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Spatial and Temporal Distribution and Biomass Trend of Planktonic Green Microalgae (Chlorophyta) in Southeast of the Caspian Sea

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ABSTRACT

Aims Green algae (Chlorophyta), as a diverse group of algae, has almost omnipresent distribution in the Caspian Sea and plays an important role in ecosystem functioning. The aim of this study was to investigate the spatial and temporal distribution and biomass trend of planktonic green microalgae (Chlorophyta) in southeast of the Caspian Sea.

Materials & Methods This experimental study was performed by sampling water from south of the Caspian Sea in Mazandaran province during the winter 2015 and summer 2016. Water sampling was done along two half-lines perpendicular to the estuaries of "Tajan" and "Babolrood" rivers in 8 stations and 4 depths (less than 1, 5, 10, and 20 m) in the euphotic zone, at 8 stations along the linear transects. Settlement method was applied to the preserved samples in formaldehyde 4%, in order to the qualitative and quantitative analysis of phytoplankton. The biomass of Chlorophyta was calculated, using the geometric shape method. The data were statistically analyzed, using PRIMER 6 and SPSS 19 software and the diagrams were drawn by Excel 2013.

Findings Of total 29 identified Chlorophyta species, 28 species were present in the summer with the highest abundance of Chlorella sp. (1395×105±671×104 N/m3) and, 19 species were observed during the winter, with the highest abundance of Binuclearia sp. (456×105±155×104 N/m3). Binuclearia sp. also showed the highest biomass in both seasons.

Conclusion The diversity of Chlorophyta is influenced by seasonal variations. Estuaries are the richest stations in terms of abundance and biomass of planktonic Chlorophyta in summer.

Keywords Phytoplankton; Chlorophyta; Distribution; Biomass; Caspian Sea

CITATION LINKS

[1] Global international waters assessment, GIWA ... [2] Biology and physiology ... [3] Freshwater algae: identification and use ... [4] Studying the diversity, density ... [5] An Introduction to ... [6] Diversity, distribution and ecology of green ... [7] Using river microalgae as indicators ... [8] A review of microalgae as indicators ... [9] Monitoring the seasonal dynamics of ... [10] Seasonal and regional distribution of ... [11] Seasonal variability of phytoplankton in ... [12] Seasonal distribution of dominant phytoplankton ... [13] Checklist of phytoplankton taxa in the ... [14] Study on stable and disturbance status ... [15] Theory and practice of water and ... [16] Distribution and composition of phytoplankton in the ... [17] Total nitrogen, total phosphorus, and nutrient limitation ... [18] Simpson diversity and the Shannon–Wiener index as special ... [19] The measurement of diversity in different types of ... [20] Caution is needed when applying Margalef ... [21] Seasonal and hydrological control of phytoplankton nutrient ... [22] Regional evaluation of nutrient transformation and phytoplankton ... [23] Phytoplankton biomass and species composition of lake Gölbaşı ... [24] A checklist of phytoplankton species ... [25] Diversity of benthic microalgae in response ... [26] Epilithic algal communities and their relationship ... [27] Phytoplankton diversity during the spring ... [28] Seasonal phytoplanktonic diversity ... [29] Diversity and seasonal variation of phytoplankton ... [30] An annotated checklist of dinoflagellates ... [31] Temporal and spatial variation of phytoplankton ... [32] Study on Bacillariophyta phylum of phytoplankton ... [33] Bacteria, algae, fungi and freshwater ... [34] Fluctuations of phytoplankton community ... [35] Phytoplankton species composition and abundance ...

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Introduction

Phytoplanktons are among the main producers of the oceans and, undoubtedly, they are considered important and valuable reserves in terms of carbon fixation into organic materials as the base of the energy pyramid [1-3]. Other organisms, while affiliated with each other in the food chain, are directly and indirectly dependent on phytoplankton; therefore, continuous monitoring the diversity and biomass of these tiny producers in aquatic ecosystem are particularly important [4].

Chlorophyta, as a diverse group of green algae, consists of Ulvophyceae, Trebouxiophyceae, and Chlorophyceae (UTC), which has many representatives in phytoplankton communities [5]. Green algae occupy a wide range of habitats, and are present almost everywhere, from wet soil to hot springs [6].

People and industries increasingly occupy the habitats of plants and animals, and human activities also have extinct numerous plant and animal species. The definitive step to identify ecosystems, where require conservation, reconstruction, and sustainable exploitation, is finding the natural distribution patterns and abundance of species as well as the processes determining these dispersions; so, the phycologists in all over the world, are seeking to evaluate distribution and abundance of microalgae to save their stock and keep marine ecosystem functioning [7-9].

Ganjian *et al*. studied the seasonal and regional distribution of phytoplankton in the southern part of the Caspian Sea, which led to the identification of 163 species of phytoplankton (including 12 genera and 31 species of Chlorophyta) [10].

Pourgholam *et al*. investigated the seasonal variation of phytoplankton in the southern Caspian Sea and 182 species of phytoplankton (including 31 species of Chlorophyta) were identified [11].

