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Benthic diatom and macroinvertebrate assemblages, a key for evaluation of river health and pollution in the Shahrood River, Iran

Moslem Sharifinia[1] · Abbas Mahmoudifard[2] · Kamyab Gholami[2] · Javid Imanpour Namin[2] · Zohreh Ramezanpour[3]

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Abstract The present study analyzed the relationships of two different biological assemblages (diatom and macroinvertebrate) when they are used to characterize a set of stations (from reference stations to impacted stations) within the Shahrood River. In particular, we examined the issue of concordance among these assemblages and the physicochemical parameters detected, using both multivariate and multimetric methods. In addition, the trophic diatom index and Hilsenhoff family-level biotic index for macroinvertebrate assemblages were used to evaluate the ecological status of the Shahrood River. Diatom and macroinvertebrate assemblages in the Shahrood River differed significantly between reference and impacted stations. ANOSIM showed a significant difference in the composition and abundance of diatoms and macroinvertebrates among reference, influence and impact stations, especially between reference and impact stations. Results of CCA ordination showed that benthic diatoms and macroinvertebrate assemblages were mainly affected by DO, TSS, NO_3^-, PO_4^{3-} concentrations and heavy metals such as Zn and Cd. Biotic indices for the Shahrood River suggested a water quality category of "fair" with fairly substantial organic pollution for the impacted stations. Finally, our results suggest that an appropriate management and restoration policy needs to be implemented for the Shahrood basin.

Keywords Aquatic pollution · Macroinvertebrates · Ecological status · Diatoms

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✉ Moslem Sharifinia
moslem.sharifinia@yahoo.com

[1] Young Researchers and Elite Club, Rasht Branch, Islamic Azad University, Rasht, Iran

[2] Faculty of Natural Resources, University of Guilan, POB: 1144, Sowmehsara, Guilan, Iran

[3] International Sturgeon Research Institute, POB: 41635-3464, Rasht, Iran

Introduction

Aquatic ecosystems are adversely impacted by human activities. For instance, surface waters suffer from strong degradation because anthropogenic activities directly impact the river system (e.g. fishing, water diversion, irrigation, and barrages), as well as those that alter the territory surrounding the watercourses (e.g. agriculture, livestock, industrial and urban complexes) (Fore et al. 1996; Malmqvist and Rundle 2002; Assessment 2005; Perkins et al. 2010; Geist 2011). In addition, rivers receive waste materials leading to eutrophication, organic pollution, acidification, and hydrological and hydromorphological alterations (Perkins et al. 2010; Geist 2011). This situation demands attentive ecological surveillance of running waters using modern methodological approaches to improve water quality and maintain biodiversity and ecosystem function (Torrisi et al. 2010).

One approach for assessing river pollution is to use bio-indicators such as benthic diatom and macroinvertebrate communities (Hirst et al. 2002; Hering et al. 2006; Hughes et al. 2012; Namin et al. 2013a, b; O'Driscoll et al. 2014; Tan et al. 2014a). The use of biotic communities for monitoring aquatic environments, especially water quality, has several widely known advantages over physicochemical monitoring (Hering et al. 2006; Sharifinia et al. 2012a; Findik 2013; Tan et al. 2014b). In running waters, where changes in hydrology are rapid and variable, biological monitoring has proved to be useful due to its integrating nature (Atazadeh et al. 2007; Miserendino 2009; Varnosfaderany et al. 2010; Smucker and Vis 2011; Namin et al. 2013a, b; Vidal-Abarca et al. 2013; Shokri et al. 2014; Nazarhaghighi et al. 2014; Tan et al. 2014b). Such methodological tools for bioassessment have improved significantly in recent years (Marzin et al. 2012; Pace et al. 2012; Chang et al. 2014; Karaouzas et al. 2015). In addition, modern multivariate statistical methods enable the identification of the major patterns in community composition and correlated factors (Armanini et al. 2011; Jyväsjärvi et al. 2014; Taherizadeh and Sharifinia 2015).

Freshwater organisms reside almost continuously in the water and respond to many environmental stressors (Morse et al. 2007; Di Veroli et al. 2010; Al-Shami et al. 2011). In the last few decades, there has been an increased interest in rapid evaluation methods for the bioassessment of water quality in several developed and developing countries (Al-Shami et al. 2010). For instance, surface waters in Iran have typically been characterized by low conductivity and high organic content (Afshin 1994). Moreover, in northwestern Iran, most of the rivers are turbid and suffer from eutrophication and leaching of suspended solids from diffuse sources, especially from agriculture (Afshin 1994). Considerable efforts have been made to analyze chemical pollution in several rivers in Iran over the past two decades: relatively less attention has been paid to include aquatic organisms for purposes of environmental biomonitoring, and only a few studies on diversity and abundance of benthic diatoms and macroinvertebrates inhabiting contaminated rivers are available (Varnosfaderany et al. 2010; Sharifinia et al. 2012b). To fill this gap, this comprehensive research was designed to investigate the effect of the different pollution inputs on the benthic diatom and macroinvertebrate assemblages. We selected the Shahrood River, a unique ecosystem with several tributaries differing in water quality characteristics. This is the first study to exclusively examine the benthic flora and fauna of the river in the region. Hence, the aims of this study were (1) to identify the main patterns of species-environment relationships and important environmental variables among two different assemblages (diatom and macroinvertebrate) using direct ordination, (2) to investigate the suitability of benthic diatom and macroinvertebrate assemblages to validate the applicability of diatom- and macroinvertebrate-based indices for water quality assessment in the Shahrood River, and (3) to examine the spatial distribution patterns of benthic diatom and macroinvertebrate assemblages using multivariate analyses.

Methods

Study area

Shahrood River is the only river of the Alborz basin in northwestern Iran, which drains into the Caspian Sea. The geographic location of the area lies between 36°49′17″–44°75′99″N latitude and 40°33′22″–40°55′52″E longitude. The region is drained by the rivers Taleghan rud and Almut rud. The Shahrood River has a catchment area of 5000 km^2 with a length of 175 km and drains a region of cold climate, with an annual mean temperature range between 2 and 18 °C. The substrate of the river is mostly composed of cobbles with low proportions of gravel and sand, and a boulder substrate is found in streams and uplands. The headwater of the river is characterized by low-population villages, and the corridor of river is surrounded by extensively developed urbanization and industry. Water quality in these areas is highly affected by industries and agricultural pesticides and herbicides.

