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Phenotypic and toxicological characterization of toxic *Nodularia spumigena* from a freshwater lake in Turkey

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ABSTRACT

Cyanobacterial blooms have been occasionally observed in Iznik lake, a freshwater body (salinity = 0.5) located in the western part of Turkey. *Nodularia spumigena* (Mertens in Juergens) was recorded in the lake in the summer months of 2005. Maximum filament concentration of the species (1.3×10^5 fil L^{-1}) was measured in August and constituted 60% of total cyanobacteria abundance. Trichomes were solitary, straight and had cells containing gas vesicles. Heterocysts were regularly spaced throughout the filaments. In the isolated filaments nodularin was detected by HPLC, ELISA and PPIA as well as LC–MS. HPLC analysis showed that gravimetric nodularin concentration in cultured *N. spumigena* cells was 578 μg of nodularin per gram dry weight (d.w.). Apart from nodularin, demethylated nodularin variant was also found in *Nodularia* cell extract. This is the first report of toxic *N. spumigena* in a European freshwater lake.

1. Introduction

The cyanobacterium, *Nodularia spumigena* is a filamentous, heterocystous, nitrogen-fixing species known to prefer saline and brackish waters. The first report of toxic cyanobacterial bloom in the world was recorded in lake Alexandrina, Australia as early as in 1800s (Francis, 1878). At that time the lake had estuarine salinities and after construction of flow control barrages, salinity decreased, however, *N. spumigena* continued to occur (Hobson et al., 1999). In recent years, the cyanobacterium has formed prominent blooms in the Baltic Sea (Kahru et al., 1994; Sivonen et al., 1989; Stal et al., 2003; Mazur-Marzec et al., 2006). There are several reports related to *Nodularia* bloom in estuaries (Blackburn et al., 1996) and brackish lakes (Heresztyn and Nicholson, 1997; Woodward and Shulmeister, 2005) of Australia and New Zealand, saline lakes and lagoons from USA (Beutel et al., 2001; Galat et al., 1990), Mexico (Falcon et al., 2002) and Uruguay (Perez et al., 1999). The presence of *N. spumigena* was previously recorded in a floristic study from a lake in Turkey without giving any information of its biomass and toxicity (Gonulol and Comak, 1992).

Blooms of *N. spumigena* are generally toxic and they usually produce a hepatotoxin called nodularin. Nodularin is a cyclic pentapeptide with general structure cyclo(-D-MeAsp[1]-L-Arg[2]-Adda[3]-D-Glu[4]-Mdhb[5]), where MeAsp[1] is D-*erythro*-β-methylaspartic acid, Mdhb stands for 2-(methylamino)-2(*Z*)-dehydrobutiric acid, Adda[5] is a (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid. The structure and biological activity of nodularin is similar to microcystin (MC), a group of heptapeptide hepatotoxins produced by freshwater cyanobacteria. A few naturally occurring variants of nodularin have been found (Namikoshi et al., 1994). The toxin can be hazardous to aquatic animals when blooms occurred and in acute cases may cause death through liver failure (Carmichael, 1994). Although no human deaths attributed to nodularin intoxication has been recorded up to now, the toxin is also potentially dangerous for the human health under long-term exposure (Ohta et al., 1994).

Several studies have been performed on bloom formation, effects of environmental variables on growth of *Nodularia*, nodularin production and degradation in nature. Most studies, however, have focused on the effect of salinity on growth and toxin production by *N. spumigena* (Hobson and Fallowfield, 2003; Lehtimaki et al., 1994; Mazur-Marzec et al., 2005), because of the conditions of presence and bloom formation characteristics of this species.

To our knowledge, this is the first report on the presence of toxic *N. spumigena* in a freshwater lake on the European continent. In this paper, three different methods were applied to characterize nodularin produced by the cyanobacterium. Phytoplankton

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composition and main environmental variables measured in Iznik lake are also discussed.

2. Materials and method

2.1. Study area

Iznik lake (40°26′N, 29°32′E), located in the southeast of Marmara region and near to Istanbul (210 km), is the biggest freshwater lake in the region and the fifth biggest lake in Turkey with a 80-m maximum depth and 313 km^2 surface area (Fig. 1). The main water input is supplied by creeks and groundwater. There is intense agricultural activity around the lake, mainly olive groves, fruit plantations and vegetable gardens where high amount of fertilisers are used. Additionally, untreated wastewaters from residential areas reach the lake (Albay, 1996). As a result of nutrient loading from catchment area, the lake's trophic status shifted from oligo-mesotrophy to meso-eutrophy in the last two decades (Table 1).

