 2011b). However, the application of diatoms for biomonitoring rivers in Iran remains understudied (Atazadeh & al. 2007; Kheiri, 2021; Panahy Mirzahasanlou & al. 2019; Safiallah & al. 2020).

Today, the heavy reliance on agricultural activities poses a significant threat to river basins in Iran (Homami & al. 2017). The Kashkan River, situated in the western region of Iran, holds critical importance as a water source. It serves dual purposes, providing essential drinking water and supporting agricultural activities (Ghasemzadeh 2012; Mostafaei 2014). This river comprises diverse geological formations, including evaporites, carbonates, calcareous rocks, dolomitic rocks, shale, and ophiolite melange. These geological constituents collectively influence river water quality (Ghasemzadeh 2012). Further research is warranted to enhance our understanding of the impact of water nutrient concentrations and heavy metal levels on diatom populations. Identifying the key factors driving population dynamics and species composition is crucial for effective diatom-based environmental monitoring and preservation of aquatic ecosystems.

In 2020, Safiallah & al. conducted a study on the taxonomy and distribution of periphytic diatoms in the Kashkan River, revealing a rich diversity of 91 taxa. This investigation underscores the utility of diatoms as proxies for assessing water quality. Specifically, the study aims to identify pollution hotspots within the Kashkan River using diatom teratological forms.

MATERIALS AND METHODS

Study Area

The Kashkan River, one of Iran's longest rivers, stretches approximately 900 km and encompasses a drainage area of 9236 km² within Lorestan Province (Fig. 1). Originating from the Zagros Range at an altitude of ca. 3140 m above sea level, the Kashkan River serves as a principal branch, which ultimately flows into the Persian Gulf. Geologically, the Kashkan River lies within the Zagros fold-thrust belt, extending from southeast Turkey to southwest Iran (Shahriyarnia & al. 2016). This river basin holds immense significance for agriculture and serves as a vital drinking water source for the residents of Lorestan Province. Four sampling stations were chosen along the Kashkan River, spanning from Kaleho to Gandabe to assess water quality and ecological conditions. These stations, situated in the central-western part of the basin, cover an elevation range of 980-1030 m. Their proximity to agricultural lands and industrial centers renders them susceptible to anthropogenic activities (Fig. 2; Table 1).

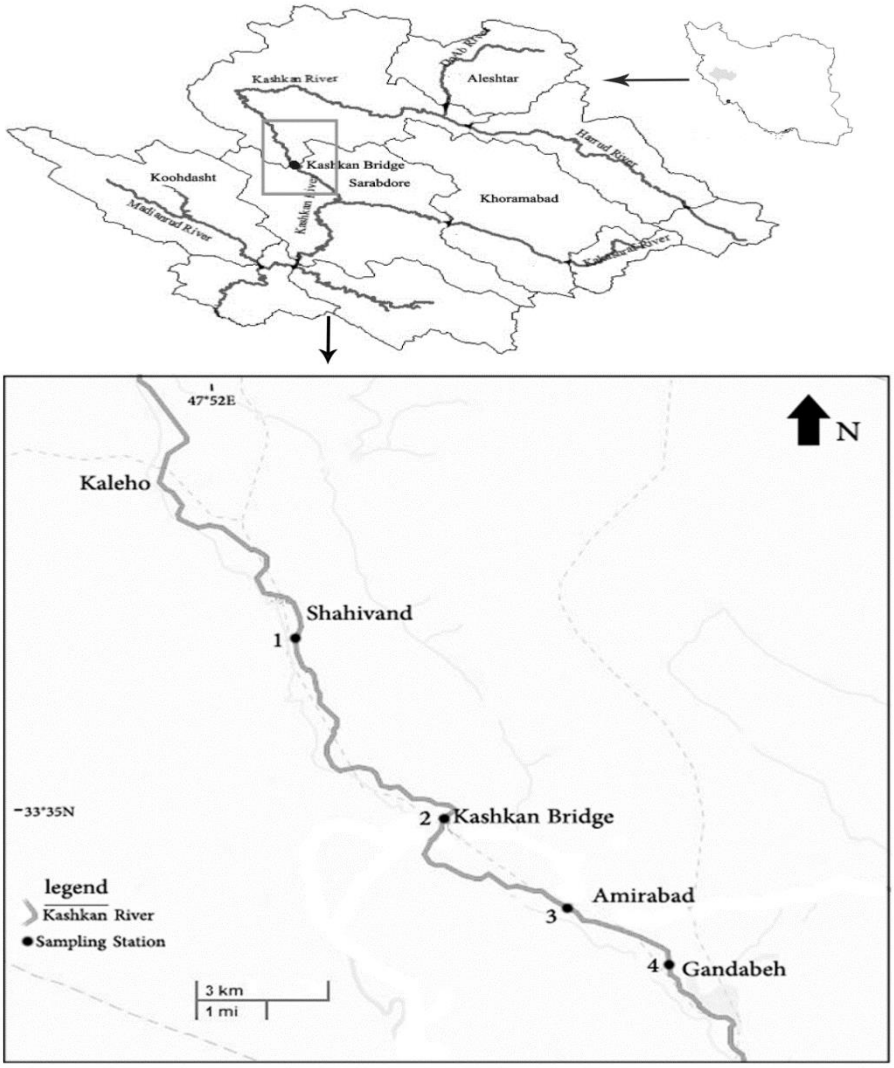

Fig. 1. Map of the Kashkan River (Lorestan Province), sampling stations are marked with numbered black dots.

Fig. 2. Images (1-4) of sampling sites along the Kashkan River. 1, Station 1, 35 Km from Koohdasht; 2, Station 2, 45 Km from Koohdasht; 3, Station 3, 55 Km from Koohdasht; 4, Station 4, 65 Km from Koohdasht.

Table 1. Location and description of the sampling stations (1-4) in the Kashkan River.

