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ORIGINAL ARTICLE

The occurrence and levels of cyanotoxin nodularin from *Nodularia spumigena* in the alkaline and salty Lake Burdur, Turkey

Fatma SAHINDOKUYUCU KOCASARI,[1*] Iskender GULLE,[2] Sukru KOCASARI,[3] Selcuk PEKKAYA,[4] Firdevs MOR[1]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Bahcelievler, 15030 Burdur; [2]Department of Biology, Faculty of Arts and Science, University of Mehmet Akif Ersoy, Bahcelievler, 15030 Burdur; [3]The Ministry of Food, Agriculture and Livestock, Burdur; [4]Veterinary Control and Research Institute, Department of Biochemistry, Ankara, Turkey
*Corresponding author: fatmasa@mehmetakif.edu.tr

ABSTRACT
The occurrence of cyanobacteria species Nodularia spumigena *and its toxin nodularin were studied in Burdur Lake, a saline alkaline lake with endemic fauna and a Ramsar site of ornithological importance. The water samples were collected from three different locations of Lake Burdur between May and November of 2011. Abundance of* Nodularia spumigena *and the nodularin levels peaked at the end of July (112,147 cells mL^{-1} and 4.82 µg L^{-1}), coinciding with the highest levels of chlorophyll* a *(Chl* a*) (27.15 µg L^{-1}) and the water temperature (29°C). Although fish or other animal deaths were not detected during the episodes, it is concluded that* N. spumigena *blooming should be monitored in Burdur Lake for the ecosystem and environmental health.*

Key words: Cyanobacteria bloom, Burdur Lake, Nodularia spumigena, Nodularin, ELISA.

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INTRODUCTION

Cyanobacteria are a group of prokaryotic organisms that occur worldwide in fresh-, brackish-, and marine waters. Under favorable conditions, cyanobacteria can multiply rapidly to form blooms in water (Kaloudis *et al.*, 2013). According to literature, over a 100 species of cyanobacteria belonging to 40 genera are reported to be toxigenic (Jayatissa *et al.*, 2006). The primary toxin-producing genera include *Microcystis*, *Nodularia*, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis* and *Planktothrix* (Nasrollahzadeh *et al.*, 2011).

Cyanobacteria are gram-negative bacteria capable of producing a wide range of potent toxins as secondary metabolites, *i.e.* the cyanotoxins (Van Apeldoorn *et al.*, 2007). Cyanotoxins can be especially detrimental to human and animal health, aquatic habitats and aquaculture enterprises (Kaloudis *et al.*, 2013). Cyanotoxins are very diverse in their chemical structure and toxicity, usually being classified as hepatotoxins, neurotoxins and dermatotoxins according to the toxic effects on animals (De Figueiredo *et al.*, 2004). The most commonly reported cyanotoxins are hepatotoxins and neurotoxins (Al-Jassabi and Khalil, 2006).

The *Nodularia spumigena* Mertens ex Bornet & Flahault belonging to Nostocales is a filamentous and dense bloom-forming cyanobacteria species mainly found in slightly saline to brackish waters such as saline inland lakes, coastal lakes, estuaries and seas (Guiry and Guiry, 2014). However, recently nodularin has also been detected in freshwater lakes (Akcaalan *et al.*, 2009; Kaloudis *et al.*, 2013). The *N. spumigena* blooms are generally toxic, and they usually produce a potent hepatotoxin called nodularin. Structurally, this toxin is a cyclic pentapeptide consisting of D-erythro-β -methylaspartic acid (D-MeAsp), L-arginine (L-Arg), 3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyldeca-4(*E*), 6(*E*)-dienoic acid (Adda), D-glutamic acid (D-Glu) and 2-(methylamino)-2-(Z)-dehydrobutyric acid (Mdhb). The structure and biological activity of nodularin are similar to cyclic heptapeptides called microcystins the most frequently encountered and best recognized group of toxins produced by freshwater cyanobacteria (Akcaalan *et al.*, 2009; Mazur-Marzec *et al.*, 2009). The characteristic feature of both microcystins and nodularin is the presence of the unusual amino acid Adda which is important for recognition of binding sites responsible for hepatotoxic effects and tumor promoters (Shan *et al.*, 2011).

