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Distribution of benthic centric diatom *Pleurosira laevis* (Compère, 1982) in different substrate type and physical and chemical variables

Distribuição da diatomácea cêntrica bentônica *Pleurosira laevis* (Compère, 1982) em diferentes tipos de substrato e variáveis físicas e químicas

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Abstract: Aim: This contribution reports the first regional occurrence of *Pleurosira laevis* in the Masuleh River, Iran and additionally describes the pattern of occurrence along the Masuleh River and among four substrate types. The aim of this study was to assess the effects of substrate type and physical and chemical variables on distribution of centric diatom *P. laevis*. **Methods:** At each station, triplicate samples were collected from 4 substrata. Epilithic (assemblages on rock), epidendric (assemblages on wood), epipsammic (assemblages on sand), and epipelic (assemblages on mud) diatom and water quality sampling was done four times at 5 stations. Physical and chemical variables including total nitrate, total phosphate, silicate, Fe^{2+}, EC, and pH were also determined. Samples preserved in 2% for formalin solution and transferred to the laboratory, boiled with acid (HNO_3: H_2SO_4; 2:1), centrifuged, identified, and counted. Cluster analysis was performed to indicate the main differences and similarities in diatom abundance amongst substrates sampled and amongst sampling stations. **Results:** The highest (12.54 ± 1.54) and lowest (0.74 ± 0.10) abundance was obtained from wooden and muddy substrates at stations S5 and S4, respectively. The highest abundance was observed in the wooden substrate among all of sampling stations where showed significant differences ($P < 0.05$) with the other substrates. The hierarchical clustering based on relative abundance of *P. laevis* distinguished two clusters amongst the four substrate types. Rougher and more stable substrates (wood, sand, and stone) clearly separated from smooth and unstable substrates (mud). Based on relative abundance of *P. laevis* on rougher substrates, epidendric and epipsammic species had the highest similarity. Results of Pearson correlation showed that relative abundance of *P. laevis* had a significant correlation with EC, TN, TP, and Fe^{2+} concentrations ($P < 0.05$) whilst no significant correlation was observed with pH, temperature, and SiO_2 concentration ($P > 0.05$). **Conclusion:** We conclude that wood substrata can be substituted for one another during field surveys. Results from this study demonstrate that the type and roughness of the substrate both influence the attachment of *P. laevis* and its subsequent growth conditions.

Keywords: periphyton; ecological survey; cluster analyze; diatom distribution.

Resumo: Objetivo: Este trabalho relata a primeira ocorrência regional de *Pleurosira laevis* no rio Masuleh (Irã) e adicionalmente descreve o seu padrão de ocorrência ao longo do rio e em quatro tipos de substrato. O objetivo deste trabalho foi avaliar os efeitos do tipo de substrato e as variáveis físicas e químicas na distribuição da diatomácea cêntrica *P. laveis*. **Métodos:** Em cada estação, amostras em triplicara foram coletadas em quatro substratos. Diatomáceas epifíticas (assembléias sobre rochas), epidendricas (assembléias em troncos), episâmicas (assembléias em areia) e epipélicas (assembléias em lodo) e amostras da qualidade de água foram obtidas quatro vezes em cinco estações. Variáveis físicas e químicas incluindo nitrato total, fosfato total, silicatos, Fe^{2+}, CE, e pH também foram determinadas. As amostras foram preservadas em solução de formalina 2% e transferidas para o laboratório, fervidas em ácido (HNO_3: H_2SO_4; 2:1), centrifugadas, identificadas e contadas. Uma análise de Cluster foi aplicada para indicar as principais diferenças e similaridades na abundância de diatomáceas entre substratos e entre estações de amostragem. **Resultados:** A maior (12,54 ± 1,54) e a menor (0,74 ± 0,10) abundância foi obtida a partir de substratos de troncos e lodo nas estações S5 e S4, respectivamente. Entre todas as estações de amostragem a maior abundância foi observada no substrato de troncos, com diferenças significativas (P <0,05) em relação aos outros substratos. O agrupamento hierárquico baseado em abundância relativa de *P. laevis* distinguiu dois clusters entre os quatro tipos de substrato. Substratos mais duros e mais estáveis (tronco, areia e rocha) ficaram claramente separados dos substratos lisos e instáveis (lodo). Com base na abundância relativa de *P. laevis* os substratos mais duros, epidendricos e episâmicos tem a maior similaridade. Os resultados de correlação de Pearson mostraram uma correlação significativa entre a abundância relativa de *P. laevis* com CE, e as concentrações de TN, TP, Fe^{2+} (P <0,05), e não mostraram correlação significativa com pH, temperatura e concentração de SiO_2 (P> 0,05) . **Conclusão:** Nós concluímos que o substrato de tronco pode ser substituído por um outro durante o levantamento de campo. Os resultados deste estudo demonstram que o tipo e a rugosidade do substrato influencia a fixação de *P. laevis* e as suas condições de crescimento subsequentes.