Station	Elevation (m)	Latitude and Longitude	Location and site description
1	1030	33°32'44"N 47°56'26"E	Khoramabad, near Kaleho, Chahivand, 35 Km from Koohdasht.
2	1015	33°35'22"N 47°52'50"E	Khoramabad, Kashkan Bridge, 45 km from Koohdasht
3	980	33°33'40"N 47°55'31"E	Khoramabad, Amirabad, 55 km from Koohdasht.
4	983	33°35'16"N 47°52'55"E	Khoramabad, Gandabe, 65 km from Koohdasht.

Diatom sampling and preparation

A total of 48 samples from both epipelic and epilithic habitats along the Kashkan River were collected in May 2019. These samples were obtained from the surface of rocks and mud at four chosen stations. Sampling timing coincided with May, when anthropogenic activities significantly impacted water quality in the river (Tornes & al. 2018). We employed a plastic syringe (50 ml capacity) with the barrel end removed at the needle adapter, to collect epipelic samples. Most epilithic samples were randomly collected from submerged stones at a depth of 20 cm.

A brush facilitated the retrieval of diatoms from the stone surfaces (Kheiri & al. 2013). Upon collection, the samples were preserved in 4% formaldehyde and transported to the laboratory. Subsequent cleaning involved treatment with H_2O_2 (30%) and HCl (37%). After thorough rinsing with distilled water, the diatom slides were prepared using Naphrax as the mounting medium (Taylor & al. 2007). Diatom slides are preserved in diatom herbarium, Research Institute of Forests and Rangelands (TARI). Diatom observations were conducted using an Olympus BX53 microscope, equipped with a DP72 camera. Species identification relied on established references (Bey & Ector 2013a, Bey & Ector 2013b; Hofmann & al. 2011; Patrick & Rimer 1966). In this study, teratological forms were categorized based on deformities in valves, striae patterns, and mixed deformities, following the criteria outlined by Falasco & al. (2009a, 2009b).

Physico-chemical sampling and analysis

In May 2019, water and sediment samples were collected from four selected stations along the Kashkan River. Notably, sampling sites two and four were significantly influenced by human activities (Figs. 1 and 2). The water samples were collected using plastic bottles with a volume of 1200 cc, specifically for subsequent chemical analysis. In parallel, sediment samples were carefully obtained using a plastic syringe (50 ml capacity) and transferred to polyethylene bottles.

In situ temperature, pH, and electrical conductivity (EC) measurements were recorded during the fieldwork. Subsequently, the collected samples were transported to the AZMA Company laboratory in Tehran, Iran, for comprehensive chemical analysis. The chemical parameters assessed included Total Dissolved Solids, Total Suspended Solids, Turbidity, Ammonium, Nitrate, Nitrite, Total Nitrogen, Organic Nitrogen, and Total Phosphorus. Additionally, heavy metal concentrations (Iron, Copper, Cadmium, Lead, Zinc, and Chromium) were determined using Varian Atomic Absorption Spectroscopy, following the guidelines outlined by the American Public Health Association (APHA 2017).

Statistical analysis

Spatial variation in diversity indices including Margalef's index for species richness (d), Pielou's index for species evenness (J´), and the Shannon-Wiener index for species diversity (H´using loge) were explored in Primer v.6 software (Clarke and Gorley, 2006). To explore patterns and spatial distribution of diatom communities, an nMDS and cluster analysis was performed based on the Bray-Curtis similarity matrix built on square root transformed relative abundance data in Primer v.6 software (Clarke and Gorley, 2006**)**. Differences in the assemblage structure of diatoms among stations were assessed using PERMANOVA test in Primer v.6 software. The SIMPER (Similarity Percentage Bray-Curtis similarity) method was employed to identify key contributors to dissimilarity among the groups at stations (Clarke and Gorley, 2006**)**.

Principal Coordinates Analysis (PCO) was used to determine relationships between important environmental data and stations imported to Primer v.6 software, based on nMDS plot analysis**.** Additionally, Principal Component Analysis (PCA) was performed to determine relationships between environmental factors and diatom assemblages (Teratological forms and Normal morphological diatoms) imported to CANOCO 5 (Ter Braak and Smilauer, 2015). Before conducting the PCA analyses, we employed a Draftsman plot in Primer v.6 software (Clarke and Gorley, 2006) to investigate the correlation among environmental parameters. Subsequently, we excluded redundant variables that exhibited a correlation exceeding 90%. In doing so, some variables (i.e., T, EC, pH, NTU_W, TSS_W, TDS_W, TN_W, NH_{4W}, Cu_s) were excluded from PCA analysis**.** Also, rare species (<1%) were removed from the dataset for PCA analysis.

RESULTS

Physico-chemical water parameters

The results of the physico-chemical parameter measurements in the Kashkan River are detailed in Table 2. It was characterized by cloudy water, with high turbidity and alkaliphilous. Parameters such as temperature, TDS, EC, pH, NH4, and NO3 exhibited minimal fluctuations across stations 1 to 4. Conversely, parameters like TN, ranging from 2.56 to 3.86 mg L-1, and ON, ranging from 0.13 to 1.36 mg L-1, showed an increase from stations 1 to 3, with the highest values recorded at station 3. Notably, station 4 exhibited high NTU concentrations, ranging from 60 to 110 mg L-1. Significant variations were also observed in the concentrations of Cd (2.8 to 4.9 mg kg−1) and Fe (6520 to 12500 mg kg−1) in sediments, particularly high at station 1. The highest Cd concentration in sediments was also noted at station 4. A comparative analysis of heavy metal concentrations revealed that their concentrations in sediment were higher than those in the water.