The hepatotoxicity and carcinogenicity of nodularin is associated with the inhibition of eukaryotic protein phosphatase catalytic subunit types 1 and 2A. The toxin has been reported to have detrimental effects on numerous organisms within the ecosystem, including invertebrates and fish. The consumption of water containing toxic *N. spumigena* blooms has led to the death of domestic and native animals by massive liver hemorrhage (Pearson *et al.*, 2010).

Lake Burdur is one of the largest and deepest lakes of Turkey. Lake Burdur is salty (20 g L^{-1}) and highly alkaline aquatic system (Gülle *et al.*, 2010). However, due to the constant discharge of pollutants into the lake through Burdur city sewage, livestock farming, and industrial wastes for long years, Lake Burdur has undergone rapid eutrophication. The water level of Lake Burdur has decreased about 40% since 1971. In addition, two occurrences of cyanobacterial blooms in 2000 and 2007 caused by *N. spumigena* were reported, leading to fish mortality (Anonymous, 2000; Yıldırım *et al.*, 2008). Earlier studies prior to 2000 event regarding the lake's water quality of Lake Burdur reported no case of blooms and also no information about the presence of cyanotoxins (Arcak and Altındağ, 2000; Girgin *et al.*, 2004; Gülle *et al.*, 2010). Thus, the present study is the first survey on the presence, levels of nodularin in Lake Burdur which has a cyanobacteria-dominated system.

The aim of this study was to investigate water quality parameters, *N. spumigena* densities, and nodularin concentrations in samples from Lake Burdur between May 2011 and November 2011.

METHODS

Description of study area

Lake Burdur is located at the Lake District Region in Southwest Anatolia (37°43′351″ N, 30°10′878″ E) (Fig. 1) and has a surface area of 147 km^2 and a catchment area of 4120 km^2. One of the deepest lakes in Turkey, average depth in Lake Burdur is 30 m, while 61 m in its deepest point. Lake Burdur has high alkaline (pH 9.1) and brackish water character (20 g L^{-1}) of Na-Mg-(Cl)-SO_4-HCO_3 type and C_5S_5 class. It is unavailable for domestic, drinking, industrial or agricultural uses as it has also very high water hardness. A species of killifish *Aphanius sureyanus* and a copepod species *Arctodiaptomus burduricus* are endemic species to the lake. It is one of the lakes in Central Anatolia that does not freeze during winter, therefore Lake Burdur has great a ornithological importance due to great numbers of wintering birds, and being the main wintering site for the globally threatened duck *Oxyura leucocephala* in Turkey. Burdur Lake is a Ramsar Site since 1993 and Wild Life Reserve (Yarar and Magnin, 1997; Girgin *et al.*, 2004; Gülle *et al.*, 2010).

Samples of water from Lake Burdur were collected from May to November in 2011. Sampling stations are described in Fig. 1. Station I at 37°43′351″ N, 30°10′878″ E, station II at 37°46′849″ N, 30°16′256″ E, and station III at 37°44′517″ N, 30°13′981″ E.

Sampling and measurements

Samples were collected at a depth of 0.5-1 m in 1.7 L Nansen bottles, pre-washed with HCl and distilled water (APHA-AWWA-WEF, 1999). Temperature, pH, electrical conductivity, salinity (YSI 63 pH/SCT meter), dissolved oxygen (YSI 550A), turbidity (WTW Turb 355IR) and transparency (Secchi disc depth) were measured *in situ*