Mahmoudi *et al*. reported 129 species of phytoplankton (17 species of Chlorophyta) by the seasonal distribution of dominant phytoplankton in the southern Caspian Sea (Mazandaran coast). They also found a correlation between the distribution of phytoplankton and environmental parameters [12]. Bagheri and Fallahi presented a Checklist of Phytoplankton Taxa in the Iranian coastal water of the Caspian Sea, including 158 species of phytoplankton (29 species of Chlorophyta) [13].

Also, Nasrollahzadeh *et al*. identified 229 species of phytoplankton (including 61 species of Chlorophyta) when studied on stable and disturbance status of Iranian coasts of the Caspian Sea based on changes of phytoplankton community structure [14].

The aim of this study was to investigate the spatial and temporal distribution and biomass trend of planktonic green microalgae (Chlorophyta) in southeast of the Caspian Sea.

Materials and Methods

Study area: This experimental study was carried out by sampling water from south of the Caspian Sea in Mazandaran province during the winter of 2015 and the summer of 2016. Water sampling was done along two half-lines perpendicular to the estuaries of "Tajan" and "Babolrood" rivers in 8 stations and 4 depths (less than 1, 5, 10, and 20 m; Figure 1 and Table 1).

Water sampling and analysis: 1 l of water from each sampling station was collected, using a water sampler (Niskin-Hydrobios, 2.5L), with 3 replications (3 l) [15]. The environmental parameters (DO, Temperature, Salinity, and pH) were recorded in each station, using water quality multi-parameter device AZ-8603 (Taiwan). The samples were preserved in buffered formaldehyde 4%, kept in 500 ml bottles, and immediately moved to the laboratory for further analysis. Settlement method was applied to water samples for the qualitative and quantitative analysis of phytoplankton [16]. Chlorophyta abundance was calculated by counting the number of species individuals and multiplying them by volume factor (relative to the volume of water samples) (Equation 1) and the biomass of Chlorophyta was calculated, using the geometric shape method (Equation 2):

Equation 1:

$$Density\ (N/m3) = water\ volume \times Volumetric\ factor \times Number\ of\ samples\ counted$$ [17]

Equation 2:

$$Biomass\ \left(\frac{g}{m3}\right) = Weight\ of\ each\ sample\ (from\ the\ geometric\ shape) \times Density$$ [17]

The data were statistically analyzed, using PRIMER 6 and SPSS 19 software for windows and charts were drawn by Excel 2013. Analysis of variance comparisons (One-way ANOVA) was done to compare the stations in terms of species abundance and biomass. To evaluate the relationship between physicochemical parameters and species abundance, the Spearman correlation coefficient (r) was used. Various diversity indices, including Shannon–Wiener diversity index (H) [18], Pielou evenness index (J) [19], and Margalef's richness index (D) [20] were used to establish the seasonal variation of species in different depths (Table 2). Indices were estimated based on the abundance of species, using PRIMER 6 software. The non-Metric Multidimensional Scaling (nMDS) ordination was conducted based on the Bray-Curtis dissimilarity matrix to evaluate clustering of samples from different depths.

Table 1) Geographical characteristics of sampling stations in the area

Area	Station	Sampling depth (m)	Latitude	Longitude
Babolsar	A(Estuary)	<1	52°65´32″	36°71´33″
	B1	<1	52°65´85″	36°72´05″
	B2	5		
	C1	<1	52°65´73″	36°72´74″
	C2	5		
	C3	10		
	D1	<1	52°65´36″	36°73´28″
	D2	5		
	D3	10		
	D4	20		
Sari	E(Estuary)	<1	53°11´51″	36°81´30″
	F1	<1	53°11´73″	36°82´21″
	F2	5		
	G1	1	53°11´85″	36°83´11″
	G2	<1		
	G3	10		
	H1	<1	53°11´83″	36°84´15″
	H2	5		
	H3	10		
	H4	20		

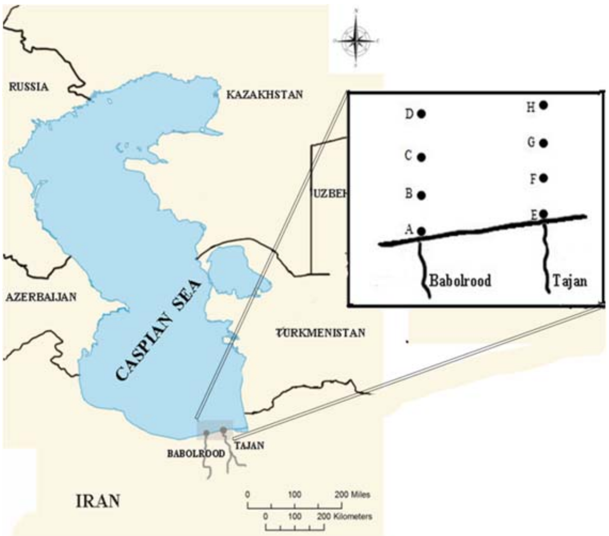

Figure 1) Location of sampling stations on the Iranian coasts of the Caspian Sea

Table 2) Equations for calculating Diversity, Richness, and Evenness indices

Index	Name	Formula
Diversity	Shannon Weiner	$H = \sum_{i=1}^{S} (P_i) LnP_i$
Richness	Margalef	$D = \frac{(S-1)}{ln\ N}$
Evenness	Pielou	$J = \frac{H}{Log(S)}$

Pi is the ratio of individuals i to the whole community; S is the number of all species in the community; ni is the number of individuals or the abundance of species i; N is the total number or the abundance of Chlorophyta in the studied area

Findings

Identified Chlorophyta consisted of 29 species (Table 3 and Figure 2). This is the first observation report of Carteria sp., Elakatothrix sp., and Tetrabaena sp. in the southern Caspian Sea coastal waters.