Diatom assemblages and physico-chemical characteristics of the study sites

The present study identified 99 taxa based on normal morphological forms and teratological forms representing 24 genera across four sampling stations in the Kashkan River (Table 3). This study identified 12 taxa from 10 genera exhibiting teratological forms in the Kashkan River (Fig. 3). Consequently, three primary types of teratological forms were categorized: 1) deformities in valve outline, 2) deformities in striations, and 3) mixed deformities. The most dominant teratological form was deformities in valve outline (Type 1). *Achnanthidium minutissimum* and *Diatoma moniliformis* were the species most affected by Type 1 at station 4, with occurrences of 0.20% and 0.15%, respectively. The most common diatoms with normal morphological forms observed in the Kashkan River were *A. minutissimum* Kützing (17.37%), *Cymbella affinis* var. *neoerosera* W. Silva (15.07%), and *D. moniliformis* Kützing (6.07%). At station 1, the highest values for species richness (d = 10.48), species diversity (H´(Loge) = 3.434), and dominance (1-λ=0.944) were observed for normal morphological forms (Table 4). In contrast, station 4 exhibited the highest diversity of diatom teratological forms, with species richness (d=3.336), species diversity (H´(Loge) =2.146), and dominance (1-λ=0.963) surpassing those at other stations (Table 5).

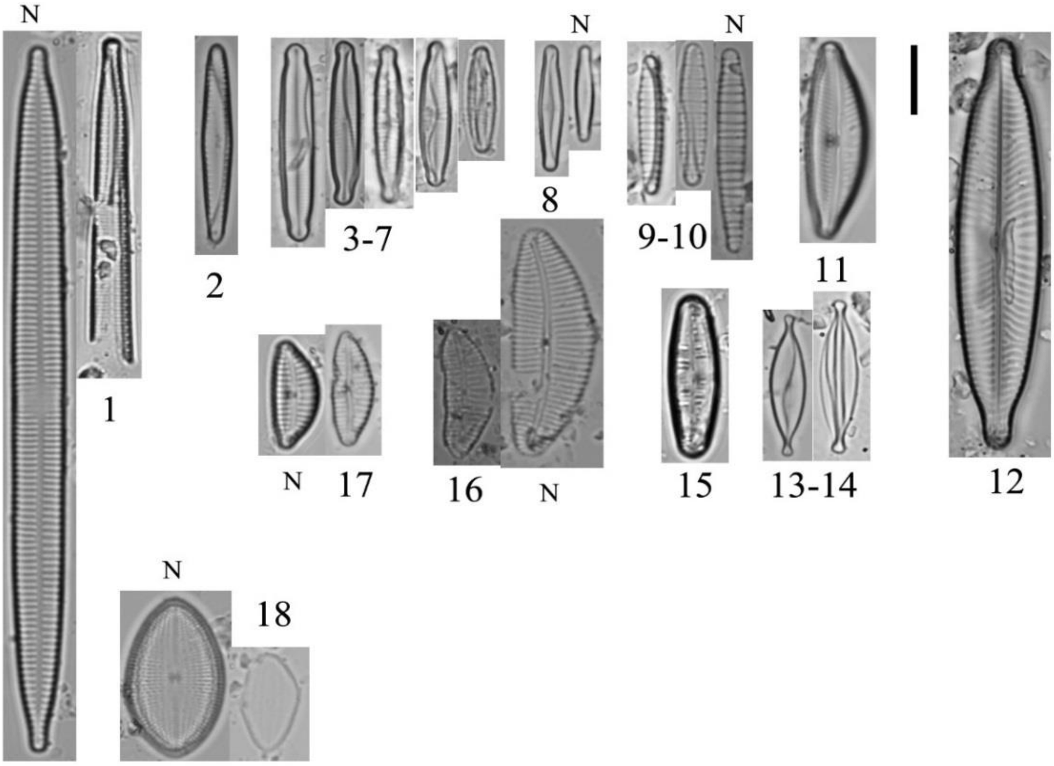

Fig. 3. Teratological forms in the Kashkan River. N, Normal diatoms: 1, *Ulnaria ulna* (diatom with deformed valve); 2, *Gomphonema* sp.(diatom with deformed striae); 3-7, *Achnanthidium* sp. (diatoms with deformed striae); 8, *Achnanthidium minutissimum* (diatom with deformed valve and raphe); 9-10, *Diatoma moniliformis* (diatoms with deformed valve); 11, *Cymbopleura* sp.(diatoms with deformed striae); 12, *Navicula viridula* (diatom with deformed striae); 13-14, *Brachysira* sp.(diatoms with deformed valve); 15, *Caloneis* sp.(diatom with deformed striae); 16, *Cymbella compacta* (diatom with deformed valve); 17, *Cymbella excise* (diatoms with deformed valve); 18, *Cocconeis placentula* var. *uglypta* (diatom with deformed valve).

Table 2. Physico-chemical parameters in the Kashkan River in spring 2019. The sensitivity to changes in parameters is shown in the bold line. Temperature (T), potential Hydrogen (pH), Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Turbidity (NTU), Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-), Total Nitrogen (TN), Organic Nitrogen (ON), Total Phosphorus (TP/ TPO_4^{-3}) and heavy metals (Fe: Iron, Cu: Copper, Cd: Cadmium , Pb: Lead, Zn: Zinc and Cr: Choromium). S: sediment and W: water. Iranian Standard Water (ISDW): Based on the physico-chemical factors reported in papers in Iran, compared to the international standard.

Parameters	**Stations**				**Range (minimum–maximum) and mean ± SE**	**World Water Standards**	**References**	**Iranian Standard Water (ISDW)**	**References**
	1	**2**	**3**	**4**					
EC (W) μScm^{-1}	330	350	337	331	330-350 337± 0.49	-	-	-	-
pH (W)	8.23	8.25	8.30	8.49	8.23-8.49 8.31 ± 0.006	6.5-9.5	WHO, 2008	6.5-8.5	Aazami & Taban 2018; Samadi & al. 2009
T (W) °C	25	21	25	26	21-26 24.25 ± 0.12	-	-	-	-
NTU (W) mg L^{-1}	**60**	**64.5**	**81.5**	**110**	60-110 79 ± 1.22	**Less than 5**	WHO, 2008	**25**	Aazami & Taban 2018; Samadi & al. 2009
TDS (W) mg L^{-1}	237	243	233	232	232-243 236.25 ± 0.27	1000	WHO, 2008	500	Aazami & Taban 2018; Samadi & al. 2009
TSS (W) mg L^{-1}	68	56	70	84	56-84 69.5 ± 0.62	-	-	-	-
NH_4 (W) mg L^{-1}	0.40	0.24	0.14	0.14	0.14-0.4 0.23 ± 0.006	1.5	WHO, 2008	-	-
NO_3 (W) mg L^{-1}	2	2.42	2.35	2.45	2-2.45 2.30 ± 0.01	50	WHO, 2008	45	Aazami & Taban 2018; Samadi & al. 2009
NO_2 (W) mg L^{-1}	**0.02**	**0.02**	**0.019**	**0.018**	0.018-0.02 0.019 ± 5.1	3	WHO, 2008	**0.004**	Aazami & Taban 2018; Samadi & al. 2009
T N (W) mg L^{-1}	2.56	3.39	3.86	3.07	2.56-3.86 3.22 ± 0.02	-	-	-	-
ON (W) mg L^{-1}	0.13	0.71	1.36	0.46	0.13-1.36 0.66 ± 0.02	-	-	-	-