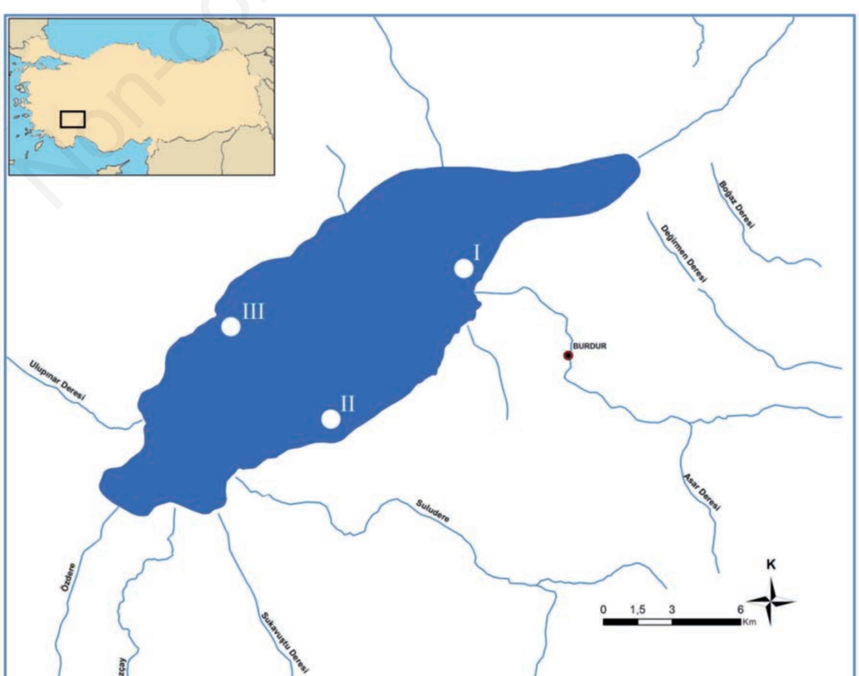

Fig. 1. Burdur Lake and sampling sites.

every sampling station. Also, total phosphorus (as P-PO_4) and nitrate (N-NO_3) determined according to the procedure described by Merck Spectroquant® Cell-test kit. The chlorophyll *a* (Chl-*a*) concentration was determined spectrophotometrically. Cyanobacteria cells were filtered through a filter (1.2 µm pore sized glass fiber filter paper), and the pigments were extracted in the 90 % acetone solution and analyzed according to APHA-AWWA-WEF (1999).

The method of Komárek *et al*. (2003) was used for the identification of phytoplankton morphospecies. Phytoplankton samples (500 mL) were fixed in Lugol's solution. The *N. spumigena* density was determined under a binocular microscope via Sedgwick-Rafter counting chambers. Shortly samples were concentrated to 10 mL by counting filaments added, and then average number of *N. spumigena* cell of randomly selected 20 filaments were used to express the cell number per milliliter (cells mL^{-1}). This procedure was repeated several times with different filaments and an arithmetic mean was established (APHA-AWWA-WEF, 1999).

Nodularin analysis was performed using an Enzyme Linked Immuno Sorbent Assay (ELISA). Extraction of samples was conducted according to the instructions of the ELISA kit (Abraxis LLC, 522015, Warminster, PA, USA). One-hundred µL of seawater sample treatment solution were added to the water samples (1 mL each), which then stirred for 1 min and incubated for 30 min at room temperature. All standards, control and lake water samples were run in duplicate. One-hundred µL standard solutions control and test samples were added to each well. Then, 50 µL of enzyme conjugate and 50 µL antibody solutions were added to each well, mixed thoroughly and incubated for 90 min at room temperature. At the end of incubation, the wells were washed three times with 250 µL of washing buffer. After, 150 µL colour solution was added to each well, mixed thoroughly and incubated for 20 min at the room temperature. After the incubation, 100 µL of the stop solution was added to each well and mixed. The absorbance was measured at 450 nm by an ELISA plate reader (ELX-800; Bio-Tek Instruments, Winooski, VT, USA).

The extracellular nodularin levels in samples were evaluated using the MS Excel spreadsheet prepared by Abraxis. The levels of nodularin standards used were 0, 0.15, 0.40, 1, 2 and 5 µg L^{-1}. According to the test preparation record, the lower detection limit was 0.10 µg L^{-1}.

RESULTS

The water quality parameters determined during the study were presented in Tab. 1. Moderately dissolved oxygen concentration was observed in surface waters of the lake having an alkaline (pH>9) and hyposaline (19.99 g L^{-1}) character (Tab. 1). According to the findings (Tab. 1), Carlson's Trophic State Index (TSI) values were calculated from Secchi's disc transparency (1.63 m), Chl *a* (14.33 µg L^{-1}) and total phosphorus (0.19 mg L^{-1}) from the measurements and found as 53, 57 and 80, respectively. Based on these values, the trophic state of the lake is determined as eutrophic according to OECD criteria and TSI values (Wetzel, 2001).

The highest *N. spumigena* cell density (St 2, mean 72.8 10^3 cells mL^{-1}) coincides with the maximum water temperature and turbidity (28°C and 18.75 NTU) values of Lake Burdur during mid-summer (on July 30th), when the cyanobacteria proliferation became visible (Fig. 2). However, the maximal Chl *a* (45.11 µg L^{-1}), turbidity (18.75 NTU) and minimum transparency (0.3 m) levels were found in St I.

