

## THE STUDY OF *NODULARIA SPUMIGENA* BLOOM EVENT IN THE SOUTHERN CASPIAN SEA

NASROLLAHZADEH, H.S.<sup>1\*</sup> – MAKHLOUGH, A.<sup>1</sup> – POURGHOLAM, R.<sup>1</sup> – VAHEDI, F.<sup>1</sup> – QANQERMEH, A.<sup>2</sup> – FOONG, S.Y.<sup>3</sup>

<sup>1</sup>*Ecology Departments, Ecological Aquatic Center of the Caspian Sea (EACCS), 961, Sari, Iran*

<sup>2</sup>*Study and Research of water resource of the Caspian Sea, Sari, Iran*

<sup>3</sup>*School of Biological Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia  
(Phone: 0098-151-356-250; Fax: 0098-151-346-2495)*

*\*Corresponding author*

*e-mail: hnsaravi@yahoo.com, hnsaravi@gmail.com*

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**Abstract.** Despite the increase in phytoplankton population in the Caspian Sea, there is few detail study regarding bloom of some species in the recent years. Previous studies have announced the bloom-forming Cyanophyta *Nodularia spumigena* Mertens to the Caspian Sea. In this study, we attempt to understand the bloom events that are involved *Nodularia spumigena* population and environmental parameters in the Iranian coastal water in 2009. The preliminary results suggested that *Nodularia spumigena* observed in the Caspian Sea in some seasons at different depths but bloom formation starting from middle of summer and reach a maximum in the early of autumn at the surface. Result of this study also showed that phytoplankton assemblage comprised of 46 species after bloom. Bacillariophyta had the highest number of species (17) follow by Pyrrophyta (14), Cyanophyta and Chlorophyta (6) and Euglenophyta (3). But, phytoplankton species of bloom sample is classified in three groups: Cyanophyta, Pyrrophyta and Bacillariophyta which Cyanophyta dominated over the other groups of algae and formed more than 98% and 96% of phytoplankton abundance and biomass, respectively. As of now, the pattern of dominant and frequent species in the Caspian Sea indicated that the health of the water body and aquatic organisms are at risk.

**Keywords:** *Phytoplankton, Cyanophyta, bloom, Caspian Sea, Iran*

### Introduction

Algal blooms that adversely affect environmental, plant, or animal health are referred to as harmful algal blooms (HABs) (Backer, 2002). HABs occur in freshwater, marine, and estuary systems and are quickly becoming a public health issue. CyanoHABs are primarily associated with surface scums from blue-green algae or Cyanophyta. There are about 150 known genera of Cyanophyta, 40 of which are known to be toxic (Saker et al., 1999). The primary toxin-producing Cyanophyta include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia* and *Planktothrix* (*Oscillatoria*).

The first report of toxic Cyanophyta bloom in the world was recorded in lake Alexandrina, Australia as early as in the 1800s (Francis, 1878). The Cyanophyta *Nodularia spumigena* Mertens (*N. spumigena*) is a filamentous, heterocystous, nitrogen-fixing species known to thrive in brackish and saline waters. There are several reports related to *Nodularia* bloom in fresh water lake of Turkey (Akcaalan et al., 2009), estuaries of Australia (Blackburn et al., 1996), brackish lakes of Australia and New Zealand (Heresztyn and Nicholson, 1997; Woodward and Shulmeister, 2005), saline lakes and lagoons of USA (Beutel et al., 2001; Galat et al., 1990), Mexico (Falcon et al., 2002), Uruguay (Perez et al., 1999) and Baltic Sea (Sivonen et al., 1989; Kahru et al.,

1994; Stal et al., 2003; Mazur-Marzec et al., 2006). In September 2005, researchers of the Caspian Environment Programme were informed by the Department of Environment of the Gilan Province (I.R. Iran) of the occurrence of Anomalous Algal Bloom (AAB) in the southern of Caspian Sea with affected area as wide as 20,000 km<sup>2</sup> (Soloviev, 2005). This phenomenon repeated within a short period of time in coastal waters of the southwestern of Caspian Sea in 2009 as reported by the Ecological Aquatic Center of the Caspian Sea (EACCS). In 2010, *N. spumigena* was again bloomed in early August in the southern of Caspian Sea (unpublished data). In the southern of Caspian Sea, phytoplankton composition during bloom and the early hours after bloom as well as factors favor the bloom of *N. spumigena* have not been fully established yet. In this paper, we attempt to study phytoplankton structure (abundance and composition) and environmental parameters at few hours after bloom. In addition, phytoplankton composition during bloom and meteorological parameter (wind) adopted from satellite which effected on spread of bloom was investigated.

**Table 1.** Longitude and latitude of Tonekabon station in the southern of Caspian Sea

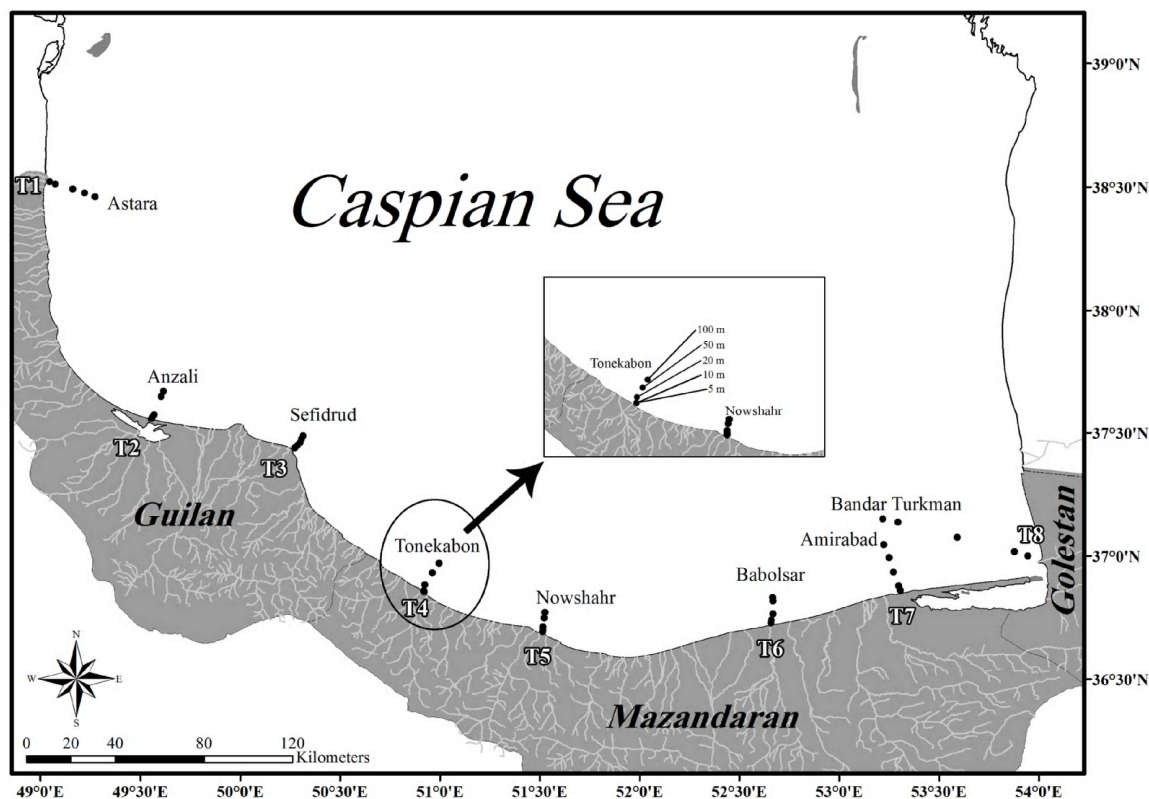
| Stations        | Longitude | Latitude  |
|-----------------|-----------|-----------|
| <b>A (5m)</b>   | 50° 54' E | 36° 49' N |
| <b>B (10m)</b>  | 50° 54' E | 36° 49' N |
| <b>C (20m)</b>  | 50° 55' E | 36° 50' N |
| <b>D (50m)</b>  | 50° 57' E | 36° 53' N |
| <b>E (100m)</b> | 50° 59' E | 36° 56' N |