Selection of reference stations

The selection of appropriate reference stations is a critical step in developing and applying biological indicators of ecological condition (Whittier et al. 2007; Feio et al. 2014; Huang et al. 2015). The sampling stations (R1, R2 and S1–S7) were distributed along 96 km of the river reach. The initial field recognition and choice of reference condition took place before the fundamental field survey. The reference stations were selected following the criteria provided by the EU Water Framework Directive (WFD) (Andersen et al. 2004). Additional factors which were considered in reference site selection included: the geology, the catchment area with respect to its physical, chemical and biological attributes, the pristine nature of the headwater streams as determined by physical and chemical analyses and the absence of any obvious sources of pollution, alien species as well as little or no commercial forestry operations (Baattrup-Pedersen et al. 2013; Lewin et al. 2013). In the present study three regions (upstream, middle and downstream) were investigated. Reference stations (R1 and R2) were located in wilderness and deep forests, within a distance of several km from the nearest hiking trails, marked tracks and paths, and above shelters and huts. The reference stations (R1 and R2) were located in mountainous areas with rocky beds, high flow rate and minimal anthropogenic activities. Sampling stations S3 and S4 were located at the headwater of Shahrood River, with a trout farm existing between R2 and S3. Sampling stations S1, S2 and S5 were influenced by agriculture effluents and

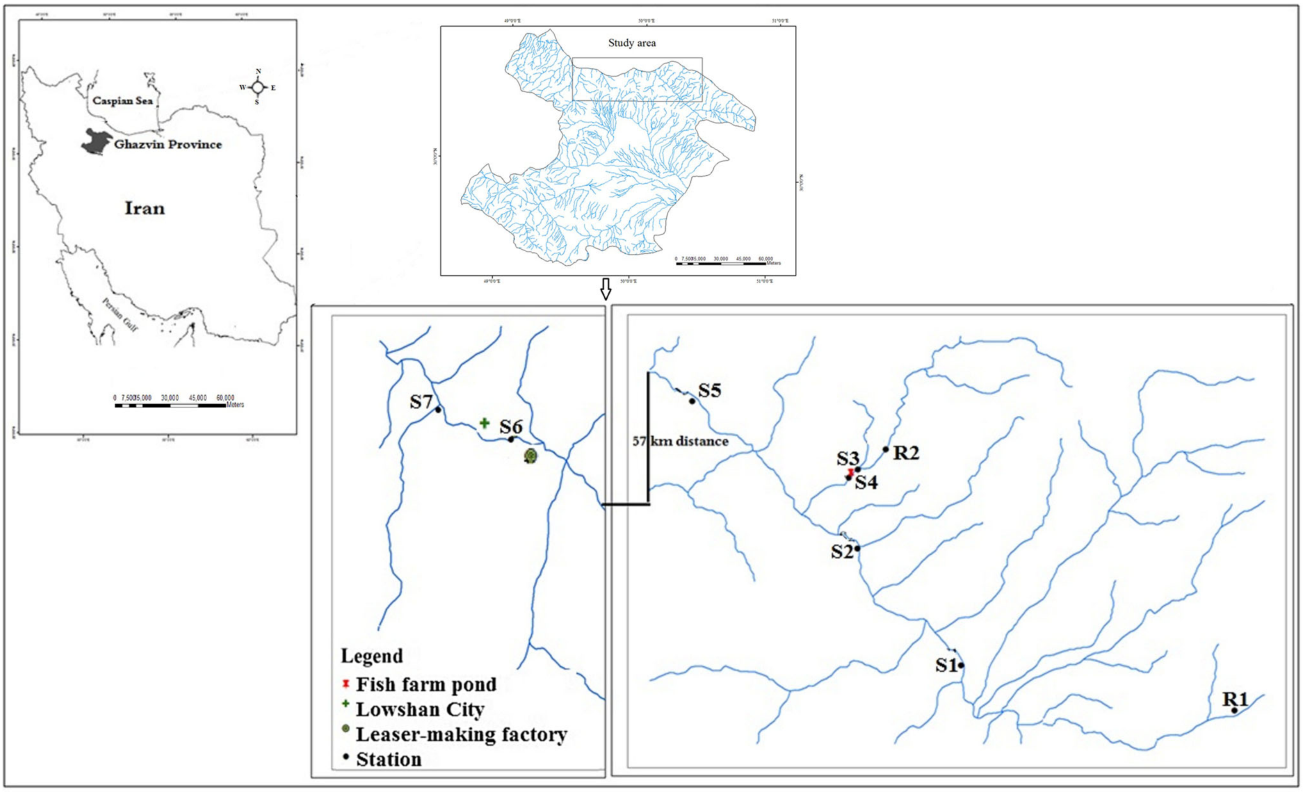

Fig. 1 Distribution of sampling stations along the Shahrood River

S6 and S7 were located downstream of a leather-making factory and Laoshan city, respectively (Fig. 1).

Sampling

Physicochemical measurements

Water physicochemical parameters were measured at the same time as diatom and macroinvertebrate sampling. A Multiline P4 (model WTW; Germany) and a TSS meter (model TSS 740 PARTECH; England) were used to measure dissolved oxygen (DO, mg l^{-1}) and total suspended solids (TSS, mg l^{-1}), respectively. The concentrations of silicate (SiO_2, mg l^{-1}), nitrate (NO_3^-, mg l^{-1}), orthophosphate (PO_4^{3-}, mg l^{-1}), Cadmium (Cd, mg l^{-1}), Fe and Zinc (Zn, mg l^{-1}) in the water were measured using an atomic absorption spectrophotometer (model KSL-AA320 N; China).

Diatom sampling

Qualitative sampling was performed once a month for a year (February 2012 to February 2013) for each station. Five cobbles (5- to15-cm sized stones) from the substratum were randomly collected at each sampling station. Cobbles were collected from the river bank if the cross section at the stations were too deep to sample. Diatoms were sampled by scrubbing the surface of stones, and the suspension was placed in a small plastic bottle (Kelly et al. 1998). Diatom samples were preserved with ethanol and cleaned from organic material in the laboratory using wet combustion with acid (HNO_3:H_2SO_4; 2:1) and mounted in Dirax or Naphrax. Three replicate slides of each sample were prepared. We identified 300–500 diatom frustules per sample (Moore 1974; Hendricks et al. 2006) and counted using phase contrast light microscopy (OLYMPUS DP12: magnification 1000×). The diatoms were identified to the lowest possible taxonomy level using Krammer and Lange-Bertalot (1986).

In the present study, trophic diatom index (TDI) was calculated for biological assessment of sampling stations (Kelly and Whitton 1995; Kelly et al. 1998; Atazadeh et al. 2007; Gudmundsdottir et al. 2013). TDI has a range of values between 1 and 100, with high values being associated with high trophic status and low water quality. We used a table of TDI categories with their corresponding water quality classes and ecological status described by Szczepocka and Szulc (2009) to assess water quality and trophic status of our sampling stations.

This index was calculated as follows (Kelly 2001):

$$TDI = (WMS \times 25) - 25,$$

where TDI = trophic diatom index, and WMS = weighted mean sensitivity, calculated as:

$$WMS = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j},$$

where a_j = abundance (proportion) of species j in sample, s_j = pollution sensitivity (l–5) of species j, and v_j = indicator value (l–3).

Macroinvertebrate sampling

Macroinvertebrate quantitative sampling was carried out based on the rapid bioassessment protocols that are used for rivers and streams (Barbour et al. 1999). A Surber sampler (40 × 40 cm aperture, 100 µm mesh size) was used for macroinvertebrate sampling (Elliott and Tullet 1978; Sharifinia et al. 2012a). Three replicate samples from each station were taken from different microhabitats in the studied riffles (length 10–50 m) to get a representative sample of the species pool at each station. Each of the three replicates was taken by positioning the hand net vertically and disturbing the pond bed by either gently rotating the heel of a boot or sweeping the substrate to dislodge the substratum and the fauna within a depth of at least 5–10 cm. Each sample was analyzed separately. The samples were preserved in 4 % buffered formaldehyde for further identification in the laboratory (Leunda et al. 2009). Macroinvertebrates were identified under a stereomicroscope with taxonomic keys (Hynes 1984; Elliott et al. 1988; Milligan 1997; Timm 1999; Pescador et al. 2004) to the family and genus level whenever possible.

