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Short communication

Phytoplankton bloom (Cyanobacteria: *Nodularia spumigena*) in the southwestern Caspian Sea off Anzali, July 2021

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Introductions

There were drastic changes in the hydrological and meteorological regimes at the end of the 1980s that affected phytoplankton communities in the Caspian Sea (Oguz *et al*., 2003). Climate change and variation in nutrient levels are significant threats to biodiversity and biological resources such as plankton and invertebrate structure in the Caspian Sea (Dumont, 2000; Daskalov and Mamedov, 2007; Bagheri *et al*., 2012, 2016; Mirzajani *et al*., 2016). Toxic phytoplankton bloom can impact on marine ecosystems and food web. Harmful bloom can cuase in oxygen reduction, impasse of light to organisms or may poisoning fish (Khan *et al*., 2021).

The *Nodularia spumigena* bloom was increased in numerous systems in the Mediterranean, Canada, USA, Brazil, South Africa, New Zealand and Australia ecosystems (Blackburn *et al*., 1996; McGregor *et al*., 2012; Rakko and Seppala, 2014). This species can produce the toxin nodularin, a hepatotoxic cyclic pentapeptide (Rinehart *et al*., 1988; McGregor *et al*., 2012), which accumulates in the animal liver and can act as a carcinogen in mammals and extremely harmful to vertebrates. The hepatotoxins were identified in bivalvia, while the nodularin was measured very low in cod and herring fish in the Baltic Sea (Carmichael, 1994; Ohta *et al*., 1994; Korpinen *et al*., 2006). The *N. spumigena* has two kind cells; heterocytes with molecular nitrogen fixation cells and akinetes with resistance spores (Silveira *et al*., 2017). Heterocyte is cased the growth of *N. spumigena* under nitrogen low levels, while the akinetes remain viable for long times in the sediment and can grow in response to variations in environmental situations (Hansson, 1993, 1996; Myers *et al*., 2010). In addition, main environmental parameters that manage

N. spumigena bloom are salinity and temperature, and nutrient concentration.

The first *N. spumigena* bloom was accrued in the southern open part of the Caspian Sea very fast during period 10-13 August in 2005 as result of wind speed weakness and surface heating. The *N. spumigena* bloom area increased and reached the 20,000 km^2 and from 1 to 17 Sep , the *N. spumigena* bloom area was transformed to the coastal area (Soloviev, 2005; Bagheri *et al.*, 2011). Furthermore, the *N. spumigena* bloom occurred again in the southwestern Caspian Sea between 2009 and 2010 in August (Nasrollahzadeh *et al*., 2011).

This study, intends to uncover the phytoplankton abundance and species diversity during bloom in the southwestern Caspian Sea off Anzali.

Materials and methods

Study area

The area under investigation was located in the southwestern Caspian Sea, an area influenced by freshwater input from the Anzali wetlands (Bagheri *et al*., 2012). The study on phytoplankton bloom was performed using samples collected at the Anzali transect. The bloom was located at 37° 31" 14' N and 49' 35" 313' E, almost 7 km from the Anzali coast in the Caspian Sea at 26 m depth. The samplings were conducted periodically in 2021 (10 April , 13 July, 20 July). The eighteen samples were taken and stations sampled by using a speedboat in one day during 9–11 am (Fig. 1).

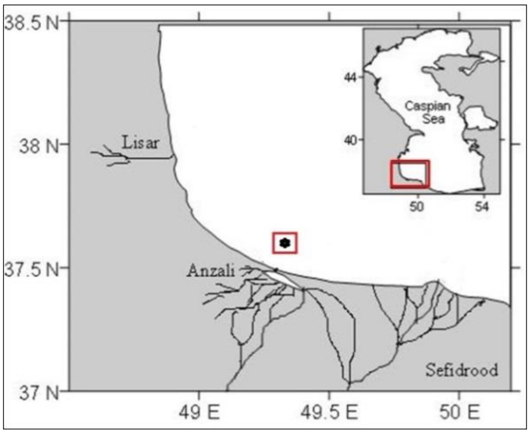

Figure 1: The location of bloom and sampling stations in the Caspian Sea off Anzali, 2021.

