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Table of Contents

Physiological and Biochemical Responses of Dinoflagellate *Symbiodinium* sp. to Different Light Intensities

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Abstract

Symbiotic dinoflagellates create valuable bioactive compounds such as carotenoids and lipids, which are used in various industrial fields. However, a small amount of their biomass can be produced by using the suspension-based closed photobioreactors. The present study tried to obtain high lipid and total carotenoid contents from *Symbiodinium* sp. by creating optimal light intensity conditions using a Twin-layer photobioreactor. In this regard, growth rate, biomass, chlorophyll a concentration, and total carotenoid and lipid contents were examined at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ for 16 days. Based on the results, biomass productivities ranged from 35.7 to 72.0 $g.m^{-2}$ at 50 to 250 µmol photons $m^{-2}s^{-1}$, respectively. In addition, the highest linear growth rates were 2.03, 3.27, and 5.85 $g.m^{-2}d^{-1}$ at the light intensity of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ between 12-16 days, respectively. Further, the maximum amount of total carotenoids, chlorophyll a, and total lipid was attained 0.85 and 0.96 $g.m^{-2}$, and 27.77% at the light intensity of 250 $\mu mol.m^{-2}s^{-1}$, respectively. The results represented that the immobilization of algal cells in the photobioreactor biofilm resulted in producing high biomass, total carotenoids, and lipids.

Keywords: Biomass, Chlorophyll a, Total carotenoids, Total lipids, Twin-layer photobioreactor, Symbiotic alga.

Introduction

The light quality and quantity affect microalgae physiology significantly, providing the energy needed for photosynthesis (Etheridge and Roesler, 2005). The light leads to variations in the growth dynamics, ultrastructure, biophysics, and physiology of algae for their adaptation to change environmental conditions (Schlöter et al., 2000). Based on the results of the various studies, algal bioactive compounds such as pigments, lipids, and fatty acids react to an increase or decrease in light intensity differently (Treignier et al., 2008; Valenzuela-Espinoza et al., 2011). For example, an enhancement in light intensity can inhibit algae growth (Parkhill and Cembella, 1999) or improve the quality of bioactive com-

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pounds such as polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in dinoflagellates (Fuentes-Grünewald et al., 2009).

The biomedical, biological, and biophysical researchers identified dinoflagellates as the most promising subject due to their two valuable compounds of carotenoids and fatty acids (Benstein et al., 2014; Sugawara et al., 2009). Peridinin is considered as one of the main carotenoids in dinoflagellates, which exists only in this group. Additionally, it is a part of the membrane attached to light receptor complexes (LHCs) in PCP and AcpP complexes. Both complexes play a role as an optical shield, as well as transferring energy to chlorophyll a, which result in increasing absorption range in optical products and transferring excited energy with 95% efficiency to chlorophyll a (Pinto et al., 2000).

Microalgae have attracted much attention due to their higher lipid content compared to the higher plants (Grünewald, 2012). Under adverse and stressful conditions, microalgae enhance lipid production and alter their lipid biosynthetic pathway to lipid formation and accumulation (Hu et al., 2008; Zhang et al., 2014). The results of the previous studies indicated that 20-70% of the total biomass of microalgae can be lipid (Fuentes-Grünewald et al., 2013). Microalgae lipids have high potential for pharmaceutical, chemical, and food industries, and are considered as a suitable source for biodiesel production (Gordillo et al., 1998). The highest percentage of total fatty acid content was observed in *Symbiodinium microadriaticum* among the assessed symbiotic algae (Mansour et al., 1999). Light intensity can improve lipid metabolism and profile (Valenzuela-Espinoza et al., 2011; Yeesang and Cheirsilp, 2011).

However, only a small amount of biomass is obtained from culturing the symbiotic dinoflagellates which can produce valuable bioactive compounds by using the suspension-based closed photobioreactors. The present study sought to produce a high lipid and total carotenoid content by providing optimal conditions for culturing *Symbiodinium* sp. by using a Twin-layer photobioreactor.

Material and methods

Preparation and culture conditions in a Twin-layer photobioreactor

Symbiodinium sp. (strain CCATM-210) was prepared from the culture collection of algae in the Department of Marine Biology, Tarbiat Modares University. In addition, culture medium ASP12 was used (Benstein et al., 2014), which was sterilized by autoclaving at 121 ˚C for 20min. Further, the suspension culture was performed in a 50-ml Erlenmeyer flask with 200×10^3 cells. ml^{-1} at a light intensity of 100 $\mu mol.m^{-2}s^{-1}$ at 23 ± 1 ˚C and photo-period was adjusted to 9h dark and 15 h light. The symbiotic algae were cultivated in a 50-ml Erlenmeyer flask until reaching the density of 8×10^6 cells. ml^{-1}. Furthermore, the algal inoculant was transferred to 2lit of the prepared medium. The biomass was centrifuged at 500rpm when culture stock in 2 liters reached 4×10^6

cells.ml^{-1} again. Finally, microalga biomass was transferred into the culture discs of a Twin-layer photobioreactor and cultivated at 23±1 ˚C at the light intensity of 50, 100, and 250 µmol.m^{-2}s^{-1} for 16 d (Benstein et al., 2014). During culturing in a Twin-layer photobioreactor, the medium was exchanged every 3 d to compensate evaporation and avoid nutrient depletion.

Determination of growth rate and cell biomass

In order to evaluate the dry weight of microalga, samples were collected every four days and dried in a freeze-drier (Model FD-5010-BT) for 2 h. The specific growth rate was calculated by using Guillard (1973) equation.

$\mu = (\ln X_t - \ln X_0)/t$, day^{-1}

where X_0 demonstrates initial cell density and X_t denotes its density after t days.

Additionally, the biomass concentration (B) of samples was obtained by using the following equation.

$B = W_t - W_{m/a}$ (g.m^{-2})

in which W_t and W_m are respectively considered as total (biomass + membrane) and membrane weight, and a illustrates inoculation area (m^2).

Determination of chlorophyll a concentration and total carotenoid content

Algal cells were extracted by pure methanol and 2% ammonium acetate solvent. To this end, 4.5 ml of pure methanol was added into extracted biomass and vortexed for 120 s. In addition, the supernatant was placed in ice bath under dark conditions for 2 h. Further, 0.5 ml of 0.5 M ammonium acetate (pH: 7.2) was poured, vortexed again, and stored at-20 ˚C overnight. Furthermore, the samples were vortexed for 10 s over three 30 min periods. In the next stage, the insoluble impurities were removed by double centrifugation at 3500 rpm at 4 ˚C for 10 min. Then, the concentration of chlorophyll a was measured by using a 5100-Vis spectrophotometer at 665 nm and calculated by using the following equation.

$A = \varepsilon\lambda.c.d$

where A represents the absorbance of chlorophyll at wavelength (λ), which can be obtained by using a UV–Vis absorbance spectrometer. Additionally, d indicates the path length of cuvette (cm), ε refers to molar extinction coefficient, and c demonstrates an accurate molar concentration (Porra et al., 1989).

The carotenoid content was determined as follows. First, 5 ml of 90% acetone solution was added to the extract and vortexed for 2 min. Additionally, the pellets were placed in an ultrasonic bath at 4 ˚C for 15 min. Further, the material was kept in an ice bath under dark conditions for 2 h. Furthermore, the samples were vortexed for 4 s over four 30-min periods and stored at 20 ˚C overnight. Finally, the acetone extract was measured using a spectrophotometer at 470 nm and obtained by some researchers (Jeffrey and Haxo, 1968; Jeffry et al., 1975; Lira et al., 2017; Prezelin and Haxo, 1976).

Measurement of total lipid content

In the study, lipid was extracted from the culture. For this purpose, 50 mg of each sample was soaked in 4 ml of distilled water

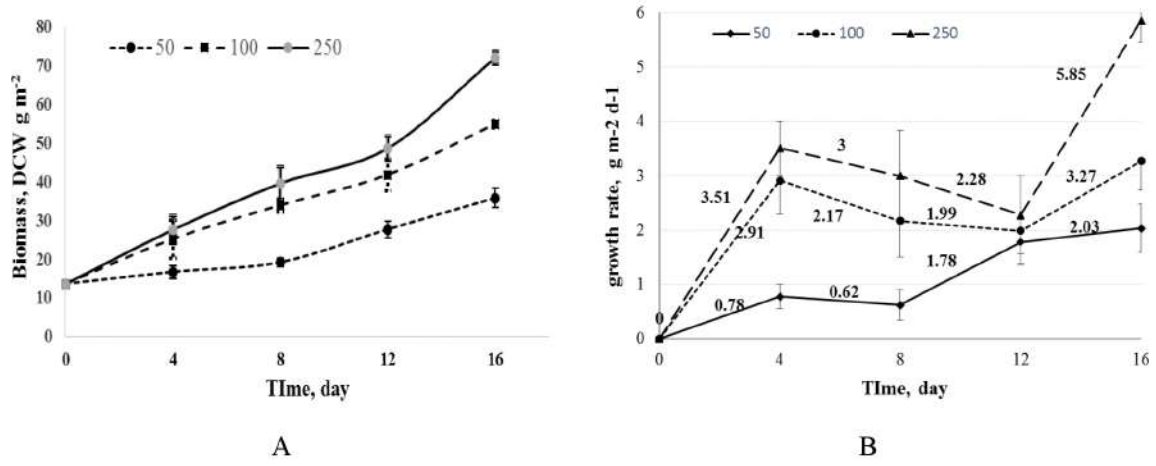
and homogenized for 1min. After pouring 10ml of methanol and 5 ml of chloroform the samples were restored for 5 min and homogenized. In addition, the mixture was rested for 15 min, 5 ml of chloroform was added, and the resultant mixture was again rested for 15 min to obtain lipid extract. Further, the sample was vigorously stirred after adding 5 ml of distilled water and transferred to a decanter container for 12 h in order to help separate two phases. Furthermore, the lower chloroform phase containing lipid was poured to the pre-weighed 50-ml Erlenmeyer flasks and the level of their solutions was equalized by adding chloroform. Then, lipid solution was dried in the boiling water by nitrogen. Finally, concentrated sediments were weighed and total lipid content was calculated (Bligh and Dyer, 1959; Nigam et al., 2011; Zhukova, 2007).

Statistical analysis

Experimental treatments were compared in three replicates, data were analyzed by SPSS and Excel software, and their homogeneity was examined through Kolmogorov-Smirnov test. Further, one-way ANOVA and Duncan statistical test were utilized to compare the differences between 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ light intensities. The significance of each parameter was reported at 95% ($p<0.05$).

Results

The effect of different light intensities on the productivity of *Symbiodinium* sp. biomass was measured at 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ on days 4, 8, 12, and 16 (Fig. 1A). Based on the results, biomass productivities increased over the time and a significant difference was observed between the obtained biomass at all three light intensities ($P< 0.05$). Additionally, the highest and lowest biomass was produced at 250 and 50 $\mu mol.m^{-2}s^{-1}$, respectively. Fig. 1B displays the linear growth rate of *Symbiodinium* sp. As demonstrated, the maximum linear



A B

Fig. 1. The effect of different light intensities on the dry biomass of *Symbiodinium* sp. ($g.m^{-2}$) (A) and linear growth rate of *Symbiodinium* sp. ($g.m^{-2}d^{-1}$) (B) under the light intensities under study by the 16^{th} day (mean ± SD, n = 6, six replicate filters)

growth rate was respectively determined 2.03, 3.27, and 5.85 $g.m^{-2}d^{-1}$ at the light intensity of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ between 12-16 days. Under 50 µmol photons $m^{-2}s^{-1}$, an increase in linear growth rate from 0.78 by 2.6 times results in decreasing biomass density to 35.7 $g.m^{-2}$ within 16 days. Further, light intensity increased up to 100 µmol photons $m^{-2}s^{-1}$ in Twin-layer photobioreactor. Furthermore, *Symbiodinium* sp. represents a linear growth rate from 3.5-5.85 g dry weight $m^{-2}d^{-1}$ to 72 $g.m^{-2}$ in vertical growth surface after 16 days at high photon fluence rate (250 µmol photons $m^{-2}s^{-1}$). Based on the Guillard's equation, mean specific growth rate was obtained 0.06, 0.08, and 0.10 d^{-1} at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$, respectively. In general, biomass density and growth rate increased by raising light intensity.

The evaluation of the bioactive compounds produced by *Symbiodinium* sp. indicated the effec tiveness of low and high light intensities on biomass production. In addition, the maximum and minimum concentration of chlorophyll a was 0.96 and 0.69 $g.m^{-2}$ at the light intensities of 250 and 50, respectively. Further, an increase in light intensity resulted in enhancing chlorophyll a concentration (Fig. 2A). Furthermore, the total carotenoid content was determined 0.36, 0.61, and 0.85 $g.m^{-2}$ at the light intensities of 50, 100, and 250 $\mu mol.m^{-2}s^{-1}$ by the 16th day, respectively (Fig. 2B). Thus, total carotenoid content developed by rising light intensity. The statistical results demonstrated a significant difference in the amount of total carotenoid content and chlorophyll a concentration at the intended light intensities by the 16th day ($P< 0.05$)

Finally, total lipid percentage was significantly different ($p<0.05$) at the light intensities under study by the day 16, the lowest and highest of which were 19.5 and 27.77%, respectively, at dry weight at 50 and 250 $\mu mol.m^{-2}s^{-1}$ light intensities (Fig. 3). Therefore, the amount of total lipid production improved by increasing light intensity.

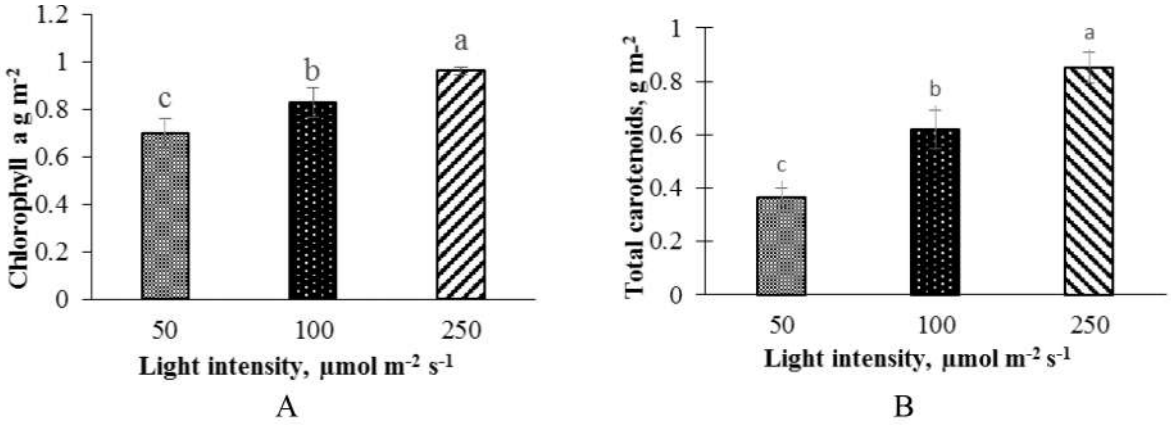


Fig. 2. (A) Chlorophyll a and (B) total carotenoid contents in *Symbiodinium* sp. at 50, 100, and 250 µmol photons $m^{-2}s^{-1}$ (a, b, c, standard deviation, n = 6, six replicate filters)

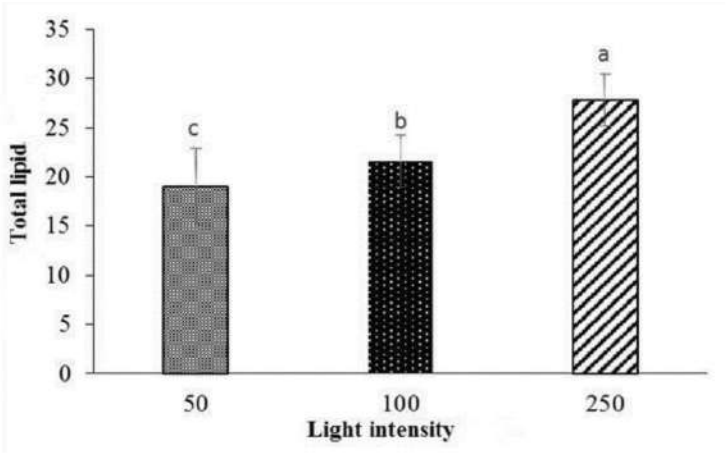


Fig. 3. Total lipid percentage in *Symbiodinium* sp. under the intended light intensities (a, b, c, standard deviation, n = 6, six replicate filters)

Discussion

The light intensity influences the growth efficiency of symbiotic dinoflagellates in vivo (Fuenes-Grünewald, 2012; Graham et al., 2010). Wilkerson et al. (1983) assessed the growth rate of *Symbiodinium microadriaticum* in different hosts and reported specific growth rates from 0.01 to 0.1 d^{-1}. Obviously, growth rate in the laboratory-controlled culture conditions can be several times higher than that in the natural environment. In the present study, the growth rate of the symbiotic microalga *Symbiodinium* sp. under study enhanced under light from 0.6 to 0.1 d^{-1}. Additionally, linear growth rate kept increasing by raising light intensity to 250 $m^{-2}s^{-1}$, which demonstrates that growth inhibition has to be occur at the light intensities above those under study. The 470 $\mu mol.m^{-2}s^{-1}$ light intensity can prevent dinoflagellates from growing (Parkhill and Cembella, 1999) although the issue was not considered in the present study. In general, the range of the biomass productivity of the *Symbiodinium* sp. under study was similar to that of other microalgae immobilized on Twin-Layers under comparable conditions (Benstein et al., 2014; Podola et al., 2017). Based on the results of the present study, biomass content enhanced linearly under all three light intensities although growth rate at the light intensity of 50 $\mu mol.m^{-2}s^{-1}$ was lower than that of two others, which may be related to the difference in biofilm division into two photosynthetic activities (Kiperstok, 2016; Li et al., 2015).

The absorption of light wavelengths by protective pigments increases under the high light intensity. When affecting microalgae such as *Symbiodinium* sp. by high light, their photosynthetic apparatus prevents damage from photosynthetic active radiation (Hoegh-Guldberg and Jones, 1999). The results of cultivating microalga under the low light illumination (50 $\mu mol.m^{-2}s^{-1}$) represented that the total carotenoid con-

tent was determined 0.36 $g.m^{-2}$, while the immobilized cells accumulated up to 0.85 $g.m^{-2}$ of total carotenoids under the higher photon fluency (250 $\mu mol.m^{-2}s^{-1}$). Further, the obtained carotenoid pattern indicated an enhancement in total carotenoid such as peridinin production because of improving light intensity. Furthermore, a relationship was observed between raising light intensity and increasing total carotenoids linearly with its protective role in maintaining photosynthetic system and biomass growth process (Khalesi and Lamers, 2010; Kiperstok, 2016; Schlöter et al., 2000).

Regarding the cultivation of most species under limited and optimal light, the concentration of chlorophyll a and photosynthetic pigments is linearly related to growth rate (Goericke and Montoya, 1998). The results of the present study reflected an improvement in growth rate and chlorophyll a by enhancing light illumination. The changes in chlorophyll a concentration are based on the variations in PSU count (Prézelin, 1976). However, the stimulating effect of light intensity on microalgae reduces the function of photosystem II, electron transfer, and chlorophyll a, and consequently decreases growth (Lesser, 1996).