Table 2. Continued.

T PO_4 (W)mg L^{-1}	0.05	0.19	0.12	0.20	0.05-0.2 0.14 ± 0.003	-	-	-	-
Cr (W) mg L^{-1}	0	0	0	0	0	0.05	WHO, 2008	0.05	Aazami & Taban 2018; Samadi & al. 2009
Cd (W) mg L^{-1}	0	0	0	0	0	0.003-0.005	WHO, 2008	0.01	Aazami & Taban 2018; Samadi & al. 2009
Cu (W) mg L^{-1}	0.02	0.001	0.03	0.06	0.001-0.06 0.02 ± 0.001	2	WHO, 2008	1	Aazami & Taban 2018; Samadi & al. 2009
Pb (W) mg L^{-1}	0	0	0	0	0	0.01	WHO, 2008	0.05	Aazami & Taban 2018; Samadi & al. 2009
Zn (W) mg L^{-1}	0	0	0	0	0	3-5	WHO, 2008	15	Aazami & Taban 2018; Samadi & al. 2009
Fe (W) mg L^{-1}	0.05	0.06	0.17	0.18	0.05-0.18 0.11 ± 0.003	0.3	CCME, 2006	1	Aazami & Taban 2018; Samadi & al. 2009
Cr (S) mg.kg^{-1}	31.6	26	26	40	26-40 30.9 ± 0.35	-	-	43.4	Burton, 2002
Cd (S) mg.kg^{-1}	**4.9**	**3.7**	**3**	**2.8**	2.8-4.9 3.6 ± 0.05	-	-	**0.99**	Burton, 2002
Cu (S) mg.kg^{-1}	26.2	22.7	21.5	26	21.5-26.2 24.1 ± 0.12	-	-	31.6	Burton, 2002
Pb (S) mg.kg^{-1}	0	0	0	0	0	-	-	35.8	Burton, 2002
Zn (S) mg.kg^{-1}	32.18	29.10	26.50	46.41	29.1-46.41 33.54 ± 0.48	-	-	121	Burton, 2002
Fe (S) mg.kg^{-1}	**12500**	**7300**	**6250**	**6520**	6250-12500 8142.5 ± 159.07	**0.3**	FEPA, 2003	>17000	Mireles & al. 2011

The non-metric multidimensional scaling (nMDS) ordination, utilizing square root-transformed abundances and the Bray-Curtis similarity matrix, identified three distinct groups of stations, with dashed lines indicating 60% similarity (Fig. 4). The first group, comprising epilithic diatoms at stations 1 and 2, formed a single cluster. The second group, consisting solely of epilithic diatoms at station 4, was the most distinct from all other groups. As illustrated in Fig. 4, the third group, including five sample stations, demonstrated a high similarity.

The PERMANOVA analysis revealed statistically significant differences between teratological forms and normal morphological diatom assemblages at the study sites (Pseudo-F=8.684, df=1, p=0.002), (Table 6). The SIMPER analysis, based on the percentage contribution at each station, indicated that the contribution of teratological forms at station 4, with 0.54% abundance and 17.88% average dissimilarity, was highly statistically significant compared to other stations (Table 7).

Principal Coordinates Analysis (PCO) was conducted for major environmental factors across all stations, as indicated by the nMDS plots (Fig. 5). The environmental variables in the PCO accounted for 43.7% of the variance on the first axis and 30.1% on the second axis. The concentrations of Zn and Cr in the sediment exhibited strong positive correlations with station 4. Additionally, the second axis showed a strong positive correlation with the concentration of Cd in sediments and a positive correlation with the concentration of Fe in both water and sediments.

A Principal Component Analysis (PCA) was conducted to examine the relationships between environmental factors and diatom communities, with a particular focus on diatom teratological forms (Fig. 6). The first and second components of the PCA explained 60% and 28% of the variance in the environmental data, respectively. The first axis was primarily influenced by the concentrations of Fe, NO_3, and NO_2 in water, and Cd in sediment. The second axis was mainly associated with the concentrations of Cr and Zn in sediment. Species such as *Cocconeis lineata*, *Gomphonella olivaceae*, and *Gomphonema* sp. aligned with the directions of the arrows for NO_2 concentration in water and Cd concentration in sediments. Teratological forms, including *Achnanthidium minutissimum* (AB3), corresponded with the arrows indicating Fe concentration in water. Additionally, other teratological forms such as *Caloneis* sp. (AB12), Cymbopleura sp. (AB11), *Caloneis placentula* var. *euglypta* (AB10), Brachysira sp. (AB7), *Ulnaria ulna* (AB6), and *Cymbella compacta* (AB1) were observed to align with the arrows for Zn and Cr concentrations in sediments and Fe and Cu concentrations in water at station 4. The results indicated that most other teratological forms exhibited strong negative relationships with heavy metals and nutrients.