## Materials and methods

### *Sampling and environmental parameters*

The Caspian Sea Investigation of hydrology and hydrobiology cruise was planned in 2009 in the southern of Caspian Sea. This cruise was carried out aboard the vessel, R/V *Gilan*. Water samples (at few hours after bloom) were collected at five sampling stations (A to E) within the 0-100 m depth along a transect perpendicular from the Tonekabon coast at 5 m (Station A), 10 m (Station B), 20 m (Station C), 50 m (Station D) and 100 m (Station E) interval (*Table 1* and *Fig. 1*). At each sampling station, physico-chemical parameters were determined within the 0-100 m depth: A(0), B(0), B(10), C(0), C(10), C(20), D(0), D(10), D(20), D(50) and E(0), E(10), E(20), E(50), E(100) with figure in bracket showing the sampling depth in meter at each station. Temperature was determined using an inverse thermometer and a salinometer (Model GM65, Russian) was used to measure the salinity levels and pH meter (WTW 320, Germany) used to measure the pH of water sample. Water samples were analyzed for nutrients concentration manually with spectrophotometer in the laboratory. Nitrate and nitrite were determined by reduction column and the standard pink azo dye method and ammonia by the hypo-phenol oxidation-blue dye method ( $\text{DIN}=\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ); phosphate (DIP) and silicate (DSi) were determined by the standard molybdenum blue and yellow method as suggested by APHA (2005). Digestion of samples for the determination of total nitrogen (TN) and total phosphorus (TP) were done following the persulphate digestion procedure of Valderrama (1981). DON was calculated as the

difference between total nitrogen and DIN concentrations and DOP was calculated as the difference between total phosphorus minus phosphate concentration (Yurkovskis, 2004).



**Figure 1.** Map of the southern Caspian Sea showing locations and depths of sampling points along the Tonekabon transects

### **Phytoplankton analysis**

Phytoplankton samples (at few hours after bloom) were collected using a 1.6 liters Ruttner sampler at five sampling stations (A to E) (Vollenweider, 1974). Totally, 15 water samples were taken as same as environmental samples. The samples were kept in 0.5 litter bottles and preserved using buffered formaldehyde to yield a final concentration of 2%. The samples were left to settle for at least 10 days following which they were concentrated to about 30 ml by sedimentation and centrifugation. The quality and quantity analysis of phytoplankton for this study was reported in detail by Nasrollahzadeh et al. 2008a. Abundance of filamentous species from the bloom sample was expressed as filamentous per ml (mean length and width of 20 filaments were considered as a filament unit).

### **Statistical analyses**

Principal component Analysis (PCA) is used to understand the correlation structure of collected data and identify the most important factors contributing to the data structure (Schoer, 1985; Buckley and Winters, 1992; Padro et al., 1993; Moncheva et al., 2001). All tests were performed at 5% significance level. Principle Component Analysis (PCA) was applied to score and narrow down the selection of parameters (Moncheva et al., 2001). The environmental data obtained from the sampling period was

used for statistical analyses, which were performed using SPSS 11.0 software. Eight environmental variables were reduced into three variable factors (PC1, PC2 and PC3) using the principal component analysis. The eigenvalues, which give the variance of the factor components, were used as criteria for determining significant changes (eigenvalues > 1). For easier interpretation, factor axes were modified by 'factor rotation' using varimax (Lau and Lana, 2002).

Species richness, Shannon-Weaver diversity index and evenness were done using the software Multivariate Statistical Package (MVSP) version 3.13d.

## Results

### *Composition of phytoplankton during bloom*

Phytoplankton species of bloom sample (2009) is classified in three groups: Cyanophyta, Pyrrophyta and Bacillariophyta. Cyanophyta dominated over the other groups of algae and formed more than 98 and 96% of phytoplankton abundance and biomass, respectively. Pyrrophyta and Bacillariophyta shared almost 2 to 4% of the phytoplankton community in term of abundance and biomass. Species richness was not very low (17), but Shannon-Weaver diversity index and evenness were very low (0.01 bits/individual and 0.04 respectively). Cyanophyta contained 2 species: *Oscillatoria* sp. and *N. spumigena*. *N. spumigena* had highest abundance and biomass (> 99%) (Table 2). Concentration of *N. spumigena* was as high as 5830 filaments/ml with 0.05 mg/ml biomass. While the concentration of the other present species was 83 cells/ml with 0.002 mg/ml biomass. *Prorocentrum proximum* from Pyrrophyta division was dominant among the eleven species. Bacillariophyta composition was consisted of four species. Maximum abundance of Bacillariophyta was found belonging to *Nitzschia acicularis* but the maximum biomass documented was *Rhizosolenia calcar-avis*.

**Table 2.** Species composition, abundance and biomass percentage of phytoplankton in the Caspian Sea during bloom in summer 2009

| Species                         | Abundance percentage of corresponded division | Biomass percentage of corresponded division |
|---------------------------------|-----------------------------------------------|---------------------------------------------|
| <b>Cyanophyta</b>               |                                               |                                             |
| <i>Nodularia spumigena</i>      | 99.96                                         | 99.99                                       |
| <i>Oscillatoria</i> sp.         | 0.04                                          | 0.01                                        |
| <b>Pyrrophyta</b>               |                                               |                                             |
| <i>Prorocentrum proximum</i>    | 44.58                                         | 73.99                                       |
| <i>Exuviaella cordata</i>       | 20.74                                         | 1.38                                        |
| <i>Peridinium latum</i>         | 11.15                                         | 11.10                                       |
| <i>Peridinium achromaticum</i>  | 6.81                                          | 6.78                                        |
| <i>Goniaulax polyedra</i>       | 6.50                                          | 2.59                                        |
| <i>Peridinium</i> sp.           | 3.41                                          | 0.88                                        |
| <i>Peridinium trochoideum</i>   | 2.79                                          | 0.74                                        |
| <i>Prorocentrum scutellum</i>   | 1.55                                          | 1.70                                        |
| <i>Glenodinium lenticula</i>    | 0.93                                          | 0.41                                        |
| <i>Goniaulax</i> sp.            | 0.93                                          | 0.37                                        |
| <i>Glenodinium behningii</i>    | 0.62                                          | 0.08                                        |
| <b>Bacillariophyta</b>          |                                               |                                             |
| <i>Nitzschia acicularis</i>     | 83.33                                         | 15.80                                       |
| <i>Nitzschia longissima</i>     | 8.33                                          | 2.69                                        |
| <i>Cyclotella meneghiniana</i>  | 6.25                                          | 11.85                                       |
| <i>Rhizosolenia calcar-avis</i> | 2.08                                          | 69.66                                       |