Algae adapt to environmental conditions by altering lipid metabolism. In general, microalgae increase lipid content under unfavorable and stressful conditions (Gordillo et al., 1998; Nigam et al., 2011). Comparatively, the biomass and total lipid contents up to 27% related to some of the dinoflagellates cultured in a photobioreactor are quite similar to those of the green algae such as *Chlorella minutisima* and *Scendesmum obliquus*, as well as the different species of *Botryococcus*, which are often used to produce biodiesel (Fuentes-Grünewald et al., 2013; Rodolfi et al., 2009; Yeesang and Cheirsilp, 2011). The results of the present study regarding the effect of light intensity on total lipid content in *Symbiodinium* sp. demonstrated that high intensity led to greater total lipid content. In addition, the maximum and minimum total lipid yield at 250 and 50 $\mu mol.m^{-2}s^{-1}$ were 27.83 and 19.31% of dry weight, respectively. Comparing the total lipid content of *Symbiodinium* sp. and *Alexandrium catenella* (27%), *Amphidinium* sp. (18.9%), *Scrippsiella* sp. (16%), *Symbiodinium microadriaticum* (15%), *Gymnodinium* sp. (22.6%), *Gymnodinium sanguineum*, and *Fragilidium* sp. (13%) of dry weight indicated that the studied species can be considered as a prospective species with fairly high lipid content (Fuentes-Grünewald et al., 2009; Islam et al., 2013; Mansour et al., 1999).

Further, light intensity particularly affects growth and bioactive compounds. Furthermore, the content of biomass, chlorophyll a, carotenoid, and total lipid improved following an enhancement in light intensity, and *Symbiodinium* sp. is considered as light intensity-resistance. At the same time, it seems that greater lipid percentage is a protective mechanism against high light intensity. Finally, the immobilization of algal cells in the biofilm of photobioreactor resulted in producing maximum biomass and bioactive compounds through biofilm culture method

because of overcoming the hydrodynamic stress of suspension culture on microalgae cells.

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Phytoplankton Population Structure in Mighan Salt Lake (Arak, Markazi Province)

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Abstract

Mighan lake with the surface area of 112 km^2 is located eight kilometers northeast of Arak city, the main phytoplankton population and food chain of which include *Dunaliella salina* (Dunal) Teodoresco. The salinity of the lake ranges between 20-120 g/l depending on season and water input. The present study evaluated the effect of salinity and physicochemical factors on phytoplankton communities in the lake. To this end, sampling was performed monthly during March to December 2019. In addition, species and phytoplankton density were determined through inverted microscopy. Based on the results, 12 algal species were identified, among which *D. salina* was 87.3% of phytoplankton composition. Indeed, salinity, as a major limiting factor, reduced phyto-plankton diversity in Mighan Lake.

Keywords: Algal composition, Diversity, Hypersaline, Mighan lake, Salinity

Introduction

Hypersaline environments are defined as the places with the salt concentration higher than that of seawater (3.5% total dissolved salts) (Das sarma and Arora, 2001). The environments are related to biogeochemical processes and considered as an integral and dynamic part of the biosphere (Mohebbi, 2010; Shadrin, 2009). Prokaryotic and eukaryotic algae can contribute to primary production in salt waters (Borowitzka, 1981). The management and protection of hypersaline lakes depend on the effects of salinity level on biological productivity and community structure. Consequently, phytoplankton composition may influence *A. partenogenetica* Bowen and Sterling, as the major macrozooplankton in hypersaline waters. A continuous reciprocal interaction was observed between *A. partenogenetica* and phytoplankton population in hypersaline environments (Mohebbi, 2010). Mighan Lake is located in central Iran, about 8km far from the Arak, as the capital of Markazi province at altitude 1660m above sea level. The annual average temperature and precipitation of the lake are 11.7 ˚C (Ghahroodi Tali et al., 2012) and 258 mm (Ansari, 2008), respectively. Additionally, the surface area of the lake fluctuates depending on the entered water

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and season (Environment Conservation Organization, 2016), and its depth is about 1-1.5 m during wet period. The main water suppliers in the lake include precipitations, floods, a few rivers, springs in its bed, and sewage water effluents from Arak. *A. partenogenetica* is a brine shrimp thriving in Mighan wetland, as well as a major food chain for migratory birds, the main food source for which is *Dunaliella salina* (Dunal) Teodorescoa, a unicellular green algae.

Hypersaline environments are considered as a significant economical, ecological, and natural habitat, the management and protection of which depend on the effect of salinity levels on biological productivity and community structure. The present study assessed the effects of salinity levels on microalgal structure and dynamics in Mighan Lake. In the hypersaline lakes, salinity is the principal abiotic variable affecting phytoplankton species richness. Further, *Dunaliella* sp. can tolerate higher salinities, which results in outcompeting other algal species (Larson and Belovsky, 2013). Furthermore, salinity is negatively correlated to species richness in all of the lakes.

Materials and Methods

In the present study, samples were monthly collected from 18 sampling sites selected in Mighan wetland during March to February 2019 (Fig. 1). Phytoplankton samples were preserved in cold and dark conditions after fixation by lugol solution. After settling phytoplankton to the bottom of 5ml-settling chambers, they were counted and identified by using Nikon TS100 inverted microscope at 400× magnification based on the Utermöhl method (1958).

At least 50 fields or 100 individuals of the most abundant species were counted in each sample (Venrick, 1978). Phytoplankton taxa were determined according to Prescott (1962), Tiffany and Britton (1971) and Bellinger (1992).

In each site species composition, and density of phytoplankton community were analyzed. Salinity was measured by a refractometer model ATAGO (Japan). Temperature was measured in situ by alcoholic thermometer. EC, TDS, and pH were measured by WTW LF 320 EC meter and a Testo 320 PH meter, respectively. Transparency of water was measured by Secchi disc (30 cm diameter).

Correlation among some variables were calcu-

Fig. 1. Samplings sites in Mighan Lake

lated (Excell, 2013). The data were standardized (mean= 0, variance= 1) and correspondent analysis was performed. Multivariant analysis was done to observe distribution of the sampled waters based on environmental parameters.

Based on distance matrix method UPGMA tree was constructed, Two-way clustering of environmental parameters was carried out using UPGMA. Indeed, the Euclidean distance was determined among standardized data. Statistical analysis were performed by PAleontological STatistics (PAST) version 3.04 (Hammer et al., 2001).

Results

In this study 12 algal species were identified in Mighan Lake. Cyclic pattern of water temperature fluctuations presented in Figure 1. Bacillariophyta were the most conspicuous taxa (5 species) (Table. 1). While Cyanobacteria and Chlorophyta and included 3 and 4 species, respectively. *D. salina* a halophilic green algae composed 87.36% of phytoplankton composition (Fig. 2).

Based on the results in Fig. 3, the maximum and minimum temperature in Mighan Lake was recorded in September and December as 32.4 and 5.7 ˚C, respectively.

As shown in Fig. 4, the salinity of the lake is

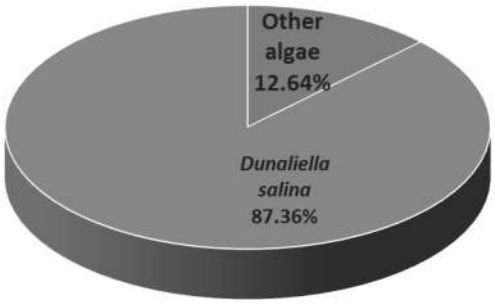

Fig. 2. Algal population structure in Mighan Lake

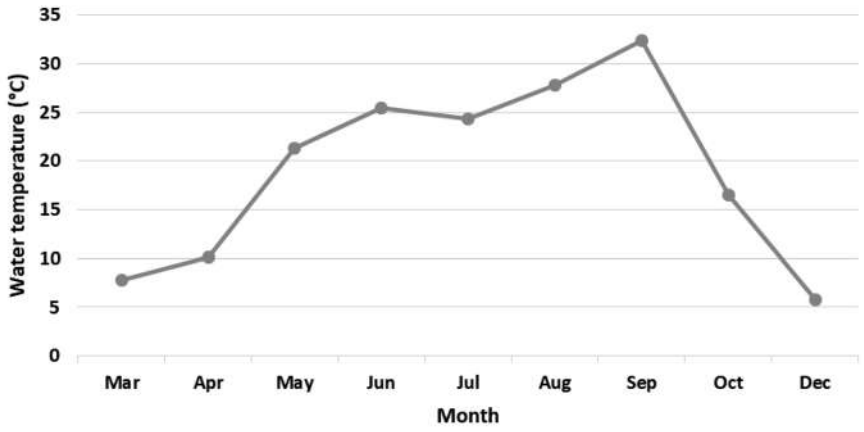

Fig. 3. Water temperature changes in Mighan Lake

Table 1. Algal species density in Mighan Lake during the study period

Density (%)	Mean Density (Ind/L)	Algal species	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Dec	Density (%)
87.36	377033	*Dunaliella salina* (Dunal) Teodoresco	+	+	+	+	+	+	+	+	+	87.36
12.64	4142	*Navicula* sp.	+	+	+	+	+	-	-	+	+	12.64
	39613	*Nitzschia* sp.	+	+	+	+	+	+	+	+	+	
	3278	*Chlorella vulgaris* Beijerinck	+	-	-	-	-	-	-	-	-	
	731	*Gomphosphaeria* sp.	+	-	-	-	-	-	-	-	-	
	365	*Symbella prostrate* (Berkeley) Cleve	+	-	-	-	-	-	-	-	-	
	2947	*Oscillatoria* sp.	+	+	+	+	+	-	-	-	-	
	365	*Oocystis crassa* Wittrock	+	-	-	-	-	-	-	-	-	
	2947	*Closterium* sp.	-	+	+	+	+	-	-	-	-	
	644	*Microcystis* sp.	-	+	+	-	-	-	-	-	-	

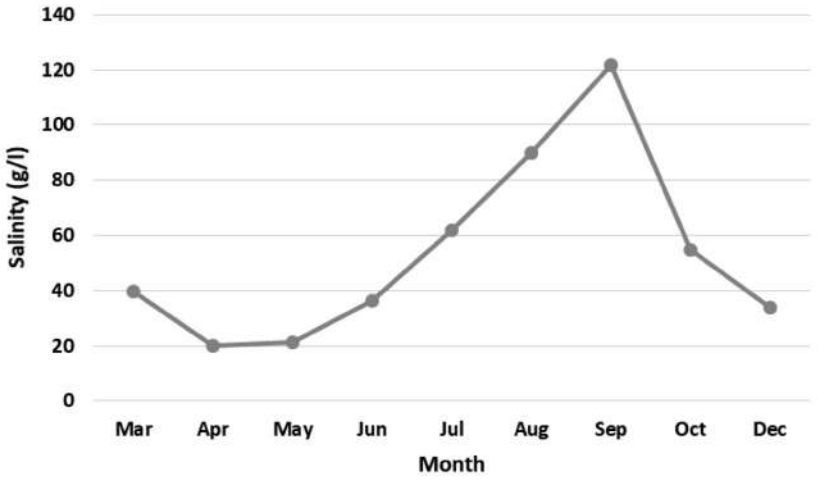

Fig. 4. Salinity fluctuations in Mighan Lake

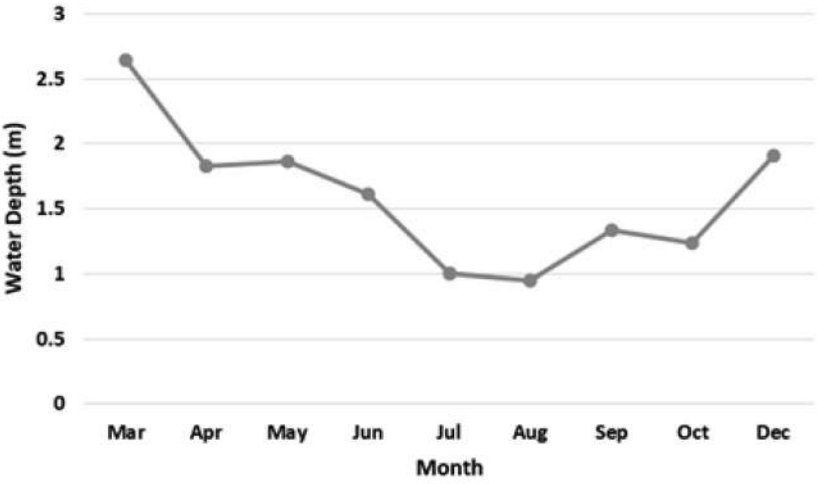

Fig. 5. Water depth fluctuations in Mighan Lake

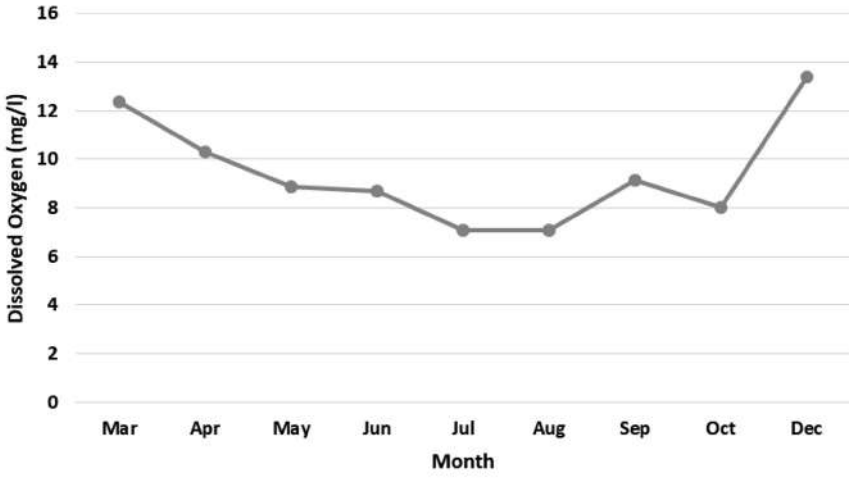

Fig. 6. Water DO fluctuations in Mighan Lake

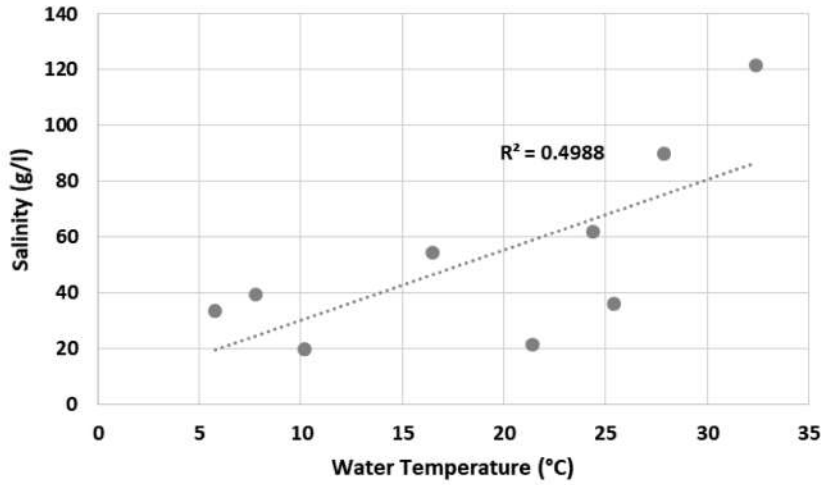

Fig. 7. Correlation between water temperature and salinity in Mighan Lake

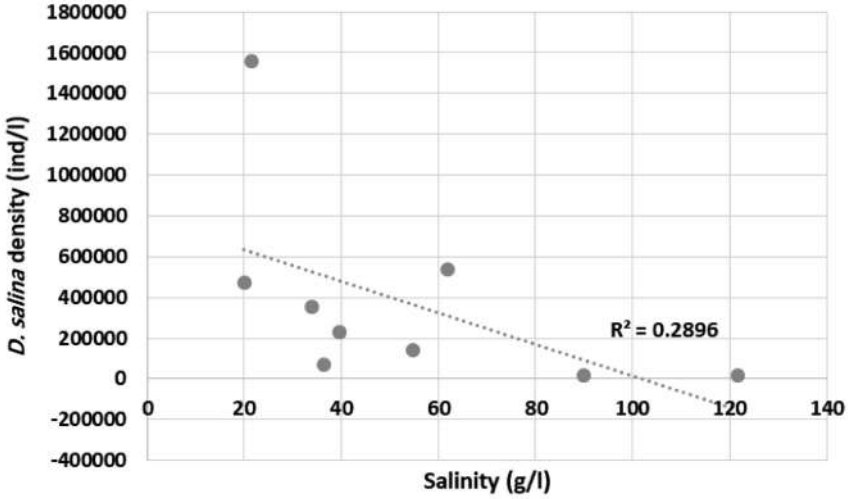

Fig. 8. Correlation between water salinity and *D. salina* density in Mighan Lake

between 19.9 g/l in April to 121.6 g/l in September, respectively (M: about 33.8 g/l).

Further, water depth was respectively measured 0.95 and 2.64 m in August and March (Fig. 5). Furthermore, the highest and lowest dissolved oxygen content (DO) was 13.3 and 7 mg/l in December and August, respectively (Fig. 6).

As displayed in Fig. 7, a significant positive correlation is observed between salinity and water temperature (R=0.71). However, the results in Fig. 8 demonstrated a relatively negative significant correlation among salinity and *D. salina* density (R= 0.54), which confirms a decrease in *D. salina* by increasing salinity. The results of PCA analysis and CA analysis that provided two distinct groups are represented in Fig. 9 and 10.