Palavras-chave: perifíton; levantamento ecológico; análise de cluster; distribuição de diatomáceas.

1. Introduction

Extinction of native species, diversity reduction, and irreversible changes of habitat are the main consequences of invade exotic algae in new areas (Kaštovský et al., 2010). *Pleurosira laevis* (Compère, 1982) is typically a halophilic and rheophilic species but can survive in freshwater environments. In first occurred in the Lake Michigan, it was found in regions with higher chloride concentrations and associated with increased nitrate concentrations. It has been recorded from hard freshwater, oligohaline, and mesohaline environments (Whitford, 1956; Crayton & Sommerfeld, 1979; Wujek & Welling, 1981; Compère, 1984). Individual diatoms of this species exhibit centric to slightly elliptical valves and are cylindrical in side view. On each valve, one ocellus produces mucilage that allows it to connect to other cells, forming zigzag, filamentous chains that grow epiphytically, epilithically, or form large mats. On individual cells, an ocellus-like process intermediate between a thickened ocellus rim and a rimless pseudocellus is apparent (Pfiester & Terry, 1978; Kociolek et al., 1983; Compère, 1984; Ferreira et al., 1999).

Using benthic assemblages such as diatom and macroinvertebrates have been developed to assess the ecological status of streams and rivers (Sharifinia, 2015; Sharifinia et al., 2016a, b). Diatoms, a key component of stream periphyton, are often the most important photosynthetic unicellular eukaryotes and primary producers in streams (Minshall, 1978; Falkowski et al., 2004). These organisms occur both in plankton and benthos of most aquatic ecosystems (oceanic, coastal, and freshwater) and are considered as one of the most successful groups among unicellular algae. The spatial distribution of benthic diatoms is determined by a set of factors such as climate, geology, and land-use at the catchment scale, to the availability of light and nutrients at the substrate scale (Leland & Porter, 2000; Passy, 2007; Sharifinia, 2015). At the spatial scale of the sample station, substrate is another potential source of diatom assemblage heterogeneity (Townsend & Gell, 2005; Sharifinia et al., 2013). Species abundance of benthic diatom assemblages sampled at the same station but from different substrata (e.g. sand, wood, rock, sand, submerged or emergent macrophytes) often differ substantially because species are better adapted to one substrate than other substrates (Potapova & Charles, 2005; Townsend & Gell, 2005; Fisher & Dunbar, 2007; Sharifinia et al., 2013).

Knowledge of the mechanisms by which nutrients and substrata affect the centric diatom *P. laevis* distribution in the rivers and streams remains limited. The central question of this paper was whether on distribution of *P. laevis* was affected by different natural substrate types (rock, wood, sand, and mud). Also, the effect of nutrient concentrations on relative abundance of this centric diatom species in the Masuleh River was investigated. Another goal of this work is to document the first record of *P. laevis* in Iran, in the Masuleh River, Guilan Province.