Hydro-Physicochemical sampling

Water samples were collected using a 1.71 L Nansen water sampler (Hydro–Bios, Germany; TPN; Transparent Plastic Nansen water sampler, No. 436201). Temperature was measured in situ by using a reverse thermometer (Hydro–Bios, TPN). Salinity was measured by a salinometer (Beckman; RS–7B, U.S. Patent, No. 2542057). Water transparency was measured with a Secchi disk. Total phosphorus (T.P) and, total nitrogen (T.N) were determined by a spectrophotometer (Hach DR/2000) using standard methods (APHA, 2005).

Sampling and laboratory analysis of Phytoplankton

Phytoplankton samples were collected with using a Nansen water sampler. The samples were kept in 500 ml bottles and preserved in 4% formaldehyde. The samples were first allowed to settle at least for 12 days and then the sample volume was reduced to 250 ml by siphoning. The subsamples were further reduced to 30.0 ml using a centrifuge (5 min at 3000; ALC-PK131R; Germany, No. 30206372). For the enumeration of the phytoplankton, tubular plankton chambers (Hyrdo-Bios) counting 5 ml was used and the samples were counted using a inverted-microscope (AE 31E series- Motic) (Prescott, 1962; Vollenweider, 1974; Newell and Newell 1977; APHA, 2005). The enumerations of phytoplankton were repeated three times. Phytoplankton taxonomic classification was performed based on Tiffany and Britton (1971), and Kasimov (1994).

Statistical analysis

Analysis of variance (One-way ANOVA) for hydrochemical factors was used to identify the importance of variables between different sampling periods. Spearman rank correlation coefficients (r) was calculated to estimate the relationships between chl-*a* and secchi depth. A statistical software (Statsoft; SPSS version 19) was used for comparisons between different sampling periods.

Results and discussion

Hydrophysical characteristics

Temporal variations of surface temperature and salinity in the southwestern Caspian Sea in the period of Apri and July 2021 are demonstrated in Table 1. The sea surface temperature (SST) ranged between 13.10 and 30.20°C due to monthly variations in weather temperature. The temperature variations were significant (ANOVA, $p<0.01$). The salinity in the south western Caspian Sea off Anzali varied between 12.11 and 12.55 PSU (Practical Salinity Unit) in 2021. High spatial variation occurred on 13 July . Variations could be related to increased water temperature and evaporation surface water during July. The surface water temperature was exceptionally up in July 2021 (average: 29.8°C) as compared to long–term observations (Roohi *et al*., 2010; Bagheri *et al*., 2012, 2014a: average<28°C). This could be related to the heat wave and drought during 2021. The remarkably high water temperature and the high salinity variation in 13-July 2021 could be related to decline rainfall and freshwater input by the Anzali wetland during this month. In 2021, the salinity was high (12.55 PSU) as compared to 2008, 2010 (Bagheri *et al*., 2012; Mertens *et al*., 2012; average: 10.24-12.20 PSU). According to Bagheri *et al*. (2014b), there was a strongly negative correlation between salinity and freshwater discharge in the southwestern Caspian Sea. Sharifi (1990) noted that the monthly average precipitation amounts exceeded monthly average evaporation levels in the southwestern Caspian Sea. The chl-*a* (measured in 5 m depth) displayed a marked monthly variation with values between 9.1 $\mu g.L^{-1}$ in April and 22.1 $\mu g.l^{-1}$ in July 2021. The Secchi disk depth, an indicator of water turbidity, the secchi disk depth was changed between 3.8 and 2.5 m, respectively in April and 13 July during the study period. Statistical variance analysis (ANOVA) showed that secchi disk depths and chl-*a* were significantly different between the months ($p<0.05$). Furthermore, the occurrence of secchi disk depths was negatively correlated with chl- *a* in this study (p<0.05). In this study, the lowest secchi depth was recorded 2.5 m in 13 July, and the highest chl-*a* was occurred as 22.1 µg L^{-1} at the same time in the southwestern Caspian Sea (Table 1).