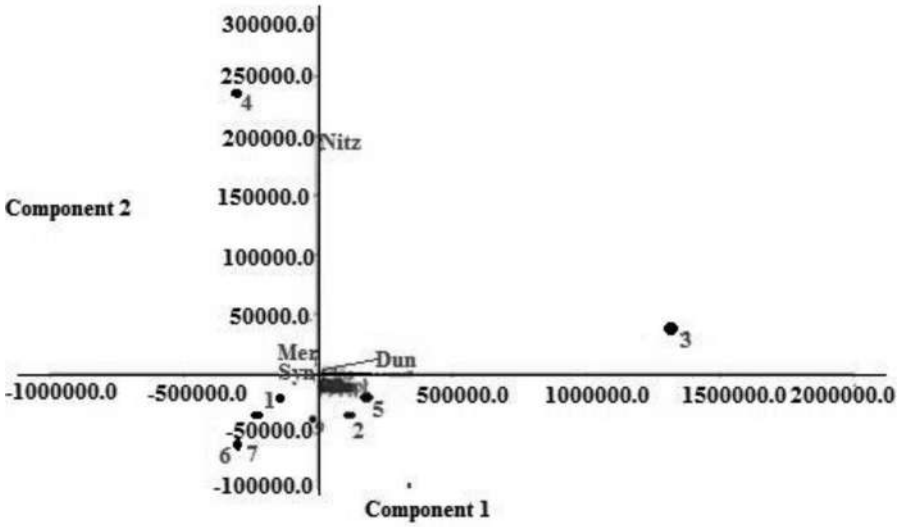

Fig. 9. PCA analysis of phytoplankton taxa and sampling months in Mighan Lake

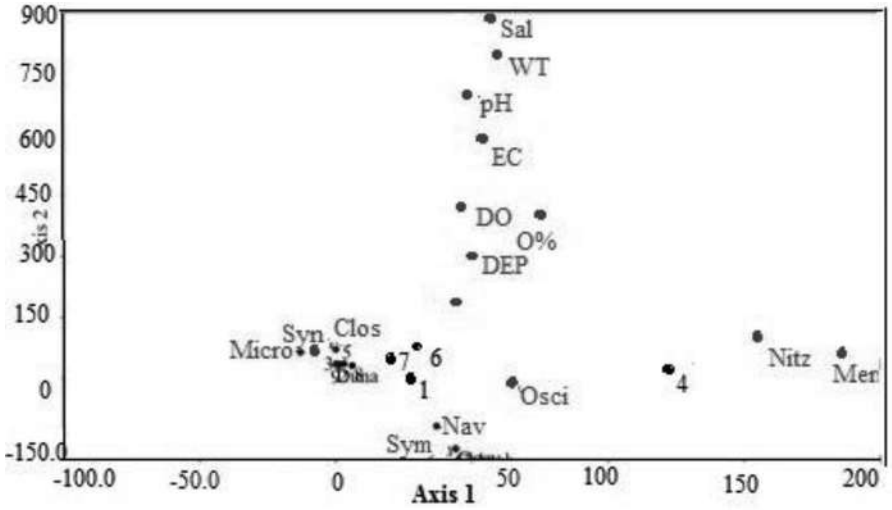

Fig. 10. CA analysis of phytoplankton taxa and physicochemical parameters in Mighan Lake

Discussion

Physicochemical factors influence the phytoplankton population of aquatic ecosystems. In the hypersaline lakes, the population is affected by salinity, as a dominant parameter. In fact, temperature and nutrients have a direct effect on algal composition. In addition, *D. salina* is observed in all hypersaline lakes although the participation of species in total phytoplankton density depends on the salinity level, which is 99 and 87% of algal composition in the Urmia Lake with salinity above 200 g/l and Mighan one with lower salinity level (M:33 g/l), respectively.

Based on the results, the highest and lowest density of *D. salina* was observed in May and September, respectively. Indeed, *D. salina* density, water level, and nutrients increased

simultaneously during spring, which is consistent with those obtained in other hypersaline lakes such as Urmia (Mohebbi, 2020). *A. parthenogenetica* feeds on *D. salina* the density of which reduces in September (Hesami et al., 2017). The results of the present study indicated that the maximum species richness was related to spring and early summer, while the minimum was achieved in late summer, autumn, and winter, which are in line with those concerning Urmia Lake (Esmaeili Dahesht et al., 2010; Mohebbi, 2020) and Great Salt Lake in the United States (Barret and Be-lovsky, 2020). However, the red color observed in Urmia Lake (Mohebbi et al., 2011) was not reported in the Mighan. Further, salinity concentration remained below the saturation level in which halobacterial density reaches 107-108 cells/ml in Urmia Lake. Ghadimi (2020) found municipal wastewater as a major source of heavy metal pollution in Mighan Lake.

The results of PCA analysis reflected that two main components included 99.94% of total vari-ance (Fig. 9) so that the first and second allocated 96.57 and 3.37% of total variance, respectively. In fact, the PC_1 was positively correlated with the main phytoplankton species (D. salina), water temperature, and salinity. Furthermore, PCA ordination represented three distinct regions indicating the connection of environmental variables and phytoplankton community changes. Most of the sampling months were included in the central region, while the upper was related to sampling in June and higher *Nitzschia* density in the month. Finally, sampling in May was pre-sented in the right end of PCA biplot because of measuring the maximum density of *D. salina* in the month. Thus, salinity and water temperature are considered as the main driving factors which contribute to the separation of three groups in PCA analysis.

The results of CA analysis provided two different groups, the first of which included sampling during June when *Nitzschia* density increased and *Merismopedia* species appeared (Fig. 10). The results of PCA analysis demonstrated that CA analysis classified the data by considering the salinity and water temperature.

Based on the results, hypersaline lakes are not a simple ecosystem. Therefore, there is a need for enhancing the knowledge about the factors affecting diversity and coexistence patterns in the unique ecosystem.

Paturej and Gutkowska (2015) examined the effect of salinity on zooplankton population in Vistula lagoon and reported that salinity influences species number and biomass, while it has no effect on species diversity. Additionally, salinity level affects ecosystem processes in wetlands such as phytoplankton diversity and biomass (Gao et al., 2008). Further, *Dunaliella*, as a halotolerant species, mainly occurs in high salinity levels such as Mighan Lake (Hesami et al., 2017). According to Kondo et al. (1990), the effectiveness of salinity levels on phytoplankton composition is more than that of temperature changes.

Finally, phytoplankton assemblages adapt to habitat properties at different salinity levels, which these levels are the main limiting factor determining phytoplankton associations. However, the presence of *Artemia* species, as

well as nutrient levels, should be considered. Thus, further studies should be conducted to explore the ecosystem structure of Mighan lake.

Acknowledgement

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High Content of Heavy Metals in Seaweed Species: A Case Study in the Persian Gulf and the Gulf of Oman in the southern coast of Iran

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Abstract

The contamination of heavy metals is a serious environmental challenge which threatens human health through food chain. This study focused on pollution level of six heavy metals (Cu, Zn, Pb, Ni, Fe^{+2}, and Cd) by investigating 12 seaweed species collected from different intertidal areas in the Persian Gulf and the Gulf of Oman. In addition, the uptakes of heavy metals in seaweed species were examined. The results confirmed the heavy metals contamination in the studied seaweed species. In addition, the uptake level was affected by the type of heavy metal ($P \leq 0.0001$). Additionally, the type of seaweed species and collection site affected the heavy metal uptake. Accordingly, the highest content of Fe^{+2} (2844 ppm) was found in *Dictyota* sp. and Nickel (Ni) was observed in *Padina gymnospora* (105.97 ppm) and *Hypnea* sp. (100.41 ppm). Furthermore, the highest concentrations of Zinc (58.46 ppm) and Copper (32.44 ppm) were found in *Sargassum angustifolium,* and *S. boveanum*, respectively. Additionally, Cadmium had the lowest concentration ranging from 4.8 ppm in *S. angustifolium* to 10.7 ppm in *Dictyota* sp. The lowest content of all tested heavy metals was observed in *Gracilariopsis persica*. Further, the results revealed that brown macroalgae (Phaeophyta) contaminated more than green (Chlorophyta) and red (Rhodophyta) macroalgae.

Keywords: Heavy metals, Persian Gulf, Seaweeds, Environmental pollution

Introduction

Contamination by oil fractions may persist in the marine environment for many years after an oil spill, depending on characteristics of oil such as type, spill size, and location (Tansel, 2014). However, the environment may recover quickly (within 2-10 years) in areas such as salt marshes and mangrove swamps. A spill can remain for more than 25 years if it is not related to the physical removal of oil (Kingston, 2007).

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Chronic oil inputs cause a range of biological effects such as repeated small spillages in coastal waters, as well as local and long-term impacts (Carpenter, 2019).

Heavy metals are considered as the most common environmental pollutants which prevent the presence of natural or anthropogenic sources (Khoshnam et al., 2017). Previous research showed that seaweeds can rapidly accumulate high concentrations of metals such as Cd and Cu (Jarvis and Bielmyer-Fraser, 2015). Further, the toxic metals may deposit in human body where enter seaweeds grown in aquatic systems. In addition, Cd, Hg, and Pb can be toxic even in very small quantities and biologically essential elements might cause toxic effects in high concentrations. Additionally, heavy metals can accumulate in fatty tissues and internal organs of the human body, which may affect the central nervous system. In another instance, arsenic as a metalloid element in organic and inorganic forms can produce different toxicities. This element causes genotoxic damage and is classified as a human carcinogen such as liver, bladder, lung, and skin cancers (Rose et al., 2007). There are some concerns about ecological and global public health issues regarding environmental contaminations caused by heavy metals (He et al., 2005). Metals can cause oxidative stress by increasing the formation of free radicals, and high levels of heavy metals in soils could make the environment unsuitable for plant growth and decrease biodiversity (Ghosh and Singh, 2005). Furthermore, in biological systems, heavy metals damage cell membrane, mitochondria, lysosome, endoplasmic reticulum system, nuclei, and some enzymes with functional roles in metabolism, detoxification, and damage reparation (Wang and Shi, 2001). Further, metal ions interact with other cell components (e.g., DNA and nuclear proteins) and cause conformational disorders and changes in DNA structure (Chang, 1996; Beyermann and Hartwig, 2008).

Despite the widespread negative effects of metal pollution on marine ecosystems and humans, this type of pollution has received less attention. Previous studies showed that it can threaten all types of life in aquatic and moist terrestrial environments (Tawfiq and Olsen, 1993; Moreno et al., 2011). Anthropogenic sources of metals are derived from mining, petrochemical industries, printing, electronic industry, and municipal waste, which are ultimately discharged into the marine environment (Wang et al., 2013). Further, heavy metals polluting water inhibit growth, body size, and reproduction of fishes (Sarnowski and Jezierska, 2007). Although many coastal countries depend on desalinated seawater as a source of potable water for domestic and industrial use, heavy metals are considered as serious pollutants of the aquatic environments due to their accumulative behavior (Abdolahpur Monikh et al., 2015; Forouhar Vajargah et al., 2018). The chemical and biological analysis methods for tracing environment pollution efficiently have increasing the importance of environmental waste management (Allah et al., 1997). Different animals and plants (e.g.,

seaweeds) are often used as bio-indicators for checking the quality of effluent and surface water (Trifanet al., 2015).

Persian Gulf, bordered by several wealthy countries, is one of the most important traditional marine regions with 800 oil and gas platforms (Srinivasan and Swain, 2007). Over the past five decades, the gulf has been the main pathway for oil transportation and was damaged by oil leakages (Kazemi et al., 2012), which may potentially have destructive effects on its marine ecosystem. Furthermore, the increasing trend of urbanization and industrialization polluted the coastline by heavy metals (Kazemi et al., 2012). Although little is known about the toxicity of metals in seaweeds and their health risks, several studies have been conducted on different aspects of seaweeds in Persian Gulf and the Gulf of Oman (Sohrabipour et al., 2004; Jasbi et al., 2013; Moein et al., 2015; Pirian et al., 2018). The present study investigated the heavy metal absorption level in 12 seaweed species collected from geographically distinct sampling sites in the Persian Gulf and the Gulf of Oman.

Material and Methods

Sampling

First, 12 seaweed species including green, brown, and red algae were collected from different intertidal regions of the Persian Gulf and the Gulf of Oman (Table 1 and Fig. 1) from May to June 2018. Species identification was carried out using standard keys (Bellorin et al., 2008; Sohrabipour and Rabiei, 2008; Kokabi and Yousefzadi, 2015).

The seaweed specimens were thoroughly washed with tap water. Then, all epiphytes and organic and inorganic debris were manually removed. Next, all samples were rinsed in distilled water and air-dried (24 ºC) for 72 h. Finally, the dried samples were stored at -20 ºC for further analyses.

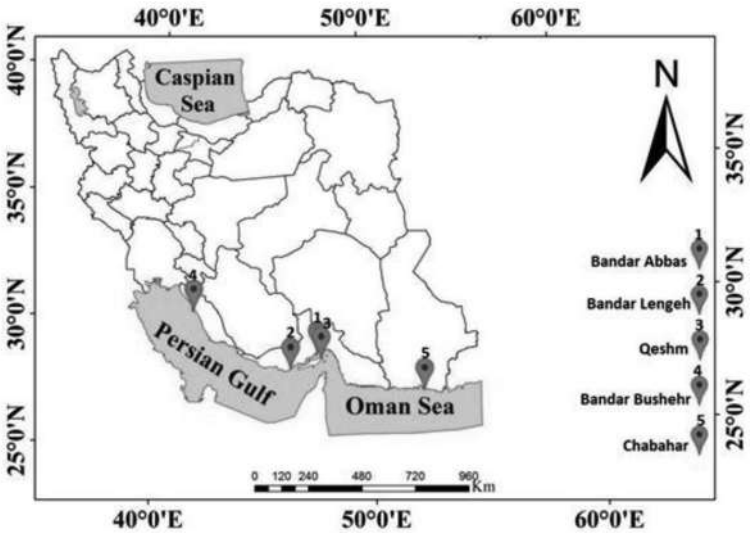

Fig. 1. Map of the five regions in the south of Iran where seaweed species were collected

Table 1. List of seaweed species, taxonomical status, and geographical data of the collection sites

No	Species	Phylum	Province	Local	Longitude (E)	Latitude (N)
1	*Hypnea* sp.	Rhodophyta	Bushehr	Bushehr	E50.83	N28.95
2	*Champia glublifera*	Rhodophyta	Bushehr	Bushehr	E50.83	N28.95
3	*Ulva* sp.	Chlorophyta	Sistan	Chabahar	E60.64	N25.29
4	*Gracilariopsis persica*	Rhodophyta	Hormozgan	Qeshm Island	E56.16	N26.57
5	*Gracilaria follijfera*	Rhodophyta	Bushehr	Bushehr	E50.83	N28.95
6	*Sargassum boveanum*	Phaeophyta	Hormozgan	Bandar-e-Lengeh	E54.30	N26.18
7	*Padina gymnospora*	Phaeophyta	Hormozgan	Qeshm Island	E56.16	N26.57
8	*Dictyota* sp.	Phaeophyta	Hormozgan	Qeshm Island	E56.16	N26.57
9	*Sargassum boveanum* var. *aterrimum*	Phaeophyta	Bushehr	Bushehr	E50.83	N28.95
10	*Padina gymnospora*	Phaeophyta	Bushehr	Bushehr	E50.83	N28.95
11	*Sargassum angustifolium*	Phaeophyta	Hormozgan	Bandar-e-Lengeh	E54.30	N26.18
12	*Sargassum boveanum*	Phaeophyta	Hormozgan	Bandar Abbas	E56.27	N27.18

Heavy metal analysis

Samples were dried in the oven at 155 ºC for 30 minutes (Rattanasomboon et al., 2018). Dried and finely ground seaweed materials (0.5 g) were transferred into poly tetra fluoroethylene digestion vessels, and 5.0 ml of concentrated HNO3 (ultrapure 65%) was added and incubated at ambient temperature for 2 hours. Then, the samples were heated for 5 hours at 100 ºC until 1 ml of the acid remained. Next, the digested cool solutions were filtered by Whatman filter paper (No 41) and transferred to polypropylene volumetric tubes. Ultra-pure deionized water was added to make up 50 ml volume for instrumental analysis in three replicates. The blank was prepared based on Trifan et al.'s method (2015). The concentration of

six heavy metals including Zn, Pb, Cu, Ni, Cd, and Fe^{+2} was measured using the atomic absorption device (Thermo Electron Corporation, S series, UK). The multiparameter analyzer (HACH, HQ 40 d, USA) and refractometer (ATAGO, S/Mill-E, Japan) were used for seawater analysis, pH, salinity, and EC parameters.

Statistics analysis

One-way ANOVA was used to compare significant differences using SAS software (Version 9.4) (SAS, 1998), and mean grouping was performed by the Least Significant Difference (LSD) test ($P < 0.05$).

Results

Average uptake concentration of Zn, Pb, Cu, Ni, Cd and Fe were significantly different among 12 studied species. The F value, df, and mean square of error for each element were as follows: $F_{11,0.005}$= 92708.9, $F_{11,0.000}$= 54626.9, $F_{11,0.001}$=91288.3, $F_{11,0.000}$= 4496553, $F_{11,0.002}$= 4626.06, and $F_{11,0.09}$= 3.66 for Zn, Pb, Cu, Ni, Cd, and Fe, respectively ($P \leq 0.0001$) (Table 2).

S. angustifolium collected from Bandar-e-Lengeh and *G. persica* collected from Qeshm Island had the highest (58.46 ppm) classified in group A, and the lowest Zn content (19.67 ppm) was placed in class L, respectively (Table 3 and Fig. 2).

The *Dictyota* sp. collected from Qeshm Island had the highest Pb content (19.17 ppm) and bunched in class A. The species *G. folliifera* collected from Bushehr with 7.41 ppm Pb concentration had the lowest content of Pb and grouped in class K (Table 3 and Fig. 3).

Table 2. ANOVA analysis data on seaweed heavy metals content

Source	Zn	Pb	Cu	Ni	Cd	Fe
MS	523.03	48.70	140.73	3085.13	11.91	3143552.95
SS	5753.36	535.79	1548.09	33936.48	131.03	34579082.42
Df	11	11	11	11	11	11
Error	0.005	0.000	0.001	0.000	0.002	0.09
F. value	92708.9***	54626.9***	91288.3***	4496553***	4626.06***	3.66***
C.V	0.17	0.22	0.17	0.07	0.77	0.02

MS, SS, Df and C.V, means: Mean of scure, Sum of Scure, Degree of Freedem, Coefficient of variation.

S. boveanum and *S. angustifolium* collected from Bandare-Lengeh had the highest (32.44 ppm) and lowest (11.17 ppm) Cu content and were grouped in class A and L, respectively (Table 3 and Fig. 4).

The highest and lowest content of Ni was

Table 3. Mean (±SE) concentration of six heavy metals in the red, green, and brown seaweed species collected from the Persian Gulf and the Gulf of Oman

Loc	Samples	Zn (ppm)	Pb (ppm)	Cu (ppm)	Ni (ppm)	Cd (ppm)	Fe (ppm)
B*	*Hypnea* sp.	53.34±0.06^{c}	17.64±0.00^{c}	26.06±0.02^{e}	100.41±0.00^{b}	6.4±0.04^{b}	2556.0±0.47^{c}
B	*C. glublifera*	57.516±0.02^{b}	14.53±0.00^{e}	24.49±0.02^{f}	32.38±0.00^{e}	6.39±0.00^{b}	2447.15±0.00^{d}
C	*Ulva* sp.	26.65±0.03^{k}	11.24±0.00^{h}	27.49±0.01^{d}	14.02±0.03^{k}	6.05±0.00^{c}	440.57±0.00^{j}
QI	*G. persica*	19.67±0.03^{l}	9.24±0.01^{i}	15.71±0.02^{j}	15.79±0.02^{i}	6.07±0.00^{c}	196.08±0.00^{l}
B	*G. folliifera*	30.16±0.04^{j}	7.41±0.00^{k}	11.74±0.01^{k}	17.37±0.00^{h}	5.75±0.00^{d}	776.65±0.00^{g}
BL	*S. boveanum*	33.37±0.00^{h}	16.90±0.03^{d}	**32.44±0.00^{a}**	29.49±0.00^{g}	5.48±0.00^{ef}	926.1±0.04^{f}
QI	*P. gymnospora*	31.53±0.01^{i}	13.32±0.00^{g}	29.15±0.01^{b}	**105.97±0.00^{a}**	5.53±0.00^{e}	644.74±0.00^{i}
QL	*Dictyota* sp.	48.8±0.04^{f}	**19.17±0.00^{a}**	19.20±0.00^{i}	35.99±0.00^{c}	**10.7±0.04^{a}**	**2843.96±0.00^{a}**
B	*S. boveanum* var. *aterrimum*	49.73±0.00^{e}	18.39±0.02^{b}	23.72±0.00^{g}	30.03±0.00^{f}	10.66±0.00^{a}	1906.16±0.00^{e}
B	*P. gymnospora*	40.01±0.04^{g}	14.36±0.00^{f}	23.21±0.00^{h}	34.34±0.00^{d}	5.19±0.00^{g}	2606.78±0.00^{b}
BL	*S. angustifolium*	**58.46±0.03^{a}**	8.37±0.00^{j}	11.17±0.01^{l}	12.89±0.00^{l}	4.8±0.04^{h}	231.3±0.04^{k}
BA	*S. boveanum*	52.48±0.00^{d}	11.21±0.00^{h}	27.86±0.03^{c}	15.67±0.00^{j}	5.41±0.00^{f}	772.6±0.04^{h}

The means with bold letters in each column show significance difference (P≤ 0.5) according to the LSD test. *means; Bushehr (B), Bandar Abbas (BA), Bandar-e-Lengeh (BL), Qeshm Island (QI), and Chabahar (C).

