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Molecular detection of hepatotoxic cyanobacteria in inland water bodies of the Marmara Region, Turkey

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ABSTRACT

Blooms of cyanobacteria are an increasingly frequent phenomenon in freshwater ecosystems worldwide as a result of eutrophication. Many species can produce hepatotoxins that cause severe health hazards to humans. The aim of this study was to identify the bloom forming cyanobacteria species by molecular methods and to amplify genes responsible for hepatotoxin biosynthesis from the environmental samples and isolated strains of cyanobacteria from Küçükçekmece Lagoon, Sapanca, İznik, Manyas and Taşkısı Lakes. A total of 10 bloom samples and 11 isolated strains were examined and *Microcystis* spp., *Planktothrix* spp., *Nodularia spumigena, Anabaenopsis elenkinii, Sphaerospermopsis aphanizomenoides*, *Cylindrospermopsis raciborskii* were identified. Hepatotoxin genes were detected in 60% of the bloom samples and 45% of the strains. Two *Microcystis* strains were obtained from Küçükçekmece Lagoon. While the strain assigned to *Microcystis flos-aquae* was non-toxic, *Microcystis aeruginosa* strain produced microcystin. According to PCR results, the *M. aeruginosa* and *Planktothrix agardhii* bloom samples of Küçükçekmece Lagoon contained the microcystin synthetase gene E (*mcy*E) indicative of microcystin production, however, no microcystin was detected by HPLC. The *mcy*E gene was also found in *Microcystis wesenbergii* isolated from Taşkısı Lake, and in all *Planktothrix rubescens* bloom samples from Sapanca Lake. To our knowledge, this is the first detailed study for identifiying different toxic cyanobacteria species and their hepatotoxin production from several waterbodies in Turkey using molecular methods.

Key words: 16S rRNA, Aminotransferase, Nodularin, Microcystin, Cyanobacteria, Cyanotoxin.

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INTRODUCTION

Blooms of cyanobacteria (blue-green algae/ cyanoprokaryotes) have increased globally in recent decades (Paerl and Otten, 2013; Harke *et al*., 2016). Due to the ability of toxin production, some species affect livestocks and high cyanotoxin concentrations were linked to animal deaths and human health hazard through drinking and recreational waters (Codd *et al.*, 1999; Carmichael *et al*., 2001; Azevedo *et al*., 2002; Backer *et al*., 2015). Cyanobacteria can produce different types of toxic compounds, which include hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (Bláha, 2009; Westrick *et al.*, 2010). The occurence of cyanotoxins have been reported in several cyanobacterial genera such as *Microcystis*, *Nodularia*, *Aphanizomenon, Planktothrix, Anabaena* and *Cylindrospermopsis* (Sivonen *et al*., 1990; Merel *et. al*., 2013; Bernard *et al.,* 2017).

The most studied group of cyanobacterial toxins are the hepatotoxic cyclic peptides, which include the microcystins and nodularins. Although they are similar in structure, nodularin has been isolated from only one species of cyanobacteria, *Nodularia spumigena* Mertens ex Bornet & Flahault, whereas microcystin can be produced by multiple cyanobacterial genera, most notably by *Microcystis*, *Planktothrix* or *Anabaena* (Sivonen and Jones, 1999; Bernard *et al., 2017*). Over 100 microcystin variants and 10 nodularin variants have been identified (Spoof *et al.*, 2001; Bortoli and Volmer, 2014).

Cyanobacterial blooms occur in Turkish inland waters, mostly lakes and reservoirs used as supplies of drinking water or recreation. *Aphanizomenon* sp. was the first cyanobacteria to cause problems in filter system of drinking water treatment plant in Kurtbogazi Dam Lake (Ankara) in 1981 (Guler Aykulu, pers. comm.). During the 1990s many cyanobacterial blooms were detected in the Marmara region. In 1994, blooms of *Anabaena* spp. resulted in fish mortality in İznik Lake (Albay *et al*., 2003a). Cyanotoxin research has started at the end of 1990s and increased in recent years (Albay *et al.*, 2003a,b; Albay *et al.*, 2005; Akçaalan *et al.*, 2006, 2014a, 2014b, 2016)

It is well known that microscopic identification of cyanobacteria is time consuming and it requires taxonomic expertise. Due to this limitation, molecular tools have been increasingly applied also to environmental studies (Kurmayer and Christiansen, 2009; Bukowska *et al*., 2014). Especially, because of the conserved nature of the 16S rRNA gene, it is used to discriminate strains at the species level (Neilan *et al*., 1997; Moffitt and Neilan,

2001). Jungblut and Neilan (2006) developed a molecular method to detect both microcystin and nodularin-producing species by amplifying and sequencing of the aminotransferase (AMT) domain of *mcy*E and *nda*F genes in the *mcy* and *nda* operons. The reason for choosing AMT domain was its important role in synthesis of all microcystins and nodularins.