The nodularin concentrations are found in our study to be ranging between 0.30 and 4.82 µg L^{-1} (Fig. 3) and generally correlated with the water temperature, Chl *a* concentration and the *N. spumigena* density (Tab. 1; Figs. 4 and 5). The *N. spumigena* densities and the nodularin concentrations regression relationship were found to be high (R^2=0.88) (Fig. 6).

DISCUSSION

The cyanobacterial blooms are common and formed mostly by *Microcystis*, *Anabaena*, *Planktothrix, Aphanizomenon* and *Nostoc* species in fresh waters, while in marine and brackish environments, toxic blooms are less common and formed by *N. spumigena* (Mazur-Marzec *et*

Tab. 1. Water quality parameters of Lake Burdur determined during May-November 2011 period (mean±SD, n=12).

Parameters	Mean±SD	Min.	Max.
Temperature (°C)	21.6±4.6	9.9	29
pH	9.04±0.04	8.97	9.12
Dissolved oxygen (mg L^{-1})	7.59±1.40	4.78	10.6
Conductivity (mS cm^{-1})	32.13±0.46	31.12	32.93
TDS (g L^{-1})	22.29±0.39	21.69	23.05
Salinity (g L^{-1})	19.99±0.27	19.20	20.40
N-NO_3 (mg L^{-1})	-	-	<0.1

TDS, total dissolved solids.

al., 2006). *N. spumigena* blooms have been reported from brackish waters of Australia (Heresztyn and Nicholson, 1997; McGregor *et al.,* 2012), America (Beutel *et al.*, 2001), Africa (Harding *et al.,* 1995), Baltic Sea (Kononen *et al.*, 1993; Mazur and Pliński, 2003; Henriksen, 2005; Mazur-Marzec *et al.*, 2006, 2009, 2013; Suikkanen *et al.*, 2007, Kankaanpää *et al.*, 2009), and Caspian Sea (Nasrollahzadeh *et al.*, 2011). The mass occurrence of *N. spumigena* in Lake Burdur was recorded in 2000 for the first time and second for the brackish waters of Turkey (Gülle *et al.*, 2010), the species now the most dominant cyanobacteria species in Lake Burdur. The first brackish water *N. spumigena* bloom record (July 1996) was from Bafa Lake (Kazancı *et al.*, 2008).

The seasonal occurrence of *N. spumigena* blooms varies according to the literature. Heresztyn and Nicholson (1997) reported the *N. spumigena* blooms during the summer/autumn of 1994-1995 in lakes Alexandrina and Albert (South East Australia). However, McGregor *et al.* (2012) observed later *N. spumigena* blooms between September and November of 2008 in Carbrook Cable Ski Lake, South East Australia. Similarly, Nasrollahzadeh *et al.* (2011) described the *N. spumigena* bloom in Caspian Sea as starting from middle of summer with a maximum in early of autumn. On the other hand, in the Gulf of Gdańsk (Baltic Sea), the intensive *N. spumigena* blooms were observed in summer (late June-early July in 2001 and in late July-early August in 2002) (Mazur and Pliński,

Fig. 2. Accumulation of *N. spumigena* filaments near the lake shore and surface during the blooms (2011.07.30 St. II). (Courtesy I. Gülle).

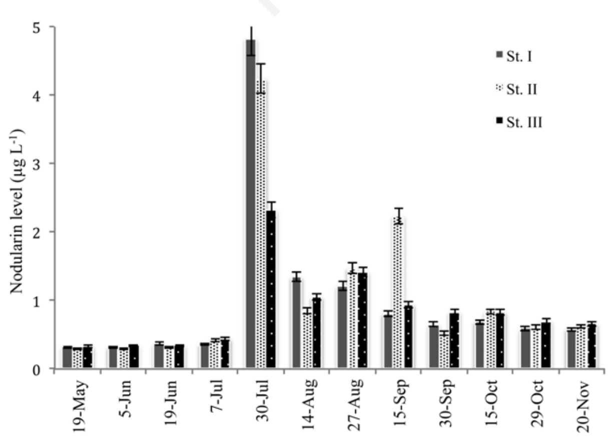

Fig. 3. Nodularin levels (μg L^{-1}) in sampling stations of Lake Burdur.

