Physical-Chemical Factors Affecting Diversity and Distribution of Blue-green Algae in the Southern Caspian

Sarvenaz Bigham Soostani[1] , Behrouz Zarei Darki[2*], Rahman Patimar[3], Eisa Jorjani[4]

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Abstract

The effect of physical and chemical factors on the species diversity, distribution and density of blue-green algae was examined along the Nour shore of the Caspian Sea. Sampling was conducted seasonally during four sampling campaigns along four transects (A, B, C, D) each bearing three sampling sites (from 1 to 3) in February, May, July and November 2014. As a result, 13 species of Cyanophyta division belonging to nine genera were identified. The highest cyanophyte cell density was observed in summer, when 38×10^5 cellsl^{-1} was observed. However, the results of variance analysis illustrated there is no significant differences between Shannon's diversity index in different seasons ($P<0.05$ and $F=0.521$). The spatial distribution of blue-green algae was non-uniform along the shore in the study area, but, along transects, the results proved that the highest density was usually at the first station of each transects, except C. The most favorable conditions for blue-green species growth were in late spring and summer, when the water temperature increased. In addition, the environmental variables such as salinity and nutrients, especially, phosphates affect the species diversity, distribution and density of blue-green algae in the investigated area according to the Pearson correlation and linear regression.

Keywords: Phytoplankton, Cyanobacteria, Density, Pearson correlation, Nour shore.

Introduction

Blue-green algae (syn. Cyanophyta, Cyanobacteria, Cyanoprokaryota) are photosynthetic Prokaryotes and oxygen producers including the features both eukaryotic algae and prokaryotic bacteria (Kalaitzis et al., 2009). They are definitely considered as the most successful organisms on the earth that have the highest genetic variety and live in a wide range of earth's habitats (De los Ríos et al., 2007). Furthermore, they play an important role in water quality since some of their species are able to generate toxins (Stewart and Falconer, 2008).

Different environmental conditions are constantly affected diversity, distribution and density of cyanobacteria (Coles and Jones,

1- School of Marine Science and Technology, Hormozgan University
2- Department of Natural Resources and Marine Sciences, Tarbiat Modares University
3- Fisheries Department, Gonbad-e-Qabus University
4- Engineering and Basic Sciences Department, Gonbad-e-Qabus University
*email: zareidarki@modares.ac.ir

2000; Moisander et al., 2002; Wetz and Wheeler, 2003; Robarts and Zohary, 1987; Ploug, 2008); nutrients, light, turbulence and temperature. In general, temperature is considered as one of the important factors affecting the growth of blue-green algae (Ward and Castenholz, 2000). The warm standing water may cause the increasing blue-green algae density right up to bloom (WHO, 1999). Moreover, nutrients, pH and salinity also impact on the growth of blue-green algae (Hakanson et al., 2007). Cyanophyta can adapt towards high salinity and mostly, they are found in environments with high salinity (Anagnostidis and Pantazidou, 1991; Roussomoustakaki and Anagnostidis, 1991).

In terms of morphology, these algae have a wide range of diversity (Ward and Castenholz, 2000). In Iran, 198 intraspecific taxa from the different aquatic ecosystems were reported (Zarei Darki, 2011). In the Caspian Sea, according to the investigated data, 102 species of Cyanophyta were totally identified (Heydari et al., 2018). The Iranian researchers conducted the several surveys on the study of cyanobacterial flora including their seasonal distribution, cell abundance and biomass in the Southern part of the Caspian Sea (Golaghai et al., 2010; Emtiazjoo et al., 2012; Pourghola et al., 2014; Heydari et al., 2018). Furthermore, Emtiazjoo et al. (2012) also assessed the trophic status in the Iranian waters of the Caspian Sea. Mahdavi et al. (2014) studied the effect of temperature and salinity on the cyanobacteria in the Southern Caspian (the stretch between Tonekabon and Amirabad). Naghdi et al. (2018) examined the effects of cyanobacteria bloom through the MODIS-L2. Their work covered the location from Astara to Bandar Torkaman, but the vicinity of the Nour shore was overlooked.

Thus, the changes in diversity, distribution and density occur under the influence of the numerous ecological factors in the algal flora structure of an aquatic ecosystem. The aim of the present study was to assess the impact of physical and chemical factors on diversity and distribution of blue-green algae along the Nour coast, Mazandaran province.