Due to the increased frequency of algal blooms in Turkish lakes, it is important to understand the distribution of toxin-producing cyanobacteria in this area. The aims of the present study were to determine the bloom-forming cyanobacteria species using the 16S rRNA gene as well as the potential toxicity using the *mcy*E and *nda*F genes indicative of microcystin/nodularin biosynthesis, occurring in lakes around the Marmara region (Küçükçekmece, Sapanca, İznik, Manyas and Taşkısı).

METHODS

Sampling sites

Cyanobacterial blooms have been collected from five lakes in Marmara region (Fig. 1). İznik Lake, located in the southeast of Marmara region, is the fifth biggest lake in Turkey. Cyanobacterial blooms occured because of heavy nutrient loading (Albay *et al.,* 2003a; Akçaalan *et al.,* 2006; Tas and Gonulol, 2007). The first bloom was formed by *Anabaena* sp. in 1994. *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek and *Nodularia spumigena* were also detected (Akçaalan *et al*., 2006; Akçaalan *et al*., 2009). Sapanca Lake is an oligo-mesotrophic lake and *Planktothrix rubescens* blooms have been observed in the metalimnion of the lake since the 1980s (Akçaalan *et al*., 2006). The other studied area, Küçükçekmece Lagoon (Istanbul, Turkey), has a connection to the Marmara Sea via a narrow channel. The lagoon is in hypereutrophic conditions and *Microcystis aeruginosa* (Kützing) Kützing blooms were observed from late spring to mid-autumn (Albay *et al*., 2005)

Manyas Lake is a eutrophic lake which is an important bird sanctuary, and in 1998 it was listed in the Ramsar Convention (Çelik and Ongun, 2006).

Taşkısı Lake is a small, shallow lake situated in the eastern part of the Marmara region (Aykulu *et al*., 1999) (Tab. 1).

Cyanobacteria identification

Freshly collected bloom samples were identified by inverted microscopy (Axio Observer Z1, Carl Zeiss GmbH, Jena, Germany). 1-2 drops of fresh sample were

Fig. 1. Location of sampling lakes in Marmara Region.

investigated according to taxonomical keys using filament/colony traits, presence and structure of mucilage, cell shape and size, whether having a specialized cell or not. Cyanobacterial identification was done according to Whitton and Potts (2007), Komárek (2013), Komárek and Anagnostidis (1986; 1999; 2005) and Anagnostidis and Komárek (1988).

Environmental samples

During 2004-2009, ten bloom samples were collected from five lakes of Marmara region (Tab. 2). For cyanotoxin and molecular analysis, samples were collected using plankton net (20 µm mesh size, Hydro-Bios) and lyophilised and conserved at -20°C.

Cyanobacterial strains

Cyanobacterial strains used in the present study (Tab. 3) were collected from blooms. Single filaments and colonies of cyanobacteria were isolated by repeated washing with sterile media from a Pasteur pipette and transferred 96-well plates filled with 200 µL BG 11 medium with or without nitrate according to presence or absence of heterocytes (Rippka *et al.*, 1979).

DNA extraction

DNA extraction from fresh cell pellets and lyophilized bloom samples was performed using XS extraction buffer containing 1% potassium-methylxanthogenate (800 mM ammonium acetate; 20 mM EDTA; 1% SDS; 100 mM Tris-HCI, pH 7.4) (Tillett and Neilan, 2000). DNA was dissolved in Tris-EDTA buffer (10:1). Concentrations of DNA were determined using a Nanodrop® ND-1000 spectrophotometer and DNA extracts were stored at -20°C.