The total abundance of phytoplankton in summer was 7481×10^6±623 N/m^3 that Chlorophyta accounted for 7% of this amount; and in winter, the total abundance of phytoplankton community was 3410×10^6±223 N/m^3, of which 5% belonged to Chlorophyta.

The maximum diversity of Chlorophyta (28 species) was recorded in summer; when Chlorella sp., Binuclearia sp., and Selenastrum sp. were dominant species, respectively (Diagram 1-A). Also, 20 species were identified in winter, when Binuclearia (27%) sp. constituted the bulk of the population, following with Chlamydomonas sp. (22%) and Chlorella sp. (18%; Diagram 1-B). In summer, Chlorella sp. showed the highest abundance percentage (27%) in all depths, while in winter, Binuclearia sp. (27%) was dominant (Diagram 2).

Binuclearia sp. had the most biomass in both seasons. The total biomass of Chlorophyta was 335±4 g/m^3 in summer, of which 158±2 g/m^3 (47%) belonged to Binuclearia sp., followed by Scenedesmus sp. (102±17 g/m^3 [31%]) and Chlorella sp. (25±1 g/m^3 [8%]; Diagrams 3-A and 4).

The total biomass of Chlorophyta in winter was 117±4 g/m^3, of which 82±8 g/m^3 (70%) belonged to Binuclearia sp., and Scenedesmus sp. (12±1 [11%]) and Chlamydomonas sp. (11±4 g/m^3 [9%]) were ranked second and third (Diagrams 1-B and 4). Meanwhile, the highest abundance and biomass of planktonic Chlorophyta was recorded at the stations A and

E (Estuaries).

The highest value of Shannon-Weiner biodiversity index (1.87±0.02) and Margalef richness index (0.73±0.39) of Chlorophyta belonged to the estuaries and the surface layer (<1m) (Margalef index=0.48±0.099; Shanon–Weiner index=1.62±0.153), while Evenness index showed no significant difference between different depths and stations (0.89± 0.04; diagram 5). In estuaries and surface layer, the Margalef index in summer was significantly higher than in winter.

The result of correlation tests showed the highest correlation (+0.816) between Shannon-Weiner and Margalef indices in sampling stations. Salinity showed the highest correlation (-0.601) and depth a relatively acceptable correlation coefficient (-0.533) with Shannon-Weiner index. There was a significant difference between Shannon-Weiner and Margalef indices with depth variable and consequently the station variable. In contrast, the Pielou index did not show any significant differences among different depths.

The nMDS positioned the depth in the ordination space based on the species abundance. Depth of water sampling was relatively expressed in species abundance in summer (Diagram 6-A), and nMDS also revealed a strong statistically significant separation in abundance of Chlorophyta species of the estuaries and 20m layer with other marine stations in summer (p=0.0012), while there was no recognizable clustering in the winter (Diagram 6-B).

The seasonal changes of environmental parameters were presented (Table 4). There was a negative correlation (-0.528) between Chlorophyta biomass and water salinity, while there existed relatively weak negative correlation with depth (-0.458), stations (-0.341), and season (-0.381). There was a significant difference between the biomass and abundance of Chlorophyta in different depths (p<0.05). The Tukey test also confirmed this significant difference.

Table 3) The presence/absence of identified Chlorophyta, depending on depth and season in the studied area (2016)