2. Material and Methods

2.1. Study area

Masuleh River, with a catchment area of approximately 228 km^2, is located at the southwest of Guilan province, on the northern slopes of the West Alborz (Talesh Mountains). The geographic location of the area lies between 37°22′-37°23′N latitude and 49°16′-49°19′E longitude. Based on Emberger's Climatic Indices, the regional climate is humid and mild. The average monthly temperature recorded by stations within the basin suggests a maximum temperature of 18.8°C in April and a minimum temperature of 3.7°C in February. The average annual relative humidity is 85% which represents a very humid climate. Agricultural activities of the basin comprise rice and horticultural fields (Nezami, 2012). Five sampling stations (S1-S5) were selected along the Masuleh River (Figure 1). The five sampling stations were selected longitudinally on the basis of various forms of impacts and predominant human activities along the Shahrood River. Two sampling stations (S1 and S2) that were likely to be of a high ecological status and that were situated in the headstream of river. These stations placed in the mountainous area and characterized by steep slopes, high water flow, prevailing coarse substrate, riparian vegetation and minimal external stress influence. Sampling stations S3, S4 and S5 were impacted by agricultural wastewater. The stations were selected according to the following criteria: (a) ease of vehicle access, (b) the presence of riffle or river run habitat, (c) the presence of four substrata, and (d) a benthic sample depth of no more than 60 cm (Figure 1).

2.2. Benthic diatom sampling and laboratory procedures

Benthic diatoms were collected during dry seasons of 2011, in the summer and autumn, and more than 5 weeks after the last storm runoff event of the preceding rainy season that may have disturbed the assemblages. This period of undisturbed flow exceeds the 3 week delay before sample collection to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations, which affect diatom development, especially growth rate and relative abundance of different species (Round, 1991; Stevenson & Bahls, 1999).

At each station, epilithic, epidendric, epipelic, and epipsammic diatoms were sampled separately, avoiding mixing as much as possible. Epidendric and epilithic samples were first gently shaken under the water, to remove any loosely attached sediments