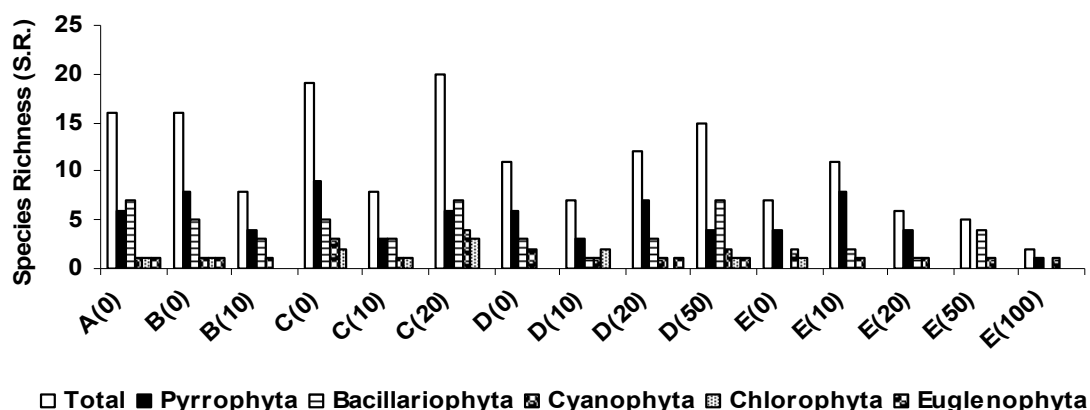
The microscopic observation of *N. spumigena* showed straight, branchless and olive-green to yellowish color filaments with cylinder or barrel shape cells (length less than width). The cell length:width ratio was 1:1.8. Each filament contained 12-200 cells. Heterocyst usually observed with every 10-12 vegetative cells. Table 3 summarizes the results of microscopy measurement of *N. spumigena*.

**Table 3.** Microscopic measurements of *Nodularia spumigena* in the Caspian Sea collected during bloom in summer 2009

|                            | Minimum (μm) | Maximum (μm) | Mean ± S.D. (μm) |
|----------------------------|--------------|--------------|------------------|
| Length of filaments        | 44           | 732          | 297±163          |
| Length of vegetative cells | 3.0          | 4.4          | 3.7±0.5          |
| Width of vegetative cells  | 6.0          | 9.0          | 6.7±1.0          |

### Composition of Phytoplankton after bloom

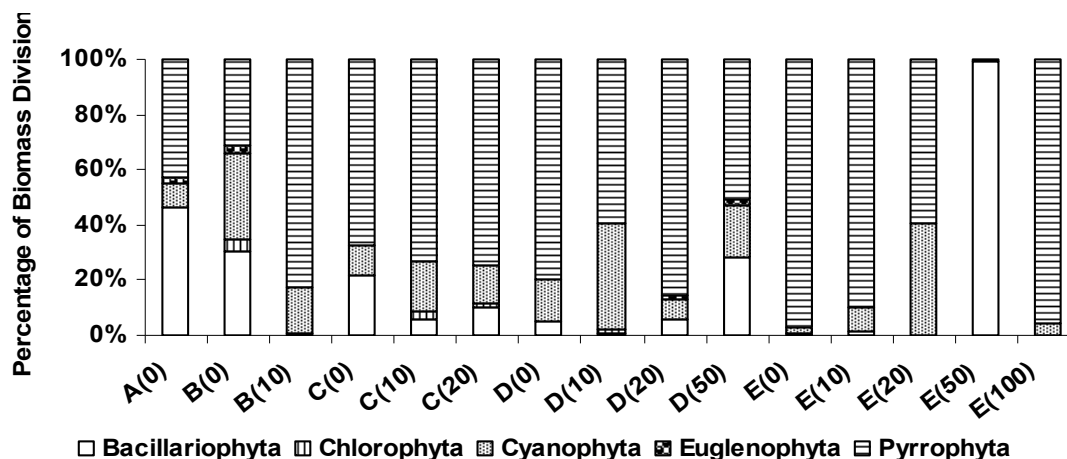
Phytoplankton assemblage comprised of 46 species after the 2009 bloom. Bacillariophyta had the highest number of species (17) follow by Pyrrophyta (14), Cyanophyta and Chlorophyta (6) and Euglenophyta (3). The number of species represented at each station is shown in Fig. 2. This figure shows that the species richness increased from station A to station C and then decreased toward station E. Species richness decreased from surface to bottom at stations B and E while it increased in water column at stations C and D.



**Figure 2.** The number of Phytoplankton species represented at different depth (sampling depth in meter in bracket) of the Tonekabon transect (5 sampling station, A-E) in the southern of Caspian Sea after the 2009 bloom

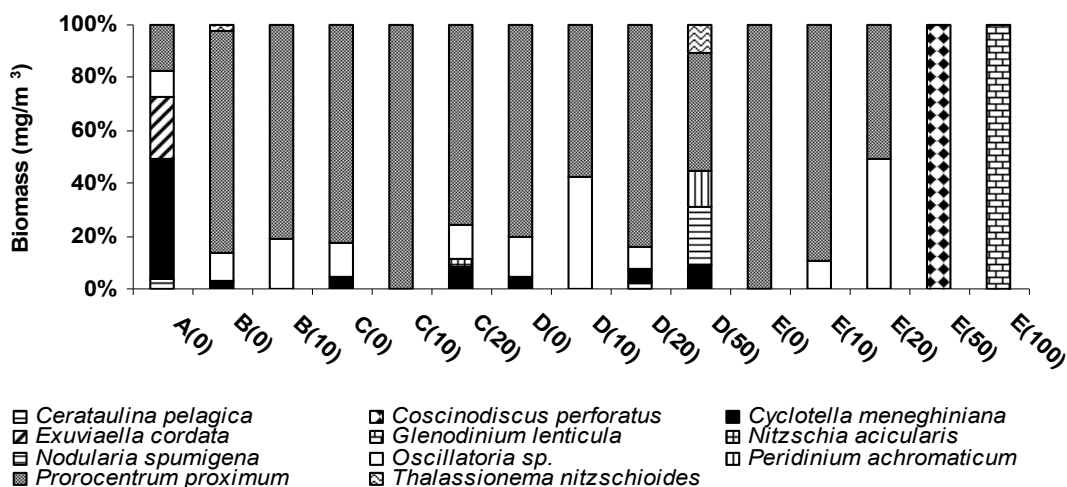
The highest and lowest value of total phytoplankton biomass recorded at station E at 10 m depth (413 mg/m<sup>3</sup>) and 100 m (1.4 mg/m<sup>3</sup>) depth, respectively. Pyrrophyta showed the highest biomass (78 mg/m<sup>3</sup>) while the Chlorophyta and Euglenophyta recorded a minimum biomass (0.4 mg/m<sup>3</sup>). Biomass of each phytoplankton division showed decreasing trend between the two depths (surface to bottom). However, there were some incongruities in these biomass patterns fluctuating from surface to bottom. Where biomass of Pyrrophyta increased from surface to 10 m and then decreased toward bottom while biomass of Bacillariophyta showed sharp decrease from surface to 10 m, then slightly increased at 20 and 50 m depths and reached thereafter to zero

biomass at the bottom. Bacillariophyta played the main role in the formation of the phytoplankton biomass at the surface and 10 m depth while at the deeper depths Pyrrophyta dominated the phytoplankton biomass. As the Fig. 3 shows, Chlorophyta and Euglenophyta were only recorded in low biomass at some depths of the offshore water.