Materials and Methods

According to the current research, 4 transects and 12 sampling sites were established along the Nour shore in the southern Caspian Sea and samples were seasonally taken during four sampling campaigns in February, May, July and November 2014. Transects were specified perpendicular to the shore and in parallel with the estuaries of Sabze-rud River (A), Nour River (B) and Lavij River (D). The third transect was right in front of Department of Natural Resources and Marine Sciences of Tarbiat Modares University (C) (Table 1 and Figure 1). The different sampling tools such as a plankton net, Secchi disk, a Ruttner bathometer, the containers with different volumes, GPS and a boat were used for sampling of phytoplankton. In parallel with sampling, the factors including temperature, salinity, oxygen saturation (O%), dissolved oxygen (DO) as well as pH were measured in each site. The phytoplankton samples were transported to the laboratory in dark cool containers in the shortest possible time (Fathi and Flower, 2005).

Blue-green algae were prepared and recognized according to standard methods mentioned in monographs and identification keys (Proshkina-Lavrenko, 1974; Kondratyeva, 1968; Komarek and Anagnostidis, 1999; Komárek and Hauer, 2009).

Measurement of chlorophyll-a and pheophytin were carried out immediately after sam-

Table 1. Geographic coordinates and depths of the investigated sites in February, May, July and November 2014.

	Transect	Site	Depth	Northern latitude	Eastern longitude
1	Sabze-rud River	A1	0.5	36°34’55	52°59’24
		A2	5.0	36°34’40	52°59’82
		A3	12.0	36°35’99	52°59’38
2	Noor River	B1	0.5	36°35’18	52°01’07
		B2	5.0	36°35’82	52°01’98
		B3	12.0	36°35’37	52°01’02
3	Tarbiat Modares University	C1	0.5	36°35’22	52°02’05
		C2	5.0	36°35’62	52°02’11
		C3	12.0	36°35’90	52°02’12
4	Lavij River	D1	0.5	36°35’74	52°05’89
		D2	5.0	36°35’95	52°05’72
		D3	12.0	36°36’21	52°05’08

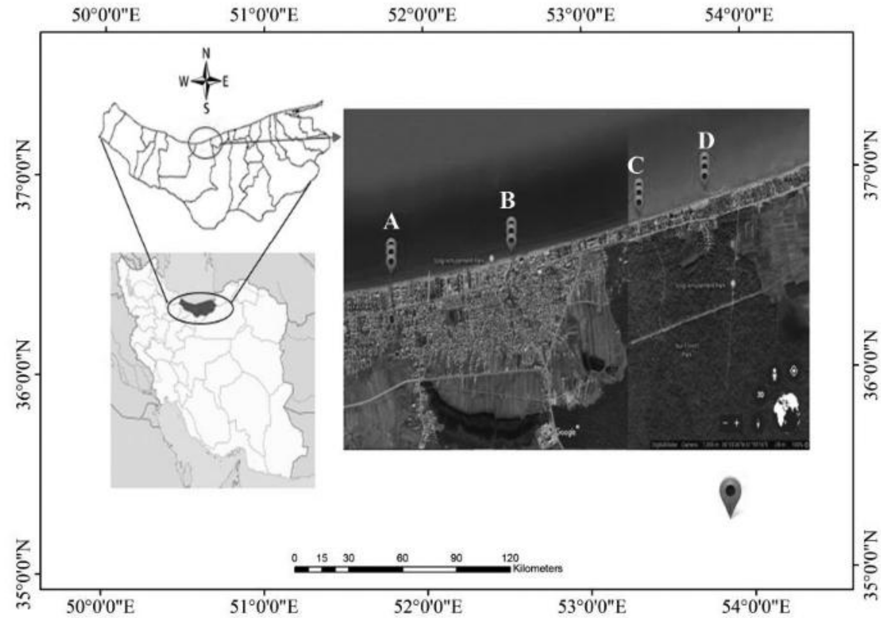

Fig.1. Map showing the sampling transects (A, B, C, D) each bearing three sites (1, 2, 3) along the Nour shore of the Southern Caspian.

pling. Acetone and citric acid were used for extraction, and results were interpreted using a BioTek ELISA microplate reader (model 264852) at wavelengths of 430, 450, 480, 630, 647, 664, 665 and 750 (Lorenzen, 1967; APHA, 1999). To analyze the species diversity of the blue-green algae, the Shannon-Wiener Index (SWI) was estimated using the PRIMER 5.0 program. To determine differences among seasons and stations, the SWI was applied with the SPSS (ver. 20) software. The Chi-square test ($\chi 2$) was used to compare species and cell abundances among stations and seasons. To determine the relationships between blue-green species and environmental variables, the Pearson correlation and linear regression were applied using SPSS (ver. 20) and Excel 2013 softwares.