The Hilsenhoff family-level biotic index (FBI) was calculated by multiplying the number of individuals of each family by an assigned tolerance value for that family. Assigned tolerance values range from 0 to 10 for families and increase as water quality decreases (Hilsenhoff 1988). This index was calculated as follows:

$$FBI = \sum [(TV_i)(n_i)]/N,$$

where TV_i is the tolerance value for family i, n_i is the number of individuals in family i and N is the total number of individuals in the sample collection. High FBI community values are an indication of organic pollution, whereas low values indicate good water quality.

Statistical analyses

Principal component analysis (PCA) was used to ordinate physicochemical data among the sampling stations during the 1-year sampling period. Prior to PCA, physicochemical variables were log transformed and normalized. Canonical correspondence analysis (CCA) was used to elucidate the relationship between the macroinvertebrate assemblage and the measured physicochemical parameters (TSS, DO, SiO_2, NO_3^-, PO_4^{3-}, Cd, Fe and Zn) in order to determine important parameters/variables responsible for the observed spatial distribution of macro-invertebrate taxa (Ter Braak 1986). PCA and CCA ordination were performed using program PC-ORD version 5 (McCune and Mefford 1999). Univariate measures for benthic diversity estimation included Shannon-Weiner diversity and Pielou's diversity evenness indices (Pielou 1966; Washington 1984; Shannon and Weaver 2002).

Non-parametric multivariate techniques (NMDS, ANOSIM and SIMPER) were used to compare the composition of species of each study area. A one-way analysis of similarity (ANOSIM) test was used to test whether composition and abundance of macroinvertebrates and diatoms differed among the sampling stations. When significant differences were found, a similarity percentage (SIMPER) analysis was used to identify the taxa responsible for the observed dissimilarity between the stations. Non-metric multidimensional scaling (NMDS) was used to provide a graphical summary of the relationships in ANOSIM and SIMPER (Clarke and Gorley 2001; Clarke and Warwick 2001). NMDS, ANOSIM and SIMPER analyses were performed using PRIMER version 5 (Primer-E) (Clarke and Gorley 2001). Non-parametric Kruskal–Wallis analysis by ranks was used to evaluate differences in benthic diatoms and macroinvertebrate indices among the sampling stations using SPSS version 19.

Results

Spatial variation of physicochemical parameters

The physicochemical data obtained at each sampling station are shown in Table 1. The dissolved oxygen (DO; mean ± SD) ranged from 8.50 ± 1.50 to 17.65 ± 3.60 mg l^{-1}. The mean total suspended solids (TSS; mean ± SD) was 80.64 ± 2.77 mg l^{-1} with the highest value being recorded at S7 (downstream station) and the lowest at R1 and R2 (reference stations), respectively. The concentration of cadmium (Cd) did not differ among the stations throughout the year, ranging from 1.08 to 1.37 mg l^{-1}. The nitrate concentrations (NO_3^-) ranged from 2.37 ± 0.48 to 2.95 ± 0.39 mg l^{-1} (Table 1).

PCA analysis explained 88 % of the variation in the first two principal components. The first PCA axis (PC1) explained most of the variation in the hydrochemical data

Table 1 The characteristics of physicochemical parameters (mean ± SD) in the Shahrood River for the period 2012–2013

Sampling stations	SiO_2 (mg l^{-1})	$NO3^-$ (mg l^{-1})	PO_4^{3-} (mg l^{-1})	TSS (mg l^{-1})	DO (mg l^{-1})	Cd (mg l^{-1})	Fe (mg l^{-1})	Zn (mg l^{-1})
R1	5.51 ± 0.63	2.58 ± 0.24	0.001 ± 0.008	37.38 ± 5.63	17.65 ± 3.6	1.09 ± 0.07	112.12 ± 32.81	1.70 ± 1.03
R2	5.29 ± 0.59	2.41 ± 0.22	0.007 ± 0.005	29.73 ± 3.44	17.3 ± 1.88	1.08 ± 0.07	12.13 ± 14.89	0.93 ± 0.04
S1	7.81 ± 0.95	2.5 ± 0.47	0.012 ± 0.009	70.05 ± 6.84	11.02 ± 3.63	1.4 ± 0.3	29.13 ± 19.85	6.40 ± 4.2
S2	7.53 ± 0.84	2.37 ± 0.48	0.017 ± 0.009	70.78 ± 4.65	11.75 ± 2.85	1.09 ± 0.02	128.13 ± 22.1	5.26 ± 3.2
S3	5.19 ± 0.77	2.54 ± 0.15	0.013 ± 0.001	31.65 ± 6.32	16.15 ± 2.22	1.08 ± 0.08	13.13 ± 0.15	0.93 ± 0.3
S4	5.15 ± 0.61	2.69 ± 0.36	0.011 ± 0.008	29.84 ± 4.67	16 ± 0.59	1.08 ± 0.07	12.13 ± 14.22	0.94 ± 0.2
S5	9.41 ± 1.18	2.67 ± 0.27	0.024 ± 0.008	75.32 ± 2.83	10.12 ± 2.34	1.23 ± 0.14	28.43 ± 18.68	6.38 ± 2.11
S6	8.80 ± 0.72	2.79 ± 0.32	0.023 ± 0.009	70.73 ± 6.15	10.32 ± 2.16	1.14 ± 0.12	102.17 ± 25.13	5.34 ± 2.17
S7	9.68 ± 1.07	2.95 ± 0.39	0.025 ± 0.019	80.64 ± 2.77	8.5 ± 1.5	1.37 ± 0.2	62.7 ± 23.65	6.61 ± 2.98

Sampling stations: R1 and R2 (references stations), S3 and S4 (upstream stations), S1, S2 and S5 (middle stations), S6 and S7 (downstream stations)

DO dissolved oxygen, cadmium, *NO_3^-* nitrate, *PO_4^{3-}* orthophosphate, *TSS* total suspended solids, *SiO_2* silicate, *Zn* zinc

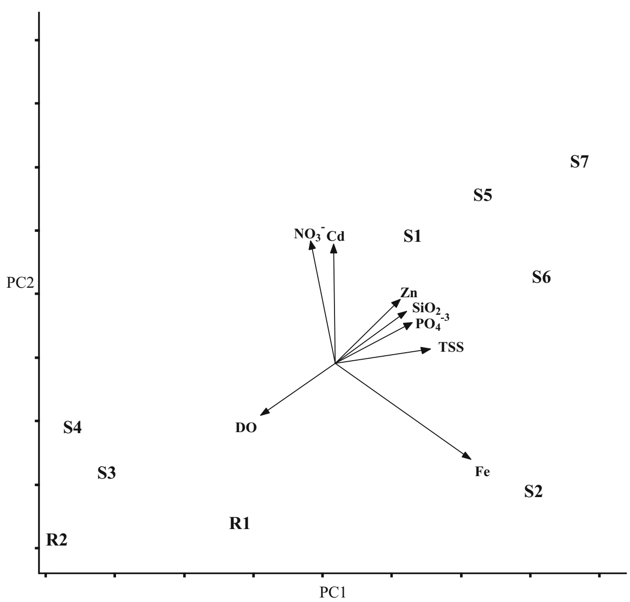

Fig. 2 Principal components analysis results (71.29 and 15.26 % of variances explained by axis 1 and 2, respectively). Dissolved oxygen (DO), cadmium (Cd), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), total suspended solids (TSS), silicate (SiO_2) and zinc (Zn). Reference stations (R1, R2), headstream stations (S3, S4), middle stations (S1, S2, S5) and downstream stations (S6, S7)

(72 %) and was represented mainly by positive loadings of water TSS, PO_4^{3-}, SiO_2 and Zn and negative loading of dissolved oxygen (DO). The second component (PC2) showed strong positive correlation with NO_3^- and cadmium (Cd) and negative correlation with Fe. The presence of dissolved oxygen allowed separating reference (R1 and R2) and tributary stations (S3 and S4). The high concentrations of nutrients, heavy metals and TSS clustered at the middle and downstream stations (Fig. 2).