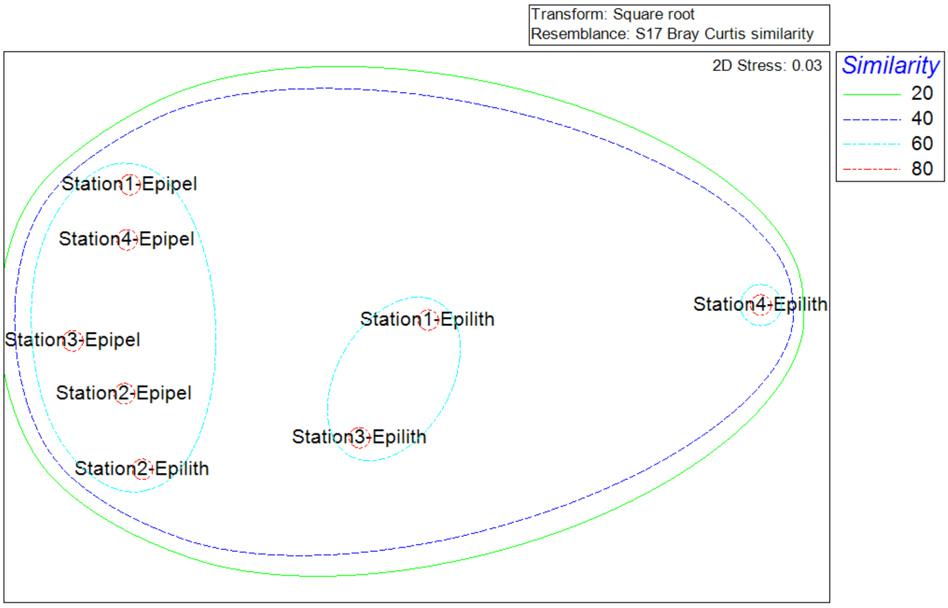

Fig. 4. Non-metric multidimensional scaling (nMDS) plots of Bray-Curtis similarity index of diatoms across all sampling stations. The plots showing the similarity of diatom communities in different sampling sites (indicating the groupings with dashed lines 60% similarity). Epili (epilithic) and Epipel (epipelic).

Table 3. List of species (Safiallah & al. 2020) and relative abundance (In this study: normal morphological diatoms and teratological forms) in the Kashkan River, RA (rare).

Diatom Taxa	Code	(RA%)
Achnanthidium cf. *pyrenaicum* (Hustedt) H.Kobayasi	Ach.py	0.75
Achnanthidium gracillimum (F. Meister) Lange-Bertalot	Ach.gr	0.35
Achnanthidium minutissimum (Kützing) Czarnecki	Ach.mi	17.37
Achnanthidium sp.1	Ach.sp1	4.17
Achnanthidium sp.2	Ach.sp2	0.14
Amphipleura pellucida (Kützing) Kützing	Amu.pel	0.46
Amphora pediculus (Kützing) Grunow	Amp.ped	0.25
Brachysira microcephala (Grunow) Compère	Bra.mip	0.10
Caloneis bacillum (Grunow) Cleve	Cal.bac	0.20
Cocconeis lineata Ehrenberg	Coc.li	1.20
Cocconeis placentula Ehrenberg	Coc.pl	0.14
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	Coc.plv	0.75
Craticula cf. *accomoda* (Hustedt) D.G.Mann	Cr.acc	0.20
Craticula sp.1	Cr.sp	0.59
Cyclotella atomus Hustedt	Cyc.at	0.36
Cyclotella meneghiniana Kützing	Cyc.mgh	0.10
Cymbella affinis var. *neoperocera* W.Silva	Cym.af	15.07
Cymbella cf. *excisiformis* Krammer	Cym.exf	0.30
Cymbella compacta Østrup	Cym.com	0.53
Cymbella excisa Kützing	Cym.esa	2.38
Cymbella exigua Krammer	Cym.exg	4.31
Cymbella lange-bertalotii Krammer	Cym.lb	0.52
Cymbella tumida (Brébisson) Van Heurck	Cym.tum	0.13
Cymbopleura cf. *angelica* (Lagerstedt) Krammer	Cyb.ang	0.20
Denticula kuetzingii Grunow	Den.ku	0.20
Diatoma moniliformis (Kützing) D.M.Williams	Dia.mo	6.07
Diatoma voulgaris Bory	Dia.vu	0.64
Diatoma sp.1	Dia.sp1	0.20
Encyonema lange- bertalotii morpho1 Krammer	Enc.ty1	0.33
Encyonema minutum (Hilse) D.G.Mann	Enc.min	0.65
Encyonema silesiacum (Bleisch) D.G.Mann	Enc.ss	1.49
Encyonema ventricosum (C.Agardh) Grunow	Enc.ven	0.87
Encyonopsis microcephala (Grunow) Krammer	Enp.mic	0.05
Encyonopsis minuta Krammer & E.Reichardt	Enp.mi	0.48
Encyonopsis sp.1	Enp.sp1	0.20
Encyonopsis subminuta Krammer & E.Reichardt	Enp.sum	0.20
Gomphonella olivacea (Hornemann) Rabenhorst	Gnl.ol	2.31
Gomphonella sp.1	Gnl.sp1	0.32
Gomphonella sp.2	Gnl.sp2	0.31
Gomphonella sp.3	Gnl.sp3	0.20

Table 3. Continued.