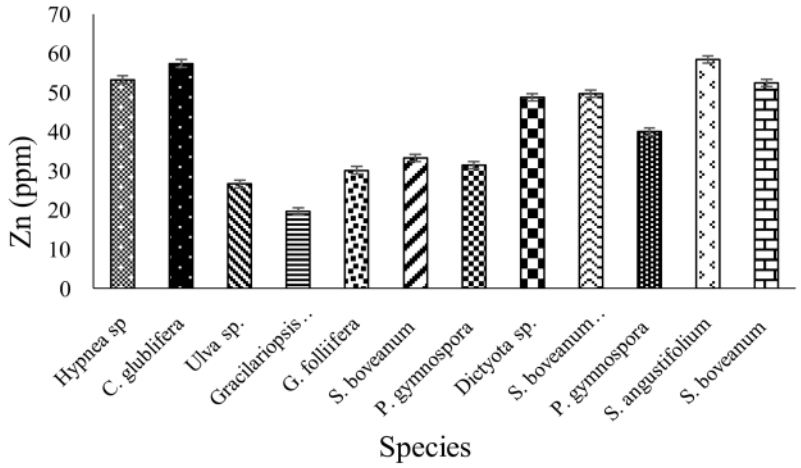

Fig. 2. Variation in Zn concentration in studied species

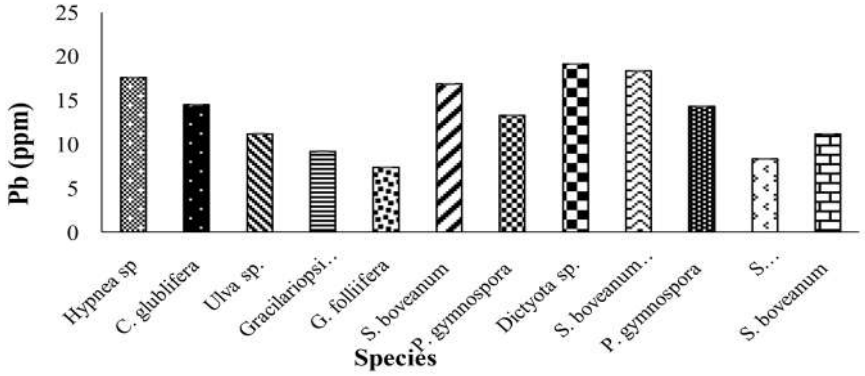

Fig. 3. Pb concentration level in studied species

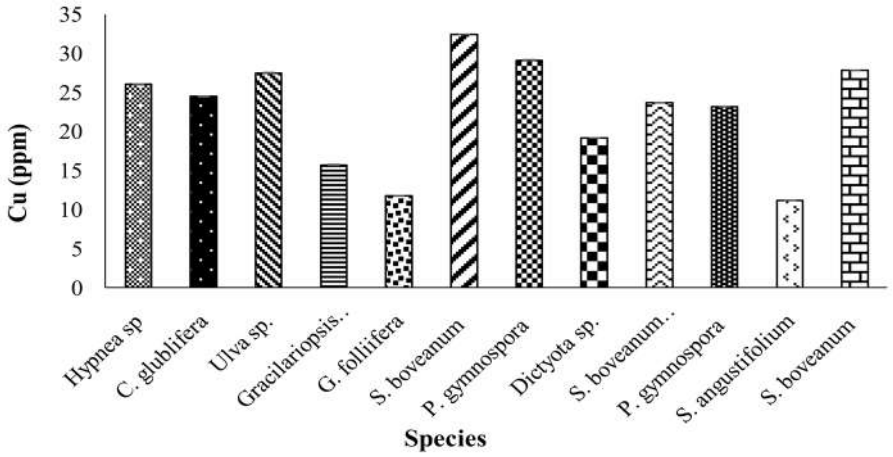

Fig. 4. Cu concentration in studied seaweeds

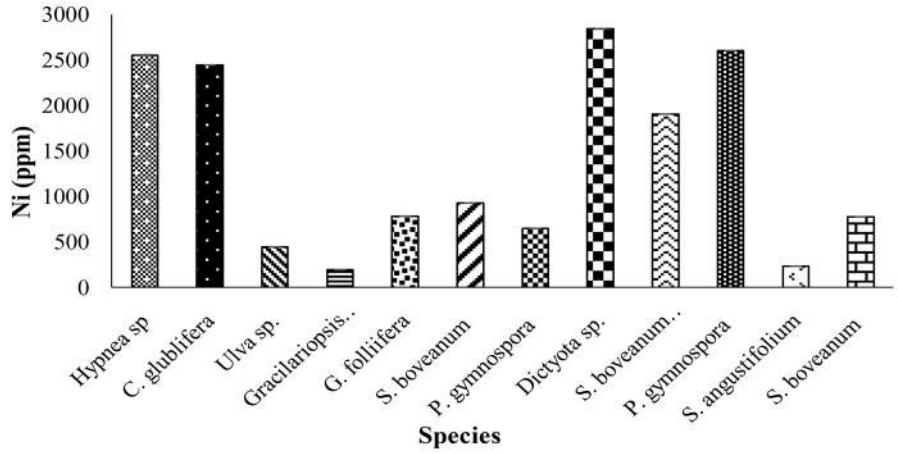

Fig. 5. Ni concentration in different seaweed species

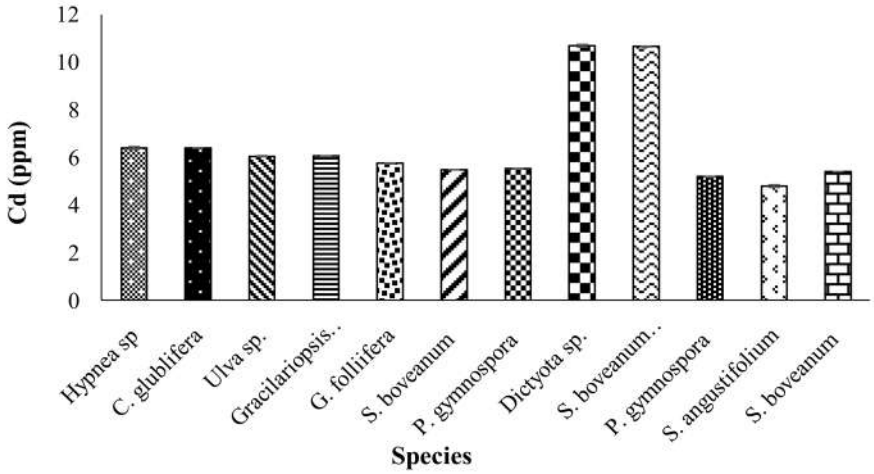

Fig. 6. Cd concentration in different seaweed species.

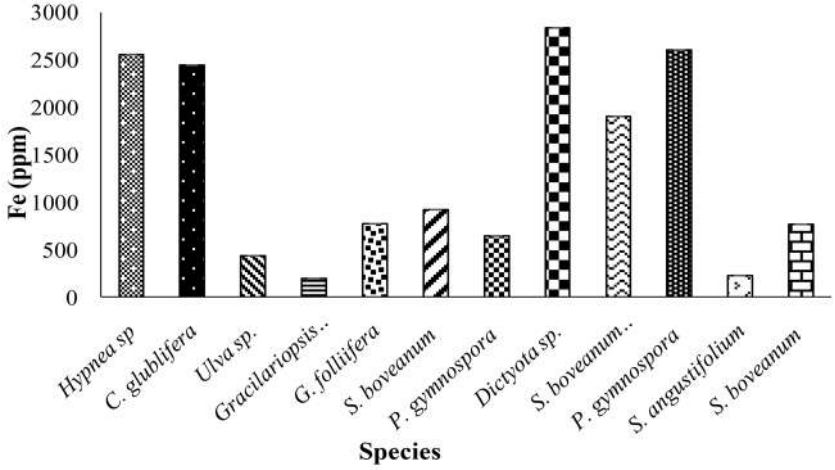

Fig. 7. Iron (Ferro) concentration in studied species

observed in *P. gymnospora* (105.97 ppm) and *S. angustifolium* (12.89 ppm) collected from Qeshm Island and Bandar-e-Lengeh, respectively (Table 3 and Fig. 5).

However, *Dictyota* sp. and *S. boveanum* var. *aterrimum* collected from Bushehr had the highest content of Cd (10.7 and 10.66 ppm, respectively) and were placed in class A. *S. boveanum* collected from Bandar Abbas (4.8 ppm) had the lowest content and was placed in class H (Table 3 and Fig. 6).

Accordingly, the descending order of Fe concentration was observed in *Dictyota* sp. (2843.96 ppm), *P. gymnospora* collected from Bushehr (2606.78 ppm), *Hypnea* sp. (2556 ppm), *C. glubifera* (2447.15 ppm), *S. boveanum* var. *aterrimum* (1906.16 ppm), *S. boveanum* collected from Bandar-e-Lengeh (926.1 ppm), *G. folliifera* (772.6 ppm), *P. gymnospora* collected from Qeshm Island (644.74 ppm), *Ulva* sp. (440.57 ppm), *S. angustifolium* (231.3 ppm) and *G. persica* (196.08 ppm), respectively (Table 3 and Fig. 7).

Among the studied heavy metals, Fe had the highest concentration and variance in concentration (196.08-2843.96 ppm) followed by Ni (12.89-105.97 ppm), Zn (19.67-58.46 ppm), Cu (11.17-32.44 ppm), Pb (7.41-19.17 ppm), and Cd (4.8-10.7 ppm) (Table 3 and Figs. 2-7). While the lowest levels of EC (52.8 ms/cm) and salinity (37/6 PPT) were measured in Chabahar, the highest levels of EC (62.7 ms/cm), salinity (42 PPT), and pH (8.27) were observed in Bushehr (Table 4). Table 5 presents the comparison of the results of the current study with previous studies.

Discussion

The present study provided valuable information related to heavy metal contamination in some marine seaweed species collected across Iranian coastlines of the Persian Gulf and the Gulf of Oman. The results showed that Fe, Ni, Zn, Pb, Cu, and Cd were found in all of the studied seaweed species. The content of metals in seaweed tissues varied from 4.8 to 10.7 ppm for Cd, 11.17 to 32.44 ppm for Cu, 8.37 to 19.17 ppm for Pb, 19.67 to 58.46 ppm for Zn, 12.89 to 105.97 ppm for Ni, and 196 to 2843.96 ppm for Fe. Juma and Al-Madany (2008) examined seawater contamination in the territorial water of the Kingdom of Bahrain (Persian Gulf) and observed 5 ppm of Cd, 5 ppm of Cu, 25 ppm of Pb, 40 ppm of Zn, 30 ppm of Ni, and 1000 ppm of Fe content in seawater. The results are consistent with the published data of the United States Environmental Protection Agency (USEPA), reporting 8.8 ppm of Cd, 3.1 ppm of Cu, 8.1 ppm of Pb, 81 ppm of Zn , 610 ppm of Ni, and 300 ppm of Fe. Al-Abdali et al. (1996) studied the bottom sediments of the Persian Gulf and found that the amounts of all metals were within the permissible range (Zn = 0-60, Pb = 15-30, Cd = 1.2-2.0, Ni = 70-80, Fe = 10,000-20,000 and Cu = 15-30 ppm). In addition, the content of Cd in shallow water was higher than those at the bottom of the sea. In another study, Janadeleh and Jahangiri (2016) investigated the heavy metal concentrations in sediments and fish

Table 4. Seawater analysis in different collection sites

Region	EC (ms/cm)	pH	Salinity (PPT)
Bandar-e-Lengeh	56.6	7.97	38.4
Chabahar	52.8	8.1	37.6
Bushehr	62.7	8.27	42/0*
Qeshm Island	56.6	7.94	38.9
Bandar Abbas	56.9	7.49	39.3

*Samples with ≥40 PPT salinity, were detected by refractometer device.

Table 5. Heavy metal concentrations (ppm) in sea sediments from different regions in the Persian Gulf compare with the present study

Area	Source	Zn	Pb	Cu	Ni	Cd	Fe	Reference
Pakistan	Sediment	-	7.9	24.40	76.40	0.44	-	(Tariq et al. 1993)
Kuwait	Sediment	-	1.03	1.20	15.00	0.77	-	(Fowler et al. 1976)
Saudi Arabia	Sediment	-	1.70	3.24	13.80	0.14	-	(Fowler et al. 1976)
Kish Island	Sediment	-	4.22	3.25	5.48	0.28	-	(Dadolahi et al. 2011)
Khoremosa	Sediment	-	39.55	27.48	26.20	0.61	-	(Karbassi 1998)
Bahrain	Seawater	40	25	5	30	5	1000	(Freije 2018)
Bushehr	Sediment	11.45	2.77	5.5	13.4	0.18	5360	(Bibak et al. 2018)
Hormuz strait	Sediment	112.3	10.12	-	42.38	0.43	22400	(Janadeleh and Jahangiri 2016)
Bushehr	Sediment and seaweed	-	1.2	3.88	9.88	0.36	-	(Amini 2020)
Present study	Seaweed	58.46	19.17	32.44	105.97	10.7	2843.96	Present study

The amounts of the metals in the table are the maximum value measured in each study.

body of the Persian Gulf (Hormuz strait) and represented that the contents of Cd, Zn, Ni, Pb, and Fe were 0.12-0.43, 25.83-112.31, 24.63-42.38, 5.32-10.12, and 10800-22400 ppm, respectively. The consistency of the recent results with other findings (Table 5) determining pollution level in seaweeds can provide more information about contamination of seaweeds, especially those used for food and cosmetic purposes.

The comparison of the findings of this study with those of the previous studies indicated that the values of Ni and Cu increased significantly by increasing moisture-proof paints (Srinivasan and Swain, 2007). Dadolahi et al. (2011) studied the heavy metal absorption in seaweed and associated sediment harvested from the Strait of Hormuz and showed that the amounts of Pb, Cd, Cu, Ni, Zn, and Fe were 13.3-30.5, 0.7-7, 6.35-16.87, 21.46-71.6, 39.65-54.93, and 7441-14867 in different seaweed species, respectively. Indeed, the results confirmed the differences in metal contamination in different classes of dry seaweeds. For instance, Pb content was 23.95, 20.53, and 22.82 in Chlorophyta, Phaeophyta, and Rhodophyta, respectively. Cd content was 3.27, 5.09, and 5.05, while it was 11.95, 11.68, and 10.92 for Cu. Regarding Ni, it was 47.04, 40.72, and 52.98, while it was 45.99, 46.53, and 51.03 for Zn. Finally, it was 11550, 6371, and 11085 for Fe. In addition, the results of this study could confirm these achievements. Qari (2015) studied heavy metal contamination in *Padina pavonia* and *P. tetrachromatic* species and found that the level of Fe, Zn, Pb, Cu, Ni, and Cd was 0.25-1.64, 0.091-0.76, 0.25-0.63, 0.047-1.22, 0.037-0.42, and 0.017-0.38 ppm, respectively. Accordingly, the Persian Gulf was more polluted than the open seas.

The results also indicated a relationship between contamination level in seaweeds and the collection site. For instance, *S. boveanum* collected from Bandar-e-Lengeh and Bandar Abbas had different levels of contamination, which were also true for *P. gymnospora* collected from Qeshm Island and Bushehr. Additionally, the results revealed that brown macroalgae (Phaeophyta) had a higher level of contamination compared with green (Chlorophyta) and red (Rdodophyta) macroalgae (Dadolahi et al., 2011; Qari, 2015). Additionally, contamination level was different based on the seaweed species. For instance, *S. angustifolium, S. boveanum* var. *aterrimum*, *Dictyota* sp., *S. boveanum*, and *P. gymnospora* showed a higher level of concentration for all six heavy metals while *G. persica, G. folliiferda,* and *Ulva* sp. had the lowest level of Fe, Ni, Pb, and Zn.

Furthermore, the findings indicated contamination variability in the same seaweed species collected from different location, while Fe and Ni had the highest rate in *Dictyota* sp., *P. gymnospora*, *Hypnea* sp., and *C. glublifera* had only high content in *Hypnea* sp. and *P. gymnospora* collected from Qeshm Island. Further, *Sargassum boveanum, S. angustifolium, Hypnea* sp., *C. glublifera, P. gymnospora*, and *Dictyota* sp. could greatly uptake Zn. Furthermore, Cd was more accumulated in *Dictyota* sp. (10.7 ppm), and high

concentration of Pb was observed in *Dictyota* sp. *Sargassum boveanum* var. *aterrimum, P. gymnospora*, and *S. boveanum* exhibited higher levels of Cu. Additionally, the brown seaweed species were more efficient biosensors for studying Cu and Pb (Figs. 3 and 4). The findings are consistent with the results of a study by Hiroyuki (2015) who found *P. gymnospora* was an effective biosensor for Cu and the concentration of heavy metals (Cu, Fe, and Pb) was different in either different seaweed species or collection sites. For instance, the concentration of Cu ranged from 1 to 20 ppm in brown seaweeds and 2 to 40 ppm in red seaweeds. Regarding the collection site, the Cu content ranged from 3 to 20 in green seaweed collected from the north and south of Kii Peninsula and 2 to 80 ppm in green seaweeds in Kanayama. Additionally, the high concentration of Cu in marine ecosystems made the environment unsuitable for fishes, seaweeds, planktons, and other marine organisms. This contamination may be caused by oil-producing and shipping companies, antifouling coating paints, and industrial and agricultural centers (Giusti, 2001; Caliceti et al., 2002).

The concentration value of Pb varied from 0.2 to 20, 0.2 to 10, and 2 to 40 ppm in the same seaweed species collected from different collection sites (Hiroyuki, 2015). This difference was observed in the present study based on seaweed species and collection site. For example, *S. boveanum* collected from Bandar-e-Lengeh absorbed more concentration of Pb, Cu, Ni, and Fe than *S. boveanum* collected from Bandar Abbas. Additionally, Zn content in *S. boveanum* from Bandar Abbas was higher than the species from Bandar-e-Lengeh. However, the concentration of Cd in the same species collected from these two sampling sites remained unchanged.