PCR amplification and sequencing

All PCR reactions were performed in 20 µL reaction volume containing PCR buffer (Bioline, London, UK), 2.5 mM $MgCI_2$, 0.2 mM dNTPs (Bioline), 10 pmol each of the forward and reverse primers and 0.2 U Taq polymerase (Bioline). The PCR amplification products were visualized using gel electrophoresis on 2% agarose, and staining with 0.5 µg mL^{-1} ethidium bromide for 10 min and documented with a Gel Doc XR camera using quantity one 4.6.1 software (Bio-Rad, Hercules, CA, USA).

16S rDNA amplification was performed using primers 27F and 809R (Jungblut *et al*., 2005) with an initial denaturation step at 92 °C for 2 min followed by 35 cycles of 94°C for 10 s, 60°C for 20 s and 72°C for 1 min and a final extension step at 72°C for 5 min (Jungblut *et al.,* 2005). *M. aeruginosa* PCC7806 was used as positive control.

Hepatotoxin (HEP) PCR reactions were performed using primers HEPF and HEPR targeting *mcy*E/*nda*F gene (Jungblut and Neilan, 2006). An initial denaturation step at 92°C for 2 min was followed by 35 cycles of 92°C for 20 s, 52°C for 30 s, and 72°C for 1 min, with a final extension step at 72°C for 5 min.

The PCR products were sent to Ramaciotti Centre for Genomics (University of New South Wales, Sydney Australia) and sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Using a PANDAseq (ver. 2.4) nucleotide sequence were reconstructed (Masella *et al,* 2012). Overlapping regions were

Tab. 1. Features of the studied lakes.

Waterbody	Surface area Max. depth	Common use	Dominant cyanobacteria
İznik Lake	300 km^2 65 m	Recreation irrigation	*Nodularia spumigena* *Planktothrix rubescens* *Cylindrospermopsis raciborskii* *Dolichospermum* sp. *Anabaenopsis* sp.
Sapanca Lake	46.8 km^2 55 m	Drinking water Recreation	*Planktothrix rubescens*
Küçükçekmece Lagoon	15.22 km^2 20 m	Recreation	*Microcystis aeruginosa* *Planktothrix agardhii* *Microcystis wesenbergii*
Manyas Lake	159 km^2 3.4 m	Fisheries activities Recreation Irrigation	*Microcystis aeruginosa* *Microcystis wesenbergii* *Sphaerospermopsis* sp. *Dolichospermum flos-aquae* *Cuspidothrix issatschenkoi*
Taşkısı Lake	0.75 km^2 4.5 m	Fisheries activities	*Microcystis* spp. *Dolichospermum* sp.

aligned and scored. Sequences were identified using the BLASTn search program (NCBI).

Hepatotoxin analysis

Microcystin/Nodularin production of environmental blooms and isolated strains were measured by high performance liquid chromatography (HPLC) with photodiode array (PDA) detector (Perkin Elmer, USA) according to Lawton (1994). Lyophilized samples (10-50 mg) were extracted in 70% (v/v) aqueous methanol with ultrasonication and centrifuged at 14,000 x g for 5 min. Clear supernatants were injected into the HPLC column (Waters Symmetry C18, 3.9 × 150 mm, 5 µm particle size). Elution mode was used: injection volume 25 µL, flow rate 1 mL min^{-1} and column temperature 40°C. Mobile phases were Milli-Q water and acetonitrile both containing 0.1% (v/v) TFA. Eluent absorbance was monitored from 200 to 300 nm and microcystins were detected at 238 nm. The limit of detection was 0.4 ng per injection corresponding to 0.001 µg mg^{-1} dw.

RESULTS

Cyanobacteria species

Species identification was done by microscopy. Since all blooms were mainly dominated by a single species,16S rDNA results are very well correlated with microscopical examination. Cyanobacteria that belong to three orders, Chroococcales, Nostocales and Oscillatoriales, were detected. A total of nine species, *Anabaenopsis elenkinii* V.V. Miller, *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, *Sphaerospermopsis aphanizomenoides* (previously denominated *Aphanizomenon aphanizomenoides* Forti), *N. spumigena*, *M. aeruginosa, Microcystis flos-aquae* (Wittrock) Kirchner*, Microcystis wesenbergii* (Komárek) Komárek ex Komárek, *P. rubescens*, and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek were identified. The 16S rDNA gene sequences obtained from both strains and environmental samples were assigned using BLASTn search of the Na-

Tab. 2. HPLC and HEP PCR results for environmental bloom samples.