Results

The mean and standard deviations as well as minimum and maximum values of physico-chemical factors during investigated time are presented in Table 2. Furthermore, the chlorophyll-a and pheophytin levels were measured. A maximum chlorophyll-a value 254.97 mgm^{-3} was pointed at transect A in summer. The lowest concentration of pheophytin was 21.8 mgm^{-3} in spring while a maximum pheophytin value of 468.81 mgm^{-3} occurred in transect C in summer.

Totally 13 species of Cyanophyta were recognized, among them only *Chrysosporum bergii* was a brackish species (Table 3). The identified species belonged to *Anabaenopsis, Chroococcus, Chrysosporum, Merismopedia, Microcoleus, Microcystis, Nostoc, Oscillatoria* and *Phormidium* genera. The most numerous species diversity was noted 8 species in transect A in summer, while, in winter, smallest one was in transect D, 4 species. The spatial distri-

Table 2. The ranges of the physico-chemical factors of the coastal water along the Nour shore in the Southern Caspian Sea over the studied period.

Season	Site	Physical and chemical factor				
		pH Mean ± SD Min-Max	DO, mg/l Mean ± SD Min-Max	O% Mean ± SD Min-Max	Salinity, ‰ Mean ± SD Min-Max	Temp.°C Mean ± S.D Min-Max
Winter	A	8.16±0.02 8.15-8.19	11.76±0.16 11.58-1.91	106.76 ±0.11 106.7-106.9	10.96±0.63 10.28-11.53	10.4±0.41 10.0-10.9
	B	8.19±0.03 8.15-8.22	10.56±0.78 9.80-11.36	97.66±4.87 94-103.2	10.97±0.99 9.82-11.56	10.9±0.17 10.8-11.1
	C	8.22±0.03 8.19-8.26	9.73±0.47 9.19-10.08	91.53±7.72 84.5-99.8	11.60±0.12 11.48-11.72	10.96±0.11 10.9-11.1
	D	8.30±0.02 8.27-8.32	9.15±0.22 8.9-9.32	83.93±1.4 82.6-85.4	11±1.07 9.76-11.65	11.1±0.49 10.8-11.7
Spring	A	8.46±0.22 8.20-8.63	7.50±0. 26 7.30-7.80	91.9±4.06 88.50-96.40	11.23±0.8 11.16-11.32	24.6±0.25 24.4-24.9
	B	8.65±0.04 8.61-8.7	7.53±0.2 7.3-7.7	91.06±1.92 89-92.8	11.1±0.18 10.96-11.32	25.4±0.1 25.3-25.5
	C	8.68±0.05 8.62-8.72	7.5±0.17 7.3-7.6	94.03±1.92 92.3-96.1	11.43±0.09 11.32-11.29	26.16±0.58 25.5-26.6
	D	8.66±0.03 8.67-8.70	7.56±0.15 7.4-7.7	92.6±1.35 91.2-93.9	11.2±0.09 11.1-11.27	25.4±0.25 25.2-25.7
Summer	A	8.38±0.09 8.31-8.49	4.56±0.87 3.60-2.30	57.5±11.4 44.7-66.8	11.6±0.01 11.58-11.6	27.9±0.63 27.2-28.3
	B	8.57±0.01 8.56-8.58	6.60±2.02 4.8-8.8	75.5±1.67 74.6-77.5	11.6±0.01 11.6-11.64	27.6±0.6 27-28.2
	C	8.67±0.03 8.63-8.69	5.50±0.30 5.2-5.8	71.9±3.58 67.9-74.8	14.1±4.25 11.6-12.07	28.3±0.17 28.1-27.4
	D	8.68±0.03 8.66-8.72	6.03±0.05 6-6.1	77.26±0.4 76.9-77.7	11.7±0.03 11.67-11.73	28.0±0.37 27.6-28.3
Autumn	A	8.60±0.24 8.32-8.77	13.04±0.8 12.3-13.9	107.93±1.18 107.2-109.3	11.27±0.05 11.22-11.33	14.7±0.55 14.1-15.1
	B	8.48±0.16 8.31-8.64	7.96±0.58 7.3-8.4	75.5±1.67 80.3-81.4	11.1±0.16 10.98-11.3	11.7±0.41 14.6-15.0
	C	8.66±0.06 8.6-8.72	12.44±2.14 10.44-14.7	107.16 ±2.01 105.3-109.3	11.14±0.16 10.96-11.25	14.7±0.15 14.6-14.9
	D	8.58±0.13 8.43-8.67	10.25±0.32 9.88-10.45	101.53 ±0.4 94-105.4	10.67±0.67 9.94-11.25	15.0±0.15 14.9-15.2