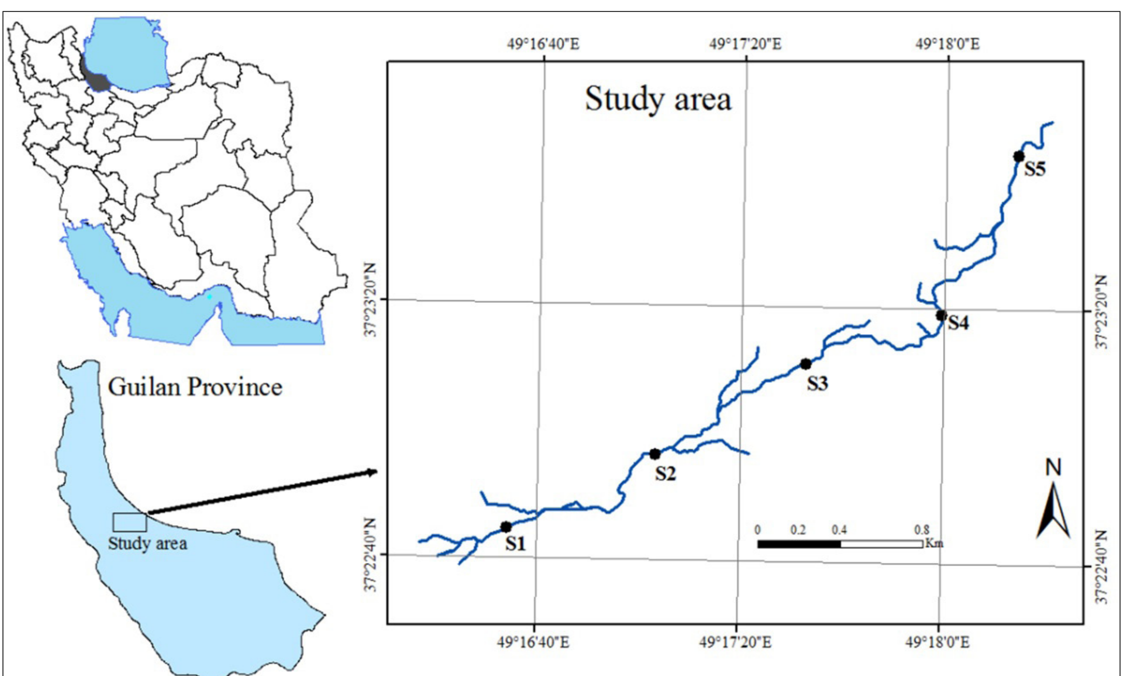

Figure 1. Location of the sampling stations along the Masuleh River.

and non-epilithic diatoms. Then, the diatom samples were sampled by brushing stones with a toothbrush or with a clean wooden spatula. At least five pebble-to-cobble sized stones were randomly collected along each sampling stretch and brushed, and the suspension was placed in a small labeled plastic bottle. Epipelic and epipsammic diatoms were sampled by pressing a Petri dish lid into the top layer of sand or silt/clay to a depth of 5-7 mm, followed by sliding a spatula blade under the Petri dish to isolate the contents in the dish that were then gently brought to the surfaces. The contents were then emptied into a labeled container. Samples from five locations in each sampling reach were pooled into a single sample (Townsend & Gell, 2005; Bere & Tundisi, 2011). 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 Naphrax. Three replicate slides of each sample were prepared. 300-500 diatom frustules per sample were identified (Moore, 1974; Hendricks et al., 2006) and counted using phase contrast light microscopy (OLYMPUS DP12: magnification 1000X using immersion oil). Species were identified using the standard taxonomic texts of Krammer and Lange-Bertalot (1991).

2.3. Systematics

Class: BACILLARIOPHYCEAE Haeckel, 1878
Subclass: COSCINODISCOPHYCIDAE Round & Crawford, 1990
Order: TRICERATIALES Round & Crawford, 1990
Family: TRICERATIACEAE Lemmermann, 1899
Genus: Pleurosira (Meneghini) Trevisan, 1848
Species: *Pleurosira laevis* (Compère, 1982)

2.4. Physical and chemical measurements

At each station, temperature and pH were measured with a multi-parameter instrument (370 pH Meter, JENWAY), and electrical conductivity (EC) by a 470 Cond. Meter, JENWAY. Water samples were analyzed in laboratory by PC MultiDirect photometer for the following parameters: total nitrate (TN), total phosphate (TP), silicate (SiO_2), and iron ion (Fe^{2+}).

To compare the relative abundance of *P. laevis* among the different substrata and different sampling dates, relative abundance data were analyzed by one-way ANOVA test using SPSS (SPSS Inc., Chicago, Illinois) software. Then, pairwise comparison between substrata was performed using Duncan test. One-way ANOVA ($\alpha = 0.05$) was applied to test significant differences among physical and chemical variables means at five sampling stations. According to the Pearson's correlation analysis, the general relationship between *P. laevis* relative abundance and physical and chemical variables could be identified. To determine relative abundance similarities of *P. laevis* between the samples, different substrates have been classified via a hierarchical cluster analysis using Ward's linkage method with Bray and Curtis distance measure.

3. Results

3.1. Morphological description of Iran population (Figure 2)

Valves shape: circular to elliptical
Valves face: slightly hemispherical
Valves size: 60-110 μm long and 35-67 μm wide
Length/width ratio: varies from 1.14 in shorter valves up to 1.44 in longer cells
Striae: radiate and number 9-12 in 10 μm
Ocelli: two ocelli (almost the same size) are present, positioned opposite one another, composed of fine rows of porelli
Rimoportulae: two rimoportulae are present, each with a small hyaline area surrounding the opening

3.2. Physical and chemical variables

Physical and chemical variables at five sampling station during the study period are summarized in Table 1. The pH decreased slightly down the agricultural to urban gradient, being slightly alkaline at sampling stations. The difference in pH amongst the five sampling stations was not statistically significant (ANOVA, $P > 0.05$). Temperature and Fe^{2+} concentration increased downstream, but as in the case of pH, the increase was not significant ($P > 0.05$). On the other hand, electrical conductivity (EC), TN, and TP concentrations increased significantly ($P < 0.05$) toward downstream (Table 1).