Genus	**Summer**					**Winter**				
Depth (m)	Est	<1	5	10	20	Est	<1	5	10	20
Actinastrum hantzschii	+	+	-	-	-	+	+	-	-	-
Ankistrodesmus flacatus	+	+	-	-	-	+	+	+	+	+
Ankistrodesmus sp.	+	+	-	-	-	-	+	+	+	+
Acutodesmus obliquus	+	+	-	-	-	-	+	-	-	-
Binuclearia laterborni	+	+	+	-	+	+	+	+	+	+
.Binuclearia sp	+	+	+	-	+	+	+	+	+	+
Carteria sp.	+	+	-	+	-	-	-	-	-	-
.Chlamydomonas sp	+	+	+	+	+	+	+	+	+	+
Chlorella sp.	+	+	+	+	+	+	+	+	+	+
.Chodatella sp	+	+	-	-	-	-	+	-	-	-
Closterium parvulum	-	-	-	-	-	+	+	+	-	-
Coelastrum sphaericum	+	+	-	-	-	-	-	-	-	-
Crucigenia tetrapedia	+	+	+	-	-	+	+	-	-	-
Dictyosphaerium pulchellum	+	+	-	-	-	-	-	-	-	-
Elakatothrix sp.	-	+	-	-	-	-	-	-	-	-
Golenkinia radiata	+	+	-	-	-	-	-	-	-	-
Kirchneriella sp.	+	+	-	-	-	+	+	+	-	-
Largerhrimia genevensis	-	+	-	-	-	-	-	-	-	-
Monoraphidium sp.	+	+	-	-	-	-	+	+	-	-
Mougeotia sp.	-	+	-	-	-	-	-	-	-	-
Oocystis sp.	+	+	+	-	-	+	+	-	-	-
Pandorina morum	-	+	-	-	-	-	-	-	-	-
Pediastrum simplex	+	+	-	-	-	-	-	-	-	-
Scenedesmus obtusus	+	+	-	-	-	+	+	-	-	-
Scenedesmus opoliensis	+	+	-	-	-	+	+	-	-	-
Scenedesmus quadricauda	+	+	-	-	-	+	+	-	-	-
Selenastrum bibraianum	+	+	+	+	-	+	+	+	-	+
Tetrabaena sp.	-	+	-	-	-	-	-	-	-	-
Tetrastrum sp.	-	+	-	-	-	+	+	-	-	-

Figure 2) Some observed species of Chlorophyta; 1.Actinastrum hantzschii

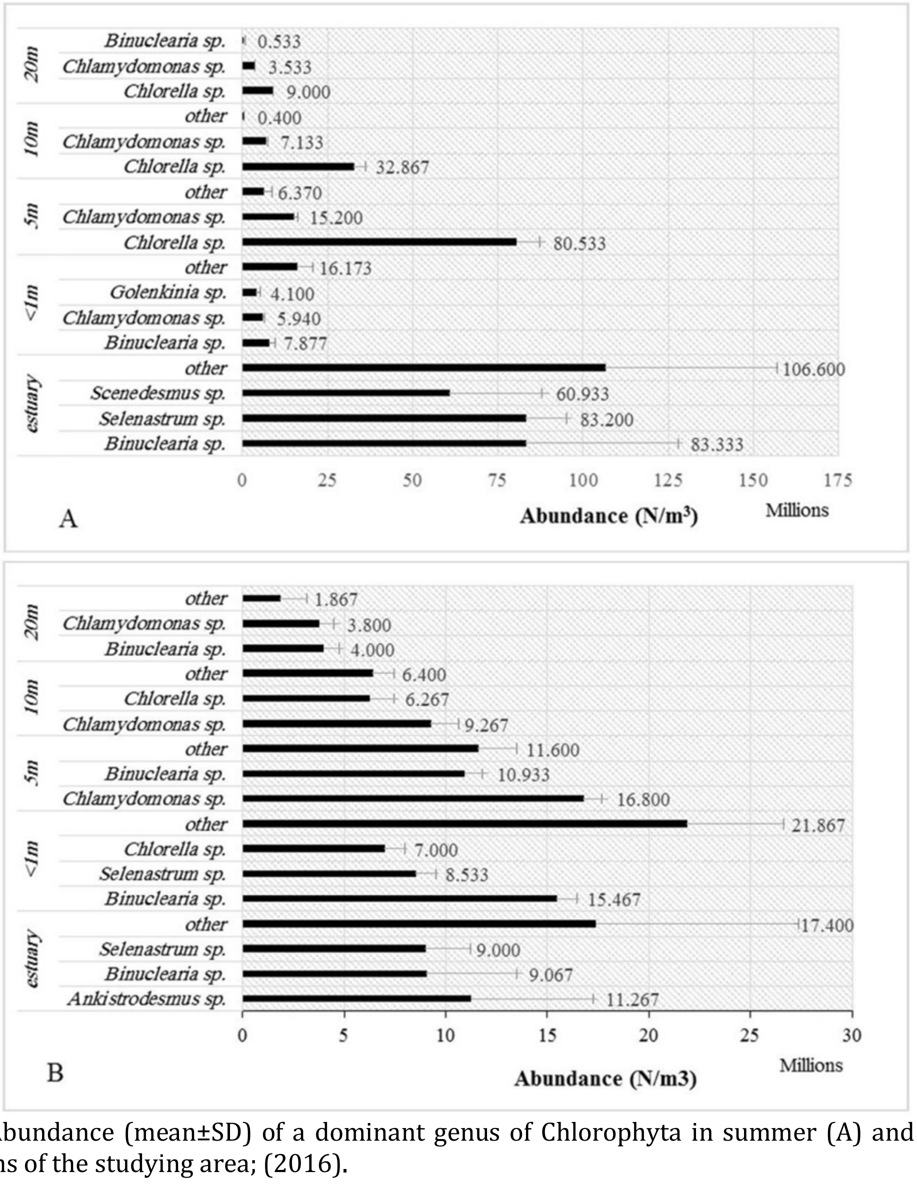

Diagram 1) Abundance (mean±SD) of a dominant genus of Chlorophyta in summer (A) and winter (B) on different depths of the studying area; (2016).