Tabel 1: Changes in sea surface temperature, salinity, chl-*a*, secchi depth and nutrient (T.P, T.N) in 26 m depth in the Caspian Sea off Anzali in April and July 2021.

Date	T.P (µM.L^{-1})	T.N (µM.L^{-1})	Chl-*a* (µg.L^{-1})	Salinity (PSU)	Temperature (°C)	Secchi depth (m)
10-Apr	0.312	5.75	9.1	12.14	13.1	3.8
13-Jul	0.589	9.91	22.1	12.55	30.2	2.5
20-Jul	0.544	6.52	13.5	12.37	29.5	3.6

The reduced secchi disk depth and raised chl-a in the bloom period related to *N. spumigena* bloom (5,120,000 cells.l^{-1}; Table 2) which occurred during the study as compared to before and after bloom period. The results of this study was similar with survey of Soloviev (2005). He reported, the chl-*a* levels was increased 76.25 µg.L^{-1} and *N.spumigena* abundance was raised sharply (more than 13,000,000 cells.L^{-1} in Aug 2005; Khatib unpublished data) during bloom in the Caspain Sea. Nutrient concentrations were high during bloom in 13 July and decreased after bloom in 20 July. The concentrations of total phosphorus varied between 0.31 and 0.59 µM.L^{-1} respectively in April and 13 July. Total nitrogen concentrations reached 9.91 µM.L^{-1} on 13 July, and decline almost 5.75 µM.L^{-1} in April (Table 1). However, total phosphorus and total nitrogen concentrations were significantly different during the study (p<0.05).

Phytoplankton compositions

Phytoplankton groups identified in 13 July (in bloom), included four branches: Ochrophyta, Chlorophyta, Cyanobacteria and Myzozoa. Cyanobacteria was the predominant phytoplankton among other phytoplankton groups, accounting for 66% (5,720,000 cells.L^{-1}) of the total phytoplankton abundance. Chlorophyta with a abundance of 19% (1,600,000 cells.L^{-1}) was the largest groups of phytoplankton after Cyanobacteria during the phytoplankton bloom. Ochrophyta and Myzozoa groups with 9 and 6%, respectively, had the lowest abundance of phytoplankton (800,000 and 520,000 cells.L^{-1}) in this study, total phytoplankton abundance during the bloom period was estimated 8,640,000 cells.L^{-1}. The phytoplankton abundance was measured as 88,200 cells.l^{-1} before bloom, on April. Ochrophyta abundance was the most dominant (97 % of total abundance; 85800 cells.L^{-1}) and the lowest abundance groups were recorded as Cyanobacteria and Myzozoa almost 2 and 1 % of total abundance (1200 cells.L^{-1}). Among the phytoplankton groups, Ochrophyta were dominant (73.0%, 83,400 cells.L^{-1}) after bloom. Cyanobacteria (15%, 12,600 cells.L^{-1}) were the second most important group, contributing to the total phytoplankton abundance after bloom, while Chlorophyta and Myzozoa (8 and 4 %, respectively) were the lowest (6600 and 3600 cells.L^{-1}, respectively) of total phytoplankton abundance. A total of 15 phytoplankton species were identified before bloom in April. Of these taxa, 12 taxa Ochrophyta (5 genera, 7 species); two taxa Cyanobacteria (2 species); one taxa Myzozoa (1 species). According to the findings on the community structure and diversity, a total of 8 phytoplankton taxa were distinguished during the bloom, of which Cyanobacteria with 3 species formed the most diversity and included; *Nodularia spumigena*, *Spirulina* sp. and *Oscillatoria* sp. (5 species in 2005; Khodaparast, 2006). The most abundant were *N. spumigena* about 5,120,000 cells.L^{-1}. In this study, the *N. spumigena* abundance were less than 2005 (13,000,000 cells.L^{-1}; Khatib unpublished data). The number of phytoplankton species raised sharply after bloom period (20 July) and species number were varied between 8 and 18, respectively in 13 and 20 July (Table 2).