3.3. Distribution of P. laevis on different substrata

Results of *P. laevis* relative abundance (mean ± SD) on different substrata are shown in Table 2. A total of four substrata were sampled at each station. The highest (12.54 ± 1.54) and lowest (0.74 ± 0.10) abundance was obtained from wooden and muddy substrates at stations S5 and S4,

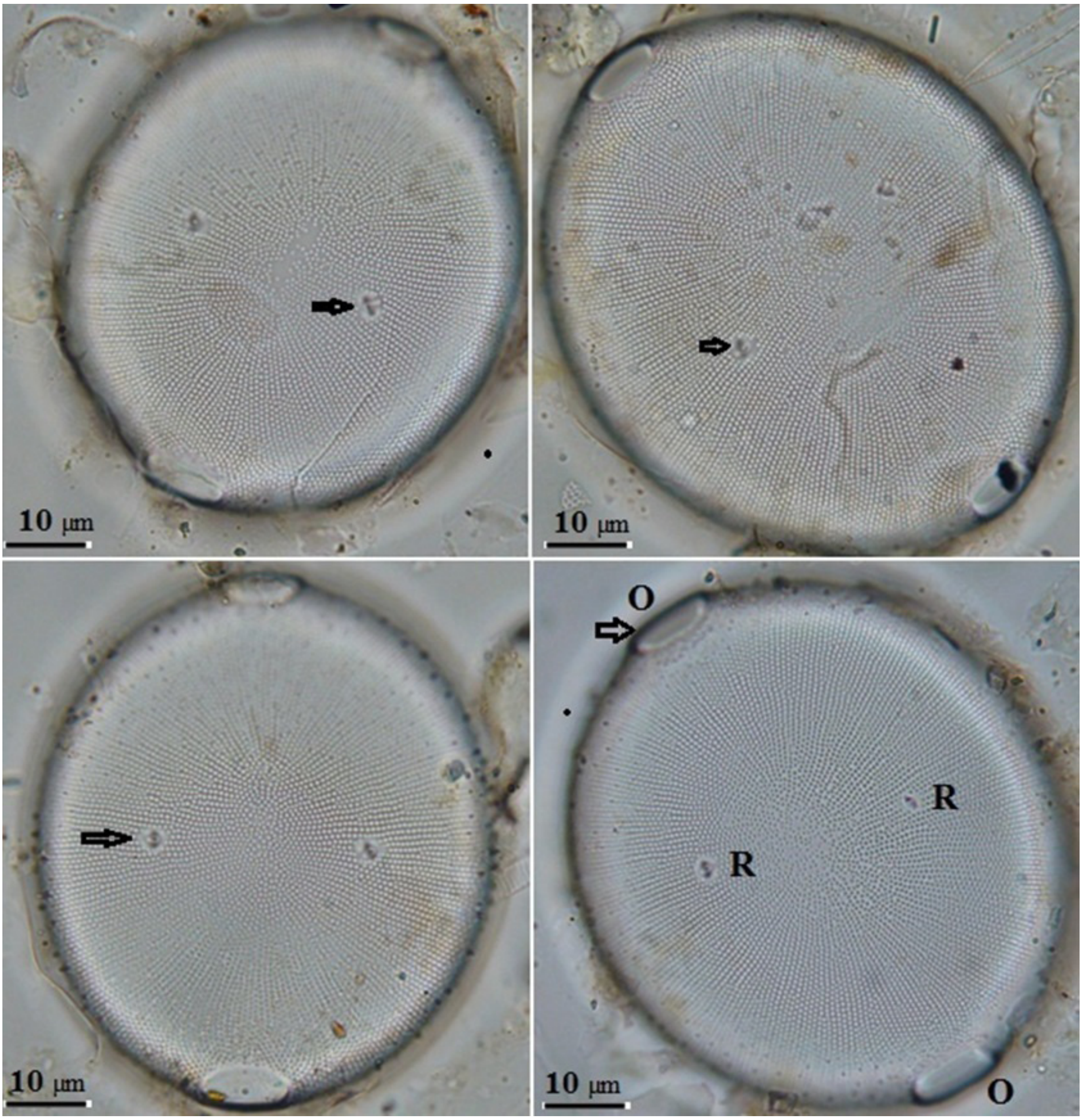

Figure 2. Light micrograph of *Pleurosira laevis* Compère from Masuleh River. (R= Rimoportulae; O= Ocelli).

Table 1. The mean values of physicochemical parameters measured at 5 sampling station.

Station	S1	S2	S3	S4	S5
Temperature (°C)	24.5	24.6	24.7	24.9	25.1
EC (µS cm⁻¹)	637.5[c]	642[c]	654[c]	856.5[b]	1000[a]
pH	8.44	8.3	8.41	8.09	8.07
TN (mg L^{-1})	1.46[d]	1.97[cd]	2.23[bc]	2.65[b]	3.11[a]
TP (mg L^{-1})	0.049[b]	0.051[b]	0.055[b]	0.057[b]	0.13[a]
Fe^{2+} (mg L^{-1})	0.011	0.012	0.015	0.019	0.021
SiO_2 (mg L^{-1})	2.39[b]	2.76[b]	2.82[b]	3.97[a]	4.55[a]

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

Table 2. Relative abundance (mean ± SD) of *P. laevis* on different substrata at five sampling stations.

Station	S1	S2	S3	S4	S5
Epilithic	0.54±0.13	0.55±0.10	0.98±0.12	2.47±0.12	3.33±0.21