In addition, some environmental factors such as salinity, pH, light intensity, and metabolic factors can affect the concentration of heavy metals in seaweeds (Zbikowski et al., 2006). For instance, Fe^{+2} contents in *P. gymnospora*, collected from Qeshm Island (644 ppm), were much lower than those collected from Bushehr (2606 ppm). In fact, the values of salinity in these two sampling sites were different, which could affect the results (Qeshm Island =36.8 PPT and Bushehr =42.0 PPT) (Table 4). Furthermore, metal pollution may affect other heavy metal contamination, which is considered as an important issue confirmed based on the results of the present study (Andrade et al., 2004). Foster (1993) reported that increased Zn contamination inhibited Cd uptake by seaweeds due to competition for binding sites. In other words, a positive or negative relationship may be observed between the uptakes of heavy metals in different seaweed species (synergistic or antagonistic interactions of ions in binding with the anionic sites). Furthermore, variation in concentration of different heavy metals may be due to the electronegativity values of metals. Fe, Ni, Zn, and Cu had higher electronegativity values and concentration than Cd. However, the high contents of Fe and Zn in the studied species could be related to the necessity of these elements for seaweeds, which is in line

with those of Dadolahi et al. (2011).

In addition, a relationship was reported between seaweed species and the concentration of heavy metals, which may be related to the morphological attributes of seaweed species. For instance, the filamentous seaweeds (*G. persica* and *G. folliifera*) represented lower metal uptake than membranous and thick-wall species such as *S. boveanum* and *P. gymnospora* (Trifan et al., 2015). Trifan et al. (2015) confirmed that filamentous seaweed species, *Enteromorpha intestinalis*, *Cladophora vagabunda*, *Ceramium rubrum*, and *Phyllophora pseudoceranoides* were able to uptake more Ni, Hg, Zn, Cd, Cu, Cr, Mn, and Pb than other morphological types of seaweed species. They suggested that these species were widely distributed and could be used as bio-indicators for assessing heavy metal contamination along sea coastlines.

During the recent decades, water sampling has been a common approach for tracing heavy metals contamination in seas and oceans (Hiroyuki, 2015). Based on this approach, a large number of sampling should be considered, which is a labor-intensive procedure. Furthermore, the results in the water sampling approach may change based on the variation in tidal range, temperature, salinity regimes, dissolved nutrients, geological structure of the study area (Dadolahi et al., 2011), sampling method, and sampling time or sampling site, which may lead to an inaccurate estimation about pollution level (Hiroyuki, 2015). Consequently, seaweed-based tracing of heavy metals is an easier and more cost-benefit approach which can be replaced by other sampling approaches. Based on the results, seaweed species can be used as worth biosensors for the assessment of heavy metal contamination. Thus, this information can be utilized as a benchmark for further studies on heavy metal contamination of the seaweed species in the Persian Gulf and the Gulf of Oman.

It is evident that the endophytic bacteria, which are related to marine plants, can induce resistance to heavy metals in hosts. For example, *Shewanella* sp. and *Idiomarina* sp., which were isolated from red seaweeds, can reduce the uptakes of several metal ions (Konishi et al., 2007; Seshari et al., 2012; YokeshBabu et al., 2014). Additionally, the association of endophytes with different seaweed species reported in previous studies confirmed the important role of these microorganisms in seaweeds (Suryanarayanan, 1992; Devarajan and Suryanarayanan, 2002; Flewelling et al., 2015; venkatachalam et al., 2015; Kaaria et al., 2015). It seems that these microorganisms could act as a deterrent and decrease metals uptake by seaweeds (YokeshBabu et al., 2014). This study provided insights into the Iranian seaweed species, which can be properly used as a metal biosensor. Based on the findings, it is strongly recommended that seaweed species be used as proper biosensors to trace other heavy metals not included in this study.

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Study of Photosynthetic System Fluidity and Long-term Growth Caused by Salinity in Cyanobacterium *Fischerella* sp. FS 18

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Abstract

The present study examined the responses of different parts of the photosynthetic system in *Fischerella* sp. FS 18 based on different concentrations (17, 80, and 160 mM) of sodium chloride and short time at regular intervals of 20, 40, and 60 min. The results revealed that salinity application (80 and 160 mM) after 20 minutes of inoculation significantly increased the yield of phycobilisome system. Increasing the time up to 40 minutes after inoculation could restore all parts of the photosynthetic system. Then, cyanobacteria can rearrange and activate photosystem II, phycobilisome, and light-collecting complex. However, the behavior of cyanobacteria at salinity of 160 and 80 mM were opposite at 20 and 60 min. Compared to the untreated sample, pretreatment application within less than one hour changed in terms of growth rate and attenuation at time intervals of 24 and 96 hours. The sample was capable of moderating the destructive effects of 160 mM for 20 min and 80 mM in 60 min treatments over 24 hours, which is incomplete. The growth rates up to 96 h in 80 mM for 20 min and 160 mM for 60 min treatments were higher than those without salinity. While the system changed its pattern after 24 hours, the initial pattern remained unaffected by time and salinity levels after this time. In general, simple salinity pretreatments and very short times increased the efficiency of energy transfer in photosystems and produced short and long-term energy and reduction, which could be considered as a major advantage for biotechnology of mass crops.

Keywords: Ecophysiology, Pretreatment, Cyanobacteria, Salinity, *Fischerella* sp. FS 18

Introduction

Cyanobacteria appeared in Precambrian (Schirrmeister et al., 2015; Gérard et al., 2018; Mloszewska et al., 2018). Precambrian can be considered chaotic in terms of environmental conditions with two characteristics: a) the unpredictable interaction including environmental factors in which the intertwined change network is formed, and b) the role of very short times in the formation of this change network. The life span of cyanobacteria, which is measured by human

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time, is short. Therefore, these organisms should complete their growth and reproduction within this short time frame. If the time is generalized to a whole chaotic period like Precambrian (i.e., when things were radically changing at any given moment), the importance of short times would become clearer (Abbasi et al., 2019; Andrault et al., 2018; Shokravi et al., 2014). Seymour et al. (2005) studied marine cyanobacteria and maintained that time in seconds and minutes could cause a complete change in the ecological behavior of these organisms. It is currently not possible to simulate a network of complex changes during Precambrian. Factors such as salinity, alkalinity, acidity, temperature, and light were introduced as environmental shocks (Amirlatifi et al., 2018; Shokravi et al., 2010). To the best of our knowledge, no study has been performed on stegonmatical cyanobacteria in the world for less than an hour. The only studies performed in this field were Amirlatifi et al. (2018) and Abbasi et al. (2019), which were related to cyanobacterum *Calothrix* sp. FS 65 and *Fischerella* sp. FS 18 and performed at 24 and 96 hours. Although they used two 24 and 96 h time periods, the results revealed that the photosynthetic system in these cyanobacteria could be affected by shorter time periods. The overlapping absorption spectra represented that the photosystem yield (especially Photosystem II and phycobilisome) significantly changed under 24-hour intervals. However, there were noticeable changes in fluorescence spectra and photosystem ratios in 24 h periods, which were particularly pronounced in combination with alkalinity (Abbasi et al., 2019). Accordingly, the present study aims to analyze the effects of time and environmental factors since pretreatments can change cyanobacteria acclimation and compatibility conditions. Nowadays, under 30-min pretreatments are often used to change the compatibility of bacteria such as Salmonella (Shigenobu et al., 2004). In the present study, the effect of pretreatment on growth behaviors of *Fischerella* sp. FS 18 and a model is considered as the first step in examining very less than 60 minutes times. The hypothesis is that 'pretreatments are recorded in the cyanobacterial memory system'. Hence, the results can be applied for changing growth behaviors and mass cultures (Amirlatifi et al., 2013, 2018; Fraser et al., 2013; Wang et al., 2011).

Materials and method

Fischerella sp. FS 18 was obtained from algal museum of Islamic Azad University of Gorgan Branch. The information on collection site and techniques was provided by Soltani et al. (2012). The identification and purity were determined using light and fluorescence microscopy based on some researchers (Soltani et al., 2009; Amirlatifi et al., 2018; Desikachary, 1959; Prescott, 1962; John et al., 2003). The species was cultured on BG0-11 solid and liquid media at 60 µmol photon.m^{-2}.s^{-1}, 28 °C, and pH 7.8 (Soltani et al., 2009). After initial growth, the isolated samples were placed under 2 µmol photon.m^{-2}.s^{-1} and at different concentrations (17, 80, and 160 mM NaCl). After 20, 40, and 60

minutes, *Fischerella* sp. FS 18 was removed from the media, centrifuged, and washed. The absorption spectra were analyzed in the visible range as an overlap by CECIL spectrophotometer model 740 CE. Extraction and full intact sample methods were used for analyzing in vivo overlap. Acetone, methanol, and ethanol were used as solvent, where the results of the primary absorption spectra confirmed the effectiveness of acetone. In order to increase the accuracy of the results, protein adsorption was measured and subtracted from total absorption, and the result was divided into protein adsorption (Tang and Wincent, 1999). The growth rate and attenuation time were measured based on Poza-Carrion et al. (2001) and Swapnil et al. (2018) by turbidity meter. Following Amirlatifi et al. (2018) and Abbasi et al. (2019), the statistical analysis was performed using SPSS (Version 11) for standard data based on Poza-Carrion et al. (2001) and RSP (Version 10) (Ghobadian et al., 2015).

Results

The absorption rates were compared at different times and salinity of 80 mM (Fig. 1). The short-term salinity (20 minutes) and medium-term salinity (40 minutes) in the photosystem II, phycobilomes, and light-collecting antennas had a stimulating effect on long-term salinity (60 minutes). No significant difference was observed the interval between 20 and 40 minutes after inoculation. However, the state of the photosynthetic system was modified 40 minutes after inoculation in 80 mM salinity. In other words, cyanobacteria are sensitive to the combination of salinity and time and alter their state of photosynthesis system 40-60 minutes after the effect of salinity. It is noteworthy that chlorophyll uptake was not abnormal in cyanobacteria at the range of 660-670 nm (Cai et al., 2015).

In addition, the 160 mM salinity increasing during two times makes the situation quite different mainly at 20 and 60 minutes after inoculation (Fig. 2). It seems that 160 mM

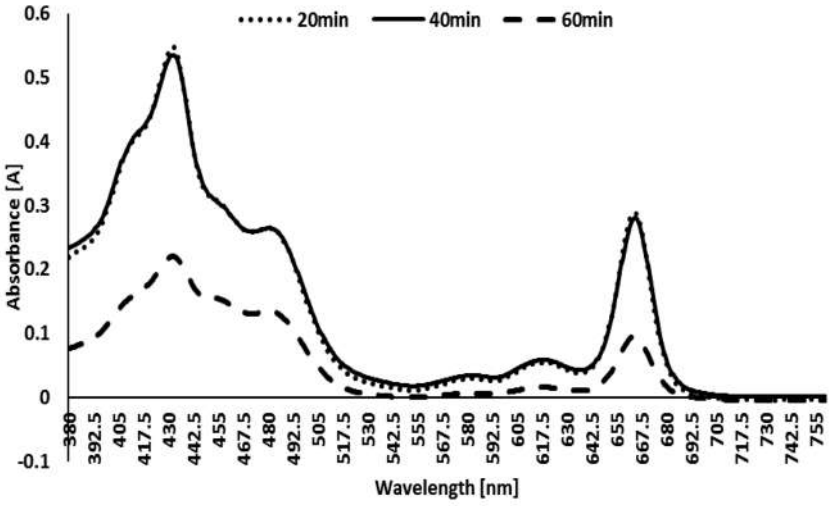

Fig. 1. Comparative study of absorption rate in acetone extract at 80 mM salinity and different times after inoculation

in a short time (20 min) can prohibit all parts of the photosynthetic system, particularly when phycobilisome is lost. Furthermore, increasing the time up to 40 minutes after inoculation can restore all parts of the photosynthetic system. After this period, cyanobacteria are able to rearrange and activate the photosystem II, phycobilisome, and light-collecting complex. However, increasing the time up to 60 minutes has a negative effect on carotenoid and a positive effect on the red area of chlorophyll. Phycobilisome and photo-systems are generally less sensitive to time fluctuations.

Thus, the photosynthetic system in the cyanobacteria was affected by the combination of time and salinity. Thus, changes depending on both variables (particularly 20 and 60 min time intervals) were affected by 80 and 160 mM salinity. The 40-minute time requires some of these two effects and brings the patterns of behavior closer together.

Table 1 shows the state of the progressive phase of growth under pre-treatment conditions. As can be seen, 60 min pretreatment at 160 mM salinity increases the production of live material by approximately 10%, which is a significant practical achievement. In addition, this property is affected by time before it is caused by salinity. When the pretreatment time decreases from 60 to 20 minutes, a decrease occurs in the pretreatment in the progressive phase of growth (Table 1). The pretreatment condition is similar to 80 mM salinity. It seems that increasing time could increase the progressive phase and the reproductive rate. The effect of time is significant at high salinity pretreatment. When the time decreases from 60 to 20 minutes, the effect is completely reversed and the highest intensity of the progressive phase reduces to the lowest level.

To study the effect of different salinity concentrations, the impact of 17 mM NaCl on growth rate was investigated and 24 (short term) and 96 hours (long term) were determined. The comparison of pre- and non-pretreatment samples is presented at two 24-hour times (Table 1) and at 96 hours (Table 2), respectively.

Table 1. The effect of different short-term pretreatments on growth rate and attenuation time after 24 hour (The number in brackets represents treatment for minutes)

Salinity (mM)	Time (min)	Growth rate (μ)	Generation time (G) (Day)
17		0.11±0.01	6.27
80	20	0.31±0.09 (0.38)	2.22 (1.81)
160		0.08±0.02	8.62
17		0.11±0.01	6.27
80	60	0.04 ± 0.01	17
160		0.28±0.06 (0.31)	2.43 (2.22)

Table 2. The effect of different short-term pretreatments on growth rate and attenuation time after 96 hours (The number in brackets represents treatment for 40 minutes)

Salinity (mM)	Time (min)	Growth rate (μ)	Generation time (G) (Day)
17	20	0.14±0.02	4.93
80		0.14 ±0.09 (0.24)	4.93 (2.87)
160		0.11±0.01	6.27
17	60	0.15±0.02	4.6
80		0.11 ± 0.01	6.27
160		0.16 ± 0.04 (0.22)	4.31 (3.14)

The results of the growth rates at two time periods (Figs 3 and 4) reveal that under non-saline condition, the growth rate is 0.11 after 24 hours then increases to 0.14 at 96 hours. Treatments Under salinity 80 mM, at 20 min for up to 24 hours revealed growth rate 0.31. Over 24 hours, growth rate reached to 0.14. Under 80 mM salinity, treatments at 60 minutes demonstrated growth rate of 0.04 at 24 hours and increased after 96 hours up to 0.11. Treatments exposed to 160 mM salinity for 20 minutes showed growth rate 0.08 after 24 hours and when the time increased to 96 hours changes to about 0.11. Under 160 mM salinity, treatments at 60 minutes growth rate was 0.28 up to 24 h and by time increasing to 96 hours was reduced to 0.16.

Based on RSP analyses (Figs. 5 and 6) and the layering pattern of distribution, time and salinity in combination affect the specific growth rate. The distribution pattern at 24 hours (Fig. 5) has a regular pattern and diversifies at 96-hour treatment (Fig.6). The transition from 20 to 30 minutes and 50 to 60 minutes at 96 hours causes a critical increase/decrease in growth rate and matter production (opposite of 24 hours). While the acclimation of *Fischerella* sp. FS 18 at 96 hours reduces the growth rate, the sensitivity to the time increases and exhibits more severe reactions to time variations. Finally, interstitial salinity levels are critical at short time periods and more effective.

Discussion

Several studies investigated the impact of salinity on cyanobacteria while neglecting short time effects (Bajwa et al., 2015; Shamim et al., 2017; Swapnil et al., 2018; Li et al., 2019). Although this hypothesis was not examined on cyanobacteria, it is suggested that the application of short-term pretreatment times can stimulate the development of new features in the cyanobacterial photosynthetic system (Affenzeller et al., 2009; Hamilton et al., 2018).

The research on one hour of photosystems and

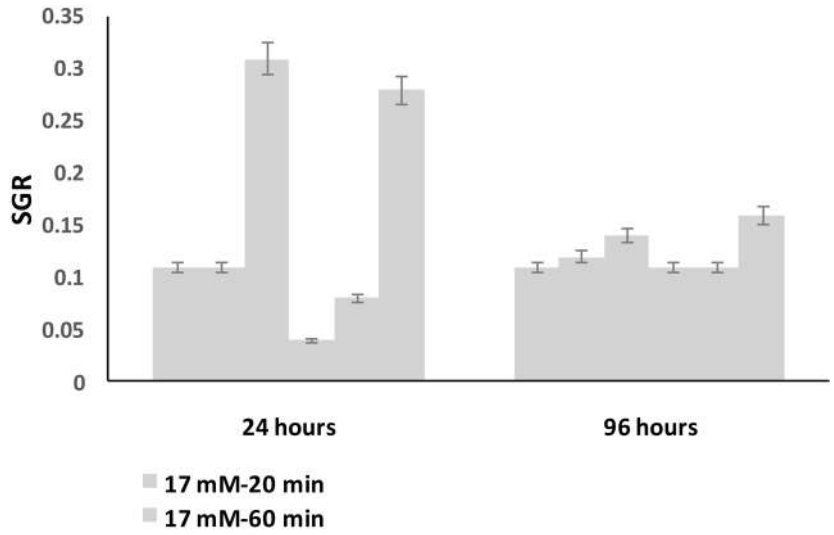

Fig. 3. Comparison of 17, 80, and 160 mM salinities on growth rate, at short-term pretreatments (20 and 60 min), after 24 and 96 hours inoculation.

