İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2022; 37(3): 129-139 • DOI: https://doi.org/10.26650/ASE20221088270

Research Article

Cyanobacterial Diversity and the Presence of Microcystins in the Küçük Menderes River Basin, Turkiye

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Cite this article as: Koker, L., Oguz, A., Akcaalan R., & Albay, M. Cyanobacterial diversity and the presence of microcystins in the Küçük Menderes River basin, Turkiye. *Aquatic Sciences and Engineering, 37*(2), 129-139. DOI: https://doi.org/10.26650/ASE20221088270

ABSTRACT

Although cyanobacteria are commonly associated with eutrophic lakes, they are the basic components of phytoplankton communities in lakes that have different trophic statuses. In inland waters, both nutrient loading from watersheds and warmer conditions promote phytoplankton growth and cause extensive cyanobacterial blooms. Certain bloom-forming cyanobacterial species can pose a health risk to humans and aquatic ecosystems through cyanotoxin production. The aim of this study was to evaluate the cyanobacterial composition and toxins in five reservoirs and two natural lakes in the Küçük Menderes River Basin, all with varying trophic statuses. Within this scope, samples were collected in autumn 2017 and spring 2018. Cyanobacterial species were enumerated according to the Utermöhl method. Cyanotoxin samples were analyzed using HPLC. To find the trophic status of the water bodies, the Trophic State Index (TSI) developed by Carlson (1977) was used and Total Phosphorus (TP), Secchi Depth (SD), and Chlorophyll-*a* (chl-*a*) measurements were performed. Cyanobacterial abundance, species composition, and cyanotoxin production differed significantly between the lakes and reservoirs. A total of 13 cyanobacteria species were identified including potential cyanotoxin producers such as *Microcystis*, *Aphanizomenon*, and *Dolichospermum*. According to the TSI, three reservoirs were mesotrophic and the other four waterbodies had eutrophic-hypereutrophic conditions. *Microcystis* is the most common bloom-forming freshwater cyanobacteria in the Küçük Menderes River Basin. However, microcystin concentrations were relatively low and the highest microcystin concentration was detected in the Tahtalı Reservoir at 9 µg/L. The Küçük Menderes River Basin is under water-stressed conditions and the cyanobacteria blooms in the region might pose another threat for wildlife and humans.

Keywords: Cyanobacteria, Cyanotoxin, Reservoir, Lake, Algal Blooms, Eutrophication

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Submitted: 19.03.2022

Revision Requested: 05.04.2022

Last Revision Received: 07.04.2022

Accepted: 10.04.2022

Online Published: 11.05.2022

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INTRODUCTION

Cyanobacteria commonly occur in many regions throughout the world from the polar regions to the tropics (Giani et al. 2020). In addition to favorable environmental conditions such as excessive nitrate/phosphate loading and temperature, urbanization, agriculture and inappropriate water policies also contribute to the occurrence of cyanobacteria. In USA and China, heavy rainfalls and floods, led to the distribution of the hepatotoxin-producer *Microcystis* into several estuaries (Preece et al. 2017).

Cyanobacteria-contaminated water bodies used for recreational activities, irrigation, or drinking water resources may cause health problems for both animals and humans since some of them produce toxic secondary metabolites including hepatotoxins, neurotoxins, and dermatotoxins (Janssen 2019). Among these toxin groups, hepatotoxic microcystin is the most commonly reported and studied toxin globally (Svirčev et al. 2019). Similar to other countries, Turkiye faces the major challenge of maintaining the quality of the available water resources. As a result of changing environmental conditions, the increasing presence of harmful algal blooms is an emerging issue (Akcaalan et al. 2009; Albay et al. 2003; Mariani et al. 2015; Wan et al. 2020). Several species of *Microcystis, Anabaena, Dolichospermum, Cylindrospermopsis*, *Aphanizomenon,* and *Nodularia* are known to dominate blooms in Turkiye (Albay et al. 2005, 2009; Koker et al. 2021; Oğuz et al. 2020). As regards the health risks associated with these groups, the guidelines for cyanobacterial toxins were prepared for drinking and recreational waters (Anonim 2019a; 2019b).