Code	Dominant species*	Place of collection	Date of collection	HPLC results (µg mg^{-1} d.w)	HEP PCR results	GenBank accession numbers
E1	*Planktothrix agardhii*	Küçükçekmece Lagoon	27/10/2004	ND	-	KY091680
E2	*Planktothrix agardhii*	Küçükçekmece Lagoon	03/11/2004	ND	+	KY091681
E3	*Planktothrix agardhii*	Küçükçekmece Lagoon	11/11/2004	ND	-	KY091682
E4	*Microcystis aeruginosa*	Küçükçekmece Lagoon	04/10/2006	2.9	+	KY091683
E5	*Planktothrix rubescens*	Sapanca Lake	06/02/2007	6.0	+	KY091684
E6	*Planktothrix rubescens*	Sapanca Lake	21/02/2007	4.7	+	KY091685
E7	*Microcystis aeruginosa*	Küçükçekmece Lagoon	28/09/2007	ND	+	KY091686
E8	*Planktothrix rubescens*	Sapanca Lake	23/01/2008	0.3	+	KY091687
E9	*Anabaenopsis elenkinii*	İznik Lake	16/05/2008	ND	-	KY091688
E10	*Planktothrix rubescens*	Sapanca Lake	28/01/2009	1.1	+	KY091689

*Species: according to microscopic identification; ND, not detected.

Tab. 3. HPLC and HEP PCR results for cyanobacterial cultures.

Code	Cyanobacterial species*	Origin	Strain	HPLC results (µg mg^{-1} d.w)	HEP PCR results	GenBank accession numbers
S1	*Microcystis aeruginosa*	Küçükçekmece Lagoon	IFCC-MA03	6.8	+	KY077257
S2	*Microcystis flos-aquae*	Küçükçekmece Lagoon	IFCC-MF01	ND	-	KY077258
S3	*Microcystis wesenbergii*	Taşkısı Lake	IFCC-MW01	2.4	+	KY077259
S4	*Anabaenopsis elenkinii*	İznik Lake	IFCC-AE01	ND	-	KY077260
S5	*Sphaerospermopsis aphanizomenoides*	İznik Lake	IFCC-AA05	ND	-	KY077261
S6	*Sphaerospermopsis aphanizomenoides*	İznik Lake	IFCC-AA01	ND	-	KY077262
S7	*Cylindrospermopsis raciborskii*	Manyas Lake	IFCC-CR01	ND	-	KY077263
S8	*Nodularia spumigena*	İznik Lake	IFCC-NS01	3.2	+	KY077264
S9	*Nodularia spumigena*	İznik Lake	IFCC-NS03	3.0	+	KY077265
S10	*Planktothrix agardhii*	Küçükçekmece Lagoon	IFCC-PA01	ND	-	KY077266
S11	*Planktothrix rubescens*	Sapanca Lake	IFCC-PR04	4.3	+	KY077267

*Species: according to microscopic identification; ND, not detected.

tional Biotechnology Information (NCBI) database (http://ncbi.nlm.nih.gov/blast/) (Tabs. 2 and 3). The BLAST search showed 98-100% similarities.

Detection of hepatotoxin genes

The HEP PCR reaction resulted in amplification of a fragment in the expected size from two of three *Microcystis* sp. strains, *P. rubescens* and two *N. spumigena* strains. No PCR product was obtained from strains assigned to *P. agardhii*, *C. raciborskii*, *A. elenkinii* and *S. aphanizomenoides* (Tab. 3). The HEP fragment was successfully amplified from five of seven *Planktothrix* sp., one of two *M. aeruginosa* dominated environmental bloom samples.

In culture samples, *M. aeruginosa* (S1) and *M. flos-aquae* (S2) strains were isolated from same bloom recorded in Küçükçekmece Lagoon. While *M. aeruginosa* strain showed a HEP-PCR product, *M. flos-aquae* was found negative (Tab. 3). The other *Microcystis* morphospecies, *M. wesenbergii* gave a positive result and showed HEP-PCR product. The Nostocalen species; *S. aphanizomenoides* and *A. elenkinii* did not give positive result as well as *C. raciborskii* strain.

In environmental samples, the HEP PCR reactions resulted in amplification of a 472-bp fragments for eight of ten samples. The *mcy*E products were obtained from one of three *P. agardhii* bloom sample (E2), while no PCR products were obtained from *P. agardhii* (E1-E3) bloom samples.