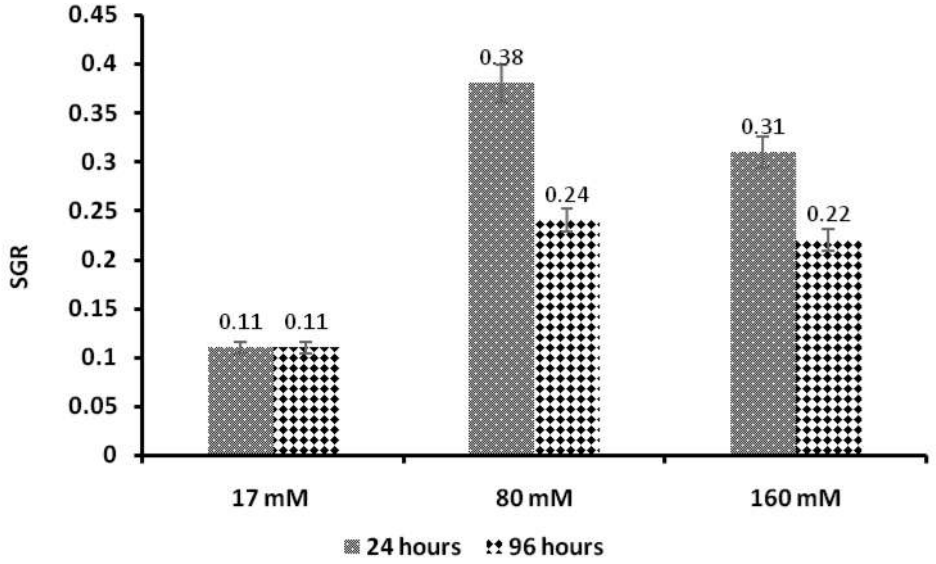

Fig. 4. Comparison the effect of 40 min pretreatments in 17, 80 and 160 mM salinities on growth rate, after 24 and 96 hours inoculation.

phycobilisome dynamics at 160 mM salinity indicated that (2x) affects the passage of time in phycobilisome and photosynthetic systems. Akulinkina et al. (2015) compared *Synechocystis* phycobilisome absorption rate at different times and found that the mutant had twice the amount of phycobilisome in comparison to the wild type. The effects of time duration were altered by increasing salinity. Furthermore, the yield was poor in the first 20 minutes, while the yield increased significantly after 20 minutes and up to 40 minutes in light-collecting antennas, phycobilisome, and photosystem II although it was unchanged at 60 minutes. However, the effect was diminished (for up to 40 minutes) in the light-collecting antennas and slightly increased in the red chlorophyll area. The phycobilisome was

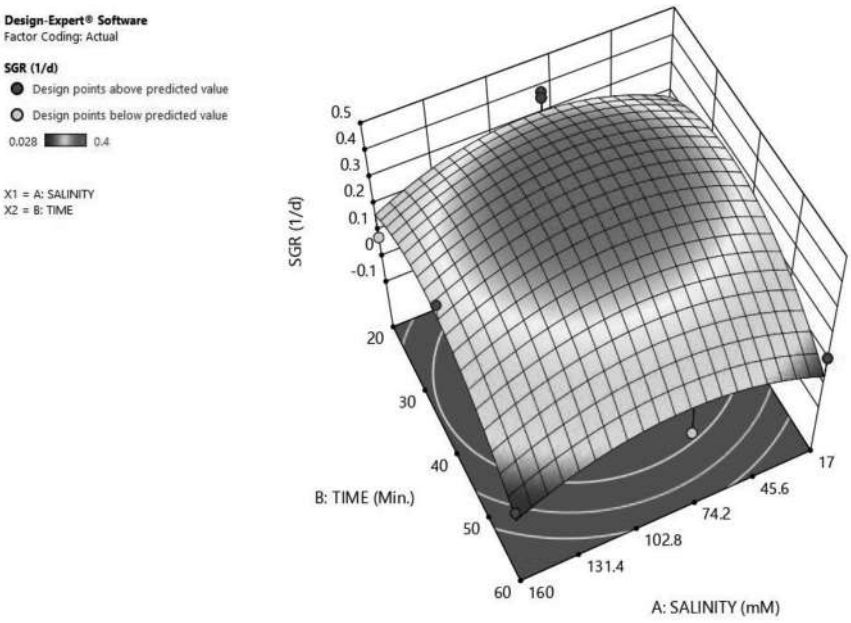

Fig. 5. RSP analysis of the growth rate at different pretreatment time and salinity after 24 hours.

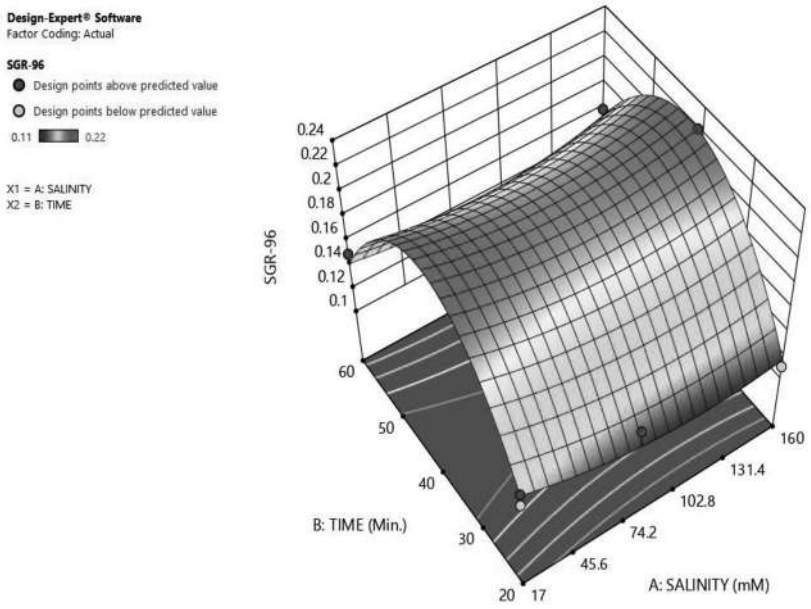

Fig. 6. RSP analysis of the growth rate in different pretreatment time and salinity after 96 hours in *Fischerella* sp. FS18.

similarly affected in 40 minutes and 60 minutes. Therefore, salinity increases at 20 minutes decreased while the yield decreased up to 40 minutes. The rearrangement is somewhat consistent in *Synechococcus* (Lefort-Tran et al., 1988). However, the phycobilisome part did not change by increasing 20 minutes to 60 minutes, and there was a slight change in the light collecting antennas in photosystem II. In general, phycobilisome, photosystem II,

and light-collecting antennas were damaged at 160 mM in the first 20 minutes. Further, photosynthesis happened by reducing the number of phycobilisomes per cell originating from reducing the level of photosynthetic thylakoids and a transformation in the internal structure of phycobilisome (Six et al., 2011). However, Six et al. (2011) studied the light problem and found that the effects on the light-collecting antennas decreased over the time and the damages were repaired. Furthermore, 160 mM salinity concentration was time dependent, especially in the first and second twenty-two minutes when the effect decreased while increased at the third. Based on the results, 40 minutes is the optimum time in 80 and 160 mM salinity concentration for photosynthesis efficacy. It seems that the behavior of cyanobacteria at salinity levels of 160 and 80 mM are different at 20 and 60 min. Further, 60 min photosynthesis is stimulated at 80, 20, and 160 mM salinity levels. The obtained results from the initial stages of the experiment were generally promising. According to Amirlatifi et al. (2018) and Abbasi et al. (2019), there is a strong reason for developing the practical use of this achievement in cyanobacteria and replacing the simple pretreatment method rather than biologics or at least in parallel should not be repeated. When less than one hour is applied compared with 24 and 96 hours, it leads to some changes in cyanobacterial photosynthesis system, which is time-, energy- and cost-effective.

The present study focused on answering the question whether changes are maintained over time or cyanobacteria was reacted based on 17 mM concentration. Further, acclimatization or adaptation was physiologically encountered. Based on the results, the destructive and stimulant effect of 160 mM for 20 and 60 min on photosynthesis system was maintained for up to 24 hours and then disappeared after 96 hours. However, the destructive effect maintained at 80 mM for 60 minutes to 24 hours did not continue after 96 hours. In fact, the stimulant effect of 80 mM for 20 minutes was maintained up to 24 hours not more than 96 hours.

Thus, *Fischerella* sp. FS 18 could modulate the destructive effect of the two salinity treatments (160 mM at 20 min and 80 mM at 60 min) after 24 hours. However, the growth rates up to 96 h were higher in the samples treated with 20 min salinity at 80 mM and 60 min salinity at 160 mM than 17 mM. In other words, the system changed its pattern over time although the initial pattern remained unaffected by time and salinity conditions until after 24 hours. Obviously, the results showed the effect of combined changes of salinity and short time on the photosynthetic system, which is consistent with those of Iranshahi et al. (2014) and Moisander et al. (2014). More specifically, some of these mechanisms were unknown to researchers (Shokravi, 2017). The findings of this study could be used in mass crop technology and applied phycology (Fraser et al., 2013). Therefore, cyanobacterial species can tolerate environmental changes and provide sustainable and economically viable biomass throughout the years (Bravo-Fritz et al., 2016; Pathak et al., 2018).

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Diversity of Thermophilic Cyanobacteria in Maragheh Mineral Springs and Variable Environmental Factors

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Abstract

Cyanobacteria or blue-green algae are photosynthetic microorganisms, found in several habitats. Hot springs are considered as extreme habitats with "sub-cosmopolitan" geographical distribution, the biodiversity of which is unique due to their special conditions. Over the last century, these natural inhabitants have attracted the attention of biologists and tourists. In the present study, cyanobacteria were collected and identified from five hot and mineral springs located in East Azarbaijan province, Maragheh city during 2019. In this regard, the spring water was analyzed physic-chemically, the effect of some environmental parameters on algal communities was assessed, and the samples were fixed in 4% formaldehyde solution. Based on the results, 30 species were detected, which the highest and least diversity of cyanobacteria were respectively observed in Isty Bulakh (15 species) and Ghare Palchigh spring. In addition, *Phormidium* sp. possessed the maximum diversity among all of the identified taxa. Finally, environmental factors such as total dissolved solids (TDS) and turbidity were determined among the factors affecting biodiversity.

Keywords: Cyanobacterial diversity, Hot spring, Maragheh, *Phormidium* sp., Physico-chemical parameters

Introduction

Cyanobacteria are a group of ancient photosynthetic organisms with 2.8 billion years age (Dadheech et al., 2013). Due to the high compatibility of the microorganisms, they are found in extreme habitats such as saline waters and hot springs (Mishra et al., 2018). In general, they are considered as free-living microorganisms, which occur in symbiotic association with some eukaryotic plants, algae, fungi, gymnosperms, pteridophytes, angiosperms, and bryophytes, as well as the lower group of animals like ascidians (Anand et al., 2019). Additionally, the microorganisms can play an important role in atmospheric nitrogen fixation (Jeevanantham et al., 2019). The use of cyanobacteria, as an environmental indicator, is suggested during the recent decade (Barinova et al., 2017). In general, cyanobacteria are studied in aquatic ecosystems for various purposes

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(Dubey, 2019) such as floristic, ecological, physiological, or applied phycological ones. Thermo-mineral springs are a special group of aquatic ecosystems with particular characteristics. The aquatic ecosystems are highlighted as interesting habitats for evaluating algal microflora including the cyanobacteria, which are often plentiful and can live in different ecological niches (Singh et al., 2018). The issue makes them an appropriate candidate for different uses such as industrial processing, bottled water, power production, and health and well-being sector (Lai et al., 2019). Therefore, academic communities highlighted the examination of the natural resources significantly over the past decades (Durowoju et al., 2018).

Cyanobacteria have been detected in the thermal-mineral springs of several countries such as China (Tang et al., 2018), Sri Lanka (Medhavi et al., 2018), India (Singh et al., 2018), Russia (Gorlenko et al., 2019), Ireland (Shiels et al., 2019), Saudi Arabia (Yasir et al., 2019), Kenya (Ngetha et al., 2019), Brazil (Ramos et al., 2019), Indonesia (Prihantini et al., 2018), Turkey (Yilmaz-Sariozlu and Yilmaz-Cankilic, 2018), Australia (McGregor and Sendall, 2017), Pakistan (Amin et al., 2017), Japan (Martinez et al., 2019), Africa (Maree et al., 2018), and Iran (Heidari et al., 2013, 2018). In Iran, 149 springs exist in Sarab and Bostanabad, and 141 ones are found in Tabriz and Azarshahr area. Maragheh, as one of the most important tourist areas, is located in the northwest of Iran, one of the natural attractions of which includes the presence of several hot springs. So far, limited studies have been published regarding algal flora in East Azarbaijan. Thus, the present study aimed to assess cyanobacterial diversity in some of the springs in the area.

Material and methods

Study area and sampling sites

Samples were randomly taken from five thermal springs with different geographical situations located in Maragheh city, East Azerbaijan (Table 1, Fig. 1) during April-October 2019.

Further, benthic taxa were collected by scraping the algae from the bottom of the springs and the surfaces of marginal rocks, muddy sediments, aquatic plants, and macrophytes. All samples were fixed in 4% formalin, labeled, and transferred to laboratory in cool containers. In order to assess some of the physical and chemical properties of each sitre, 1.5 L of water without formalin was taken by using plastic bottles. Furthermore, the temperature was measured by using a mercury thermometer, and pH was determined using a portable pH meter WTW LF 320 EC meter, a Testo 320 pH meter, and a multi-parameter analyzer. The electrical conductivity, turbidity, alkalinity, TDS (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, S^{2-}, NH_4^{+}, NO_3^{-}, NO_2^{-}) of water were determined by Arian Fan Azma Institute (WWW.AFA-Co.ir). Table 3 summarizes laboratory measurement methods.

Identification of cyanobacterial taxa

The semipermanent slides of colonies were prepared and morphometric study was per-

formed by light microscopy (Olympus, Model BH-2) based on Desikachary (1959), Prescott (1970), Komárek and Anagnostidis (1986, 2005) and Komárek (2014). The most variable morphological characteristics were color and shape of colonies; color, shape and size of thallus, vegetative cells, apical cell shape,and presence or absence of mucilage sheath.

Results

Species diversity in Maragheh springs

In the present study, 30 taxa of cyanobacteria were detected and recorded from Maragheh springs, which the highest and least diversity was respectively determined in site two (Isty Bulakh) and five (Ghare Palchigh) with 15 and 5 species (Table 2, Fig. 2). In Oscillatoriales, *Phormidium* sp. with fila-

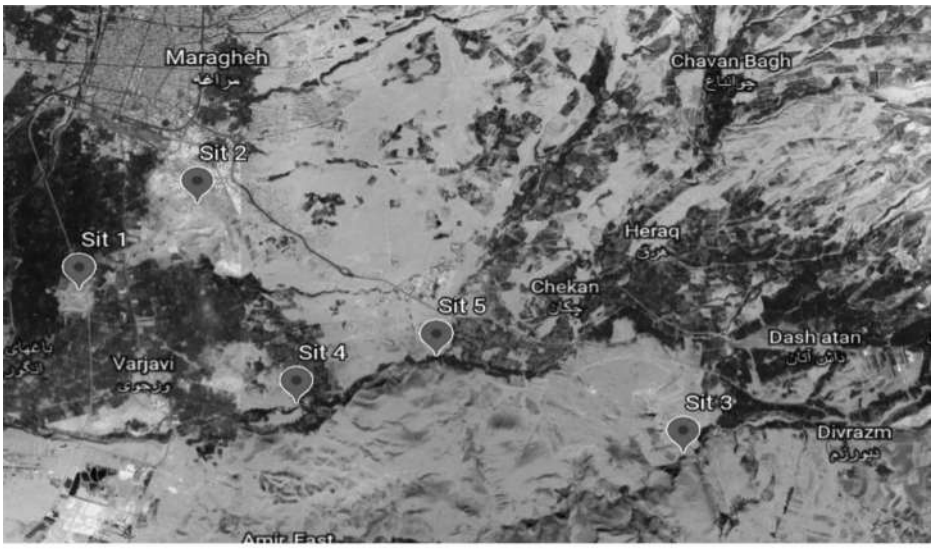

Fig. 1. Location of the springs examined in Maragheh. Sit 1 (Sari Sou); Sit 2 (Isty Bulakh); Sit 3 (Goshayesh); Sit 4 (Shour Sou); Sit 5 (Ghare Palchigh)

Table1. Geographical details of the sampling locations

Site No.	Spring name	Location	Height (m)	Temperature (°C)
1	Sari Sou	37° 20′ 57.3″ N 46° 13′ 38.3″ E	1390	45
2	Isty Bulakh	37° 20′ 14.8″ N 46° 17′ 38.4″ E	1493	31
3	Ghoshayesh	37° 23′ 12.7″ N 46° 14′ 41.1″ E	1579	25
4	Shour Sou	37° 19′ 46.1″ N 46° 16′ 5.6″ E	1495	19
5	Ghareh Palchigh	37° 21′ 52.4″ N 46° 14′ 58.0″ E	1432	28

Table 2. List of cyanobacterial species recorded from five hot springs in Maragheh

Taxon	Sit 1	Sit 2	Sit 3	Sit 4	Sit 5
Spirulina subsalsa Oersted ex Gomont	•			•	
Spirulina major Kützing ex Gomont	•				
Spirulina rosea Crouan			•		
Chroococcus turgidus (Kützing) Nägeli		•	•		
Chroococcus minutus (Kützing) Nägeli		•	•		
Chroococcus thermalis (Meneghini) Nägeli			•	•	
Chroococcus lithophilus Ercegovic		•	•	•	
Phormidum schroeteri (Hansgirg ex Hansgirg) Anagnostidis	•	•			
Phormidum lividum Nägeli in Kützing ex Gomont	•	•			
Phormidum cortianum (Meneghini ex Gomont) ex Komárek		•		•	
Phormidum toficola Gomont	•				
Phormidum lucidum Kützing ex Gomont	•	•			•
Phormidum articulatum (Gardner) Anagnostidis and Komárek		•			
Merismopedia sp.		•			
Merismopedia elegance A. Braun	•	•			
Merismopedia glauca (Ehrenb.) Nägeli		•		•	
Pseudanabena oblonga Kullberg	•			•	
Pseudanabaena limnetica (Lemmermann) Komárek		•			
Pseudanabaena firigida (Fritsch) Anagnostidis	•			•	•
Pseudanabaena minima (G.S.An) Anagnostidis					•
Geitlernema numidicum (Gomont) Anagnostidis			•		
Nodularia sp.			•	•	
Nostoc sp.			•	•	
Cyanabacterium minervae (Copeland) Komárek					•
Planktothrix isothrix (Skuja) Komárek et Komárková			•	•	
Planktolyngbya limentica (Lemmermann) Komárková - Leganerova & Cronberg		•		•	
Osillatoria subcapitata Ponomarev ex Elenkin		•			
Osillatoria prolifica (Gerv.) Gomont				•	•
Nostoc microscopicum Carmichael	•		•		
Gleocapsa punctate Nägeli		•			

Sit 1 (Sari Sou), Sit 2 (Isty Bulakh), Sit 3 (Goshayesh), Sit 4 (Shour Sou), Sit 5 (Ghare Palchigh)

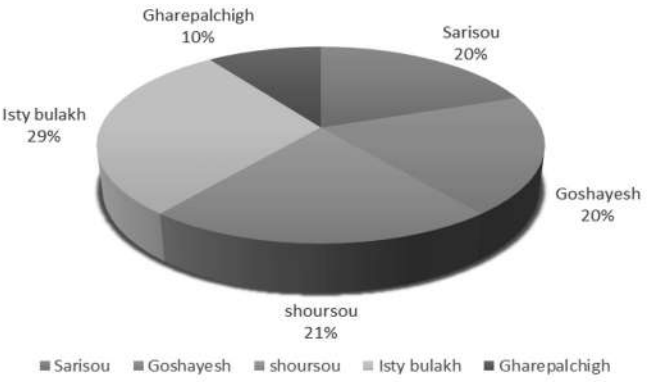

Fig. 2. Frequency percentage of cyanobacterial species in the studied mineral springs

mentous structure (six species) represented the maximum species diversity among the identified taxa.

Physicochemical parameters of water

Table 3 presents the results related to the physicochemical analysis of five springs in East Azerbaijan province (Maragheh city). The water physical parameters in aquatic ecosystems affect the life cycle and diversity of cyanobacteria. The factors provide accurate data for predicting the behavior of algae in aquatic ecosystems such as springs. Based on the results of the present study, water temperature varied between 19-45 ˚C so that the highest and least temperature was respectively observed in site one (Sari Sou) and four (Shour Sou). In addition, the maximum electrical conductivity, turbidity, pH, TDS, and salinity were obtained in Ghare Palchigh spring. Further, sulfate content was maximized and minimized in Ghare Palchigh and Shour Sou springs, respectively (Table 3).