bution of cyanophytes along transects and the shore in the study area was non-uniform and showed that, in general, the highest density was usually at the first stations (A1, B1, D1) of each transect. The transect C was the only exception, where blue-green species diversity was higher at the second and third stations.

The cell number of blue-green algae reached up to 38 $\times 10^5$ cells l^{-1} along the Nour shore. During the investigated time, the smallest cell number was recorded for *Ch. turgida* and *Nostoc* sp. The species of *A. nadsonii, M. tenuissima, O. tenuis, O. limosa* were observed quite often. Their densities in different seasons are shown in Figure 2. According to the Chi-square test ($\chi 2$), frequency comparison of blue-green algae was not similar at each station during different seasons ($\chi 2$, $P< 0.05$). On

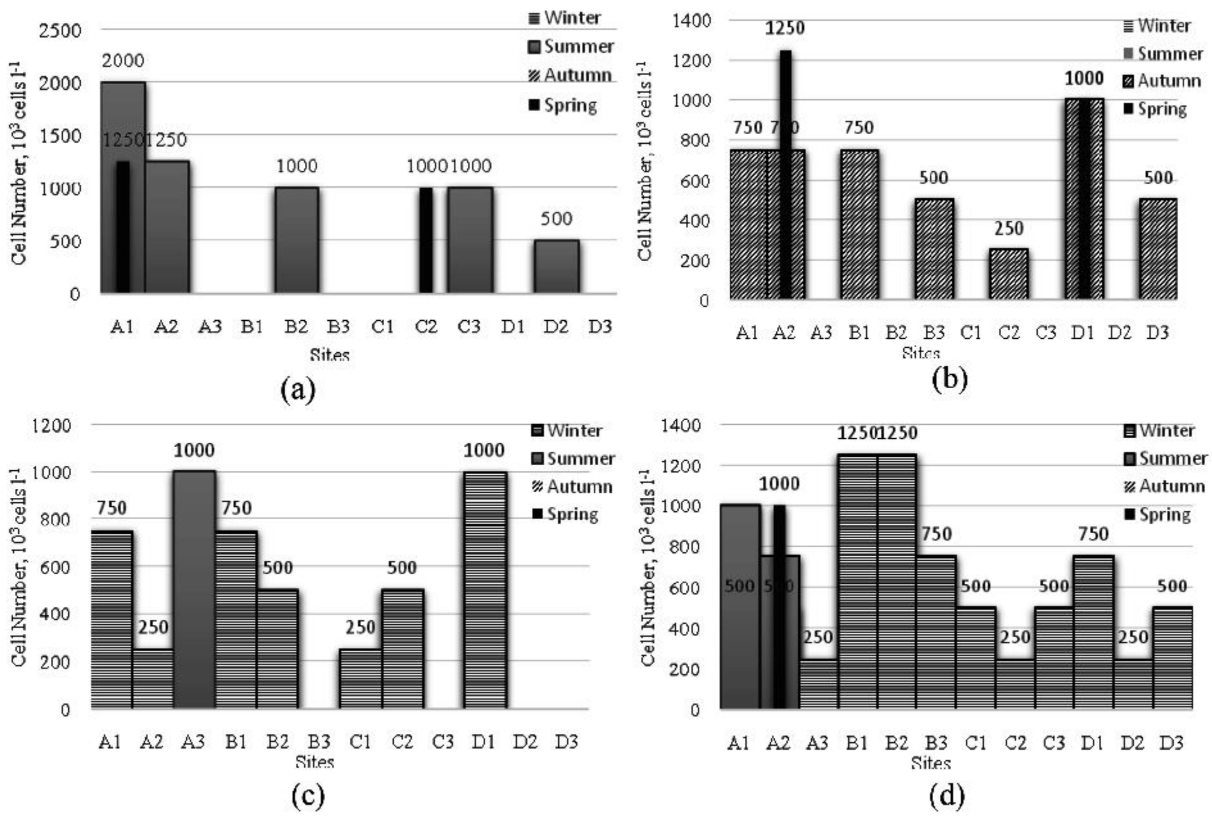

Fig. 2. The population density of the most numerous species at different sampling stations (from 1 to 3) along transects (A to D) in different seasons. (a) *A. nadsonii*, (b) *M. tenuissima*, (c) *O. tenuis*, (d) *O. limosa*.

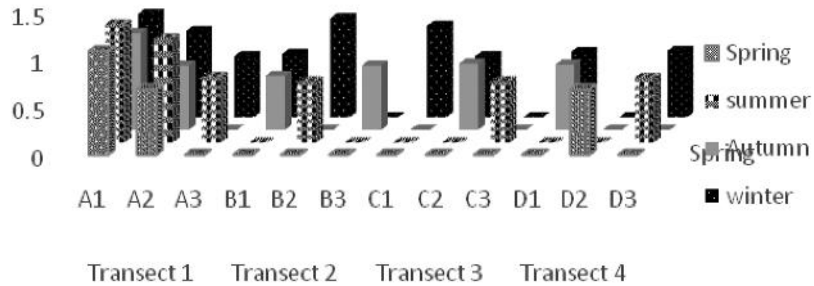

Fig. 3. Variation of the Shannon-Wiener Index applied to the planktonic cyanophyte species diversity at different sampling stations (1 to 3) along transects (A to D) in different seasons.

Table 3. Blue-green algae species composition and distribution along transects (A, B, C, D) in phytoplankton of the coastal waters from the Nour shore of the Caspian Sea over the study period.