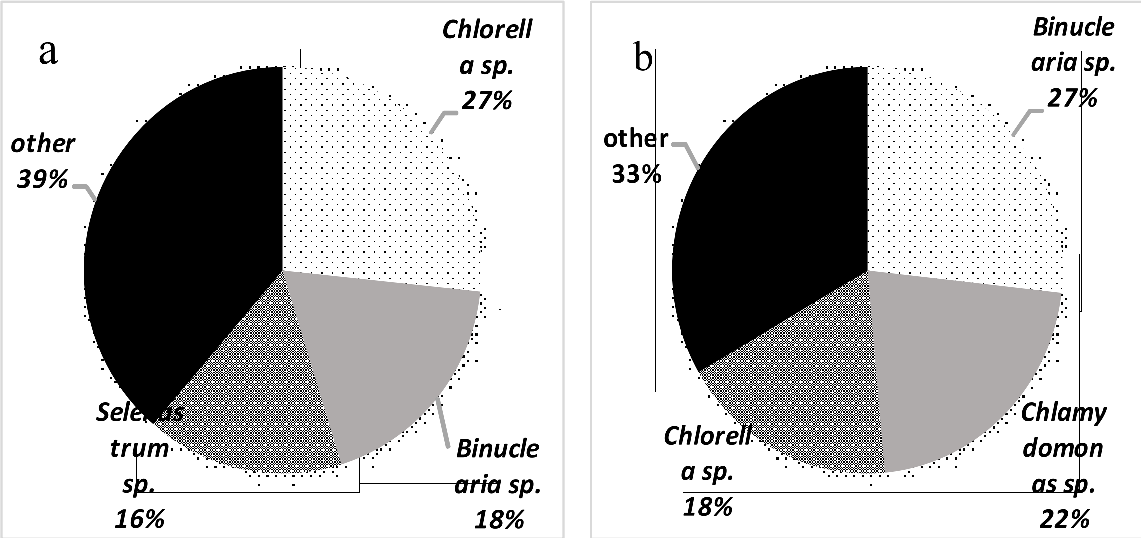

Diagram 2) Abundance percentage of the dominant genus in the summer (a) and winter (b) in the studying area (2016)

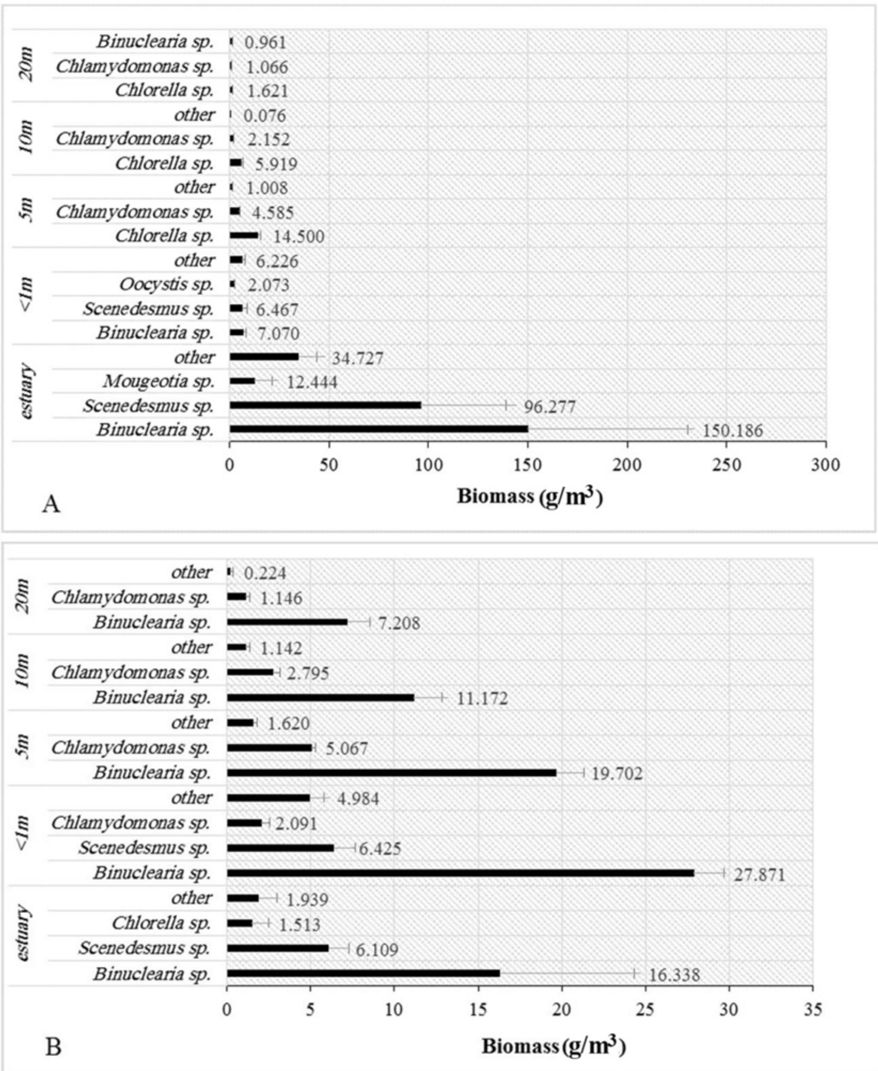

Diagram 3) Biomass (mean±SD) of a dominant genus of Chlorophyta in summer; (A) And winter (B) on different depths of the studying area; (2016)