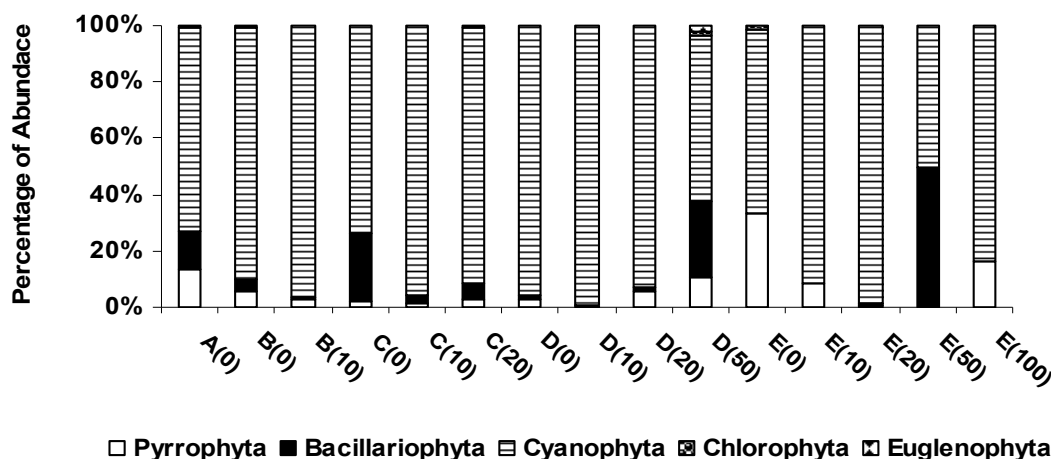
**Figure 3.** The percentage of biomass of each division at different depth of the five sampling stations (A-E) in the Tonekabon transect in the southern Caspian Sea (2009).  
Bracket shows the station depth in meter at each sampling station

Fig. 4 shows the phytoplankton species that contributed more than 70% of biomass. Biomass of the major Pyrrophyta species was more evenly distributed with *Prorocentrum proximum* and *Exuviaella cordata*. Whereas *Glenodinium lenticula* and *Peridinium achromaticum* only contributed slightly in terms of biomass. *Cyclotella meneghiniana*, *Cerataulina pelagica*, *Nitzschia acicularis*, *Thalassionema nitzschioides* and *Thalassiosira caspica* formed more biomass than the others species of Bacillariophyta. Cyanophyta biomass was represented mainly by *Oscillatoria* sp. and rarely *Nodularia spumigena*.



**Figure 4.** The percentage of biomass of phytoplankton species at different depth of Tonekabon transect in the southern of Caspian Sea (2009)

Fig. 5 shows the difference of the contribution of the phytoplankton abundance divisions at each station. Cyanophyta was the dominant division from surface to bottom in inshore and offshore stations. Pyrrophyta was present at all depths (except at some depth at offshore station). Bacillariophyta was dense at surface of inshore and certain depths of the offshore water.



**Figure 5.** Contribution of phytoplankton divisions at different depth of the five sampling stations (A-E) at the Tonekabon transect in the southern of Caspian Sea. Bracket shows the station depth in meter at each sampling station after the 2009 bloom

22 of the most frequently found phytoplankton species contributed more than 70% of abundance at each station as shown in Table 4. Cyanophyta was represented mainly by *Oscillatoria* sp. and it was the dominant species at all stations. Nine species out of the twenty-two most common phytoplankton were from Pyrrophyta. Pyrrophyta abundance was formed mostly by *Prorocentrum proximum* and *Exuviaella cordata*. The rest of the Pyrrophyta species were found at certain stations especially station D and station E. Bacillariophyta hold the maximum diversity (11 species) but with lower density as compared to Pyrrophyta. Among the dominant Bacillariophyta species, 4 species were more substantial: *Thalassionema nitzschioides*, *Cyclotella meneghiniana*, *Nitzschia acicularis* and *Chaetoceros peruvianus*. The Bacillariophyta species at 10 meter depth (both inshore and offshore) played a minimum role in phytoplankton abundance while more contribution was observed at 50 m depth of stations D and E. The presence of *N. spumigena* occurred at surface water C (0), D (0), E (0) and at 50 m (station D).

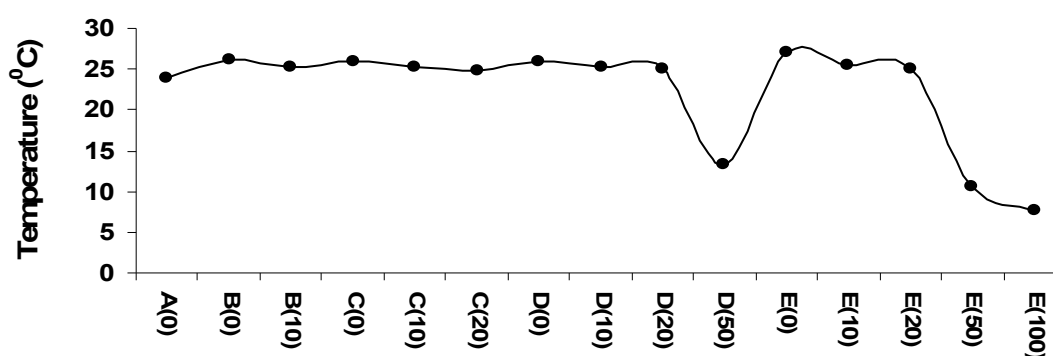
### Environmental factors

In the brackish Caspian Sea, weather temperature varied from 24.0 to 26.0°C. The surface water temperature was 24.0-26.2°C in summer 2009, after bloom of *N. spumigena* (Fig. 6).

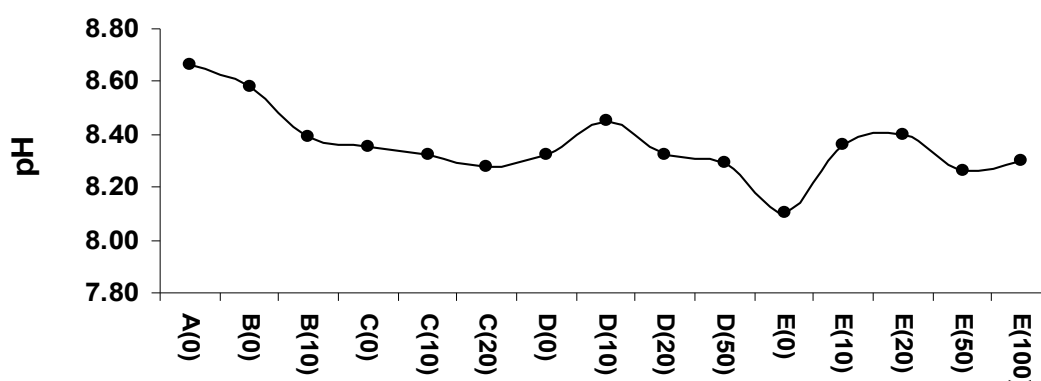
In summer 2009, the pH values for the surface layer of the southern of Caspian Sea come to 8.10-8.66 (Fig. 7). This value gradually dropped with depth especially in the inshore water and at the bottom, which came to less than 8.30.