The Küçük Menderes River Basin is located in the western part of Turkiye and covers an area of about 3.490 km^2. It has the lowest total precipitation area in Turkiye (Ministry of Agriculture and Forestry 2019). There are three natural lakes (Belevi, Çatal, and Gebekirse) in the basin. Additionally, a number of reservoirs were built for drinking water supply and irrigation to support the increase in the population of the metropolitan city, İzmir (Sac et al., 2020).

This study aims to assess the levels of eutrophication in seven freshwater bodies, to identify potentially toxic cyanobacterial genera, and to evaluate the occurrence of microcystins in the Küçük Menderes River Basin.

MATERIAL AND METHODS

Study sites

Surface water samples were collected from five reservoirs and two natural lakes in the Küçük Menderes River Basin in November 2017 and May 2018 (Fig. 1). Sampling stations were chosen to represent water bodies relative to the size of the surface area. Two sampling stations were selected for water bodies with a surface area up to 3 km^2 and three sampling stations for those larger than 3 km^2 (Tahtalı and Beydağ reservoirs). Some brief information about the studied water bodies is given in Table 1.

There are nine dams in operation in the Küçük Menderes River Basin and the largest of them is the Tahtalı Reservoir (İzmir). This reservoir supplies approximately 40% of İzmir's drinking water (İspirli, 2009). The Alaçatı Reservoir, which is located on Hırsız Stream, is a drinking water supply with a volume of 16.61 hm^3 (Tosun, 2018). Although the Beydağ Reservoir was built for irrigation purposes, with the increase in urbanization in the region, it is also used as a domestic water source with a water consumption capacity of 108.9 hm^3 (Sac et al., 2021). Also, there are several dams and ponds such as the Kavakdere and Seferihisar Reservoirs on the Küçük Menderes river that were built for general irrigation and flood control purposes .

Çatal Lake and Gebekirse Lake, which are the closest lakes to the Aegean Sea, are brackish waters. Gebekirse Lake is under protection due to its ornithological and vegetation values (Somay et al., 2008). Çatal Lake is a small shallow lake, situated to the west of Gebekirse Lake.

Physical and chemical analysis

Water temperature (WT), pH, Dissolved Oxygen (DO), and Electrical Conductivity (EC) were measured *on-site* with a portable multiparameter (6600, YSI, USA). Water transparency was measured using Secchi Disc (SD). Nitrite+Nitrate (NO_2-N + NO_3-N) and Total Phosphorus (TP) were measured spectrophotometrically following APHA-AWWA WPCF (1989). Chlorophyll-*a* (chl-*a*) was performed using the ethanol extraction method (ISO 10260, 1992).

To determine the trophic state of the water bodies, the trophic state index (TSI) developed by Carlson (1977) was used and total phosphorus (TP), chlorophyll-*a* (chl-*a*), and Secchi depth (SD) measurements were used to calculate the index. The $TSI_{(SD)}$