PCR-amplification of the AMT domain was successfully attained from all *P. rubescens* samples.

To verify that the resulting amplicons, all PCR–amplified products from various lakes were sequenced. BLAST searches were used to identify similar sequences from GenBank.

Detection of hepatotoxins

Cyanobacterial hepatotoxins were detected by HPLC-PDA. Total microcystin concentrations varied from 0.3 to 6.8 microcystin-LR equivalents μg mg^{-1} d.w. (Tabs. 2 and 3).

Nodularin concentrations in IFCC-NS01 (S8) and IFCC-NS03 (S9) were 3.2 and 3.0 μg mg^{-1}, respectively. The highest amount of microcystin (6.8 μg mg^{-1} d.w.) was found in *M. aeruginosa* (S1) strain. Microcystin content of *M. wesenbergii* (S3) was found to be 2.4 μg mg^{-1}. HPLC analyses confirmed no microcystin presence in *M. flos-aquae* (S2), *A. elenkinii* (S4), *S. aphanizomenoides* (S5, S6), *C. raciborskii* (S7) and *P. agardhii* (S10) strains.

In environmental samples, microcystins were not detected in *P. agardhii* (E1, E3) and *A. elenkinii* (E9) bloom samples. While *mcy*E products were obtained from *P. agardhii* (E2) and *M. aeruginosa* (E7), microcystin was not detected by HPLC. Microcystin content of *P. rubescens* samples varied between 0.3-6 μg mg^{-1}.

DISCUSSION

Cyanobacteria species were shown to be the main component of phytoplankton community in lakes and reservoirs. Earlier records on the algal flora of Turkish waterbodies reported taxonomic lists, which were based on the microscopical monitoring and showed a diverse cyanobacteria community (Aykulu and Obalı, 1981; Fakıoğlu *et al*., 2011). However, polyphasic approaches in classification of organisms are essential, since morphological characters are often unstable and incongruent with molecular tools. For example, the genus *Microcystis* has several morphospecies sharing rather similar characteristics and discussions on the taxonomic assignment of these morphotypes is ongoing (Bittencourt-Oliveira, 2003). Within the genus *Microcystis*, typically two morphospecies (*M. aeruginosa* and *M. flos-aquae*) are found in the same population. According to the results of molecular methods used in this study, the *mcy*E gene occurred in *M. aeruginosa* (S1) strain, but not in *M. flos-aquae* isolated from the same bloom. Tillett *et al*. (2001) also did not find *mcy*A gene occurrence among *M. flos-aquae* strains. However, *mcy*A and B genes were detected in half of the colonies assigned to *M. flos-aquae* (total number was 8) isolated from lakes in Europe. Correspondingly, *M. aeruginosa* (n=149) had a higher proportion of colonies containing the *mcy*A/B gene (Via-Ordorika *et al*., 2004). In this study, the third strain of *Microcystis* isolated from Taşkısı Lake was assigned to *M. wesenbergii* (S3) and not only it contained the *mcy*E gene but also produced microcystin (2.4 μg mg^{-1} d.w.). According to the study of Via-Ordorika *et al*. (2004) this morphospecies was found non-toxic in all colonies (n=21) from European lakes. Maršálek *et al.* (2001) showed that in Czech Republic *M. wesenbergii* contains little or no microcystin, similarly no microcystin was detected in colonies isolated from a Czech reservoir (Welker *et al*., 2007). Also, molecular and chemical analysis did not show microcystin production in 250 individual colonies and 21 strains of *M. wesenbergii* isolated from Chinese lakes (Xu *et al.*, 2008). However, Otsuka *et al* (1999) found that *M. wesenbergii* has toxic and nontoxic strains. Yosuno *et al.* (1998) also found that all *M. wesenbergii* (n=8) strains examined contained microcystin. Likewise, in Lake Kastoria (Greece), *M. wesenbergii* dominant bloom containing toxin producing genes such as *mcy*A and *mcy*B was reported (Gkelis *et al.,* 2014). Pavlova *et al.* (2014; 2015) found toxic bloom dominated by *M. wesenbergii* in Lake Dourankoulak, and highlighted that toxicity may vary between clones of the same strain. Because of these contradictory results, it is necessary to analyse higher number of *Microcystis* mor-

phospecies to determine the relationship between toxigenicity and morphological characters.