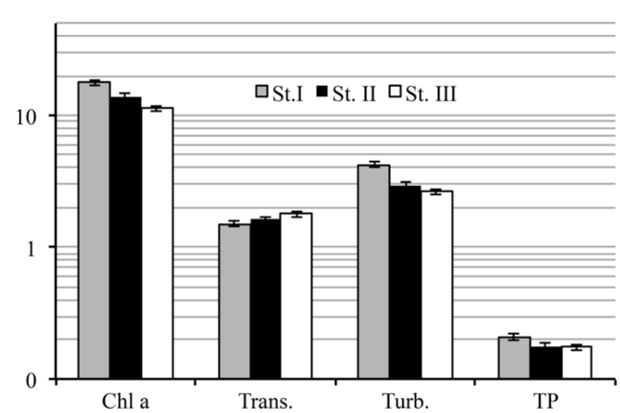

Fig. 4. The average Chl *a* (μg L^{-1}), transparency (m), turbidity (NTU) and total phosphorus (mg L^{-1}) measurements in sampling stations of Lake Burdur.

2003). Our results (late July-early August) were similar to that of Mazur and Pliński (2003). It is considered that the seasonality of the blooms could be related with environmental factors.

The several environmental factors have been considered to enhance the blooms of cyanobacteria (Ballot *et al.,* 2003). The growth of *N. spumigena* is influenced by temperature, salinity, light irradiance, low N:P ratio (Mazur and Pliński, 2003; Henriksen, 2005). Holland *et al.* (2013) reported that the *Nodularia* blooms occurred in waters with salinity between 9-20 and the water temperature above 20°C. Several study results (Mazur and Pliński, 2003; Mazur-Marzec *et al.*, 2006) demonstrated that growth of the *N. spumigena* is strongly temperature-dependent and is optimal at 25-28°C. In our study, during the bloom, water temperature was 25-28°C and salinity was 19.83-20.05 g L^{-1}. Salinity has been drastically increased (by around 50 %, 13-14 to 20 g L^{-1}) due to drying of the lake. However, this increase is not considered as an important factor since the blooms have been observed in lower salinities. The nitrogen limitation, a condition when the ratio of dissolved inorganic nitrogen (DIN) to dissolved phosphorus (filterable reactive phosphorus, FRP) is less than about 10:1, is assumed to be another factor and *N. spumigena* blooms reportedly occur when DIN:FRP is <5:1 **(**Holland *et al.*, 2013). As a general condition encountered in salty lakes (Jelison, and Melack 2001), nitrogen limitation is a natural phenomenon in Lake Burdur. The mean N-NO_3 values of the lake, <0.3 mg L^{-1} (<0.1 mg L^{-1} in our study) (Anonymous, 2013), are much lower than the minimal eutrophication limits for the fresh water systems. The increased levels of P mainly resulting from domestic pollutants during 2000s have lowered down the N:P ratio (Gülle *et al.*, 2010; Yıldırım *et al.*, 2008).

The maximum density of *N. spumigena* in our study ranged from 37.6 10^3 to 112.1 10^3 cells mL^{-1} (mean 72.8 10^3 cells mL^{-1}). The intensity of the bloom can be evaluated as mild compared with the figures of other studied lakes, Carbrook Cable Ski Lake for instance having a cell density between 164.5 10^3 and 605.2 10^3 cells mL^{-1} (McGregor *et al.*, 2012). However, the intensity of the blooms are observed to fluctuate largely among the years (Kankaaanpää *et al.*, 2009; Nasrollahzadeh *et al.*, 2011).

As mentioned, Chl-*a* concentrations are used as an important indicator for microalgae biomass production in water ecosystems. In this study, there is a strong correlation between the Chl-*a* concentrations and the density of the *N. spumigena* observed to be the dominant phytoplankton during the bloom period.

The present study is the first study to document nodularin presence in a brackish-saline lake in Turkey. The nodularin concentrations in Lake Burdur were measured between 0.30 and 4.82 µg L^{-1}. Our results are within the range of the results obtained from different studies performed in Baltic Sea in which the spectrum of the results is much wider (Kononen *et al.*, 1993; Mazur and Pliński, 2003; Henriksen, 2005). In this study, the correlation between the nodularin concentration and the *N. spumigena* density was found to be high (R^2= 0.88) and close to the findings of Mazur-Marzec *et al.* (2013) in Baltic Sea (R^2=0.895), while relatively lower than that of Henriksen (2005) (R^2= 0.95).