Characteristics of diatom and macroinvertebrate assemblages

A total of 35 diatom species were identified (Table 2). The common species identified as *Diatoma vulgaris* (33.56 %), *Cocconeis pediculus* (15.10 %) and *Nitzchia* sp. (8.62 %). A total of 34 macroinvertebrate taxa were identified belonging to 9 orders (Table 2). The most frequently found taxa in the study river were Hydropsyche (29.67 %) and Simulium (9.32 %). The Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa comprised 73.37 % of the total individuals recorded in the samples.

Trophic diatom index

The trophic diatom index (TDI) ranged from 32.36 ± 2.45 to 60.24 ± 1.67, with the lowest and highest values at R1 and S6 stations, respectively. Average and standard deviation for the TDI values for the nine sampling stations along the Shahrood River are shown in Table 3. Downstream stations had higher TDI values. TDI values at reference stations were significantly different when compared to middle ($P = 0.03$) and downstream ($P = 0.01$) sampling stations. Also, there was a significant difference ($P < 0.05$) between middle and downstream sampling stations (Table 3).

Macroinvertebrate and diatom indices

A summary of the calculated macroinvertebrate-based and diatom-based indices of the sampling stations are presented in Table 4. Following the water quality classes suggested by Hilsenhoff (1988), our calculated FBI indices showed that the Shahrood River was comprised of three categories.

Table 2 Taxonomic composition of diatoms [taxon sensitivities (s*) and indicator values (v*) used for the TDI] and macroinvertebrates taxa (FTV: FBI tolerance value) during sampling period (2012–2013)

Diatoms taxa			Macroinvertebrate taxa			
Species (Code)	s*	v*	Order	Family	Genus	FTV
Gomphonema olivaceoides (Gom oli)	5	2	Tricladida	Planariidae	*Phagocata*	1
Achnanthes lutheri (Ach lut)	3	1	Tubificida	Naididae	–	8
Achnanthidium reimeri (Achn rei)	2	2	Prosobranchiata	Valvatidae	*Valvata*	8
Achnanthidium minutissimum (Achn min)	2	2	Amphipoda	Gammaridae	*Gammarus*	6
Cocconeis pediculus (Coc ped)	4	2	Ephemeroptera	Baetidae	*Baetis*	5
Cocconeis placentula (Coc pla)	3	2		Ecdyonuridae	*Ecdyonurus*	4
Cyclotella ocellata (Cyc oce)	0	0		Heptageniidae	*Cinygmula*	4
Cymatopleura solea (Cym ple)	4	1		Ephemerellidae	*Serratella*	2
Cymbella sp. (Cym sp.)	2	1		Siphlonuridae	*Ameletus*	0
Cymbella lanceolate (Cym lan)	2	1	Trichoptera	Hydropsychidae	*Hydropsyche*	4
Cymbella tumida (Cym tum)	1	3		Rhyacophilidae	*Rhyacophila*	0
Diatoma moniliformis (Dia mon)	2	1		Glossosomatidae	*Glossosoma*	1
Diatoma vulgare (Dia vul)	5	3		Polycentropodidae	*Polycentropus*	6
Diatoma vulgaris (Dia vulgatis)	2	1		Helicopsychidae	*Helicopsyche*	3
Didymosphenia geminate (Did gem)	2	3		Limnephilidae	*Dicosmoecus*	1
Encyonema minutum (Enc min)	3	2		Leptoceridae	*Nectopsyche*	3
Fragilaria sp.(Fra sp.)	2	1	Plecoptera	Perlidae	*Hesperoperla*	2
Fragilaria tenera (Fra ten)	2	1			*Acroneuria*	4
Gomphonema	3	1		Capanidae	*Allocapnia*	3
Gomphonema sp. (Gom sp.)	3	1	Diptera	Tipulidae	*Tipula*	4
Gomphonema sp1. (Gom sp1.)	3	1			*Prionocera*	5
Gomphonema olivaceum (Gom oli)	2	3			*Dicranota*	3
Gomphonema sp3. (Gom sp3.)	3	1		Tabanidae	*Tabanus*	5
Gyrosigma spencerii (Gyr spe)	5	2			*Chrysops*	8
Hannaea arcus (Han arc)	1	3		Simuliidae	*Simulium*	6
Meridion circulare (Mer cir)	2	3		Chironomidae	*Diamesinae*	2
Navicula capitatoradiata (Nav cap)	4	1			*Orthocladiinae*	5
Navicula radiosa (Nav rad)	4	1		Ceratopogonidae	*Bezzia*	6
Navicula tripunctata (Nav tri)	4	2		Psychodidae	*Pericoma*	10
Neidium dinoformis (Nei din)	4	1		Thaumaleidae	*Thaumalea*	9
Nitzchia sp.(Nit sp.)	4	1		Sciomyzidae	*Pteromicra*	10
Nitzchia sp1. (Nit sp1.)	3	1		Anthomyiidae	–	6
Surirella brebissonii (Sur bre)	3	1		Blephariceridae	*Bibiocephala*	0
Synedra ulna (Syn ulna)	3	1		Muscidae	–	6
Tryblionella apiculta (Try api)	4	1	Odonata	Gomphidae	*Ophiogomphus*	3
				Coenagrionidae		8
				Aeshnidae		3

Based on the results, stations (R1, R2, S3, and S4) were categorized as ''Very good'' and slight organic pollution; S2, S5, and S6 in ''Good'' water quality classes and S1 and S7 stations were categorized as ''Fair'' and fairly significant organic pollution. The highest Shannon-Wiener index was recorded for station R1, whereas station S7 had the lowest value. The FBI and Shannon-Wiener indices varied significantly among the sampling sites (Table 4). Although the diatom-based Pielou index was statistically different among the sites, no significant variations were recorded for the index calculated on macroinvertebrate communities (Table 4).

Table 3 Trophic diatom index (TDI) ranges (mean ± SD) in the Shahrood River

Sampling stations	TDI	Water quality	Water quality class	Ecological status	Trophic status
R1	32.36 ± 2.45d	Very good	I	High	Oligotrophic
R2	34.13 ± 1.65d	Very good	I	High	Oligotrophic
S1	40.73 ± 2.12c	Good	II	Good	Oligo/mesotrophic
S2	39.93 ± 3.31c	Good	II	Good	Oligo/mesotrophic
S3	43.35 ± 3.74c	Good	II	Good	Oligo/mesotrophic
S4	49.43 ± 2.14b	Good	II	Good	Oligo/mesotrophic
S5	49.34 ± 2.36b	Good	II	Good	Oligo/mesotrophic
S6	60.24 ± 1.67a	Unsatisfactory	IV	Mediocre	Eutrophic
S7	58.73 ± 3.13a	Satisfactory	III	Average	Mesotrophic

Different letters within the same column show significant differences ($P < 0.05$)

Table 4 Macroinvertebrate-based and diatom-based indices (mean ± SD) in the Shahrood River