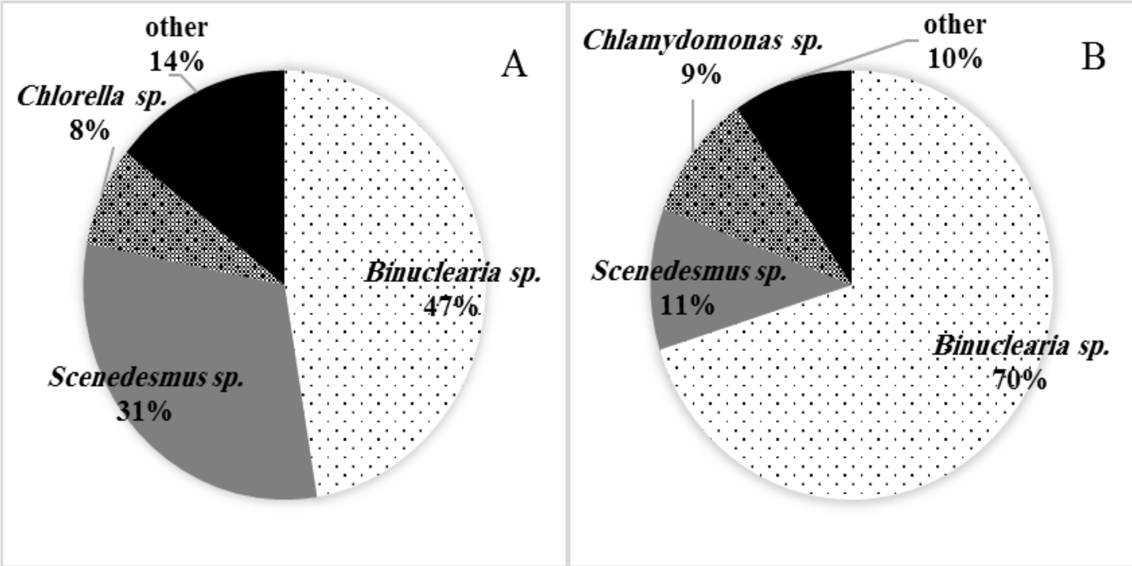

Diagram 4) Biomass percentage of the dominant genus in the summer (A) and winter (B) in the studying area (2016)

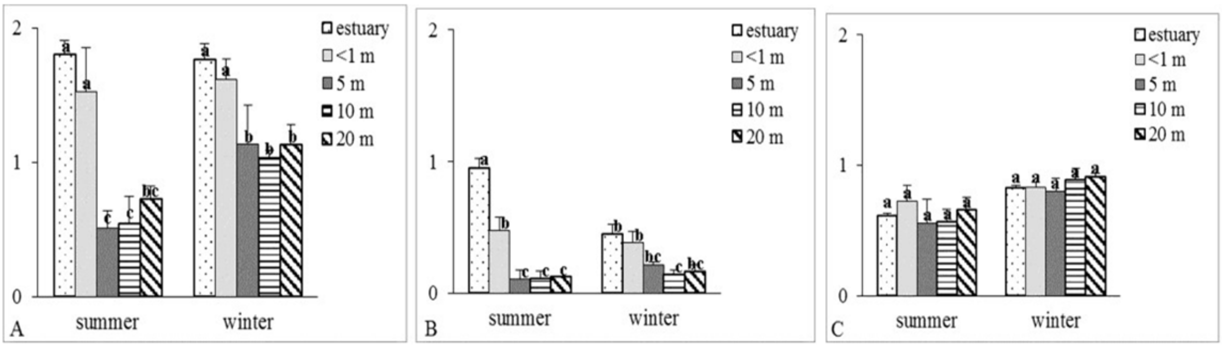

Diagram 5) Comparison of ecological indices, depending on depth and season, Shannon- Weiner (A), Margalef (B) and Pielou (C) in the studied area (2016)