Nodularin is a potent hepatotoxin in humans and animals (Pearson *et al.*, 2010). The LD_{50} of nodularin is approximately 50 µg kg^{-1} body weight in mice when given intra-peritoneally (Carmichael *et al.*, 1988). With such a low LD_{50}, nodularin is one of the most potent natural toxins. At doses below this concentration, nodularin may act as a carcinogen via the initiation and promotion of liver cell division (Pearson *et al.*, 2010). Several cases of death and poisoning of aquatic and terrestrial animals (sheep,

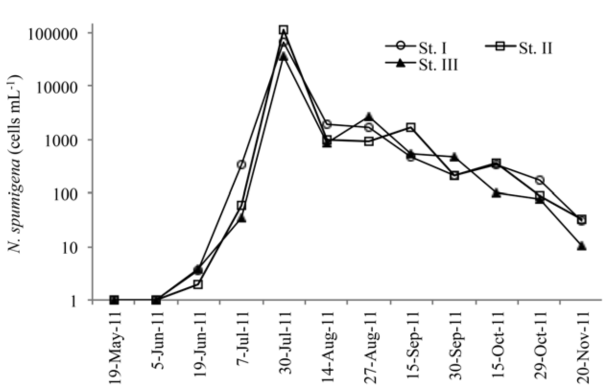

Fig. 5. Spatial and temporal change of *N. spumigena* density (cells mL^{-1}) in sampling stations of Lake Burdur.

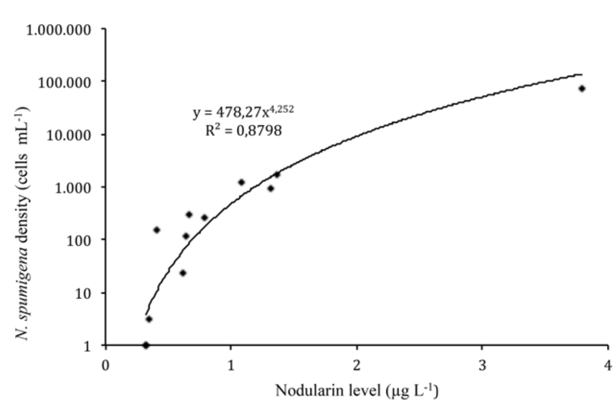

Fig. 6. The relationship between *N. spumigena* density and nodularin concentration.

lambs, horses, dogs, pigs and prawns) following contact with *N. spumigena* were recorded (Edler *et al.*, 1985; Nehring, 1993; Steffensen *et al*., 1999; Kankaanpää *et al.*, 2005). So far there have been no confirmed reports of human fatalities attributable to the *N. spumigena.* However, people who have bathed in an area with a cyanobacteria bloom have suffered from allergic reactions, skin irritation and indigestion-related illnesses (Mazur and Pliński, 2003).

The World Health Organization has provided a provisional guideline value of 1 μg L^{-1} microcystins for drinking water (WHO, 1998). Although microcystins concentrations more than 1 μg L^{-1} in drinking water is regarded as unsafe by WHO, there is no any value set by WHO for nodularin concentration.

One of the two endemic organisms of Lake Burdur is a copepod, *A. burduricus*, the dominant zooplankton of the lake. Regarding the interaction between copepods and *N. spumigena*, direct grazing effect or toxic effect have been vigorously debated (Koski *et al.*, 1999, 2002; Sellner *et al.*, 1996). During our study, no mortality in endemic fish *A. sureyanus* and the aquatic birds were observed after the blooms as well as during the blooms. This can be rough evidence about absence of toxic effect of *N. spumigena* blooms even during the maximum nodularin levels of the study period (up to a 4.82 μg L^{-1}).

CONCLUSIONS

The Lake Burdur is a large saline lake and an important Ramsar site. Even if blooms of *N. spumigena* have been recorded, very few information is available on cyanobacteria and their toxicity in the lake. The results of this study could be important in terms of presence of cyanobacteria and cyanotoxins in Lake Burdur (from May to November 2011), and could contribute to the knowledge on cyanobacterial blooms in brackish-saline lakes.

The effects of the *Nodularia* blooms on the endemic fauna of Burdur Lake and the food chain should be studied in the future studies. The negative trend of the changes in and around of the lake is likely to remain in the future, thus more severe blooms may be expected. Therefore, how the drying process of Lake Burdur and its possible consequences as increased salinity and increase in trophic level affects the *Nodularia* proliferation could be studied in detail to understand the nature of recent blooms in the lake.

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