N	Taxa	Seasons															
		Winter				Spring				Summer				Autumn			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	*Anabaenopsis nadsonii* Woronichin 1929	-	-	-	-	+	-	+	-	+	+	+	+	-	-	-	-
2	*Chroococcus turgidus* (Kützing) Nägeli 1849	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
3	*Chroococcus* sp.	-	-	-	-		-	-	-	+	-	-	+	-	-	-	-
4	*Chrysosporum bergii* (Ostenfeld) E.Zapomelová, O.Skácelová, P.Pumann, R.Kopp & E.Janecek	-	-	-	-		-	-	-	+	-	-	-	+	-	-	-
5	*Merismopedia tenuissima* Lemmermann 1898	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-	-
6	*Merismopedia* sp.	-	-	-	-	-	-	-	-	-	+	-	-	+		+	+
7	*Microcoleus amoenus* (Gomont) Strunecky, Komárek & J.R.Johansen in Strunecky et al. 2013	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
8	*Microcystis* sp.	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
9	*Nostoc* sp.	-	-	+	-	-	-	+	-		-	-	-	-	-	-	-
10	*Oscillatoria limosa* C.Agardh ex Gomont 1892	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-
11	*Oscillatoria tenuis* C.Agardh ex Gomont 1892	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-
12	*Oscillatoria* sp.	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-
13	*Phormidium* sp.	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-

Table 4. Results of the determination coefficients (R^2) and linear regression equation (Y) revealing relationships between species density and physical-chemical factors.