Gomphonella sp.4	Gnl.sp4	1.58
Gomphonema cf. *cymbelliclinum* E.Reichardt & Lange-Bertalot	Gnm.cli	0.29
Gomphonema cf.*dichotomum* Kützing	Gnm.dic	1.32
Gomphonema parvulum (Kützing) Kützing	Gnm.par	1.37
Gomphonema productum (Grunow) Lange-Bertalot	Gnm.pro	0.60
Gomphonema sp.1	Gnm.sp1	0.75
Gomphonema sp.2	Gnm.sp2	2.49
Gomphonema sp.3	Gnm.sp3	4.09
Gomphonema tergestimum (Grunow) Fricke	Gnm.trg	0.13
Hantzschia abundans Lange-Bertalot	Han.abu	0.23
Melosira varians C.Agardh	Mel.var	0.07
Navicula broetzii Lange-Bertalot & E.Reichardt	Nav.bez	0.19
Navicula capitatoradiata H.Germain ex Gasse	Nav.cap	3.62
Navicula caterva Hohn & Hellermann	Nav.cat	1.65
Navicula cf. *antonii* Lange-Bertalot	Nav.ant	0.20
Navicula cryptotenella Lange-Bertalot	Nav.cry	1.10
Navicula gregaria Donkin	Nav.gg	0.19
Navicula radiosa Kützing	Nav.rad	0.31
Navicula sp.1	Nav.sp1	0.30
Navicula sp.2	Nav.sp2	0.40
Navicula sp.3	Nav.sp3	0.27
Navicula tripunctata (O.F.Müller) Bory	Nav.tpu	0.28
Navicula tripunctata sensu Bey & Ector 2013	Nav.tri	0.47
Navicula trivialis Lange-Bertalot	Nav.trv	0.20
Navicula viridula (Kützing) Ehrenberg	Nav.trd	0.45
Navicula vandamii sensu Bey & Ector 2013	Nav.vas	1.42
Nitzschia cf. *acicularis* (Kützing) W.Smith	Nit.aci	0.20
Nitzschia cf. *desertorum* Hustedt	Nit.des	0.35
Nitzschia dissipata (Kützing) Rabenhorst	Nit.di	1.43
Nitzschia dissipata var. *media* (Hantzsch) Grunow	Nit.dim	1.43
Nitzschia flexa Schumann	Nit.flx	0.50
Nitzschia heufleriana Grunow	Nit.heu	0.59
Nitzschia linearis W.Smith	Nit.li	1.23
Nitzschia palea (Kützing) W.Smith	Nit.pa	2.65
Nitzschia paleaeformis Hustedt	Nit.pf	0.53
Nitzschia recta Hantzsch ex Rabenhorst	Nit.rec	0.17
Nitzschia sp.1	Nit.sp1	0.20
Pantocskiella iranica Nejadsattari, Kheiri, Spaulding & Edlund	Pan.ir	0.80
Surirella angusta Kützing	Su.ag	0.29
Surirella lacrimula J.D.English	Su.la	1.54
Surirella librile (Ehrenberg) Ehrenberg	Su.li	0.20
Surirella sp.1	Su.sp1	0.20

Table 3. Continued.

Tryblionella sp.1	Try.sp1	0.83
Ulnaria acus (Kützing) Aboal	Ul.ac	0.31
Ulnaria biceps (Kützing) Compère	Ul.bce	0.24
Ulnaria cf. *rhombus* D.M.Williams	Ul.rho	0.13
Ulnaria contracta (Østrup) E.A.Morales	Ul.con	0.89
Teratological forms		
Cymbella compacta	AB1	0.05
Cymbella excisa	AB2	0.04
Achnanthidium minutissimum	AB3	0.20
Achnanthidium sp.	AB4	0.28
Diatoma moniliformis	AB5	0.15
Ulnaria ulna	AB6	0.05
Brachysira sp.	AB7	0.05
Navicula viridula	AB8	0.05
Gomphonema sp.	AB9	0.05
Cocconeis placentula var. *euglypta*	AB10	0.05
Cymbopleura sp.	AB11	0.05
Caloneis sp.	AB12	0.05

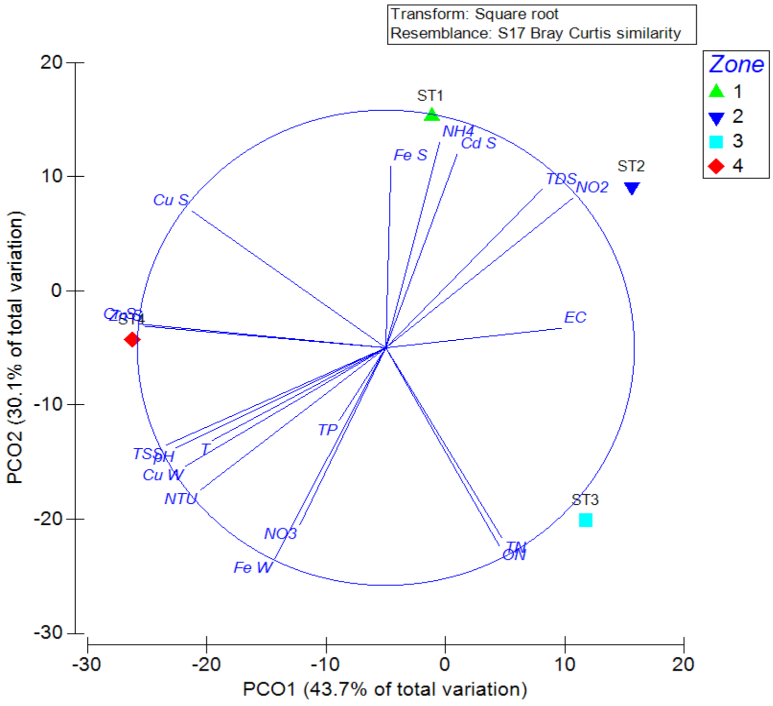

Fig. 5. Principal Component Analysis (PCO) derived from the contribution of parameters at all stations (Zone) in the Kashkan River. The circle is a unit that each vector begins at the center and ends at the coordinates consisting of the correlations between the physico-chemical and stations respectively. The importance of each variable's contribution to each of the two axes PCO1 and PCO2. * Station 4: Zn and Cr in sediments.

DISCUSSION

Aquatic ecosystems such as wetlands, rivers, and lakes are increasingly subjected to anthropogenic pressures, notably from agricultural and industrial sectors (Liu & al. 2020). Consequently, the implementation of biological and physico-chemical monitoring methodologies is essential to ascertain the impact of human activities on these water bodies (Solak & al. 2020; Mastiha 2020). The present study investigated the recorded data across various reports concerning physico-chemical parameters, with an emphasis on the teratogenic manifestations observed in diatoms. Earlier studies indicate that alterations in environmental conditions are capable of modulating diatom morphology, which is an immediate adaptive response to the surrounding environmental factors (Falasco & al. 2009a; Falasco & al. 2009b; Pandey & al. 2014; Pandey & al. 2017; Pandey & al. 2018). It is noted that morphological deformities in diatom frustules may arise due to a multitude of factors, encompassing changes in flow rate, light, population density, pH levels, nutrient accessibility, and a range of abiotic stressors (Falasco & al., 2009a; Lavoie & al. 2012).