Macroinvertebrate-based indices			
Sampling stations	FBI	Shannon-Wiener	Pielou
R1	3.54 ± 0.41d	1.96 ± 0.34a	0.83 ± 0.06
R2	3.70 ± 0.15d	1.94 ± 0.31a	0.87 ± 0.02
S1	5.34 ± 0.15ab	1.27 ± 0.22d	0.78 ± 0.04
S2	4.65 ± 0.11bc	1.40 ± 0.29c	0.79 ± 0.14
S3	4.07 ± 0.25cd	1.68 ± 0.27b	0.80 ± 0.17
S4	3.66 ± 0.36d	1.74 ± 0.15b	0.83 ± 0.03
S5	4.63 ± 0.34bc	1.44 ± 0.34c	0.84 ± 0.04
S6	5.24 ± 0.28ab	1.24 ± 0.24d	0.81 ± 0.11
S7	5.84 ± 0.44a	1.22 ± 0.10d	0.88 ± 0.06
Diatom-based indices			
R1	–	2.35 ± 0.11a	0.78 ± 0.03a
R2	–	2.33 ± 0.07a	0.83 ± 0.02a
S1	–	1.62 ± 0.11c	0.67 ± 0.04c
S2	–	1.78 ± 0.18bc	0.70 ± 0.05c
S3	–	2.20 ± 0.17a	0.79 ± 0.06ab
S4	–	2.25 ± 0.12a	0.82 ± 0.03a
S5	–	1.96 ± 0.21b	0.77 ± 0.05b
S6	–	1.96 ± 0.14b	0.76 ± 0.04b
S7	–	1.57 ± 0.27c	0.69 ± 0.10c

Different letters within the same column show significant differences ($P < 0.05$)

Relationships between benthic diatoms, macroinvertebrates and physicochemical parameters

Canonical correspondence analysis indicated that the eigenvalues of the first two axes were 0.29 and 0.06, respectively, accounting for 66.70 % of the total variance in the diatom composition. The species-environment correlations were high for both axis 1 ($r = 0.99$; $P < 0.001$) and axis 2 ($r = 0.98$; $P < 0.001$). Loadings on axis 1 and axis 2 were considerably larger than those on succeeding axes, and they principally expressed variation in DO concentrations, TSS, SiO_2, PO_4^{3-} and heavy metals. The assemblages of benthic diatoms were mainly affected by DO concentrations, TSS, SiO_2, PO_4^{3-} and heavy metals such as Zn, Cd and Fe (Fig. 3). The DO concentration contributed positively to the distribution of *Achnanthidium* spp. (*A. reimeri* and *A. minutissimum*), *Cocconeis* spp. (*C. pediculus* and *C. placentula*), *Navicula* spp. (*N. capitatoradiata*, *N. radiosa* and *N. tripunctata*) and *Gomphonema* spp. Comparatively, *Diatoma* spp. (*D. moniliformis* and *D. vulgaris*), *Neidium dinoformis* and *Gyrosigma spencerii* were explained mainly by TSS, SiO_2, PO_4^{3-}, and heavy metals concentrations (Fig. 3).

The environmental variables with significant influence on the macroinvertebrate assemblage distribution are depicted as arrows in the CCA ordination plot (Fig. 4). The eigenvalues for CCA axes 1 and 2 were 0.33 and 0.22,

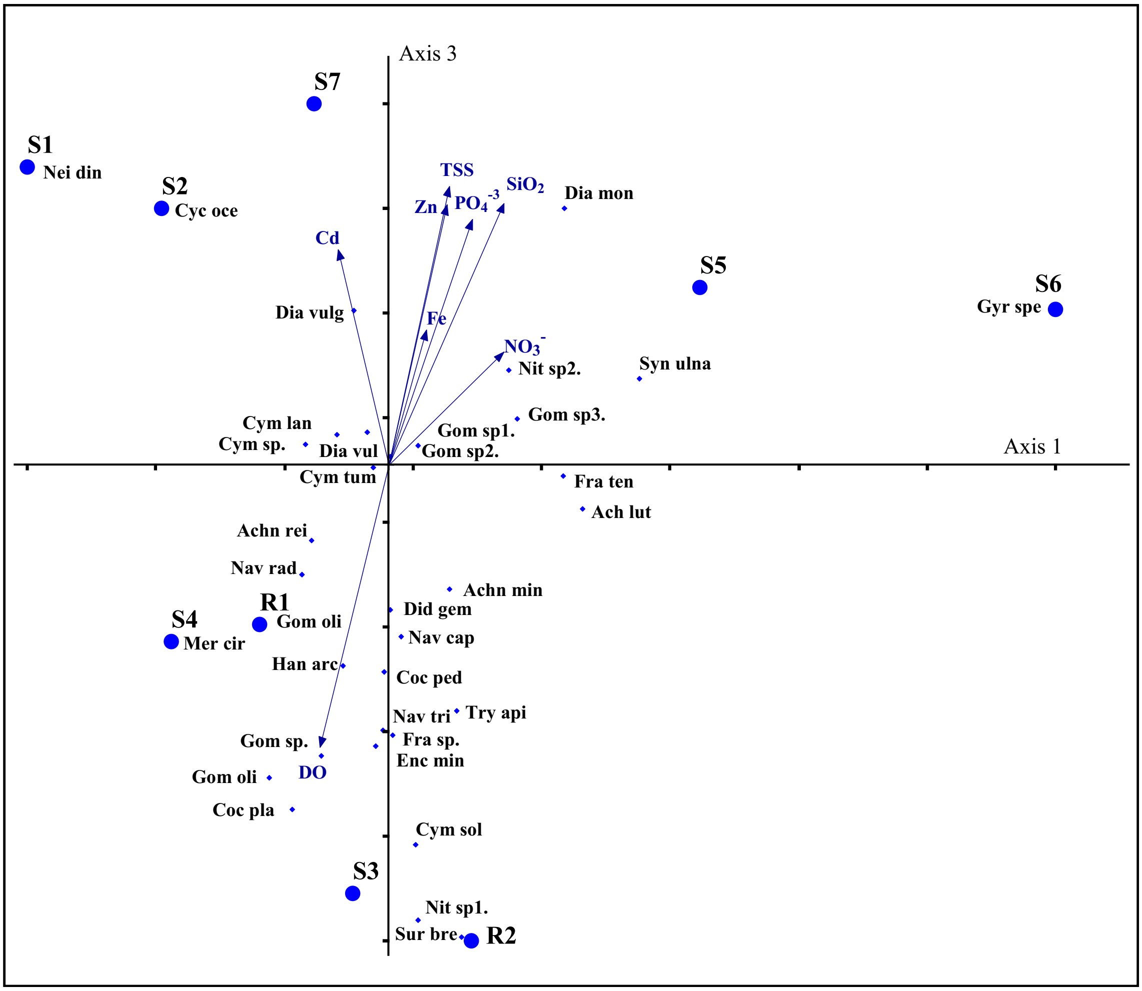

Fig. 3 Canonical correspondence analysis (CCA) showing the relationship between the 8 environmental variables selected with a Monte Carlo permutation test, and the benthic diatom assemblages in the Shahrood River. Dissolved oxygen (DO), cadmium (Cd), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), total suspended solids (TSS), silicate (SiO_2) and zinc (Zn). Reference stations (R1, R2), headstream stations (S3, S4), middle stations (S1, S2, S5) and downstream stations (S6, S7). *Vectors* represent the strength and direction of relationships between diatom assemblage and water quality parameters

respectively, thus capturing 50.80 % of the total variance. In Fig. 4 there is a constant shift to the right, in positive correlation with the increasing values of the chemical parameters, of many species that prefer or tolerate more or less elevated values of mineral nitrogen (NO_3^-), orthophosphate (PO_4^{3-}), SiO_2, TSS and heavy metals (Zn and Cd), such as Simulium, Naididae, and Orthocladiinae. On the left side, there are taxa that occupy environments with high concentrations of dissolved oxygen (DO) such as *Allocaphia*, *Glossosoma*, *Dicosmoecus*, and *Baetis* (Fig. 4).