Correlation	sig	Season	Line equation	F	R^2
AN – ChT	**	spring	Y= 90909.09+ 0.0733X	13.54	0.533
AN – chlorophyll-a	**	spring	Y= 21906.85 + 1.135X	15.53	0.569
ChT– MA	*	spring	Y= 1.286E-10 + 0.750X	8.33	0.400
ChT – chlorophyll-a	**	spring	Y= -81654.59 + 1.417E8X	588.41	**0.982**
OL – MT	**	spring	Y= 90909.09 + 1.159X	13.54	0.533
MT – phosphate	**	spring	Y= -1.774E6 + 2.159E7X	14.17	0.545
OL – phosphate	*	spring	Y= -1.238E6 + 1.455E7X	16.43	0.584
MA chlorophyll-a	*	spring	Y= 35515.95 + 8.993E7X	9.61	0.439
CB ChT	**	summer	Y= 90909.09 + 2.545X	36.74	**0.765**
CB – OL	*	summer	Y=75697.21 + 1.195X	7.83	0.383
ChT OL	**	summer	Y= -8964.14 + 0.490X	15.56	0.570
ChT – phosphate	**	summer	Y= -279326.79+ 1.224E6X	16.65	0.587
Osp. – pH	*	summer	Y= 1.14+(-1.32)X	6.31	0.326
Osp – O%	**	summer	Y= 1857245.78+(-25149.69)X	24.33	**0.680**
Osp. – chlorophyll-a	**	summer	Y= -71492.52 + 4196.24X	5810.64	**0.998**
Osp – pheophytin	**	summer	Y= -72637.98 + 2284.18X	5111.90	**0.998**
Chsp – pheophytin	**	autumn	Y= -1145242.38 + 45661.60X	13.21	0.526
Msp – Psp	**	autumn	Y= -3.95 + 5X	.	**1.00**
OT salinity	**	Winter	Y= 4.55 + (-379109.14)X	15.70	0.572
OT – temperature	*	Winter	Y= -5736867.46 + 559036.14X	6.16	0.320
OT transparency	**	Winter	Y=1.007E6 + (-399938.23)X	15.59	0.570
OT chlorophyll-a	*	Winter	Y= -490050.61 + 18437.54X	5.26	0.279
MA – DO	*	Winter	Y= -2098252.01 + 233940.03X	5.22	0.277

*Correlation is significant at p = 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed).

Abbreviations: AN, *A. nadsonii*; ChT, *Ch. turgidus*; MA, *M. amoenus*, OL, *O. limosa*; MT, *M. tenuissima*; CB, *Ch. bergii*; Osp, *Oscillatoria* sp.; Chsp, *Chroococcus* sp.; Msp, *Merismopedia* sp.; Psp, *Phormidium* sp; OT, *O. tenuis*.

the other hand, increase in temperature caused increase in diversity and population density. The results of the Shannon's diversity index showed that the highest variety of species was evaluated 1.25 in summer and the least diversity of species was 0.56 in autumn (Figure 3). The variance analysis of these information illustrated that there is no significant differences between Shannon index in different seasons ($P<0.05$, $F=0.521$). The identified species were correlated with some of the measured environmental factors (Table 4).

M. tenuissima as well as Ch. turgidus density were moderately correlated (up to 50%) with phosphate ($P < 0.01$) in spring and summer, respectively. Fifty percent of the variation in

O. tenuis density could be ex plained by salinity and transparency which had a negative impact on *O. tenuis* while temperature affected less on its cell number (32%). Moreover, the strong negative correlation was found between the number cells of *Oscillatoria* sp. and O%. The highest coefficients of determination (99%-100%) were observed between a species and chlorophyll-a or pheophytin. This clearly shows the current species formed this pigment concentration. It should be noted that some following species: *A. nadsonii* with *Ch. turgidus; O. limosa* with *M. tenuissima* and *Ch. turgidus; Merismopedia* sp. with *Phormidium* sp grew well in tandem with each other or alternate through the year.

Discussion

In the Southern Caspian, blue-green algae contribute only slightly to the algological composition from 8 to 17% (Tahami et al, 2012; Heydari et al. 2018), in contrast to the northern Caspian, where cyanophytes can take a leading position both the cell number and biomass (Abdurakhmanov et al., 2010). The reason for that is, on the one hand, the higher water salinity in the Southern Caspian and, on the other hand, the higher biogenic element content because of anthropogenic press in waters of the north Caspian (Kosarev, 2005). The study of the quantitative and qualitative composition of blue-green algae along the Nour shore proved that a relatively little species diversity of blue-green algae is distributed in the investigated area, representing by 13 species. Totally, their density reached 38× 10^5 cellsl^{-1} in summer that characterizes waters as moderate probability of adverse health effects (WHO, 1999). It is lower than the maximum cyanobacteria number of 80.18× 10^5 cells1^{-1} that was recorded, so far, in the Southern Caspian (Mahdavi et al., 2014). Species of *O. limosa* was the most frequent species across the entire southern coast of the Caspian Sea in the previous study during summer (Tahami et al., 2012; Mahdavi et al., 2014; Heydari et al., 2018).