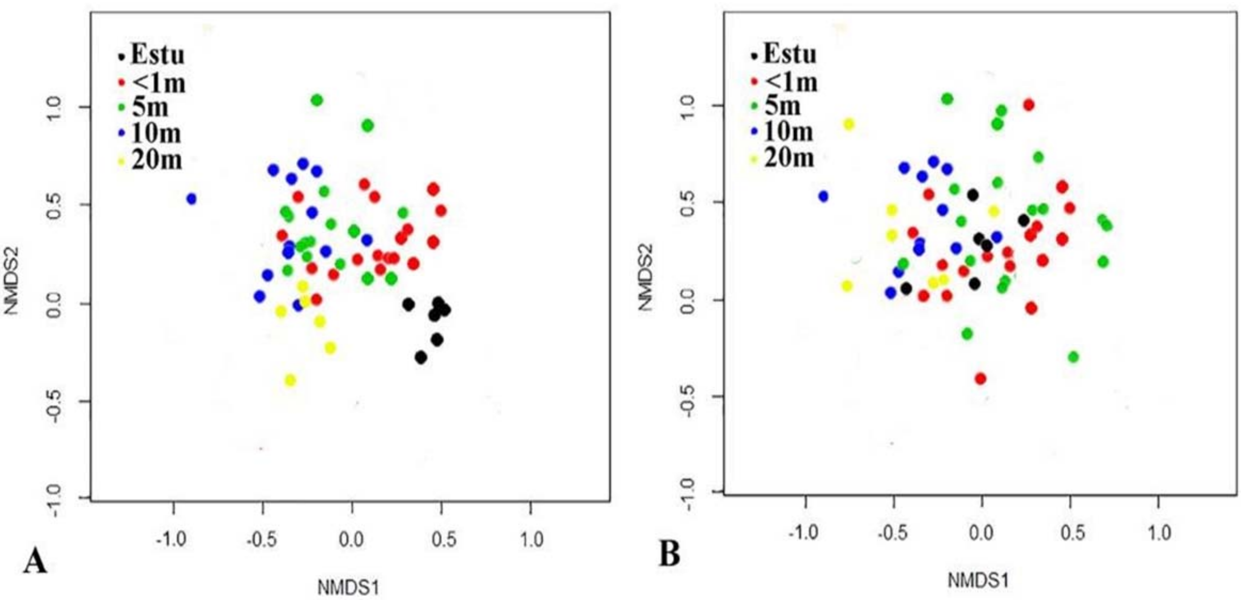

Diagram 6) nMDS was run with Chlorophyta species abundance results of Summer (A) and Winter (B) in the studied area (2016)

Table 4) Environmental data recorded in the studied area (2016)

Parameter	Depth (m)		Summer	Winter
Water temp. (˚C)	Estuary<1		19.78±0.212^{a}	15.08±4.03^{a}
	Coastal water	<1	20.57±0.350^{a}	13.96±2.52^{a}
		5	22.43±1.017^{a}	13.82±2.59^{a}
		10	21.83±1.159^{a}	13.83±2.64^{a}
		20	17.42±0.306^{b}	14.10±2.40^{a}
DO (mg/l)	Estuary<1		4.90±0.00^{a}	8.00±0.92^{a}
	Coastal water	<1	5.90±0.59^{ab}	8.85±0.27^{a}
		5	6.17±0.80^{ab}	8.69±0.34^{a}
		10	6.64±0.13^{b}	8.85±0.30^{a}
		20	4.58±0.21^{a}	8.99±0.21^{a}
pH	Estuary<1		7.15±0.02^{a}	7.34±0.47^{a}
	Coastal water	<1	7.10±0.00^{a}	8.24±0.20^{a}
		5	7.28±0.18^{a}	8.29±0.07^{a}
		10	7.23±0.33^{a}	8.29±0.13^{a}
		20	6.33±0.19^{a}	8.34±0.05^{a}
Salinity (ppt)	Estuary<1		9.92±0.07^{a}	0.41±0.16^{a}
	Coastal water	<1	10.86±0.63^{a}	9.41±0.66^{b}
		5	12.29±1.03^{ab}	10.35±0.43^{b}
		10	13.12±0.19^{b}	10.39±0.45^{b}
		20	13.28±0.07^{b}	10.42±0.52^{b}

Discussion

Human activities can significantly affect the richness and diversity of plankton by increasing nutrients inputs from urbanization, agriculture, and industry to aquatic systems [4]. The value of Shannon-Weiner and Margalef indices showed that the diversity and richness of species in both estuaries and near estuaries stations (5m) are significantly more than marine stations. The study area is heavily influenced by recreational activities and both rivers (Tajan and Babolrood) are used as a water source for agriculture (widespread use of fertilizers and chemical pesticide), and due to lack of sewage network, urban and rural wastewater directly enters the rivers and eventually the sea, leading to increases in the concentration of nutrients such as phosphates and nitrates. Considering the high sedimentation rate in estuaries, it can play a significant role in increasing the abundance and diversity of phytoplankton [21, 22].

According to the results, diversity of Chlorophyta at marine stations during the summer was lower than the winter, while estuaries showed the same diversity in both seasons (Diagram 5). Diversity of Phytoplankton is highly influenced by seasonal variations, which is due to the direct impression of physical (light, temperature, and flow), chemical (salinity, pH, oxygen, acidity, and essential nutrients), and biological (Growth rate and grazing) parameters on phytoplankton communities [23-26].

Percopo *et al*. mentioned the same seasonal diversity changes in phytoplankton communities in the northwestern part of the Mediterranean Sea, as the diversity and abundance increased during the winter due to decreasing the grazing pressure as well as in the spring due to the increase of nutrients in last winter [27].