Assemblage patterns

The two-dimensional NMDS configuration, based on species abundances, clearly differentiated differences among reference, influence and impact stations, especially between reference and impact stations, with the points for the middle stations showing limited overlap with those for the upstream and downstream stations (Fig. 5). ANOSIM revealed significant differences between the groups (Diatom assemblage: Global $R = 0.26$, $P = 0.01$). The effects of human activities were reflected in the Shahrood River macroinvertebrate composition and ANOSIM detected significant differences (Global $R = 0.32$, $P = 0.01$) among impact, influence and reference stations, especially between impact stations S6 and S7, and reference stations R1, R2, S3, and S4 (Fig. 5).

Bray–Curtis similarity (SIMPER) followed by an ordination through NMDS categorized the stations into three groups (upstream stations: R1, R2, S3 and S4; middle stations: S1, S2 and S5; downstream stations: S6 and S7). The analysis (SIMPER) showed three benthic diatom zones in the Shahrood River with *Nitzchia* sp. (22.68 %), *Diatoma vulgaris* (12.98 %) and *Cocconeis pediculus*

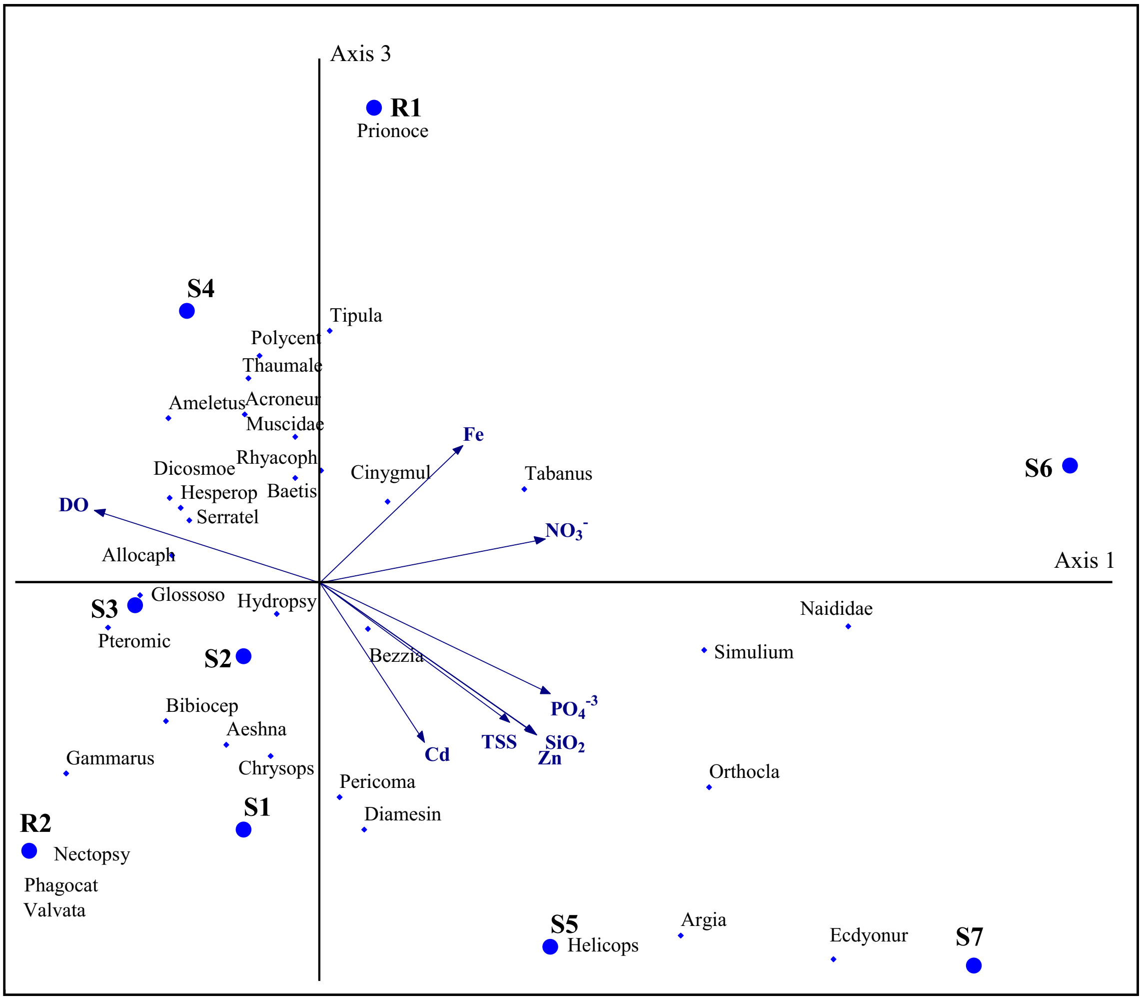

Fig. 4 Canonical correspondence analysis (CCA) showing the relationship between the 8 environmental variables selected with a Monte Carlo permutation test and the macroinvertebrate assemblages in the Shahrood River. *Vectors* represent the strength and direction of relationships. See the legend of Fig. 3 for the abbreviations

(12.50 %) dominating at upstream stations, *Nitzchia* sp. (34.64 %), *Cymbella* sp. (15.90 %) and *Diatoma vulgaris* (14.41 %) frequently occurring at middle stations, and downstream stations being characterized by *Diatoma vulgaris* (27.15 %), *Cymbella lanceolate* (26.82 %) and *Nitzchia* sp. (13.49 %) (Fig. 5). Further analysis revealed that macroinvertebrate compositions of downstream sites were significantly different from those of compositions at middle and upstream stations as confirmed by ANOSIM (Macroinvertebrate: $R = 0.32$, $P = 0.01$). Throughout the sampling period, upstream stations were dominated (70 %) by *Hydropsyche* (51 %), *Allocaphia* (10 %), and *Cinygmula* (9 %), whereas at middle stations *Hydropsyche* (48 %), *Rhyacophila* (25 %), and *Baetis* (14 %) dominated (85 %). Lastly, 80 % of individuals at the polluted downstream stations were comprised of *Simulium* (48 %) and *Cinygmula* (31 %).