The findings confirm that this species is more commonly found in planktonic form during winter. *O. limosa* is a representative of microphytobenthos and fouling developing in large number and breaking away from the substrate, their cells enter the water column, where they can grow again (Terenko and Nesterova, 2015). The mixing of water masses in the Caspian Sea occurs more in winter due to winter storms (Caspian Sea - State of Environment, 2011), so that causes the entry of the periphyton species to plankton at this time of year. In summer, *A. nadsonii*, that is a structure-forming species of the algal flora in the Black sea (Terenko and Nesterova, 2015), was the most abundant species. In autumn, a brackish and fresh water species of *Merismopedia* dominated in the coastal water along the Nour shore. The spatial distribution of cyanophytes along transects was non-uniform and the highest density was usually observed at the first stations (A1, B1, D1) of each transect, except C. It was because the first stations of transects A, B and D were located in estuaries of the rivers, where the salinity are low and the pollution are high, created a good conditions for cyanobacteria growth (Vincent, 2009). Furthermore, Shannon's diversity index which was calculated in the present study, demonstrated that there is a significant difference between Shannon index in different seasons ($P<0.05$, $F=0.521$) as it was shown in previous studies (Tahami et al., 2012).

The quite high temperature levels and light intensity cause cyanobacteria development and growth during warm seasons of a year (Khosravi, 1999). The temperature regime of the Caspian Sea is rather unusual (Kosarev, 2005). Studies of physicochemical factors of southern beaches of Caspian Sea in several stations (Astara, Anzali Port, Sefid-rud, Tonekabon, Nowshahr, Amirabad, Torkaman Port) showed the maximum temperature 20.4°C during summer and the minimum temperature 9.66°C in winter (Pourgholam et al., 2014). In comparison with it, the temperature values, were higher both in winter and summer. Increase temperature increases diversity and density of cyanobacteria along the Nour shore of the Southern Caspian. Emtiazjoo et al. (2012) also mentioned higher abundance and distribution of cyanobacteria in south coast of the Caspian Sea during summer. On the contrary, minimum population density was observed in winter when the temperature went down (Mahdavi et al., 2014). Furthermore, dynamic, spatial and temporal variations of cyanophyte abundance mainly depend on intensity of light throughout the year (Cloern, 1987). There is also a direct correlation between transparency and abundance of the blue-green algae (Howerton, 2001). According to the conducted *statistical analysis*, transparency affected on density of *Oscillatoria tenuis* while *Oscillatoria* sp. grew at oxygen deficiency.

Another ecological parameter that impacts on diversity and abundance of cyanobacteria in the Caspian Sea, is salinity as major abiotic factor (Aladin and Plotnikov, 2004). Among all species, which were recorded, *Ch. bergii* was only brackish; twelve others were freshwater species due to the fact that samples were taken mainly in estuaries in order to trace the salinity influence on them. The salinity, as shown by correlation analysis, affected, firstly, the distribution and density of freshwater species of *O. tenuis*. The correlations between salinity and another twelve recorded species were not established. However, the salinity value from 5 to 20 ppt is regarded as the ecological factor of species abundance of *M. aeruginosa* in the Baltic Sea (Maestrini et al., 1999) as well as *Nodularia* sp. and *Anabaenopsis* sp. (Moisander et al., 2002). Algal growth usually correlates with nutrients, which in turn decrease population density (Canfield, 2002). In the present research, a significant factor affecting the density of blue-green algae was phosphate concentration. It impacted primarily on the population density of *Merismopedia tenuissima* as well as *Chroococcus turgidus* according to the conducted *statistical analysis*. It is necessary to note that *statistical analysis* carried out by Khosravi Rineh (2011) also illustrated a positive and strong correlation between population density especially cyanobacteria and phosphate concentration.

In conclusion, the allochthonous pathway was dominated in the development of the blue-green flora in the coastal water along the Nour shore of the Caspian Sea. The basis of their species diversity is formed by algae carried by the waters of small rivers flowing into the Caspian Sea. As a result, a freshwater complex of blue-green algae dominates in the coastal waters along the Nour shore. The most favorable conditions for the river water flowing into the Caspian Sea are made in the late spring and summer, when the greatest diversity and highest cell number was recorded. In addition, the ecological factors such as temperature, first of all, salinity and nutrients as phosphates, affect the species diversity, distribution and density of blue-green algae in the investigated area according to the conducted *statistical analysis*.

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