Epidendric	1.35±0.11	3.62±0.22	4.78±0.32	5.02±0.19	12.54±1.54
Epipsammic	2.11±0.23	2.55±0.14	2.21±0.16	2.86±0.15	7.05±0.86
Epipelic	0	0	0	0.74±0.10	3.07±0.13

respectively. The highest abundance was observed in the wooden substrate among all of sampling stations where showed significant differences ($P < 0.05$) with the other substrates (Figure 3).

The hierarchical clustering based on relative abundance of *P. laevis* distinguished two clusters amongst the four substrate types (Figure 4a). Rougher and more stable substrates (wood, sand, and stone) clearly separated from smooth and unstable substrates (mud). Based on relative abundance of *P. laevis* on rougher substrates, epidendric and epipsammic species had the highest similarity (Figure 4a). Based on cluster analysis carried out to demonstrate the similarities in distribution of *P. laevis* amongst the 5 stations sampled, two major groups of stations were observed and downstream stations (S4 and S5) clearly separated from upstream stations (S1-S3) (Figure 4b).

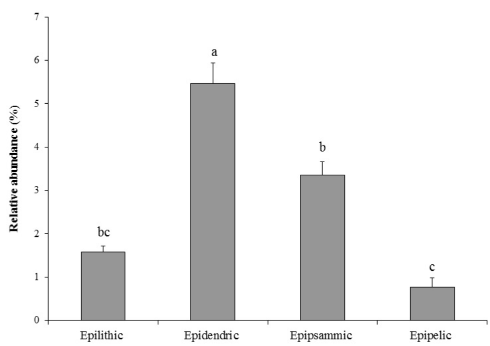

Figure 3. Relative abundance (mean ± SD) of *P. laevis* on different substrates (χ^2=11.16; $P < 0.05$).