Discussion

Selection of reference stations

In our study, we acknowledge that identifying truly undisturbed reference stations was not practical because of well-established human activities and disturbance to the ecosystems in the region. Therefore, we defined our reference stations as those with the least disturbed conditions (Sánchez-Montoya et al. 2009; Kosnicki et al. 2014). Our preliminary investigations showed that the reference stations (R1 and R2) tended to have low concentrations of nutrients and low levels of disturbance (Tables 1, 3). However, the biotic indices based on two assemblages constructed for the Shahrood River successfully reflected the structure and characteristics of regional benthic diatoms and macroinvertebrates, and

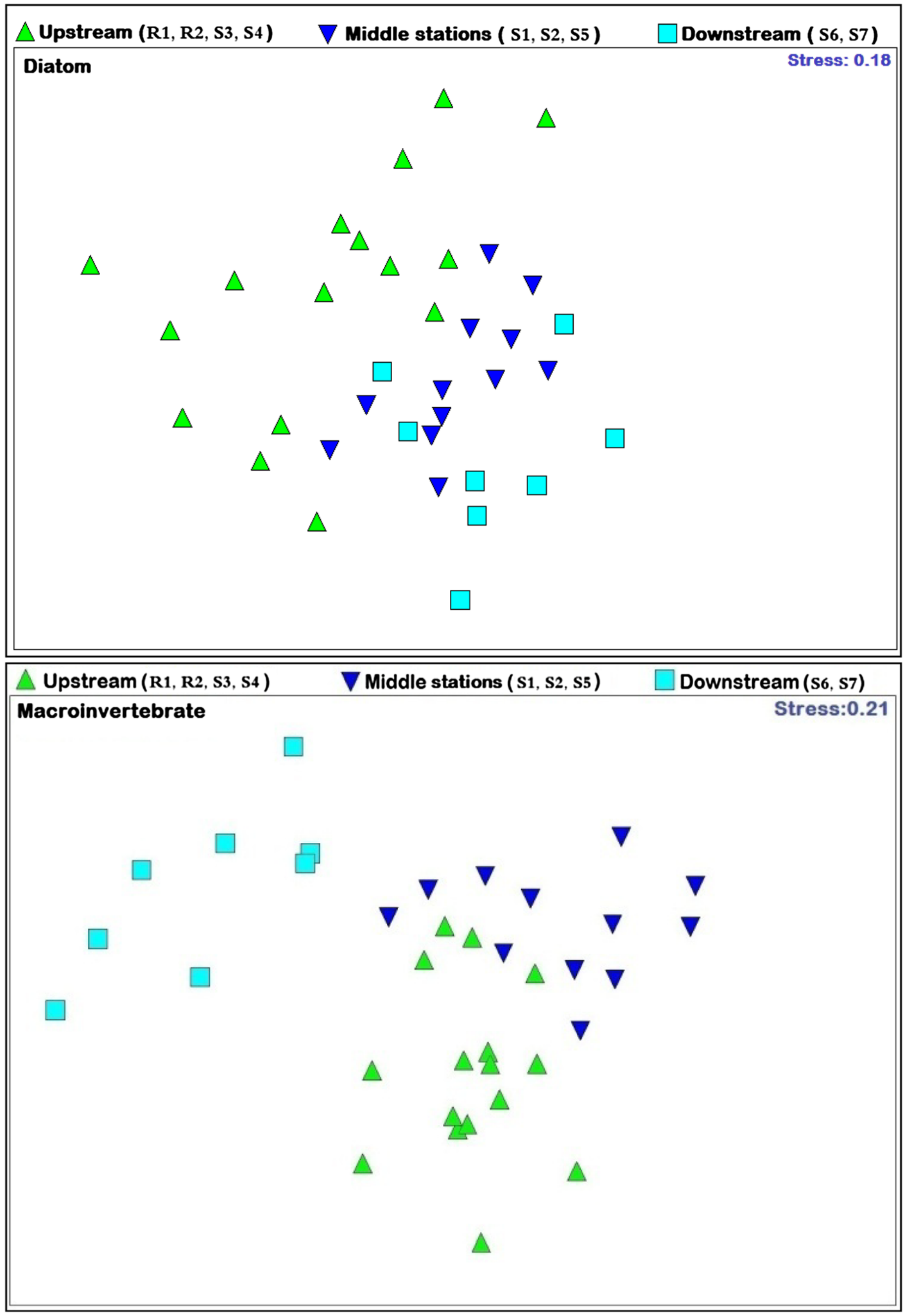

Fig. 5 Ordination station scores in two of the dimensions generated by non-metric multidimensional scaling (NMDS) using the Bray Curtis similarity metric of diatom and macroinvertebrate species relative abundances. Stations are coded by community groups and sampling period groups. Upstream and downstream stations are significantly different in ANOSIM and are designated as: upstream (R1, R2, S3, S4); middle (S1, S2, S5); downstream (S6, S7)

were sufficient, robust and sensitive in their ability to discern reference stations from impacted stations.

Spatial variation of physicochemical parameters

The results of the physicochemical analyses showed fluctuations among the sampling stations during the period of study (February 2012 to February 2013). Dissolved oxygen, TSS, zinc (Zn), orthophosphate (PO_4^{3-}) and SiO_2 were the most important parameters in distinguishing impacted (middle and downstream stations) from reference or least impacted stations as shown by the principal components technique. There was not a clear distinction among the reference and tributary stations with middle and downstream stations, indicating different water physicochemical compositions.

Dissolved oxygen showed visible spatial variations in the Shahrood River. The lowest concentrations of DO were found at middle and downstream stations, which receive untreated domestic and industrial wastewaters and agricultural effluents. Dissolved oxygen is probably the most important parameter in natural surface water systems for determining the health of aquatic ecosystems (Yang et al. 2007). The high TSS values at middle and downstream stations clearly demonstrate the significance of the agricultural effluents and urban loads to the Shahrood River while passing through the city of Laoshan. High values of total dissolved solids might have resulted from the effluent's higher concentration of soluble salts and other components (Shakir et al. 2013).

The highest concentrations of NO_3^- were found at stations S6 and S7 because of wastewater discharges and agricultural runoff. Nitrate concentrations at all stations were below the maximum permissible level of 10 mg l^{-1} for drinking water (USEPA 2009). Like other nutrients, station S7 showed the highest values for PO_4^{3-} because of wastewater discharges and agricultural activities. In the present study, mean PO_4^{3-} concentrations at all stations were found below 0.075 mg l^{-1}; the threshold above which eutrophication likely occurs (Dodds et al. 1998). Phosphorus is frequently the limiting nutrient for plant growth in freshwater systems and plays a key role in eutrophication. The increase in phosphorus concentrations in running waters leads to eutrophication and depletion of DO concentrations (Kannel et al. 2007).

Concentration of dissolved Cd and Zn were low at most stations. The frequency of metals detected in the samples was Zn > Cd at different stations. The results agreed with the Cheung et al. (2003) that Zn was the most abundant in the river water, followed by Cd. The metal concentrations of all water samples were mostly below or close to the maximum permitted concentration for protection of aquatic life and drinking water (Cheung et al. 2003; USEPA 2009). In general, the concentrations of Zn and Cd in the Shahrood River at downstream stations were higher than those at the other stations. This may be attributed to the nearby municipal or industrial activities (Cheung et al. 2003).

Biotic indices

In this study, four biological metrics based on diatoms or macroinvertebrates (TDI, FBI, Shannon-Weiner and Pielou) were tested for ecological assessment of the Shahrood River. Most of them are widely recognized as being sensitive to a range of anthropogenic stressors and they are used in the bioassessment of rivers and streams (Atazadeh et al. 2007; Sharifinia et al. 2012b; Clews et al. 2014; Ma et al. 2014; Richardson et al. 2014).