**Table 4.** Abundance percentage of dominant species (>70%) of phytoplankton in the southern of Caspian Sea (Tonekabon transect) after bloom in summer 2009

| Station                         | A (0) | B (0) | B (10) | C (0) | C (10) | C (20) | D (0) | D (10) | D (20) | D (50) | E (0) | E (10) | E (20) | E (50) | E (100) |
|---------------------------------|-------|-------|--------|-------|--------|--------|-------|--------|--------|--------|-------|--------|--------|--------|---------|
| <b>Species</b>                  |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <b>Cyanophyta ( %)</b>          |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Oscillatoria</i> sp.         | 72    | 90    | 97     | 96    | 95     | 90     | 95    | 99     | 93     | 50     | 63    | 91     | 99     | 50     | 83      |
| <i>Nodularia spumigena</i>      |       |       |        |       |        |        |       |        |        | 9      | 2     |        |        |        |         |
| <b>Pyrrophyta ( %)</b>          |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Exuviaella cordata</i>       | 12    | 2     | 1      |       |        | 1      | 1     |        | 2      | 4      | 6     | 6      |        |        | 16      |
| <i>Glenodinium lenticula</i>    |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Goniaulax digitale</i>       |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Goniaulax polyedra</i>       |       |       |        |       |        |        |       |        |        |        | 5     |        |        |        |         |
| <i>Goniaulax</i> sp.            |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Gymnodinium</i> sp.          |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Peridinium</i>               |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>achromaticum</i>             |       |       |        |       |        |        |       |        |        | 2      | 2     |        |        |        |         |
| <i>Peridinium trochoideum</i>   |       |       |        |       |        |        |       |        |        | 2      | 2     |        |        |        |         |
| <i>Prorocentrum proximum</i>    |       | 2     | 1      | 1     | 1      | 1      | 1     |        | 2      | 4      | 21    | 2      |        |        |         |
| <b>Bacillariophyta ( %)</b>     |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Cerataulina pelagica</i>     |       | 1     |        |       |        |        |       |        |        | 7      |       |        |        |        |         |
| <i>Chaetoceros peruvianus</i>   | 2     |       |        |       | 2      | 1      |       |        |        |        |       |        |        |        |         |
| <i>Chaetoceros convolutus</i>   |       | 1     |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>Coscinodiscus</i>            |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>perforatus</i>               |       |       |        |       |        |        |       |        |        |        |       |        |        |        | 10      |
| <i>Cyclotella meneghiniana</i>  | 4     |       |        |       |        | 1      |       |        | 1      | 4      |       |        |        |        | 10      |
| <i>Navicula</i> sp.             |       |       |        |       |        |        |       |        |        | 2      |       |        |        |        |         |
| <i>Nitzschia acicularis</i>     | 1     | 1     |        |       |        | 3      |       |        | 1      |        |       |        |        |        |         |
| <i>Nitzschia</i> sp.            |       |       |        |       |        |        |       |        |        | 2      |       |        |        |        |         |
| <i>Pseudo-nitzschia seriata</i> |       |       |        |       |        |        |       |        |        |        |       |        |        | 20     |         |
| <i>Thalassionema</i>            |       |       |        |       |        |        |       |        |        |        |       |        |        |        |         |
| <i>nitzschoides</i>             |       |       |        |       |        |        |       |        |        | 7      |       |        |        |        |         |
| <i>Thalassiosira caspica</i>    |       |       |        |       |        |        |       |        |        | 4      |       |        |        |        |         |



**Figure 6.** Vertical distribution of temperature at different depth of Tonekabon transect [Five sampling stations, A-E with sampling depth in bracket (meter)] in the southern of Caspian Sea, summer 2009

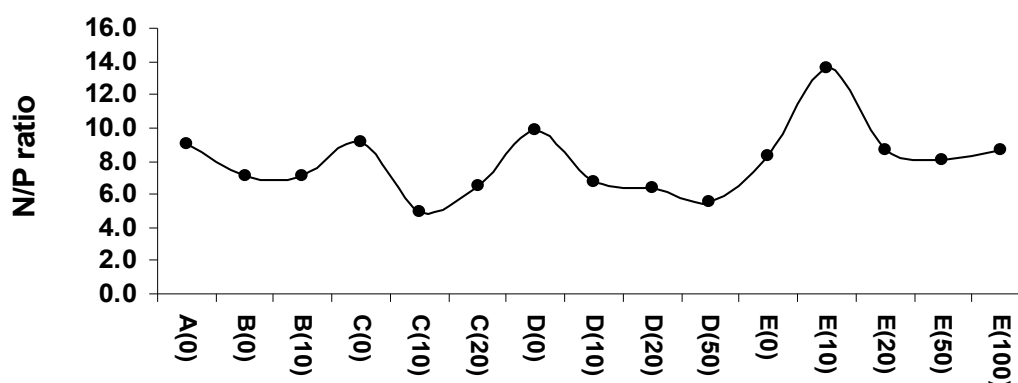


**Figure 7.** Vertical distribution of pH at different depth of Tonekabon transect. Five sampling stations, A-E with sampling depth in bracket (meter) in the southern of Caspian Sea, summer 2009