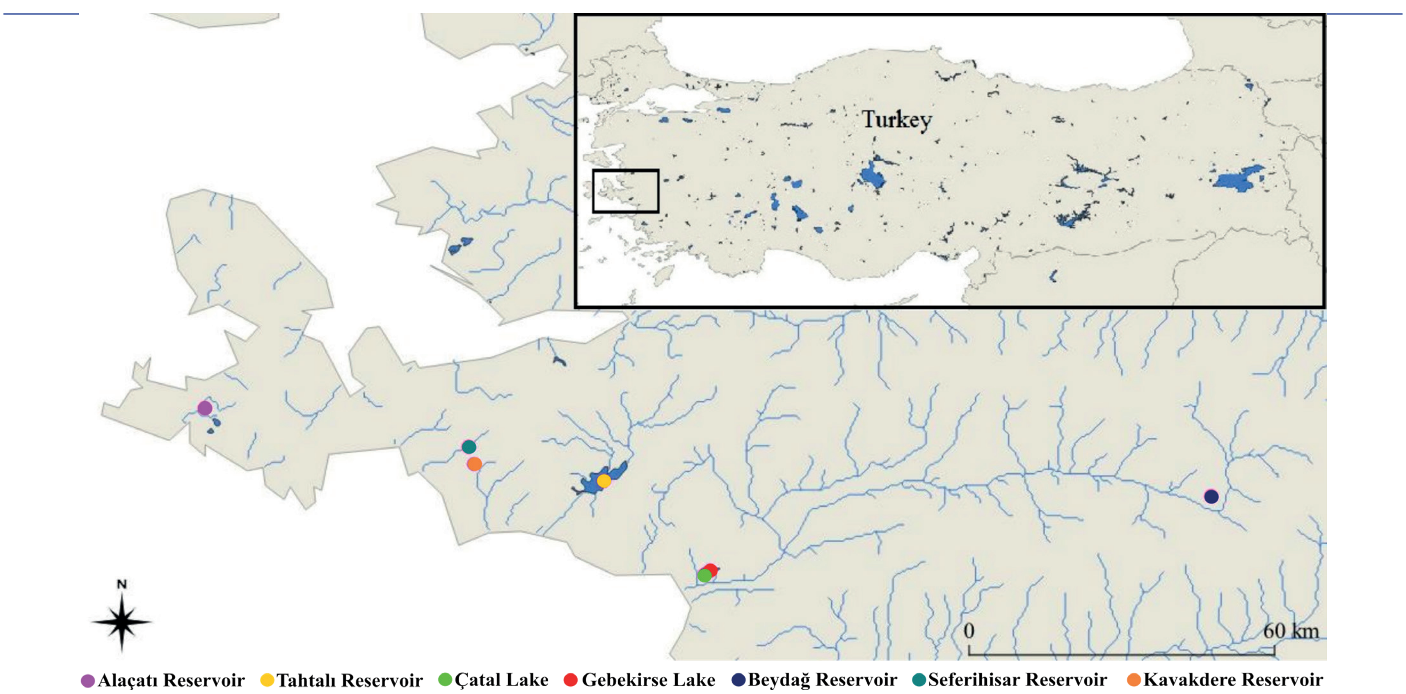

Figure 1. Sampling Sites in Küçük Menderes River Basin.

Table 1. Location and characteristics of the studied reservoirs and lakes in the Küçük Menderes River Basin.

	Coordinates	Surface Area (km²)	Maximum depth (m)	Purpose of use
Alaçatı Reservoir	38°17′20.12″N 26°24′47.29″E	2.6	4.5	Drinking supply
Beydağ Reservoir	38°7′ 2.74″ N 28° 13′ 10.4″E	12	22	Irrigation
Tahtalı Reservoir	38° 8′ 33.91″N 27° 6′ 42.81″E	23	55	Drinking supply
Seferihisar Reservoir	38°13′0.62″N 26°52′16.06″E	1.8	24	Irrigation
Kavakdere Reservoir	38°10′50.47″N 26°54′19.98″E	0.96	8	Irrigation
Çatal Lake	37° 59′ 25.38″N 27° 19′ 1.57″ E	0.74	4.5	Recreational
Gebekirse Lake	37° 59′ 12.96″N 27° 18′ 16.31″E	0.75	5	Recreational

values of the Alaçatı Reservoir, and Çatal Lake and Gebekirse Lake were not used to calculate the trophic status since they were shallow (~3-5m depth) and mixing throughout the year (Zou et al., 2020).

Microscopy analysis

Samples for the determination of phytoplankton composition were fixed by Lugol's Iodine solution. Phytoplankton was enumerated using a Zeiss Axiovert (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) inverted microscope according to Utermöhl (1958). Identifications of the sampled taxa were based on relevant literature (John et al. 2002; John 2005; Komarek and Anagnostidis 1989; 2007; 2008; Krammer & Lange-Bertalot, 1986). The phytoplankton biovolume was calculated according to the geometric equations of Hillebrand et al. (1999).