The results indicated that a decrease in total dissolved solid (TDS) resulted in increasing the total hardness (TH), electrical conductivity (EC), and turbidity diversity of cyanobacteria. The results indicated that a decrease in total dissolved solid (TDS), the total hardness (TH), electrical conductivity (EC), and turbidity resulted in increasing diversity of cyanobacteria. Furthermore, the highest species diversity was determined in Isty Bulakh (TDS: 1380 $mg.L^{-1}$, TH: 721 $mg.L^{-1}$, Ca^{+2}: 437 $mg.L^{-1}$, Mg^{+2}: 284 $mg.^{L-1}$, Na^{+}: 79.9 $mg.L^{-1}$, K^{+}: 7.20 $mg.l^{-1}$, Cl^{-}: 65.3 $mg.L^{-1}$) and the lowest was recorded in Ghare Palchigh (TDS: 4310 $mg.L^{-1}$, TH: 1524 $mg.L^{-1}$, Ca^{2+}: 1011 $mg.L^{-1}$, Mg^{2+}: 513 $mg.L^{-1}$, Na^{+}: 690 $mg.L^{-1}$, K^{+}: 78 $mg.L^{-1}$, Cl^{-}: 891.8 $mg.L^{-1}$).

Discussion

The cyanobacterial diversity has been studied in several aquatic ecosystems of Iran, but there are limited reports on the diversity of these microorganisms in thermo-mineral springs of our country. Based on the results, the maximum diversity of taxa was obtained in Isty Bulakh at 31 ˚C. The thermophilic al-

Table 3. Physicochemical parameters in the studied springs

Parameters	Analytical Method	Scale	studied Sites				
			Site 1	Site 2	Site 3	Site 4	Site 5
Temperature	Laboratory and field	℃	45	31	25	19	28
EC	Platinum electrode	$\mu S.cm^{-1}$	4990	1825	3690	3260	6010
Turbidity	Nephelometric	NTU	51.5	0.50	3.37	3.23	187
pH	Electrometric	------	6.88	6.87	6.58	6.50	6.93
TDS	Electrical conductivity	$mg\ L^{-1}$	3680	1380	2815	2505	4310
TH	EDTA titrimetric	$mg\ L^{-1}$	1400	721	1570	1443	1524
Salt	Electrical conductivity	%	0.101	0.020	0.030	0.022	0.174
Ca^{2+}	EDTA titrimetric	$mg\ L^{-1}$	732	437	1057	960	1011
Mg^{2+}	EDTA titrimetric	$mg\ L^{-1}$	868	284	513	483	513
Na^{+}	Flame emission spectrometric	$mg\ L^{-1}$	401	79.9	117	85.9	690
K^{+}	Flame emission spectrometric	$mg\ L^{-1}$	57.30	7.20	16.25	10.20	78.00
OH^{-}	Titrimetric	$mg\ L^{-1}$	1765	730	1519	1463	1673
SO_4^{2-}	Turbidimetric	$mg\ L^{-1}$	208	93	186	70	380
Cl^{-}	Argentometric	$mg\ L^{-1}$	356.6	65.3	95.4	52.3	591.8
S^{2-}	Methylene blue	$mg\ L^{-1}$	3.28	-----	----	----	----
HCO_3^{-}	Titrimetric	$mg\ L^{-1}$	1765	730	1519	1463	1673

Sit 1. Sari Sou; Sit 2. Isty Bulakh; Sit 3. Goshayesh; Sit 4. Shour Sou; Sit 5. Ghare Palchigh; EC (Electrical Conductivity), TDS (Total Dissolved Solid), TH (Total Hardness).

gae can be divided into hypothermophilous (15-26 ˚C), mesothermophilous (26-45 ˚C), euthermophilous (45-65 ˚C), and hyperthermophilous (65˚C and more) communities. Additionally, the cyanobacterial taxa distributed in Sari Sou, Isty Bulakh, and Ghare Palchigh were mesothermophilous community, while those in Goshayesh and Shour Sou included hypothermophilous one. Temperature is an environmental factor which plays an important role in development of algal communities. Due to the impact of temperature on enzymatic reactions of living organisms, this environmental factor affects many critical biological mechanisms such as photosynthesis, respiration and cell division. In addition, temperature can also affect species diversity of algae in aquatic ecosystems (Aghashariatmadary et al. 2017).

Further, 30 cyanobacterial taxa were detected, and the results revealed that identified taxa belong to the several orders: Oscillatoriales (36.6%), Chroococcales (30%), Synechococcales (13.3%), Spirulinales (10%) and Nostocales (10%), respectively. Regarding Oscillatoriales, six species of *Phormidium* formed 20% of total taxa, which is the maximum diversity among the identified species. Accordingly, *Oscillatoria, Phormidium,* and *Lyngbya* genera seemingly adapted to the habitat and are the most dominant species of Maragheh springs. These cyanobacteria are filamentous and successful in mat-forming. In addition, species of the order Oscillatoriales appear in springs with the temperature ranging 25-45 ˚C (Pentecost, 2003, 2014) and their exopolysaccharide sheath is probably considered as the

backbone of developed microbial mat (Abdelwahab and Amin, 2017).

The electrical conductivity of water indicates the amount of solutes in the water, which is a function of temperature and ions in the water. Among the studied stations, the lowest electrical conductivity is related to the station of Isty Bulakh with 1825 ($\mu S\ cm^{-1}$) and the highest electrical conductivity is related to Ghare Palchigh station with 6010 ($\mu S\ cm^{-1}$). Based on the results obtained in this study, there is an inverse relationship between EC and species diversity. This negative correlation between variability and EC was reported in some previous studies (Sheath and Cole,1992).

Moreover, salinity reduces productivity (Srivastava et al., 2005), as well as the phylogenetic diversity of some cyanophytes (Wang et al., 2011). According to the water analysis report, highest water hardness was 180 mg/L and classified as hard water (Li et al., 2017).

According to World Health Organization (WHO) standard, the highest desirable and permissible levels of water calcium content are 75 and 200 $mg.L^{-1}$, respectively (Cotruvo and Bartram, 2009). In the present study, the calcium content ranged 437-1057 $mg.L^{-1}$ in Maragheh springs, which is higher than the level of WHO standard (200 $mg.L^{-1}$) (Daghara et al., 2019).

Sulfate is relatively non-toxic, the range of which in the drinking water is up to 200 $mg.L^{-1}$ so that the level more than 500 $mg.L^{-1}$ produces a bitter taste (Gaglioti et al., 2019). Regarding the present study, sulfate level was determined 70-380 $mg.L^{-1}$ in Maragheh springs, while that of Ghare Palchigh and Sari Sou was obtained 380 and 208 $mg.L^{-1}$, respectively. Thus, the water of Ghare Palchigh and Sari Sou is not considered as suitable for drinking because of having sulfate content above the WHO standard level.

Based on the guideline for the chloride level allowed in water, the chloride range is set between 0 to 142 $mg.L^{-1}$, which is appropriate for irrigation, while the level above 355 $mg.L^{-1}$ is inappropriate for irrigation. Finally, the chloride content of the springs under study was measured 52.3-591.8 $mg.L^{-1}$. Consequently, the springs of Ghare Palchigh and Sari Sou are non-suitable for irrigation (Shafikhani et al., 2019).

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Epilithic Diatom Diversity in Golestan Waterfall

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Abstract

Waterfalls are considered as aquatic ecosystems, of which limited study was conducted on their biodiversity. Golestan waterfall is one of the outstanding waterfalls in eastern edge of Golestan National Park. Since no research was performed on algal flora in the area, the present study aimed to identify the epilithic diatoms of the waterfall, as a component of this ecosystem biodiversity. The samples were collected from stony substrates at each season. The results represented 24 genera including 47 species, which all taxa belonged to Bacillariophyceae. In addition, Cymbopleura with five species and *Gomphonema*, and *Navicula* with four species had more species, respectively. Further, *Achnanthidium minutissimum*, *Amphipleura pellucida*, *Cymbella affinis*, *Cymbopleura kuelbsii*, and *Gomphonema pumilum* were determined as the most abundant taxa. Furthermore, *Delicatophycus verenae*, *Stauroneis separanda* and *Tryblionella brunoi* were found in the diatom flora of Iran for the first time. Due to the few diatom studies in Iran, conducting detailed and local studies can improve data on diatom flora of Iran.

Keywords: Bacillariophyceae, *Cymbopleura*, Diatom Flora, Golestan Province, Iran

Introduction

Biodiversity plays a major role in ecosystem function and stability and is the life support system (Rawat and Agarwal, 2015). Diatoms are considered as an extremly diverse group of algae occurring in almost all aquatic systems. Additionally, they are a systematic group characterized by their siliceous wall. Taxonomic diversity in diatoms is important, which reflects the biodiversity and stability of an aquatic ecosystem (Andrejic et al., 2012). Further, their rapid response to environmental changes can represent the ecological conditions of the living environment, which makes them useful in water quality assessments (Delgado et al., 2012; Kelly et al., 2007; Martin et al., 2010; Noga et al., 2013).

Since 2016, some studies focused in Golestan procince regarding diatoms in Zarringol

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River (Dadgar, 2016), Chehal Chay River in Minudasht (Lakzaie et al., 2018), Gharah Chay River in Ramian (Bayani, 2019), three springs in Ramian (Ahmadi et al., 2019), and Khorrmarud River (Aghatabay et al., 2018). However, few studies have conducted on algal flora and diatom so far. Thus, the Further studies can enhance the knowledge on diatom flora in local and country scales. Waterfalls are interesting ecosystems, which are considered as vertical wetlands, and kept cool in summer and mild in winter. Many outstanding waterfalls exist in Golestan province, the algae of which have not been assessed floristically so far.

Material and methods

Golestan province with the area of 22033 km^2 is located in northeast Iran, southeast Caspian Sea, which has diverse climate and aquatic ecosystems due to its geographical position. Golestan waterfall is placed 47 km away from Galikesh city at the eastern edge of Golestan National Park (Fig. 1). The present study sought to recognize the diatoms of the waterfall as a basic study. The diatoms were sampled from stony substrates by using toothbrush seasonally in three different sites and transferred to laboratory after fixation with 4% formalin (Bellinger and Sigee, 2010). In addition, the samples were cleaned through acid digestion method (Taylor et al., 2007). Further, permanent slides were prepared using Canada Balsam and diatoms were identified by using a light microscope and considering available flora (Bahls, 2006, 2012, 2013; Krammer and Lange-Bertalot, 1986, 1988, 1991a, b; Krammer, 2002, 2003; Lange-Bertalot, 2001). Taxa names were checked in www.algaebase.org. In each slide, 300-400 valves were counted and the relative abundance of each taxon was calculated.

Along with algal samples, water ones were added into 1L containers and transferred to laboratory in order to analyze nitrate, am-

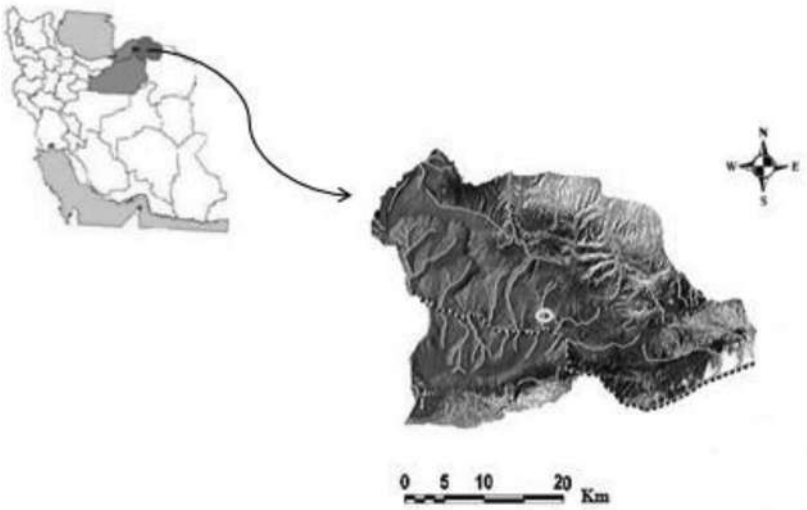

Fig. 1. Location of Golestan waterfall

monium, silicate, and phosphate (Clesceri et al., 1999.(Furthermore, dissolved oxygen (DO), temperature, EC, salinity, pH, and TDS were measured in situ by using a HQ40d portable device.

Results

Table 1 summarizes the physicochemical results related to water in different seasons. A total of 47 taxa belonging to 24 genera were identified in Golestan waterfall (Table 2, Plates I and II), all of which were related to the class Bacillariophyceae. Based on the results, the most species-rich genus included *Cymbopleura* with five species and *Gomphonema* and *Navicula* each with four species, followed by *Diploneis*, *Surirella*, and *Tryblionella* with three species (Fig. 2). Additionally, *A. minutissimum*, *Amphipleura pellucida, Cymbopleura kuelbsii, Gomphonema pumilum,* and *Cymbella affinis* were charecterized as the most abundant taxa.

Based on the morphological groups, the identified taxa were classified into six groups, the highest number of which belonged to asymmetrical biraphid with 18 species and 8 genera, symmetrical biraphid with 13 species and 7 genera. Nitzschioid with 7 species and 3 genera, and Monoraphid with 3 species and 2 genera, respectively. Further, the least number was observed in Surirelloid with 3 species and 1 genera, and Araphids with 3 species in 3 genera, respectively.

In the present study, three species were determined as new for the diatom flora of Iran, which are provided as follows.

Table 1. Results related to water physicochemical parameters in Golestan waterfall during different seasons

Parameteres	Spring	Summer	Fall	Winter
PO_4 ($mg.l^{-1}$)	0.02	0.016	0.015	0.02
SiO_2 ($mg.l^{-1}$)	0.57	0.74	0.95	0.39
NO_3 ($mg.l^{-1}$)	0.61	2.33	1.42	0.55
NH_3 ($mg.l^{-1}$)	0.011	0.015	0.011	0.016
T (ºC)	21.3	14.8	6.8	7
DO ($mg.l^{-1}$)	7.97	8.89	11.53	11.01
EC ($\mu s.cm^{-2}$)	444	476	497	447
TDS ($mg.l^{-1}$)	216	230	239	216
Air T (ºC)	31.4	23.8	11.4	9.7
pH	7.75	7.67	7.81	7.75
Salinity	0.21	0.23	0.24	0.22

Table 2. Taxa identified in Golestan waterfall during seasons of 2019 (L: Length, W: Weight, S: Striae, C: Costae, F: Fibula)

Scientific name	Dimensions
Bacillariophyceae	
Achnanthidaceae	
Achnanthidium grasillimum (F.Meister) Lange-Bertalot	L: 17.5-21 µm W:3-4 µm S:14-16
Achnanthidium minutissimum (Kützing) Czarnecki	L:7.5-13 µm W:2.5- 3 µm S:27-30
Amphipleuraceae	
Amphipleura pellucida (Kützing) Kützing	L:60-93 µm W:9-11 µm
Frustulia vulgaris (Thwaites) De Toni	L:48-49 µm W: 9-10 µm
Basillariaceae	
Hantzschia amphioxys (Ehrenberg) Grunow	L: 35 µm W:6 µm F:6
Nitzschia commutatoides Lange-Bertalot	L: 112 µm W: 15 µm F:9-11
Nitzschia dissipata (Kützing) Rabenhorst	L:20-42 µm W:3-4 µm F:7-11
Nitzschia sp.	L: 58-120 µmW:3-6 µm F:10-11
Tryblionella angustata W.Smith	L: 44-52 µm W: 6-7µm S:17-18
Tryblionella apiculate W.Gregory	L:44-47.5 µm W:5 µm S:16-17
Tryblionella brunoi (Lange-Bertalot) Cantonati & Lange-Bertalot	L:79-83 µm W:10 µm S:13
Catenulaceae	
Amphora inariensis Krammer	L:12-16 µm W:2.5-4 µm S:15-17
Amphora pediculus (Kutzing) Grunow	L:10-12.5 µm W:2.5 µm S:18
Cocconeidaceae	
Cocconeis pediculus Ehrenberg	L:27.5 µm W:19-21 µm S:16
Cymbellaceae	
Cymbella affinis Kutzing	L:27-28.5 µmW:7.5-8 µm S:9-11
Cymbella compacta Østrup	L: 44 µm W:14 µm S:11
Cymbopleura amphicephala (Nageli) Krammer	L:30-32.5 µm W:10-11µm S:8-10
Cymbopleura citrus (J.R.carter & Bailey-Watts) Krammer	L: 30 µm W:10 µm S: 10
Cymbopleura kuelbsii Krammer	L:38-32 µm W:6-7.5 µm S:11-13
Cymbopleura sp.	L:30-35 µm W:7-7.5 µm S:8-10
Cymbopleura cf. *vrana* Krammer	L: 36 µm W:10.5 µm S: 9
Diploneidaceae	
Diploneis calcilacustris Lange-Bertlot & A.Fuhramann	L:17-20 µm W:10-12 µm S:11-13
Diploneis krammeri Lange-Beterlot & E.Reichardt	L: 45 µm W:17-17.5 µm S: 12
Diploneis separanda Lange-Beterlot	L:25 µm W:12 µm S:12
Fragilariaceae	
Fragilaria recapitellata Lange-Bertalot & Metzeltin	L:17-18 µm W:5 µm S:13-14
Gomphonemataceae	
Delicatophycus sinensis M.J.Wynne	L:27.5 µm W:5 µm S:16
Delicatophycus verenae M.J.Wynne	L:33-42 µm W:6-7.5 µm S:12-14
Encyonopsis minuta Krammer & E. Reichardt	L:12.5 µm W:2.5-3 µm
Gomphonema micropus Kützing	L: 25 µm W: 7 µm S: 11
Gomphonema parvulum (Kützing) Kützing	L:12-23 µm W:5-6 µm S:12-13
Gomphonema pumilim (Grunow) E.Reichardt & Lange-	L:16-30 µm W:3-5 µm S:10-13

Bertalot	
Gomphonema subclavatum (Grunow) Grunow	L:37-52 µm W:7-10 µm S:10-12
Naviculaceae	
Gyrosigma sp.	L:120 µm W:24 µm S:13
Navicula capitatoradiata H.Germain ex Gasse	L: 37 W:7.5 S:15-17
Navicula cryptotenella Lange-Bertalot	L:16-22 µm W:5-6 µm S:12-14
Navicula rostellata Kützing	L: 38 µm W: 9 µm S:12
Navicula tripunctata (O.F.Müller) Bory	L:46-52 µm W:8-9 µm S:11-12
Rhoicospheniaceae	
Rhoicosphenai abbreviata (C.Agardh) Lange-Bertalot	L:27.5-40 µm W:4-5 µm S:12- 14
Stauroneidaceae	
Craticula buderi (Hustedt) Lange-Bertalot	L:20-26 µm W:5-6.5 µm S:18-19
Stauroneis separanda Lange-Bertalot & Werum	L: 15-16 µm W: 3.5-4.5 µm
Stauroneis smithii Grunow	L:27 µm W:7.5 µm
Surirellaceae	
Surirella angusta Kützing	L:23 W:7.5 S:25
Surirella sp.	L:36 W:24 F: 5-6 C:18
Surirella librile (Ehrenberg) Ehrenberg	L:60-88 µm W:20 µm
Tabellariaceae	
Diatoma moniliformis (Kützing) D.M.Williams	L:12.5-16 µm W:3.5-4 µm C:7
Halamphora veneta	L:19-22 µm W:4-5 µm S:16-17
Ulnariaceae	
Ulnaria ulna (Nitzsch) Compere	L:102-140 µm W: 6 µm S:10

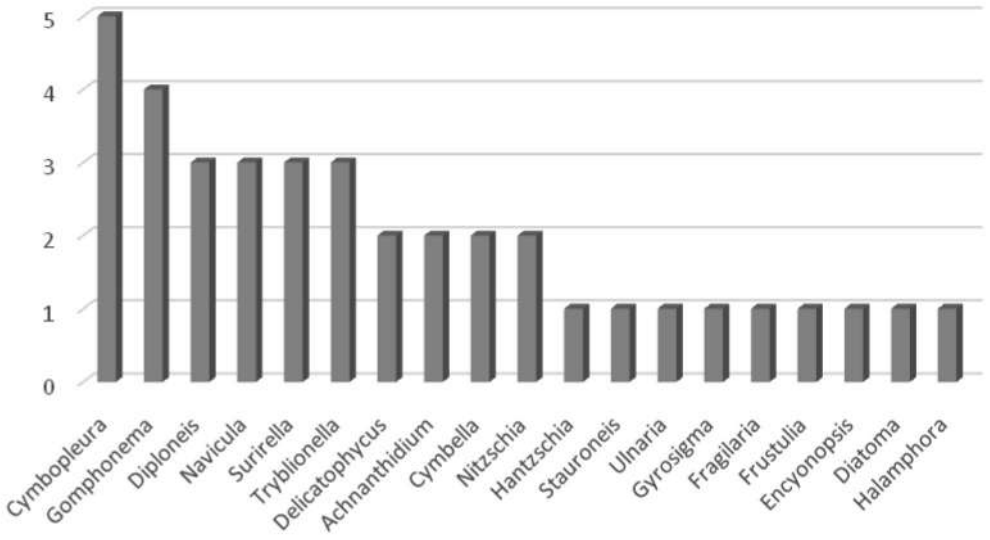

Fig. 2. Number of taxa in each of the genus recognized in the study

Order: Cymbellales

Family: Gomphonemataceae

Genus: *Delicatophycus*

Delicatophycus verenae M.J.Wynne

Synonyms: *Delicata verenae* Lange-Bertalot & Krammer

Reference: as *Delicata verenae* in Krammer, 2003, Plate 137, Figs. 1-15.