It is known that *P. agardhii* and *P. rubescens* have specific ecological niches. While *P. rubescens* occurs in oligo- to mesotrophic physically stratified lakes (Akçaalan *et al*., 2014a), *P. agardhii* become dominant in shallow, eutrophic and polymictic water bodies (Kurmayer *et al*., 2004). In this study, *P. rubescens* was isolated from Sapanca Lake, which is a moderately deep, oligo-mesotrophic lake. In contrast, *P. agardhii* formed a bloom in a hypereutrophic lake in late autumn and polymictic conditions. Similar to *Microcystis* both toxic and nontoxic strains can be found in the same population of *P. agardhii* and *P. rubescens* (Kurmayer *et al*., 2004; Akçaalan *et al*., 2006). In general, the share of strains containing the *mcy*A/B gene is highest in *P. rubescens* populations in contrast to *P. agardhii*. Accordingly, our results showed that *P. rubescens* has active microcystin genes, while the strain isolated from *P. agardhii* bloom was found nontoxic.

The strain of *A. elenkinii* was isolated from a bloom sample of İznik Lake which was dominated by this species. Both the bloom sample and isolated strain were found negative for the *mcy* genes as well as no microcystin was detected by HPLC. This species generally cooccurs with other Nostocalen cyanobacteria and toxicity is attained to all of them (Maršálek *et al*., 2000; Papadimitriou *et al*., 2013). However, there is no record of microcystin production of a isolated strain of *A. elenkinii*.

C. raciborskii has been shown to produce hepatotoxic cylindrospermopsin and neurotoxic saxitoxins (Wood and Stirling, 2003; Molica *et al*., 2005). This species originates from tropical regions and currently expands its distribution in temperate regions, therefore it may be considered an invasive species in European waterbodies (Padisák, 1997; Moreira *et al.,* 2015). In this study *C. raciborskii* was isolated from shallow hypereutrophic Manyas Lake but did not contain the *mcy*E gene. Also, no cylindrospermopsin was detected according to molecular and analytical analysis (*data not shown*).

There are some contradictory results between molecular and analytical methods. *M. aeruginosa* (E5) and *P. agardhii* (E2) contained the *mcy*E gene, but did not produce microcystin as revealed by HPLC. Studies showed that cyanobacteria strains with *mcy* genes lacked detectable microcystins as a result of inactivation of the genes (Neilan *et al.,* 1999; Nishizawa *et al.,*1999; Kaebernick *et al*., 2001; Tillett *et al.,* 2001; Mikalsen *et al.,* 2003).

Samples used in this study were collected from waterbodies with different morphological and physicochemical characteristics. Some cyanobacteria species have been found in both shallow and moderately deep lakes, some others prefer deep waterbodies. However, the distribution of species is governed mainly by trophic situation of the lakes. *Microcystis* species together with *P. agardhii* formed blooms in eutrophic environment, such as Manyas, Küçükçekmece and Taşkısı Lake. Nostocalen Cyanobacteria species, on the other hand, prefer alkaline, meso-eutrophic waters of İznik Lake (Akçaalan *et al.*, 2009, 2014b). Especially *Nodularia spumigena* is an euryhaline species living in hyposaline to brackish waters in Turkey (Kocasari *et al.,* 2015; Kızılkaya *et al.,* 2016). Similarly, *A. elenkinii* is also known as a hyposaline species (Kemp, 2009; Kotut and Krienitz, 2011). The growth of these species might have been supported by high conductivity of the lake water. On the other hand, in typical freshwater Sapanca Lake, which is used for drinking water and has low nutrient concentration, toxic *P. rubescens* form massive blooms. The most important factors are the high water transparency, thermal stratification, a long water residence time and low nutrient availability, which have negative effect on other phytoplankton species in the lake (Legnani *et al.,* 2005; Akçaalan *et al.,* 2014a)

CONCLUSIONS

In conclusion, applications of molecular and DNA amplification methods provide a great advantage for monitoring toxic cyanobacterial blooms in the aquatic environments. It has a potential to identify the organisms and to detect their cyanotoxin production. This study, using different methods collaboratively, shows that toxic cyanobacteria blooms are very common in Turkish inland waterbodies with different trophic levels. To our knowledge, this is the first detailed study identifying different toxic cyanobacteria species and their hepatotoxin production in Turkey using molecular methods.

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