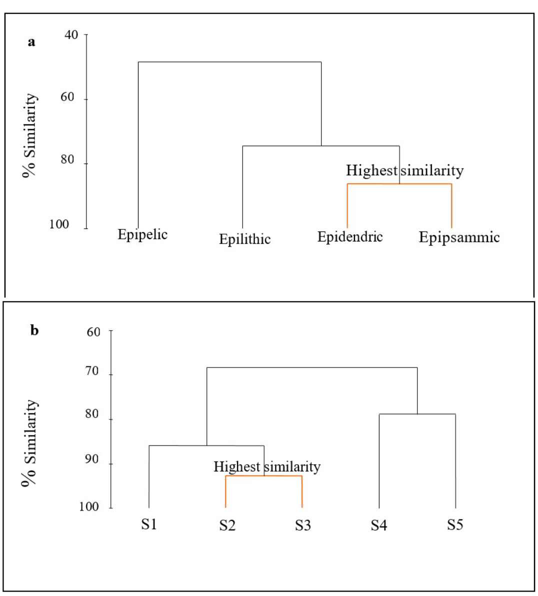

Figure 4. Hierarchical clustering (Euclidean distance; Ward algorithm) of the substrate types (a) and sampling stations (b) based on relative abundance of *P. laevis*.

3.4. Pearson's correlation analysis

A Pearson's correlation analysis of *P. laevis* relative abundance and all measured physical and chemical variables was conducted (Table 3). The results showed that relative abundance of *P. laevis* had a significant correlation with EC, TN, TP, and Fe^{2+} concentrations ($P < 0.05$) whilst no significant correlation was observed with pH, temperature, and SiO_2 concentration ($P > 0.05$).

4. Discussion

Our observations on *P. laevis* in the Masuleh River basin (Guilan Province-Iran) are the first record of initial detection of the centric diatom *P. laevis* in this basin. This species has also been reported in Iran (Soltanpour-Gargari et al., 2011) and other regions (e.g. Asia, Europe, and North America) (Kociolek et al., 1983; Bahls, 2009; Perez et al., 2009; Karthick & Kociolek, 2011). In most instances, these occurrences have been from arid regions in rivers (Czarnecki & Blinn, 1978; Crayton & Sommerfeld, 1979). The morphology of *P. laevis* cells found in the Masuleh River is more matches to images that depicted by Krammer & Lange-Bertalot (1991) than those that were prepared by Kociolek et al. (1983) and Karthick & Kociolek (2011). More research and investigations are needed to elucidate the relationships of these populations.

Pleurosira laevis is most commonly associated with marine or brackish water (Kociolek et al., 1983). Wujek & Welling (1981) refer to this diatom species as a halophil and state that it possibly is indicative of increasing near shore chloride levels. How this alga found its way into the Masuleh River is still unknown. The translocation of used fishing or water recreation equipment may be the

Table 3. Pearson correlation coefficients between *P. laevis* relative abundance and all measured physicochemical parameters.

		pH	EC	TN	TP	SiO_2	Fe^{2+}	T
P. laevis	Coefficients	0.666	**0.921**	**0.815**	**0.895**	-0.191	**0.928**	0.578
	Sig. (2-tailed)	0.220	0.026	0.043	0.040	0.758	0.023	0.222

Significant correlations ($P < 0.05$) are given in bold.

primary means for *P. laevis* introduction and also secondary spread but the natural wildlife vectors, including birds, mink, and fish are also possible vectors. Two ways of transportation for these algae can be epizoochoric (on animal body surface) and ergastochoric (on a boat). In the Masuleh River all of these ways are possible and it suggested translocation of this benthic diatom through fishing boats is more plausible in downstream stations. In addition, epizoochoric transportation (especially through migratory fishes) may be a possible vector for *P. laevis* spreading upstream stations in the Masuleh River.