Based on the trophic diatom index (TDI), water quality in reference stations (R1 and R2) was oligotrophic; in the sampling stations in tributary (S3 and S4) and middle stations in the main stream (S1, S2 and S5) it was oligo/mesotrophic; and in the downstream stations (S6 and S7) was eutrophic or mesotrophic. The significant difference between water qualities in the three sections of the Shahrood River could result from the following: (1) nutrients from agricultural pollution in the middle stations, and (2) large cities with dense human activities are generally located before the downstream stations. The trophic diatom index showed that the Shahrood River can be classified as water quality class I-IV. The TDI classified the river water quality in the oligotrophic to eutrophic zone, and stations were mostly in oligo/mesotrophic status (Table 3). The trophic diatom index indicated differences among reference stations (R1 and R2) with middle (S1, S2 and S3) and impacted stations (S6 and S7). Hence, the trophic diatom index (TDI) seems a suitable diatom-based index for the assessment of the quality of rivers and streams (Atazadeh et al. 2007; Kelly 2013; Szczepocka et al. 2014). Our results further support the claim that TDI would be a suitable biological index in assessing quality of rivers in Iran (Köster and Huebener 2001; Jüttner et al. 2003; Atazadeh et al. 2007; Kelly 2013; Szczepocka et al. 2014) as the stations in the current study varied significantly in TDI values. High TDI values at middle and downstream stations confirmed that rivers in Ghazvin province and its surroundings were negatively impacted by changes in land use to intensive agriculture, residential and industrial areas. It is worth noting that Köster and Huebener (2001) and Jüttner et al. (2003) found that some indices could not accurately reflect water quality because the response of particular taxa to water chemistry might vary between geographic region or taxa, indicating different ecological conditions are being combined under a single name. Nevertheless, the use of the TDI index offers a good compromise between the exact estimation of trophic parameters and the need to simplify the processing (especially taxonomic) effort.

The Hilsenhoff family-level biotic index calculated for macroinvertebrates (FBI) showed that the lowest class III was obtained for station S1 and downstream stations (S6 and S7), and the highest class I was recorded for reference stations (R1 and R2). The FBI values did differ significantly among the reference and tributary stations with downstream stations ($P < 0.05$). Based on our results and other studies, the FBI index seems suitable for the assessment of the quality of rivers in Iran (Lydy et al. 2000; Shokri et al. 2014). The water at the upstream stations of the Shahrood River was reasonably clean, and a suitable habitat for many sensitive macroinvertebrates such as stoneflies and caddisflies (Cole 2002; Storey and Quinn 2011).

FBI, TDI and diversity indices reflected changes in water quality of the Shahrood River due to the anthropogenic impacts and land use changes in the river catchment. Although biotic indices based on diatom and macroinvertebrate assemblages accurately reflected variation in the environmental condition of our study river and elsewhere (Frankovich et al. 2006; Hering et al. 2006; Yoshimura et al. 2006; Gudmundsdottir et al. 2013), more research is needed to determine the true optima and tolerance levels of diatoms and macroinvertebrates in least studied tropical waters such the Shahrood River. Such information will be vital for understanding the consequences of human population growth and urbanization on lotic systems (e.g. eutrophication and saprobication) and how it can be mitigated, particularly in less developed countries where environmental monitoring is seldom done. We recommend that an appropriate management and restoration policy is needed for the Shahrood River to improve its ecological integrity.

Bio-indicators in relation to physicochemical parameters

Benthic diatom and macroinvertebrate composition has been reported to be determined by the interaction of proximate determinants, such as nutrients, with intermediate and ultimate factors (i.e., land use) (Biggs 1995; Ponader et al. 2007; Sharifinia et al. 2012b; Tan et al. 2014a). Major nutrient concentration (i.e., $PO_4^{3-}$and NO_3^-) in an ambient aquatic environment was the primary factor explaining variation in benthic assemblages (Ponader et al. 2007). In this study, CCA showed that proximate determinants such as DO, PO_4^{3-}, NO_3^-, TSS and major ions were vital in explaining the variation in the diatom and macroinvertebrate assemblages. These findings, as well as previous work (Potapova et al. 2004; Pan et al. 2013; Delgado and Pardo 2014; Kilonzo et al. 2014), confirm that diatom and macroinvertebrate distribution along a watercourse, or in different water bodies, is strongly dependent on the environmental parameters. In general, benthic diatom and macroinvertebrate assemblages at upstream stations (reference and tributary) were strongly influenced by oxygen, and the assemblages at middle and downstream stations were driven by NO_3^-, TSS, SiO_2, PO_4^{3-} and heavy metals (Cd and Zn). Studies done elsewhere concurred with our predictions that water quality in agricultural areas is characterized by high NO_3^-, NO_2 and total suspended solids (Leland and Porter 2000), whilst rivers in residential and industrial areas have elevated levels of total dissolved solids, PO_4^{3-}, conductivity, total dissolved solids, alkalinity, COD and temperature (Lobo et al. 1995; Jüttner et al. 2003; Namin et al. 2013a). The same influential pattern of dissolved oxygen on diatom and macroinvertebrate assemblages at a local scale in the current study has been reported by others elsewhere (Potapova and Charles 2002; Connolly et al. 2004).

Analyses of CCA identified diatom taxa closely associated with the three site groups characterized by different environmental conditions and were comparable to other findings (Lobo et al. 1995; Leland and Porter 2000; De Fabricius et al. 2003; Ndiritu et al. 2006). Our results indicated that diatom species associated with the reference and tributary stations were different from those species at the impacted stations with higher concentrations of NO_3^-, TSS, SiO_2, PO_4^{3-}, and heavy metal concentrations, in agreement with the report by Lobo et al. (1995). Similarly, high SiO_2 concentrations were observed to be closely related to the suspended solids from agricultural fields and sewage effluents where concentrations of SiO_2 >4 mg l^{-1} would not be growth-limiting to benthic diatoms (see Leland and Porter 2000). Our findings were consistent with Leland and Porter (2000) that prostrate diatoms, notably *Nitzschia* spp. and *Gomphonema* spp. were abundant in rivers draining agricultural areas that had high phosphates and nitrate levels. Similarly, De Fabricius et al. (2003) found that *Navicula subminuscula* and *Nitzschia* spp. (*N. palea* and *N. umbonata*) were associated with high conductivity and higher nutrient concentrations (e.g. NO_3), whereas streams in urban areas were characterized by *Nitzschia palea*, and streams in catchments with low agricultural intensity by *Gomphonema parvulum* and *Achnanthes minutissima*. Van Dam et al. (1994) and Köster and Huebener (2001) reported that *Gomphonema parvulum* has wide ecological ranges and tolerates considerable organic pollution, whereas *A. minutissima*, *Nitzschia perminuta*, *N. palea* and *Navicula subminuscula* have been found to tolerate different pollution levels from different sources (e.g. agricultural, residential and industrial areas).

The sum of canonical eigenvalues for the two axes principally expressed variation in DO, TSS, SiO_2, PO_4^{3-} and heavy metals. In this study, high total variance explained <60 % (Økland 1999), indicating that the measured physicochemical variables (8 parameters) adequately satisfied the CCA unimodal model assumptions. In addition, the selected environmental variables likely fitted the CCA model, which had the ability to identify their interaction with diatom and macroinvertebrate assemblages in streams of the Shahrood River basin. However, the relationship between environmental factors and benthic biota is worthy of future research.

We found evidence that heavy metal concentration such as Zn and Cd had critical effects on most aspects of distribution and diversity of riverine diatom and macroinvertebrate assemblages. Many macroinvertebrate and diatom community characteristics were altered at the nine study stations with Zn and Cd concentrations, although the effects of other physicochemical parameters cannot be excluded. Similar conclusions have been drawn by Iwasaki et al. (2012). One of the limitations with this study is that our estimation was derived from a limited number of study stations in a restricted area. It is uncertain whether our findings can be applied to other areas that have different physicochemical characteristics and benthic assemblages (diatom and macroinvertebrate) as compared with our study stations. We also recommend future studies to include other potential factors such as shade ratio, flow, substrate size, riffle depth, temperature, and riparian vegetation that have been found to influence diatom and macroinvertebrate assemblage structure in riverine systems, but were not investigated in the current study.

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