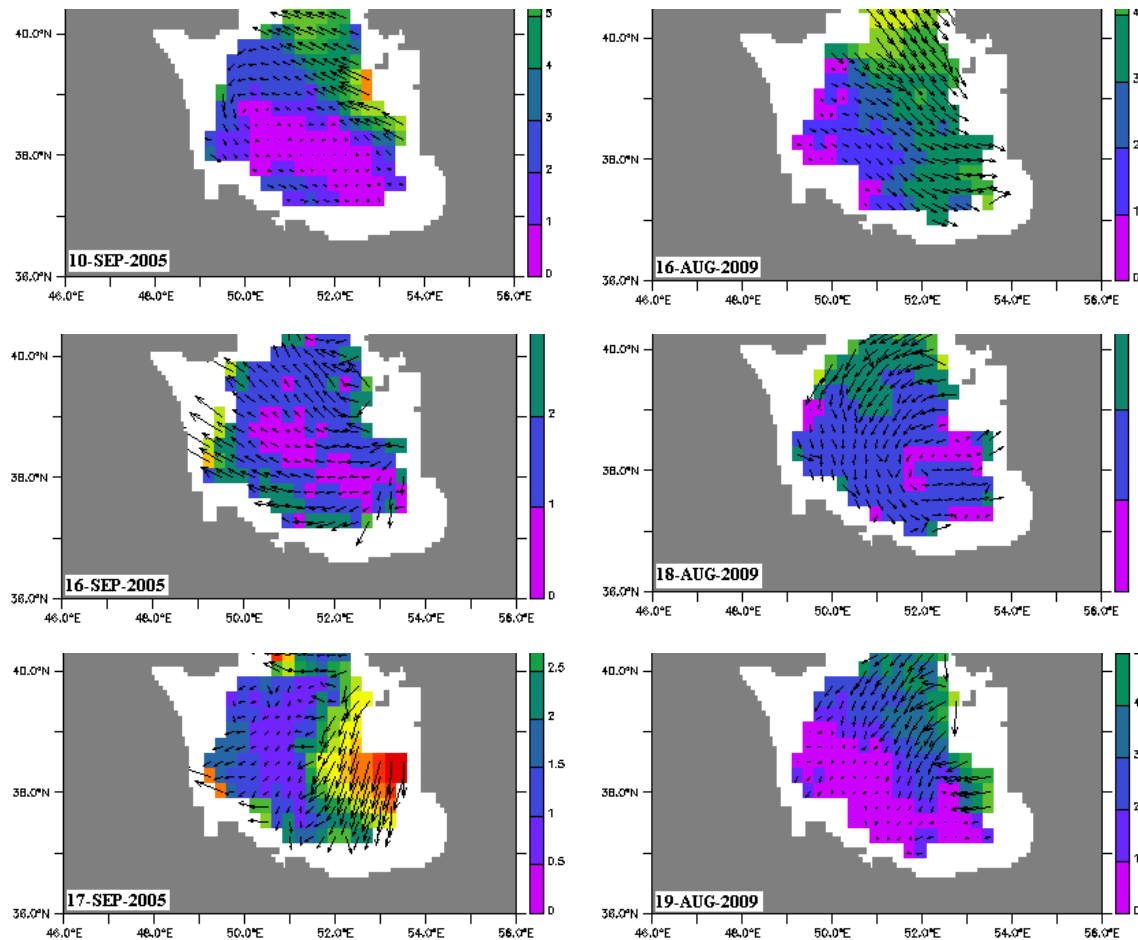
We applied PCA to the eight variables collected during cruises of the year 2009 ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , DIN, DON, DIP, DOP, DSi). In summer 2009 (after bloom of *N. spumigena*), the PC1 accounted for 51.94% of total variance comprised of DIP, DOP, TP, TN, DON and DSi, representing variations in inorganic and organic compounds (P and Si) in the water. PC2, consisted of 20.03% of total variance, represents the nutrient enrichment, including the dissolved inorganic nitrogen. PC3 with 14.04% of the total variance represents the  $\text{NH}_4^+$ .

In summer 2009, molar ratio of dissolved inorganic nitrogen/dissolved inorganic phosphorus (DIN/DIP) was less than 10 (Fig. 8).

In 2009, wind speed for August 15 ranged from 2 to 4 m/s and did not register significant scattering. However, in 16 August 2009, (with wind speed ranged from 0 to 2 m/s) wide area with strong scattering was observed (QUIKSCAT). Overall, maximum concentrations of chlorophyll (Soloviev, 2005) as estimated from wind speed were observed in August 16, 18, and 19 (wind speed ranged 0 to < 2 m/s). This trend was also happened during development of the bloom process in 2005 (Fig. 9).



**Figure 8.** Vertical distribution of N/P ratio at different depth of Tonekabon transect. Five sampling stations, A-E with sampling depth in bracket (meter) in the southern of Caspian Sea, summer 2009



**Figure 9.** Wind speed (m/s) during peak phytoplankton bloom in September 2005 and August 2009 in the southern of Caspian Sea

## Discussion

During eutrophication, when phytoplankton biomass increases, there are concurrent changes in taxonomic structure. Most notably is the relative biomass of Cyanophyta that increases with eutrophication. This relationship has been noted in boreal, temperate, subtropical and tropical ecosystems (Auer et al., 2004). Nasrollahzadeh et al. (2008a) reported that the southern of Caspian Sea's (as a subtropical system) status shifted from oligotrophy to meso-eutrophy in the last decade (after the alien introduction of *Mnemiopsis leidyi*). According to their study, the summer abundance of phytoplankton was 10,664 (cells/l), where Cyanophyta, Pyrrophyta and Bacillariophyta abundance were 9, 23 and 22 percent respectively before the invasion of *Mnemiopsis leidyi*. Whereas after the invasion, phytoplankton abundance reached 22,433 (cells/l) in summer and the abundance percentage of Cyanophyta, Pyrrophyta and Bacillariophyta were 52, 7 and 32, respectively. They also mentioned that the number of species and abundance of Cyanophyta increased after *Mnemiopsis leidyi* invasion in 1999.

In 2000s, we found blooms occurrences that coincided with changes in trophic status in the southern of Caspian Sea. Cyanophyta blooms displayed a range of temporal dynamics. The southern of Caspian Sea had Cyanophyta (*N. spumigena*) blooms that started in summer and last into autumn in 2005. Then again in 2009, we observed

blooms that occurred as extreme peaks but crashed after lasting just for days or weeks. In our current study, *N. spumigena* abundance was more than 98% of the of phytoplankton abundance in the 2009 bloom sample.

In the last decades, dramatic and intensive summer blooms of Cyanophyta (*N. spumigena* and *Aphanizomenon flos-aquae*) were also observed in several regions of the Baltic Sea (Gorokhova, 2009). In this study, *N. spumigena* blooms recorded a stunning 112,000 filaments/ml and 5830 filaments/ml in 2005 and 2009 respectively, while in Iznik Lake (freshwater), the *N. spumigena* bloom recorded a 130 filaments/ml that consisted 53% of the total abundance (Akcaalan et al., 2009). In Finland Gulf, *N. spumigena* and *Aphanizomenon flos-aquae* made 35.8% of the total phytoplankton biomass in August 2000-2005 (Raateoja et al., 2010). The results showed bloom of this species in the Caspian Sea was many more fold in terms of number of filament /ml than recorded in other seas and lakes. This also showed that the bloom of this species during 2005 was many fold more than what it recorded in 2009.

The vertical distribution of the coexisting nitrogen-fixing *N. spumigena* in the southern of Caspian Sea was distinctive as compare to systems elsewhere. *N. spumigena* usually prefers near-surface water at the top 5 meter of the water mass as observed in the Baltic Sea. In the Caspian Sea, *N. spumigena* existed at water surface during the bloom but was also found in water column as deep as 50 m after the bloom.

The results showed that species richness, abundance and biomass of Bacillariophyta declined during the bloom. Pyrrophyta and Cyanophyta are able to out-compete other groups to build massive abundance and formed mono/low species structure of phytoplankton during the bloom. This is due to the physiological and toxicity features of these species. For instance, Cyanophyta has the ability to fix atmospheric nitrogen whereas Pyrrophyta can tap into heterotrophic nutrition. These phenomena during the bloom not only will impact the water quality but also cause the quality of food for consumers to declines, for the reason that a diverse phytoplankton population is of importance to support a healthy ecosystem (Karlson, 2010). When comparing the current result with the long term study of Nasrollahzadeh et al. (2008a), the shift of phytoplankton functional group from Bacillariophyta and Pyrrophyta to Cyanophyta and Pyrrophyta is an evidence of the up-ward alteration in trophic state of the Caspian Sea. Some dominant species (*Oscillatoria* sp., *Prorocentrum proximum* and *Nitzschia acicularis*) in the Caspian Sea are indicators of high trophic state, organic pollution and with the potential to form bloom and adversely affect the water quality (Hutchinson, 1957; Palmer, 1980; Hernandez et al., 2000; Wei et al., 2008; Cabecinha et al., 2009).