Toxic blooms of cyanobacteria have occurred sporadically in the lake. A severe *Anabaena* sp. bloom was recorded in 2001 with microcystin concentrations reaching 7.2 $\mu g\ L^{-1}$ of MC-LR equivalent measured by HPLC. Up to the present, some other cyanobacteria species, *Aphanizomenon aphanizomenoides*, *Anabaenosis sp.*, *Cylindrospermopsis raciborski*, *Planktothrix rubescens* and *N. spumigena,* reached high numbers in this lake water (Unpublished data).

2.2. Chemical analysis

Samples were collected from the surface waters of the lake at one site during the June, July and August 2005. Water samples for nutrients analysis (NO_3-N + NO_2-N, SRP, TP, SRSi) were kept cool and in the dark before being brought to the laboratory and analysed according to APHA (1989).

Table 1
Measured mean (±S.D.) physical, chemical and biological features of Iznik lake during June–August 2005.

Temperature (°C)	26 (±1.4)
pH	8.99 (±0.05)
TDS (mg L^{-1})	478 (±3.1)
NO_2-N + NO_3-N ($\mu g\ L^{-1}$)	81.9 (±107)
SRP ($\mu g\ L^{-1}$)	10.3 (±3.9)
SO_4 (mg L^{-1})	21 (±7.1)
Chlorophyll-*a* ($\mu g\ L^{-1}$)	11.4 (±1.4)
Nodularia biomass ($\mu g\ L^{-1}$)	933 (±485)

Temperature, dissolved oxygen, conductivity, pH were measured *in situ* using a multi-parameter probe (Radiometer, Pioneer 65).

2.3. Phytoplankton analysis

Phytoplankton subsamples (100 mL) were fixed in Lugol's Iodine solution and kept in glass bottles in the dark. Phytoplankton cell concentration was measured by counting specimens until 400 individuals of the most abundant species were enumerated (Utermöhl, 1958). The abundance of each species was based on cell counts, only filamentous species were considered as a unit and abundance was expressed as individual per liter. The biovolume of *N. spumigena* was calculated following the formula of Hillebrand et al. (1999) and converted to biomass assuming a specific density of phytoplankton cells of 1 g cm^{-3}.

The following parameters were considered for identifying and describing *N. spumigena*: the shape and size of trichomes, length (l) and width (w) of vegetative cells and heterocytes, the corresponding $l : w$ ratio, presence and absence of gas vesicles. A minimum number of 20 individual trichomes, cells and heterocysts were measured using a stage micrometer to calculate the range and average sizes.

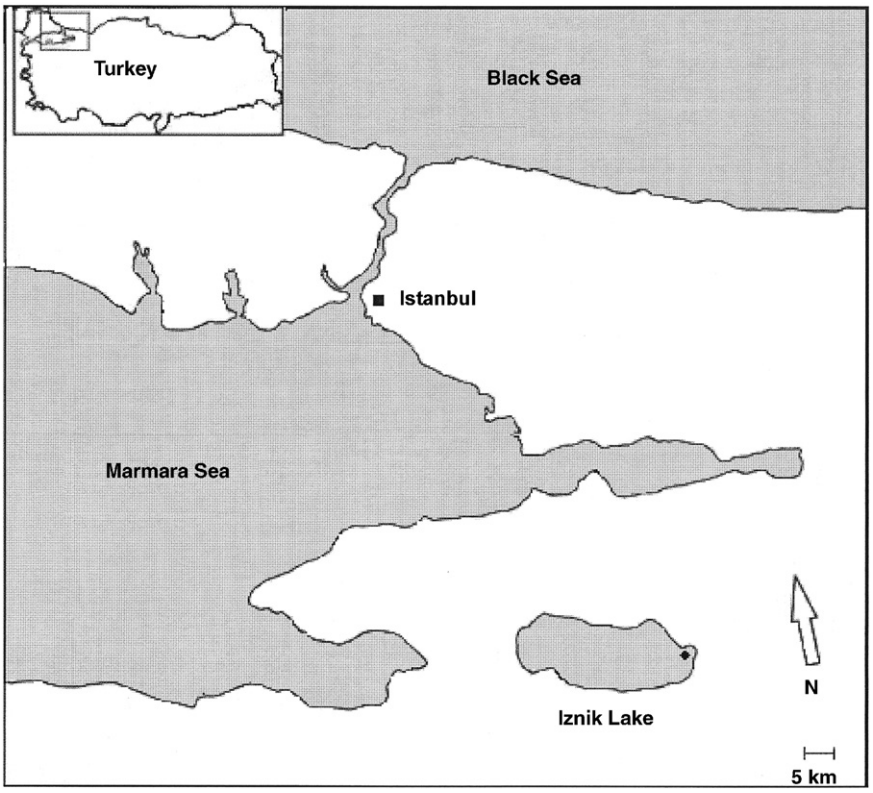

Fig. 1. Map of Iznik lake. Sampling station was shown by a diamond (◆).