Description: Valves slightly dorsiventral, elliptic-lanceolate with round not protract-

ed apices, with 33-42.5 µm length and 6-7.5 µm width; axial area widening towards the middle of valve; central area almost absent; raphe lateral, narrowing towards the distal ends; striae radiate, 12-14 /10 µm in middle of valve.

Order: Bacillariales

Family: Bacillariaceae

Genus: *Tryblionella* W. Smith

Tryblionella brunoi (Lange-Bertalot) Cantonati & Lange-Bertalot

Synonyms: *Nitzschia brunoi* Lange-Bertalot (Powers, 2018; as *Nitzschia brunoi* in Lange-Bertalot and Metzeltin, 1996, Plate 101, Figs. 11-15)

Description: Valves linear with wedge-shaped ends with 78-82.5 µm length and 10-11 µm width; striae are parallel distinctly punctate 13/10 µm.

Order: Naviculales

Family: Stauroneidaceae

Genus: *Stauroneis* Ehrenberg

Stauroneis separanda Lange-Bertalot & Werum

No Synonym

Reference: Bahls, 2012; Levkov et al., 2016, Figs. 48-63.

Description: Valves linear- lanceolate, wider at the center, with triundulate margins, apices rostrate, with the length of 15-16 µm and 3.5-4.5 µm width; central stauros is narrow and linear; pseudoseptum present at the apices; striae fine.

Discussion

In general, most of the previous studies on waterfalls have primarily focused on tourism, geology, and hydrology, while limited ones have highlighted biodiversity in the ecosystem (Offem and Ikpi, 2012). Therefore, waterfalls are sometimes considered as lifeless zones (Chernicoff et al., 1997). The present study aimed to identify epilithic diatoms in Golestan waterfall.

Reviewing the studies conducted on diatom in Golestan province demonstrated 80% similarity between the results of the present study with those of Ahmadi et al. (2019) regarding the diatoms of three springs. In addition, the taxa of *Amphipleura pellucida*, *Diploneis calcilacustris*, *D. krammeri, D. separanda*, *Fragilaria recapitellata*, *Frustulia vulgaris*, *Gomphonema subclavatum, Stauroneis smithii*, and *Surirella angusta* were found only in these two studies. However, the others except for new records were reported in the previous research.

Among the most abundant taxa, *A. minutissimum*, and *C. affinis* were abundant in most ecosystems of the province (Aghatabay, 2018; Bayani, 2019; Dadgar, 2016; Lakzaie, 2016). Further, *A. minutissimum* is considered as one of the most frequently recorded taxa worldwide (Falasco et al., 2012, 2016; Kelly et al., 2007; Kheiri et al., 2019; Krammer and Lange-Bertalot, 1991b;) from oligo- to hypertrophic in alkaline to acidic waters (Potapova and Hamilton, 2004). Although Van Dam et al. (1994) classified *C. affinis* as eutrophic taxon, BCG classification indicated human disturbance (Davies and Jackson,

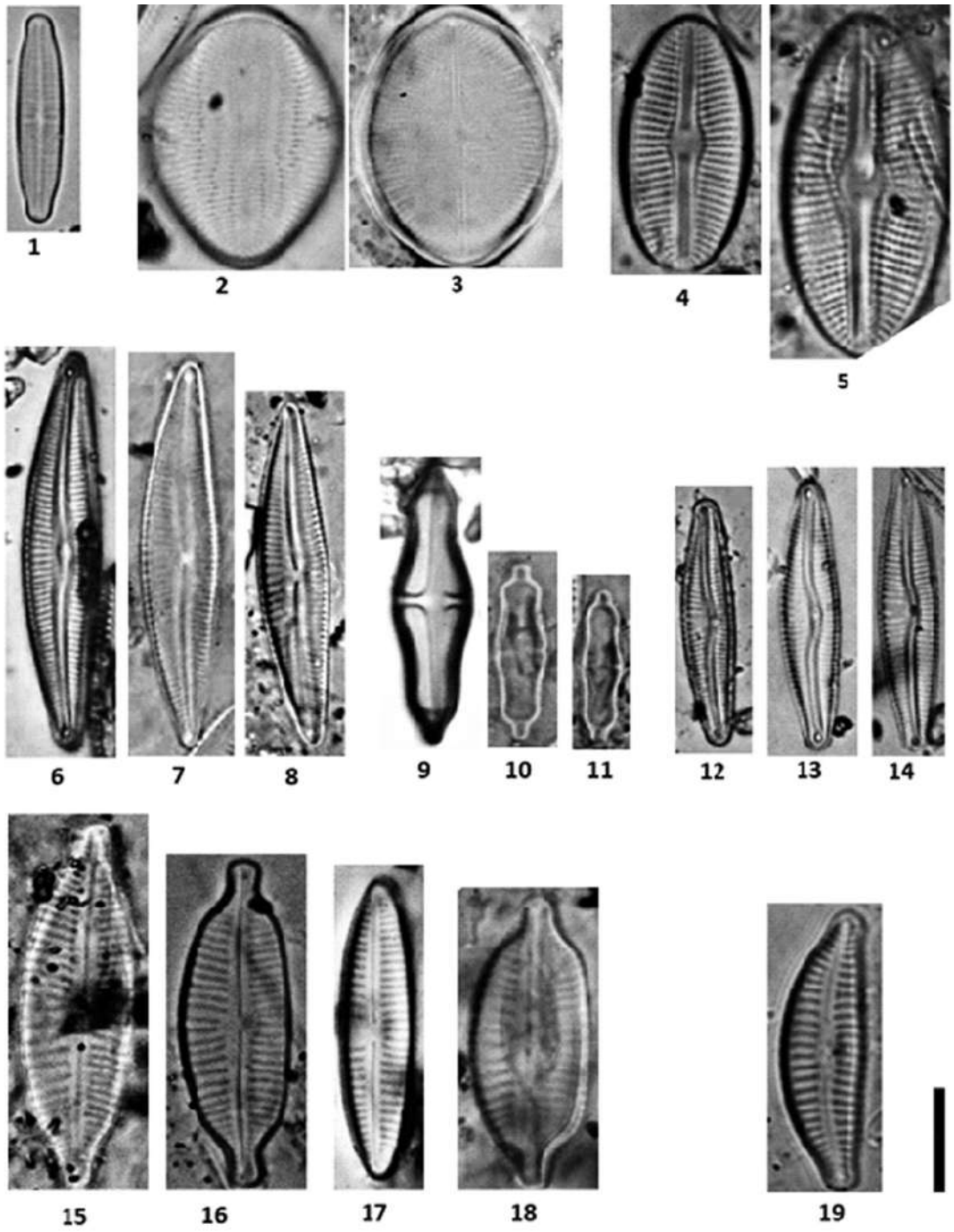

Plate I. (1-20). (1) *Achnanthidium gracillimum*, (2,3) *Cocconeis pediculus*, (4) *Diploneis separanda*, (5) *Diploneis krammeri*, (6-8) *Delicatophycus verenae*, (9) *Stauroneis smithii*, 10, 11) *Stauroneis separanda*, (12-14) *Delicatophycus sinensis*, (15) *Cymbopleura* cf. *rana*, (16) *Cymbopleura amphicephala*, (17) *Cymbopleura kuelbsi*, (18) *Cymbopleura citrus*, (19) *Cymbella affinis*. Bar: 10 µm.

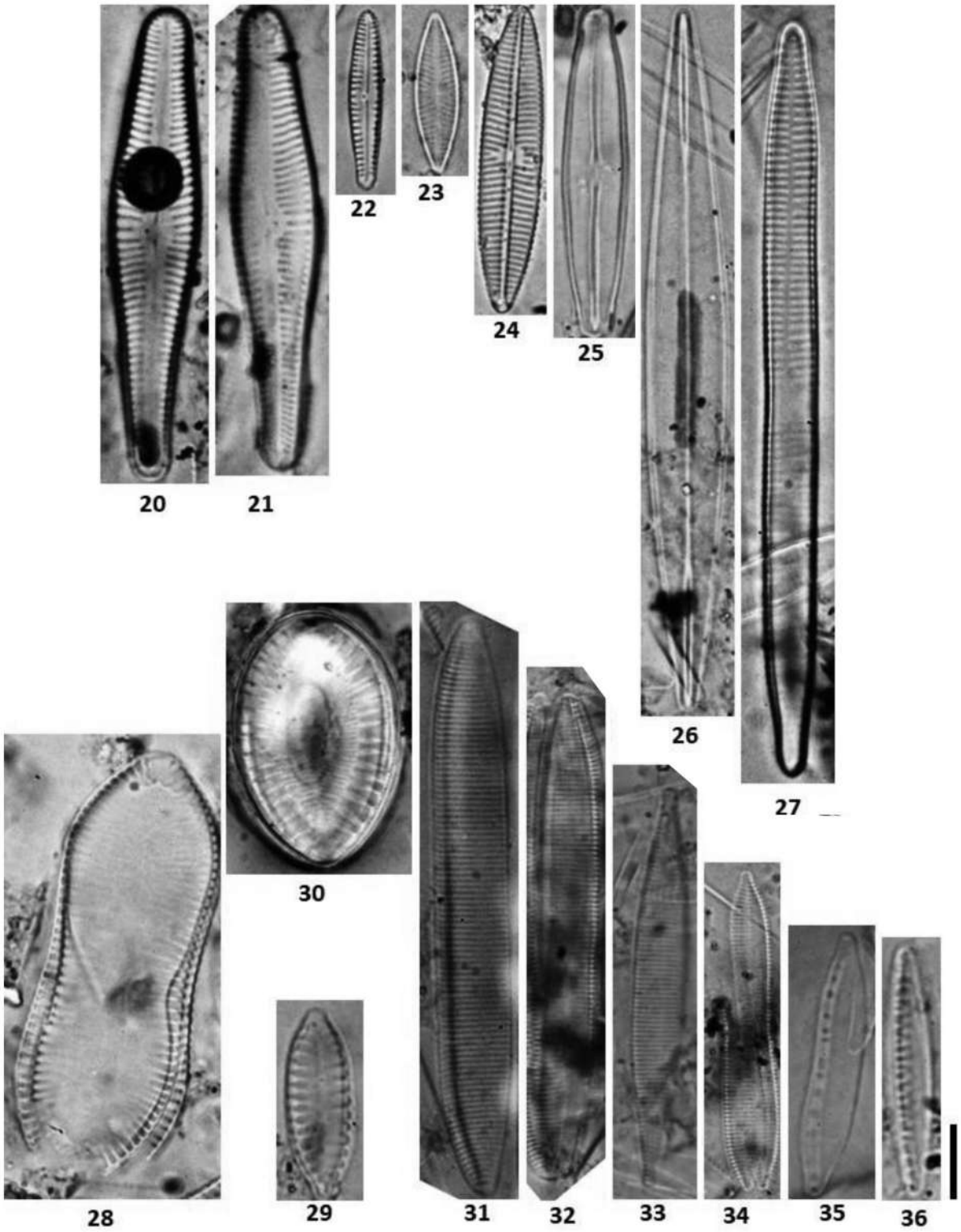

Plate II. (20-36). (20, 21) *Gomphonema subclavatum*, (22) *Gomphonema pumilum,* (23) *Navicula cryptotenella*, (24): *Navicula tripunctata,*(25) *Frustulia vulgaris*, (26). *Amphipleura pellucida* (27) *Ulnaria uln*, (28) *Surirella libril*, (29) *Surirella angusta*, (30) *Surirella* sp., (31,32) *Tryblionella brunoi* (33) *Tryblionella angustata*, (34) *Tryblionella apiculata*, (35) *Hantzschia amphyoxys* (36) *Nitzschia dissipata.* Bar: 10 µm.

2006) characterized *C. affinis* as highly sensitive species (Potapova, 2011) in California State. *C. affinis*, a common taxon both in rivers and lakes (Krammer and Lange-Bertalot, 1986; Patrick and Reimer, 1975), is abundant in oligotrophic and mineralized systems (Tornes, et al., 2007) and neighbor countries (Khazal et al., 2018). Further, *A. pellucida* is an oligo-mesotrophic species (Van Dam et al., 1994), as well as a cosmopolitan alkaliphilous taxon, which is found in pH 6.2-8 (Lowe, 1974). Additionally, the species can be observed as planktonic despite its existence as benthic taxon in most cases (Krizmanic et al. 2008). Although *A. pellucida* is a widely-distributed taxon, it was only reported from Kashkan River (Safiallah et al., 2020) and Gole-Ramian spring with low abundances in Iran (Ahmadi et al., 2019). Furthermore, *Cymbopleura kuelbsii* firstly recorded by Kheiri et al (2019), this taxon is newly initiated species (Krammer, 2003) and there is not more data on its ecology, however in our study *C. kuelbsii* was abundant only in winter. The water was well oxygenated with low nutrient and moderate conductivity.

Assymetric biraphid was determined as the most diverse group, which is in line with the results of the prevoius research in Golestan province (Aghatabay, 2018; Bayani, 2019; Dadgar, 2016; Lakzaie, 2016), while they are inconsistent with those related to Kashkan River in Zagros (Safiallah et al., 2020). In the present study, the genera of the group included *Amphora*, *Cymbella*, *Cymbopleura*, *Delicatophycus*, *Encyonopsis*, *Gomphonema*, *Rhoicosphenia* and *Halamphora*. Further, symmetrical biraphid (*Amphipleura*, *Craticula*, *Diploneis*, *Frustulia*, *Stauroneis*, and *Navicula*) Araphids (*Diatoma*, *Fragilaria*, and *Ulnaria*), Nitzschioid (*Hantzschia*, *Nitzschia*, and *Tryblionella*), monoraphids (*Achnanthidium* and *Cocconeis*), and Surirelloid (*Surirella*) were present in the waterfall. Unlike the previous studies, centric group had no representative in our study along with Epithemioid and Eunotioid groups.

Among the diatom genera, *Nitzschia*, *Navicula*, and *Gomphonema*, as large genera with many species, have been usually reported as the most species-rich genera in the studies conducted in Iran (Ahmadi et al., 2019; Mehrani Adl, 2020; Panahy et al., 2018) and the world (Jakivljevic et al., 2016; Noga et al., 2013; Solak, 2011). Furthermore, *Cymbopleura* was obtained as the most species-rich genus, which is not in line with the results of other studies in Golestan province, and above-mentioned ones. Among the species of this genus identified in our study *C. citrus* for the first time introduced by Aghataby (2018) and Ahmadi et al (2019) to diatom flora of Iran from Golestan Province. Other species of *Cymbopleura* in-

cluding *C.* cf. *vrana* have not been reported from Golestan Province yet, even though were recorded from Karaj River by Kheiri et al (2019). *Gomphonema* and *Navicula* were among the major genera too, however unlike their studies, *Nitzschia* was less important in our work.

In this research, three taxa were recorded for the first time for diatom flora of Iran. *Delicatophycus* established as a new genus, for substituting genus *Delicata* which was declared invalid by Wynee (2019). Indeed, *Delicatophycus* sinensis that were recorded by Kheirei et al (2019) as *Delicata sinensis* from Karaj River as new record for Iran, and *Delicatophycus* in which there is no record in diatom flora of Iran. Additionally, *D. verenae* was reported as a new species only by Krammer (2003) and no data are available on the ecology of the species. The results of the present study demonstrated the above-mentioned species of the genus in alkaline water with low conductivity, and nutrients, both of which were abundant in samples, especially *D. Sinensis*. *D. verenae* was introduced for the first time.

Further, genus *Tryblionella* was determined as another new record, which was described by Smith (1853), and many of its species were derived from the large genus *Nitzschia* by Round et al. (1990). Regarding *Tryblionella*, raphe is eccentric, similar to that of *Nitzschia*, while *Tryblionella* has longitudinal undulations on valve face. Furthermore, *Tryblionella brunoi* (T. brunoi) was reported in meso-to oligotrophic waters, as well as eutrophic ones in a few cases (Powers, 2018).

Stauroneis is a genus from symmetrical biraphid group with naviculoid cells. Further, the main characteristics of the genus are presence of stauros in the valve center and punctate striae. Additionally, Most species of this genus are characteristic of oligotrophic waters in the tepmerate zone (Lange-Bertalot and Metzeltin, 1996). However *S. separanda* is distinguished by smaller valves and low undulation in outline, is similar to *S. smithii*. Furthermore, this taxon was recorded firstly by Werum and Lange-Bertalot (2004), as widely distributed in calcareous springs with high conductivity in Europe. While Levkov et al. (2016) recorded this species from mesotrophic calcareous waters from Macedonia. Moreover, this taxon was observed in co-occurrence with *S. smithii* that is in accordance with present study.

Given that Iran is considered as a vast country with diverse aquatic systems, such local and detailed studies can result in improving the knowledge on diatom flora in Iran. In fact, the present study is the first research on the diatom diversity of waterfall in Iran. In general, the diatom flora of Golestan Waterfall included some cosmopolitan taxa found in various habitats, along with taxa with

narrow distribution. The presence of taxa with limited distribution worldwide indicated the importance of conserving waterfalls. Accordingly, further studies should be conducted in such ecosystems. Due to the tourism value of waterfalls, they are subjected to human impacts and environmental disturbances. Thus, the protection of waterfall habitats is important for conserving aquatic diversity.

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