High pH and scarcity of free CO<sub>2</sub> theoretically favor the growth of Cyanophyta that have a low K<sub>s</sub> for CO<sub>2</sub> uptake. In return, Cyanophyta can and use bicarbonate as a C source (Dokulil and Teubner, 2000). In the Caspian Sea, carbonate alkalinity and pH values were significantly greater than in the water of other region by the actions of river discharge that changed the chemical composition of the sea and also peak of photosynthesis in summer (Kosarev and Yablonskaya, 1994; Kostinave and Kosarev, 2005). Our results confirmed that water pH of slight alkaline is a favorable condition for Cyanophyta growth in summertime.

There is an ongoing debate on whether nitrogen or phosphorous is the limiting factor for eutrophication in the Caspian Sea. Studies have shown that the limiting factor is area-specific and changes over time. In the Caspian Sea can be phosphorous-limiting near the river mouth, but at all seasons are nitrogen-limited in the offshore area (Kosarev and Yablonskaya, 1994). During all seasons (1994-2005) phytoplankton

productivity was nitrogen limited (4.47 to 5.78) while the levels of P and Si always remained high (Nasrollahzadeh et al., 2008b). In the summer 2009, the low ratio of dissolved inorganic nitrogen/dissolved inorganic phosphorus DIN to DIP (less than 10) of the surface water, in combination with a period of calm and sunny weather that increase the stability of the water column, lead to nutrient-depletion euphotic zone that is isolated from nutrient-rich water mass below. These conditions favor the nitrogen-fixing Cyanophyta.

Recent research has pointed to the role of dissolved organic compounds in algal nutrition, competitive interactions and determination of community structure (Antia et al. 1991; Seitzinger and Sanders, 1997). In particular, certain harmful (*i.e.* toxic, hypoxia/anoxia-inducing, food web-altering) algal bloom groups, including dinoflagellates and blue-green algae (Cyanophyta), are known to contain species capable of growing in either autotrophic or heterotrophic modes (Antia et al., 1991), enabling them to exploit both inorganic and organic nutrient enrichment. In the present study, the percentage of DON was observed to be more than 90% of the total nitrogen of which is the compound available for Cyanophyta and dinoflagellates to grow. In addition, PCA analysis showed that organic nutrients such as DON and DOP were represented at PC1 with total variance more than 30%, which confirmed our aforementioned discussion.

The direction and strength of wind blowing over the Caspian Sea are determined by three factors: the distinctive general circulation of the atmosphere, the temperature field that is created by the sea itself and the relief of its coastline. The average wind speed over the sea is 5.7 m/s. The greatest average speeds are observed in the middle part of the sea and are on the average of 6 - 7 m/s per year. The average wind speeds are significantly lower in the southern of Caspian Sea: 4 - 5 m/s, 3.5 – 4.0 m/s on the eastern coast, and 2.5 – 3.0 m/s in the southeast. Low wind speeds (2.2 – 3.0 m/sec) are observed on the southwestern coast, in Iranian waters and on the southern coast of the Caspian Sea (Caspian Scientific Network, 2003). Strong Cyanophyta bloom occurred in the southern of Caspian Sea at the beginning of the second decade. These blooms developed in August and existed till the end of September in 2005 (Soloviev, 2005). Soranno (1997) also documented short-lived surface blooms throughout the summer and fall, and linked them with periods when there was a combination of low wind velocity, absence of rainfall and higher than average solar radiation. Surface blooms collapsed when wind velocities increased with cloudy weather or rainfall occurred. Result of our study showed that surface blooms collapsed when wind speed increased to more than 2.0 m/s.

As a conclusion, with considering increased load of nutrients, a slightly alkaline pH, a low N:P ratio and global warming may favor the growth of Cyanophyta, the role of light wind (calm weather) is most important to the summer bloom in the Caspian Sea. Research also must continue to focus on defining the underlying conditions that promote the blooms, to ascertain if controllable variables including P and N loading rates, N:P loading ratios, flushing rate, etc. can be manipulated to reduce the overall risk of blooms, even when atmospheric conditions are favorable for their occurrence.

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## REFERENCES

- [1] Akcaalan, R., Mazur-Marzec, H.M., Zalewska, A., Albay, M. (2009): Phenotypic and toxicological characterization of toxic *Nodularia spumigena* from a freshwater lake in Turkey. – *Harmful Algae* 8: 273-278.
- [2] American Public Health Association publication (APHA) (2005): Standard Methods for the Examination of Water and wastewater, 21<sup>th</sup> edi. – Washington DC, USA.
- [3] Anita, N.J., Harrison, P.J., Oliveira, L. (1991): The role of Dissolved Organic Nitrogen in the Phytoplankton Nutrient, Cell Biology and Ecology. – *Phycologia* 30: 1-89.
- [4] Auer, B., Elzer, U., Arndt, H., (2004): Comparison of pelagic food webs in lakes along a trophic gradient and with seasonal aspects: influence of resource and predation. – *Journal of Plankton Research* 26: 697-709.
- [5] Backer, L.C. (2002): Cyanobacterial harmful algal blooms (cyanoHABs): Developing a public health response. – *Lake Reservoir Management* 18: 20-31.
- [6] Beutel, M.W., Horne, A.J., Roth, J.C., Barratt, N.J. (2001): Limnological effects of anthropogenic desiccation of a large, saline lake, Walker lake, Nevada. – *Hydrobiologia* 466: 91-105.
- [7] Blackburn, S.I., McCausland, M.A., Bolch, C.J.S., Newman, S.J., Jones, G.J. (1996): Effect of salinity on growth and toxin production in cultures of the bloom-forming cyanophyta *Nodularia spumigena* from Australian waters. – *Phycologia* 35: 511-522.
- [8] Buckley, D.E., Winters, G.V. (1992): Geochemical characteristics of contaminated surficial sediments in Halifax Harbor: Impact of waste discharge. – *Canadian Journal of Earth Sciences* 29: 2617-2639.
- [9] Cabecinha, E., Cortes, R., Cabral, J.A., Ferreira, T., Lourenço, M., Pardal, M.Â. (2009): Multi-scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs. – *Ecological Indicators* 9(2): 240-255.
- [10] Caspian Scientific Network (CSN) (2003): Scientific Report on Caspian Sea Environment, 122 pp. [Accessed 5 January 2010]. Available from World Wide, Web: [http://www.caspio.net/caspian\\_seafacts/climate/content.htm](http://www.caspio.net/caspian_seafacts/climate/content.htm)
- [11] Dokulil, M.T., Teubner, K. (2000): Cyanobacterial dominance in lakes. – *Hydrobiologia* 438: 1-12.
- [12] Falcon, L.I., Escobar-Briones, E., Romero, D. (2002): Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico. – *Hydrobiologia* 467: 71-78.
- [13] Francis, G. (1878): Poisonous Australian lake. – *Nature* 18: 11-22.
- [14] Galat, D.L., Verdin, J.P., Sims, L.L. (1990): Large-scale patterns of *Nodularia spumigena* blooms in Pyramid lake, Nevada, determined from Landsat imagery: 1972-1986. – *Hydrobiologia* 197: 147-164.
- [15] Gorokhova, E. (2009): Toxic cyanobacteria *Nodularia spumigena* in the diet of Baltic mysids: Evidence from molecular diet analysis. – *Harmful Algae* 8: 264-272.
- [16] Heresztyn, T., Nicholson, B.C. (1997): Nodularin concentrations in lakes Alexandrina and Albert, South Australia, during a bloom of the cyanophyta (blue-green alga) *Nodularia spumigena* and degradation of the toxin. – *Environmental Toxicology Water Quality* 12: 273-282.
- [17] Hernandez-Becerril, D.U., Cortés Altamirano, R., Alonso R.R. (2000): The dinoflagellate genus *Prorocentrum* along the coasts of the Mexican Pacific. – *Hydrobiologia* 418: 111-121.
- [18] Hutchinson, G.E. (1957): A treatise on limnology. – John Wiley and Sons, INC, USA.