Present study is the first attempt to investigate the effects of substrate type on distribution of *P. laevis*. Results from one-way ANOVA test showed (Figure 3) that the relative abundance of *P. laevis* was significantly correlated to substrate type. The smooth or rough substrate surface showed significant influence on the relative abundance increment in shallow temperate river, regardless of colonization time. On the other hand, algal assemblages were more sensitive to increased surface roughness. The influence topography and type of substrate on the periphytic community structure was well demonstrated in lotic ecosystems (Bergey, 2005; Murdock & Dodds, 2007; Bere & Tundisi, 2011; Wojtal & Sobczyk, 2012). Overall, our results generally support the previous findings that rougher substrates (wood, stone, and sand) collect more algae compared to smooth substrata (Townsend & Gell, 2005; Murdock & Dodds, 2007). The highest relative abundance of *P. laevis* was recorded on the wooden substrate and significantly differed from other substrates. It is tempting to speculate that more diatom abundance was adapted to the surface roughness of the woods, and that is because the peak abundance accrual occurred at a roughness approximately equal to sand and rock roughness. A possible reason for this could be capable of wooden substrates in minerals absorption from water.

Previous studies on biological and hydrochemical parameters reveal that Masuleh River is oligo-mesotrophic (Imanpour Namin et al., 2013). The downstream stations with mesotrophic status have the more favorable environmental characteristics for the growth of *P. laevis*: high conductivity and high nutrient content (mesotrophic). From the Pearson's correlation results, relative abundance of *P. laevis* were highly positively correlated with EC, TN, TP, and Fe^{2+} concentrations whilst no significant correlation was observed with pH, temperature, and SiO_2 concentration. Some researchers suggested that this species would be found in high conductivity, nitrate, and mineral content (Whitford, 1956; Wujek & Welling 1981; Compère, 1984), and our analysis of the water chemistry of the Masuleh River supports this ecological niche (downstream stations and wooden substrates) for the species. Periphyton assemblages have been linked to other physical substratum characteristics, such as size (Watermann et al., 1999) and stability (Cattaneo et al., 1997). Furthermore, Murdock & Dodds (2007) state that roughness is equally important in regulating a substratum's physical effect in streams. According to discussions depicted by Jones et al. (2000) and Biolo et al. (2015), there is a remarkable controversy about factors that estimate the species abundance of the periphytic algal communities, especially with respect to the selective influence of type and shape of substrates. Findings from this study also indicated that the type of substrate and physical and chemical variables affect on the relative abundance of *P. laevis*. Furthermore, the development of benthic diatoms such as *P. laevis* is influenced by the properties of the substratum. For example, benthic diatoms assemblages have been found to grow better on rougher surfaces (Souza & Ferragut, 2012). This impact of surface roughness on periphytic assemblage growth can affect the comparability between different surfaces.

The hierarchical clustering based on relative abundance of *P. laevis* revealed that the rougher and more stable substrates (wood, sand, and stone) clearly separated from smooth and unstable substrates (mud). Based on relative abundance of *P. laevis* on rougher substrates, epidendric and epipsammic species had the highest similarity. Also, the downstream sampling stations clearly separated from upstream stations. The long period of low flows preceding sample collection, rapid reproduction, and ongoing immigration of species onto substrata may have favoured the domination by commonly occurring, seemingly better adapted, diatoms. Moreover, the formation of a complex periphyton matrix, with its own biological and physicochemical characteristics, may have mediated or even negated any substrate influence on the diatom assemblage (Townsend & Gell, 2005).

5. Conclusion

The results underpin the importance of substrate type and the effect of physical and chemical variables on distribution of *Pleurosira laevis* in the Masuleh

River. Multiple substrate sample collection, however, is a sound principle for the assessment of diatom distribution, as it may result in the collection of a small number of taxa that are substrate specific. We agree that sampling standard substrates is an appropriate way to alleviate the possible influence of substrate, and that standard substrates are necessary in small-scale studies carried out within single water bodies or small watersheds. Nevertheless, this effect is unavoidable if we want to test the substrates under the same conditions. Moreover, we generally have to be aware of the large heterogeneity of species composition and density on different surfaces in nature. Also, we conclude that wood substrata can be substituted for one another during field surveys. Results from this study demonstrate that the type and roughness of the substrate both influence the attachment of *P. laevis* and its subsequent growth conditions.

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