Metal-induced stress is recurrently associated with the induction of morphological abnormalities in diatom species (Morin & al. 2012). However, such morphological abnormalities have been extensively reported globally (Falasco & al. 2009a; Falasco & al. 2009b; Pandey & al. 2014; Pandey & al. 2015; Pandey & al. 2017; Pandey & al., 2018; Topia 2008). In contrast, there have been instances where what were presumed to be new variants of known species were, in fact, distinct taxa, leading to the recognition and description of teratological form within the scientific literature (Falasco & al. 2009a; Round & al. 1990; Krammer & Lange-Bertalot, 1991; Hofmann & al. 2011).

Within the scope of this study, the Kashkan River was found to harbor three distinct teratological manifestations: anomalies in valve morphology, striation irregularities, and composite deformities. Specifically, we identified *A. minutissimum* and *D. moniliformis* as species with deformed valves. Moreover, species such as *Caloneis* sp**.**, *Cymbopleura* sp**.**, and *Navicula viridula* were identified with aberrant striation patterns. This study also entailed an analysis of diversity indices among the teratological assemblages present in the Kashkan River, which include metrics of species diversity, richness, and dominance. It is noteworthy that these indices were elevated at station 4 in comparison to other sampling locations. Likewise, the study assessed the diversity indices among diatoms exhibiting normal morphologies within the same riverine system.

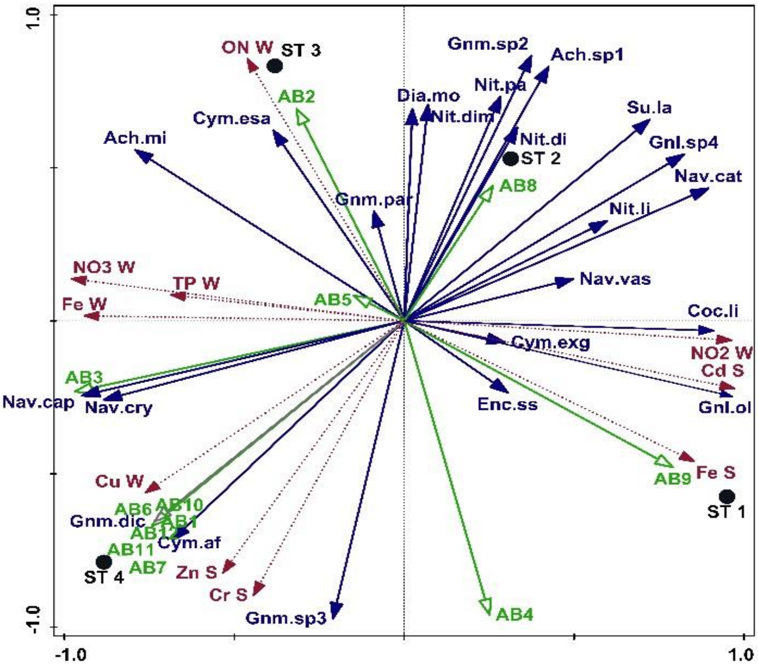

Fig. 6. Principal Component Analysis (PCA) diagram showing the relationship between diatom communities and environmental factors. Teratological diatoms marked with green color (AB). All diatom species codes are based on Table 2.

A high diversity of normotypical morphological forms was detected in the part of the river closer to the upstream at station 1, whereas teratological variants were predominantly encountered downstream at station 4. Station 1 was distinguished by higher water quality relative to the remaining stations. The downstream segment, in proximity to agricultural zones, agricultural machinery, roads, and parking lots, is suggested as the principal sources for contamination stemming from human activity. It is vital to acknowledge that anthropogenic influences exert a significant impact on diatom populations in fluvial systems, mediated through variables such as agricultural effluents, eutrophication, metallic pollutants, hydrocarbons, and pesticidal substances (Tapia 2008; MacDougall & al. 2017).

Alterations in the structural composition of diatom communities, visible at both community and individual levels, are increasingly attributed to rising anthropogenic interventions, alongside elevated concentrations of heavy metals and nutrients (Falasco & al. 2009a; Falasco & al. 2009b; Falasco & al. 2021). The application of non-metric multidimensional scaling (nMDS) in this study has explicitly delineated the compositional taxonomy of all surveyed stations. To investigate whether there are significant differences in community compositions, the researchers have performed two-dimensional non-metric multidimensional scaling (Panahy Mirzahasanlou & al. 2019; Teittinen & al. 2021; Van de Vijver & al. 2020). Furthermore, the PERMANOVA test revealed that the assemblages of teratological forms significantly differ from those with normal morphological forms, as evidenced by a highly significant index and a p-value of 0.002 at station 4, based on physico-chemical factors. Panahy Mirzahasanlou (2019) found significant statistical differences among stations concerning benthic diatom communities, that are primarily influenced by chemical constituents.

Table 4. Diversity indices of the diatom communities at the station level. d: Margalef's index for species richness; J0: Pielou's index for species evenness; H0 (loge): log2 Shannon-Wiener index for species diversity; Lambda: Simpson's index (measure of dominance). The highest results are marked in bold.

Stations	d	J'	H'(loge)	1-Lambda'
ST1	**10.48**	**0.835**	**3.434**	**0.944**
ST2	8.477	0.794	3.168	0.931
ST3	9.922	0.798	3.320	0.928
ST4	9.672	0.752	3.081	0.909

Table 5. Diversity indices of the teratological communities at species level. d: Margalef's index for species richness; J0: Pielou's index for species evenness; H0 (loge): log2 Shannon–Wiener index for species diversity; Lambda: Simpson's index (measure of dominance). The highest results are marked in bold.