- [19] Kahru M., Horstmann U., Rud O. (1994): Satellite detection of increased cyanobacterial blooms in the Baltic Sea: natural fluctuation or ecosystem change? – *AMBIO: A journal of the Human Environment* 23(8): 469-472.
- [20] Karlson, A.M.L. (2010): Benthic use of phytoplankton blooms: Uptake, burial and biodiversity effects in a species-poor system, Doctoral thesis in Marine Ecology. – Department of Systems Ecology, Stockholm University, Sweden.
- [21] Kosarev, A.N., Yablonskaya, E.A. (1994): The Caspian Sea. – SPB academic Publication, Moscow, Russia.
- [22] Kostinave, A.G., Kosarev, A.N. (2005): The Caspian Sea Environment, pp.271. [http://books.google.com/books?id=C1ajChzI9OEC&dq=pH+in+Caspian+Sea&source=gb\\_s\\_navlinks\\_s](http://books.google.com/books?id=C1ajChzI9OEC&dq=pH+in+Caspian+Sea&source=gb_s_navlinks_s).
- [23] Lau, S.S.S., Lane, S.N. (2002): Biological and chemical factors influencing shallow lake eutrophication: a long-term study. – *The Science of the Total Environment* 288: 167-181.
- [24] Mazur-Marzec, H., Krezel, A., Kobos, J., Plinski, M. (2006): Toxic *Nodularia spumigena* blooms in the coastal waters of the Gulf of Gdan' sk: a ten-year survey. – *Oceanologia* 48: 255-273.
- [25] Moncheva, S., Gotsis-Skretas, O., Pagou, K., Krastev, A. (2001): Phytoplankton Blooms in Black Sea and Mediterranean Coastal Ecosystems Subjected to Anthropogenic Eutrophication: Similarities and Differences. – *Estuarine, Coastal and Shelf Science* 53: 281-295.
- [26] Nasrollahzadeh, H.S., Din, Z.B., Foong, S.Y., Makhloogh, A. (2008a): Trophic Status of the Iranian Caspian Sea Based on Water Quality Parameters and Phytoplankton Diversity. – *Continental Shelf Research* 28: 1153-1165.
- [27] Nasrollahzadeh, H.S., Din, Z.B., Makhloogh, A. (2008b): Variations in Nutrient Concentration and Phytoplankton Composition at the Euphotic and Aphotic Layers in the Iranian Coastal Waters of the Southern Caspian Sea. – *Pakistan Journal of Biological Science* 11(9): 1179-1193.
- [28] Padro, R., Barrado, E., Castrillejo, Y., Valasco, M.A., Vaga, M. (1993): Study of the contents and speciation of heavy metals in river sediments by factor analysis. – *Analytical Letters* 26: 1719-1739.
- [29] Palmer, C.M. (1980): Algae and water pollution. The identification, Significance, and Control of Algae in water Supplies and in Polluted Water. – Castle House Publication, London.
- [30] Perez, Del, M.C., Bonilla, S., De Leon, L., Smarda, J., Komarek, J. (1999): A bloom of *Nodularia baltica-spumigena* group (Cyanobacteria) in a shallow coastal lagoon of Uruguay, South America. – *Algol Study* 93: 91-101.
- [31] Raateoja, M., Kuosa., J., Flinkman., H., Pääkkönen., J.-P., Perttilä., M. (2010): Late summer metalimnetic oxygen minimum zone in the northern Baltic Sea. – *Journal of Marine Systems* 80: 1-7.
- [32] Saker, M.L., Thomas, A.D. Norton. J.H. (1999): Cattle mortality attributed to the toxic cyanophyta *Cylindrospermopsis raciborskii* in an outback region of north Queensland.– *Environmental Toxicology* 14: 179-182.
- [33] Schoer, J. (1985): Iron-oxo-hydroxides and their significance to the behavior of heavy metals in estuaries. – *Environmental Technology Letters* 6: 189-202.
- [34] Seitzinger, S., Sanders, R. (1997): Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. – *Marine Ecology Progress Series* 159: 1-12.
- [35] Sivonen, K., Kononen, K., Carmichael, W.W., Dahlem, A.M., Rinehart, K.L., Kiviranta, J., Niemela , S.I. (1989): Occurence of the hepatotoxic cyanophyta *Nodularia spumigena* in the Baltic Sea and structure of the toxin. – *Applied and Environmental Microbiology* 55(8): 1990-1995.
- [36] Soloviev, D. (2005): Identification of the extent and causes of Cyanobacterial bloom in September–October 2005 and development of the capacity for observation and prediction of HAB in the Southern Caspian Sea using Remote Sensing Technique. – WWW Page

[http://www.caspianenvironment.org/newsite/DocCenter/2006/HABrepFinalFull\\_corrected\\_compressed\\_pictures.doc](http://www.caspianenvironment.org/newsite/DocCenter/2006/HABrepFinalFull_corrected_compressed_pictures.doc).

- [37] Soranno, P.A. (1997): Factors affecting the timing of surface scums and epilimnetic blooms of blue-green algae in a eutrophic lake. – Canadian Journal of Fisheries and Aquatic Sciences 54: 1965-1975.
- [38] Stal, L.J., Albertano, P., Bergman, B., Broćkeć, K., Gallon, J.G., Hayes, P.K., Sivonen, K., Walsby, A.E. (2003): BASIC: Baltic Sea cyanobacteria, an investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea – responses to a changing environment. – Continental Shelf Research 23(17-19): 1695-1714.
- [39] Valderrama, J.C. (1981): The simultaneous analysis of total nitrogen and total phosphorus in natural waters. – Marine Chemistry 10: 109-122.
- [40] Vollenweider, R.A. (1974): A Manual on Methods for Measuring Primary Production in Aquatic Environment. – Blackwell Scientific Publication, Oxford, London.
- [41] Wei, G., Tang, D., Wang, S. (2008): Distribution of chlorophyll and harmful algal blooms (HABs): A review on space based studies in the coastal environments of Chinese marginal seas. – Advance in Space Research 41: 12-19.
- [42] Woodward, C.A., Shulmeister, J. (2005): A Holocene record of human induced and natural environmental change from lake Forsyth (Te Wairewa), New Zealand. – Journal of Paleolimnology 34: 481-501.
- [43] Yurkovskis, A. (2004): Long-term land-based and internal forcing of the nutrient state of the Gulf of Riga (Baltic Sea). – Journal of Marine Systems 50: 181-197.