Table 2: Phytoplankton taxa abundance (cells.l^{-1}) in the southwestern Caspian Sea off Anzali, April, and July 2021.

Taxa	Before bloom			Bloom			After bloom		
	Abundance	A(%)	Taxa	Abundance	A(%)	Taxa	Abundance	A(%)	Taxa
Ochrophyta	85800	97	12	800000	19	2	60600	73	9
Chlorophyta	0	0	0	160000	9	1	6600	8	3
Cyanobacteria	1200	2	2	*5720000	66	3	12600	15	4
Myzozoa	1200	1	1	520000	6	2	3600	4	2
Tatal	88200	100	15	8640000	100	8	83400	100	18

* *N. spumigena*: 5,210,000 cells.L^{-1}

The bloom of *N. spumigena* was originally observed as a thick bright olive-green surface scum concentrated by wind-driven advection along the Caspian shore. Trichomes were lonely, tubular, straight to slightly spiral and generally<500 µm in length (Fig. 2). Vegetative cells were discoid, containing many aerotopes. Heterocytes were also compacted discoid, intercalary and commonly spaced. Akinetes were common, discoid-subspherical, mostly single or in pairs, occasionally 2–5 in series.

The first *N.spumigena* bloom occurred in the southern Caspian Sea in Sep 2005 (Soloviev, 2005), and second *Nodolaria* bloom observed in the Tonekabon coast of Caspian in August 2009 (Nasrollahzadeh *et al*., 2011). The last bloom was recorded in the southwestern Caspian Sea off Anzali in July 2021.

Vertical distribution of *N. spumigena* at the bloom region in the depth range of 5–0 m, 10–5 m and 20-10 m revealed the most abundant of *N. spumigena* in the upper layer (Fig. 3). The highest abundance was measured more than 5,000,000 cell.L^{-1} at the 0-5 m layer depth and the lowest was recored 240,000 cell.L^{-1} at the 10-20 m depth as reported by Nasrollahzadeh *et al*. (2011) in the southern Caspian Sea.