Stations	d	J'	H'(loge)	Lambda'
ST1	0.910	0.918	0.636	0.666
ST2	2.526	0.975	1.748	0.952
ST3	1.443	1	0.693	1
ST4	**3.336**	**0.976**	**2.146**	**0.963**

Table 6. Results of Permutational Multivariate Analysis of Variance (PERMANOVA) using tests between normal morphological diatoms and teratological forms in different sites. Significant results are indicated in bold.

Groups	**df**	**SS**	**Pseudo-F**	**P(Perm)**
Diatoms and teratological forms	1	13895	8.684	**0.002**

The SIMPER analytical procedure is instrumental in discerning the congruencies amongst diatom communities, as well as furnishing insights into the mean dissimilarities between diatom clusters when contrasting distinct groups (Schuch & al. 2015). This test routine is pivotal for pinpointing key species and differentiating the benthic diatom community within contaminated aquatic environments (Petrov & al. 2010). The SIMPER test facilitated the observation of a pronounced elevation in average abundance (Av. Abund), average dissimilarity (Av. Diss), and dissimilarity standard deviation (Diss/SD) among teratological forms at station 4, in contrast to other stations along the Kashkan River. It has been observed that diatom community compositions are susceptible to alterations in the face of minor stressors, with the degree of variability in these compositions being more pronounced under conditions of low stress as opposed to high stress (Falasco & al. 2021; Pandey & al. 2017; Pandey & al. 2018).

A notable stressor is the contamination of riverine systems by heavy metals, including Cu, Zn, Fe, Cd, Cr, Hg, and Co (Falasco & al. 2009a). The prevalence of heavy metals within aquatic ecosystems has seen an uptick, attributable to both human activities and natural geological processes (Ghasemzadeh 2012). The current study highlights the concentrations of Cd, Fe, and Cr in sedimentary deposits as significant environmental variables correlating with elevated heavy metal levels in the Kashkan River. Concurrently, instances of diatom deformities have been documented within the river's samples. Species such as *Achnanthidium minutissimum*, *Cymbella compacta*, *Cymbella excisa*, *Cocconeis placentula* var. *euglypta*, and *Diatoma moniliformis* have been identified with valve deformations. In addition, *Caloneis* sp., *Cymbopleura* sp., and *Navicula viridula* have demonstrated deformed striae. Within this study, *Achnanthidium* emerged as the predominant genus exhibiting teratological forms. The PCA analysis conducted herein revealed a positive correlation between certain teratological forms and environmental factors, particularly metallic constituents, at the research sites. Moreover, diatoms with normal morphological configurations exhibited a marked association with heavy metal parameters, indicating a potential for morphological evolution over time in response to these elements.

Table 7. Summary of SIMPER test and the abundance and contribution of species belonging to diatom at all the stations. The analysis showed the average dissimilarity between the groups diatoms and teratological forms.
Abund: average abundance, Av. Diss: average dissimilarity, Diss/SD: ratio of average contribution divided by standard deviation, Contrib. %: percent contribution, Cum. %: cumulative percent contribution.

Group diatoms & Teratological forms Average dissimilarity=64.91	Group Diatoms	Group Teratological forms				
Stations	Av.Abund	Av. Abund	Av. Diss	Diss/SD	Contrib%	Cum.%
1	0.70	0.13	16.70	1.25	25.73	53.27
2	0.64	0.23	15.52	1.10	23.91	77.18
3	0.72	0.11	14.81	0.98	22.82	100.00
4	0.66	0.54	17.88	1.41	27.54	27.54

This study delineates a pronounced correlation between teratological forms in diatoms and the presence of heavy metals within sediment matrices, as opposed to aqueous environments. Additionally, the incidence rates of teratological forms are highly correlated with sedimentary concentrations of heavy metals, specifically Cd, As, Pb, Hg, Zn, Cr, Cu, and Ni. Literature about anthropogenic contamination in aquatic ecosystems has documented distinct morphological anomalies in genera such as *Achnanthidium*, *Cocconeis*, *Caloneis*, *Diatoma*, *Fragilaria*, *Gomphonema*, *Navicula*, *Nitzschia*, *Pinnularia*, and *Reimeria*. These anomalies are manifested in the structural components of diatoms, including striations, valves, rimoportulae, areolae, frustules, and in mixed morphological patterns, as reported in various studies (Cichon 2016; Ciszewski & al. 2011; Corcoll & al. 2012; Falasco & al. 2009a; Falasco & al. 2009b; Ferreira da Silva & al. 2009; Gautam & al. 2017; Kim Tiam & al. 2019; Laird & al. 2015; Lavoie & al. 2012; Lavoie & al. 2018; Lavoie & al. 2019; León & al. 2018; Mu & al. 2017; Pandey & Bergey 2016; Pandey & Bergey 2018; Pandey & al. 2014; Pandey & Bergey, 2016; Pandey & al. 2017; Pandey & al. 2018; Renzi & al. 2014; Salusso & Morana 2015; Sienkiewicz & Gąsiorowski 2016; Sierra & Gómez 2010; Simić & al. 2018; Topia 2008).

In the Kashkan River, sedimentary Chromium concentrations were observed to be at the threshold of the permissible limits as delineated by Iranian water quality standards (Burton 2002; Aazami & Taban 2018; Samadi & al. 2009). Furthermore, the levels of Iron and Cadmium were identified as critical elements surpassing both national and international water quality benchmarks. Comparative assessments of the Kashkan River's water quality against the Iran Water Quality Index and the Global Water Quality Index were conducted, drawing upon pivotal references (refer to Table 2, based on Aazami & Taban 2018; Burton 2002; CCME 2006; Samadi & al. 2009; Shanbehzadeh & al. 2014; WHO 2008). To improve water quality in the Kashkan River, it is essential to develop a comprehensive water quality management program.

ACKNOWLEDGMENTS

The authors are grateful to the Research Institute of Forests and Rangelands in Tehran for providing the microscopy facilities. Additionally, the authors express their gratitude to Dr. Mohammad Reza Shokri, a professor at Shahid Beheshti University, for conducting and designing most of the analyses using PRIMER v.6 and CANOCO v.5 software.

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