After two years of assessment of seasonal phytoplankton' diversity variations in Kitham Lake, Tiwari and Chuhn reported two peaks of the population in winter and summer [28]. They also mentioned that it may be due to a higher concentration of nutrients, high dissolved oxygen, and slow water current. The study on Santraachi Lake (West Bengal) also showed that the density of phytoplankton was increased by increasing the temperature and nutrient contents [29].

Similarly, Gómez *et al*., who provided a checklist of black sea dinoflagellates, argued that a high diversity of phytoplankton is due to the high inputs of nutrients from the rivers [30]. Nasrollahzadeh *et al*. also stated the correlation between phytoplankton' biomass variation and environmental parameters as well as the concentration of nutrients and grazing rate [31].

Our recorded data of temperature confirmed the formation of a weak thermocline layer at depth of 20 meters in summer (Table 3); this phenomenon could limit upraising the nutrients from the beneath and led to reducing the diversity and richness of Chlorophyta.

Mahmoudi *et al*. showed a decrease in the biomass and abundance of Binuclearia laterbornii in summer due to the presence of the thermocline layer and its effect on the concentration of nutrients [12]. Thermocline layer was disappeared in winter, so nutrients upraised to the surface layers by the effect of waves and currents, which could have an important role in increasing the diversity of green microalgae (Chlorophyta) at marine stations compared to the summer season. The negative pressure of grazing by zooplankton can also prevent the increase of green microalgae in summer. However, winter's short days, as well as the lack of adequate light, are the main inhibitors of microalgae growth and Net-production. Nevertheless, there is permanent vertical mixing and circulation of nutrients across the water column in estuaries, due to low depth, recreational and fishing activity, and also the influence of coastal waves and currents, which can improve the richness of phytoplankton. Also in summer, more agricultural and recreational activities increase the wastewater and nutrient inputs to the rivers and estuaries and it may significantly contribute to increase the phytoplankton' richness in estuaries.

The estuaries winter diversity of Chlorophyta was less than the summer, which could be due to low water temperature, high turbidity, and reduced nutrient inputs from sewage source in winter. Tahami and Pourgholam considered the environmental parameters as the main factor to seasonal fluctuations of Bacillariophyta (diatoms) diversity in the southern Caspian Sea [32]. They mentioned a winter diversity increase due to the lack of the thermocline layer, increased silica ions, and low temperature.

This contradiction with the results of the present study may arise from the very important role of silica ion in the growth and reproduction of diatoms and also the interest of diatoms in cold water flows due to the facilitating of silica uptake in their shells. Chlorophyta belongs to mesotrophic waters, and they can tolerate cold water and deficiency of nutrients because of the large storage of starch, oils, and carotenoids [33].

In the current study, Chlorophyta included a low percentage of the total phytoplankton in both seasons (7% in summer and 5% in winter); even Pourgholam *et al*. estimated the abundance of Chlorophyta, 4.57% to 13.2% during their studies (2006 till 2010), but they also mentioned a downward trend of the abundance [11]. Therefore, the presence of Chlorophyta in both summer and winter seasons with relatively high density is expected. We identified 29 species of Chlorophyta in this study, while Bagheri *et al*. [16, 34, 35], using the same research method, reported 11 species of Chlorophyta in the southwestern part of the Caspian Sea. Also, Ganjian *et al*. reported 13 genera of Chlorophyta, mostly the same as our results (except Schroederia sp.)[10].

In comparison to the results of Nasrollahzadeh *et al*. that identified 25 genera and 63 species of Chlorophyta [14], the observed genera in the current study were much less. This is due to the extent of the sampling area in their study, which included up to 100 m depth, while in the present study, sampling was carried out up to a depth of 20 m. There were no observation reports of Carteria sp., Elakatothrix sp., and Tetrabaena sp. in previous studies, and this is the first report in the southern parts of the Caspian Sea.

Considering the great value of these microorganisms, we wish more assessment about microalgae in the Caspian Sea, providing applied studies on Chlorophyta, which can be achieved, using the results of this study.

Conclusion

Within two seasonal sampling, 29 species of Chlorophyta are identified, accounting for 5-7% of the total amount of phytoplankton community. Binuclearia sp. and Chlorella sp. are the most dominant genera in winter and summer, respectively; while Binuclearia sp. shows the most biomass in both seasons. Chlorophyta diversity is increased by increasing the depth in summer, where the biomass is reduced strongly by depth. The depth of water sampling is relatively expressed in species abundance only in summer. Habitat condition is going more favorable for green microalgae.

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Ethical permission: Our study was not carried out in a protected or private area and no specific permission was required. We confirmed that the field study did not involve endangered or protected species. Only water samples were collected (no animals), therefore, not subject to regulation.

Conflict of Interests: We state that there is no conflict of interest.

Authors' Contribution: Mirhasannia S.D. (First author), Introduction author/Assistant/Discussion author (45%); Akhoundian M. (Second author), Methodologist/Original researcher/Statistical analyst/Discussion author (45%); Taghavi H. (Third author), Assistant (10%).

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