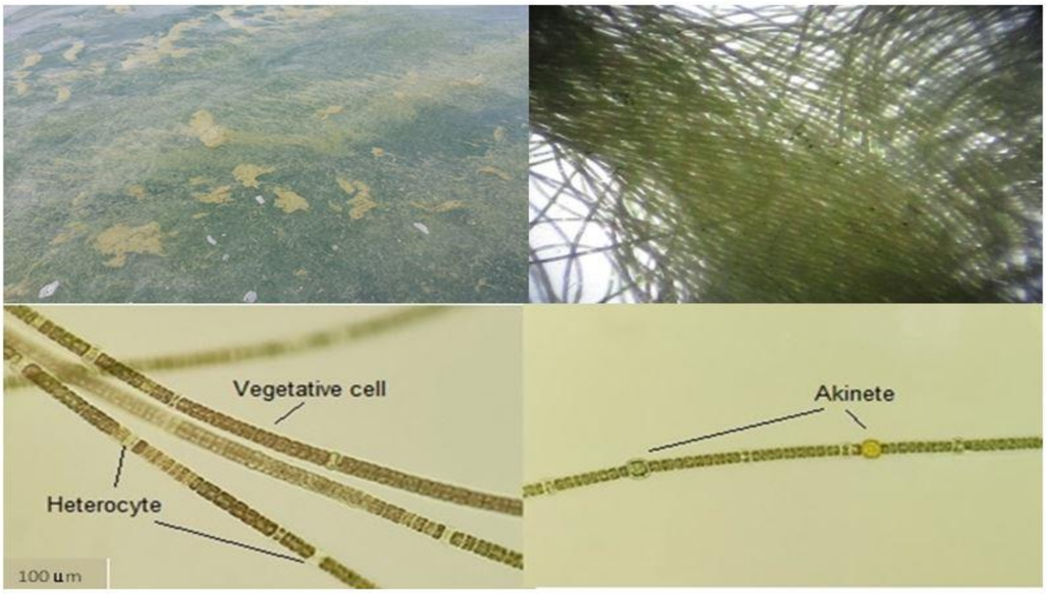

Figure 2: *Nodularia spumigena*: Arrows indicates vegetative cell, heterocyte and akinete in the southwestern Caspain Sea off Anzali, July 2021 (40× magnification).

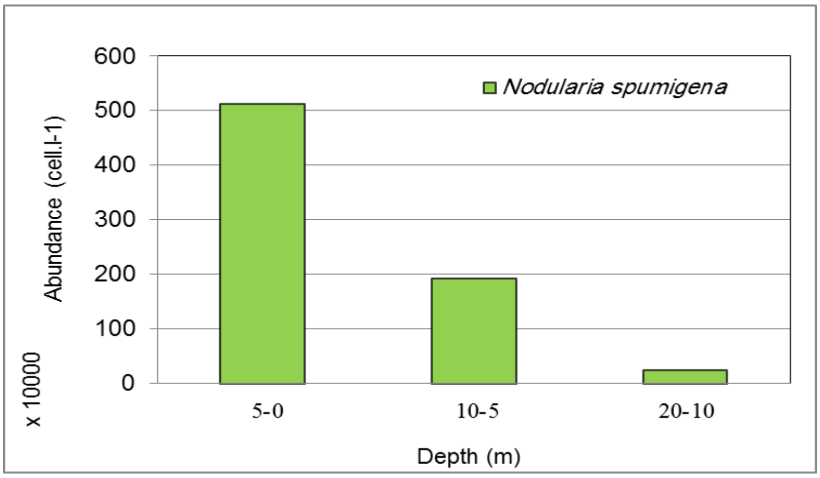

Figure 3: Vertical distribution of *N. spumigena* in the southwestern Caspian Sea off Anzali, July 2021.

The intensive *N.spumigena* blooms were occurred in the Baltic Sea, Mediterranean Sea and Australia ecosystems, Great Salt Lake in US (Gorokhova, 2009). The *N. spumigena* bloom recorded among 53% of the total abundance in the Iznik Lake (Akcaalan *et al*., 2009).

The *N. spumigena* and *Aphanizomenon flos-aquae* made 35.8% of the total phytoplankton biomass in August 2000–2005 in the Finland Gulf (Raateoja *et al*., 2010; cited by Nasrollahzadeh *et al*., 2011). Tha abundance of cyanobacteria was 66 % in the present study (Table 2), while the Cyanobacteria abundance were 98 % and 93 % in 2009 and 2005, respectively in the southwestern Caspian Sea (Khodaparast, 2006; Nasrollahzadeh *et al*., 2011).

The wind speeds are considerably lower as 4–5 m/s in the southern Caspian Sea, low wind speeds 2.20–3.0 m/s were observed in the southwestern coast of Iranian waters (Nasrollahzadeh *et al*., 2011). Strong Cyanobacteria bloom occurred in the southern Caspian Sea in 2005. These blooms developed in August and existed till the end of September. (Soloviev, 2005). In present study, the very low wind speed almost 0.0-3.0 m/s (Wundergroud, 2021), high sea surface water temperature (SST; 30.2ºC; Table 1) and high nutrients levels (T.P: 0.59 and T.N: 9.91 $\mu M.L^{-1}$; Table 1) in the Caspian Sea off Anzali were the most reason to bloom of *N. spumigena* in the beginning of July 2021. A bulk of *N. spumigena* from surface water came to the shore of Anzali with raised wind in the sea during a week after bloom.

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