Microcystin analysis

The microcystin concentrations were measured using high-performance liquid chromatography (HPLC) with a photodiode array (PDA) detector (Perkin Elmer, USA) according to Lawton (1994). Elution mode was used: injection volume 25 µL, flow rate 1 mL min^{-1}, and column temperature 40°C. The mobile phases were Milli-Q water and acetonitrile both containing 0.1% (v/v) TFA. All the reagents were of high-performance liquid chromatographical (HPLC) grade. The eluent absorbance was monitored from 200 to 300 nm and microcystins were detected at 238 nm.

RESULTS & DISCUSSION

The trophic status of a waterbody is generally a good indicator for the possibility of cyanobacteria blooms. The trophic statuses of the water bodies are given in Table 2. The Tahtalı, Seferihisar, and Kavakdere reservoirs were classified as mesotrophic. Aksu et al. (2015) reported that the Tahtalı Reservoir was found oligotrophic according to $TSI_{(chl-a)}$ between 2006 and 2007. The calculated $TSI_{(chl-a)}$ and $TSI_{(TP)}$ values measured in the waterbodies indicated both nutrient- and phytoplankton-rich conditions (Fernandez et al., 2021). Based on $TSI_{(chl-a)}$, only the Seferihisar Reservoir showed no sign of eutrophication.

In the Küçük Menderes River Basin, three of the seven lakes and reservoirs were classified as eutrophic, three of the reservoirs were mesotrophic and Çatal Lake was hypereutrophic (Figure 2).

Figure 2. Cyanobacterial blooms in the reservoirs.

Table 2. Trophic Status of studied water bodies in the Küçük Menderes River Basin.

	Alaçatı Reservoir	Beydağ Reservoir	Tahtalı Reservoir	Seferihisar Reservoir	Kavakdere Reservoir	Çatal Lake	Gebekirse Lake
$TSI_{(TP)}$	65.2	59.8	56.8	45,8	41.4	79.9	54.3
$TSI_{(chl-a)}$	49.5	63.5	41.8	39.2	43.5	69.0	57.8
$TSI_{(SD)}$	-	53.9	43.7	40.4	51.8	-	-
TSI	57.4	59.1	47.4	41.8	45.6	74.5	56.1

Oligotrophy Mesotrophy Eutrophy Hypereutrophy

Table 3. Environmental variables and chl-*a* values of studied water bodies.

		Alaçatı Reservoir		Beydağ Reservoir		Tahtalı Reservoir		Seferihisar Reservoir		Kavakdere Reservoir		Çatal Lake		Gebekirse Lake	
		Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018
WT	**°C**	15.5	24.6	15.3	24.2	16.5	24.2	15.9	24.3	15.2	18.5	15.8	26.8	16.7	25.3
TP	**µg/L**	31.7	69.0	30.4	59.6	20.2	38.9	14.9	22.6	14.2	25.6	103.9	268.6	22.6	32.4
TN	**µg/L**	1037	787	1620	934	882	699	752	282	675	284	8475	3284	430	1110
TN:TP		32.7	11.4	53.3	15.7	43.7	18.0	50.6	12.5	47.7	11.1	81.6	12.2	19.1	34.3
SDD	**m**	0.43	0.15	0.87	2.21	2.73	3.23	0.9	2.7	3.4	4.5	1.00	0.50	1.23	0.83
Chl-*a*	**µg/L**	13.5	10.7	94.0	28	16.7	2.9	1.4	2.8	3.4	3.7	176.4	22.5	55.5	15.8

WT: Water Temperature; TP: Total Phosphorus; TN: Total Nitrogen; Secchi Disk Depth: SDD; Chl-a: Chlorophyll-a

TN:TP ranged from 11.1 to 81.6. Based on these ratios, in spring, all water bodies were potentially co-limited with a range of 10<TN:TP<17, except for Gebekirse Lake (34.3) and the Tahtalı Reservoir (18.0). In autumn, the ratios were significantly high and found to be potentially P-limited (TN:TP > 17) with the range of 19.1-81.6 (Wan et al., 2020) in all water bodies. The maximum TN:TP ratio was recorded during autumn in Çatal Lake (81.6) where the chl-*a* concentration was the highest, which reflects a significantly higher nutrient level (Table 3).

The phytoplankton compositions were generally dominated by Cyanobacteria, Bacillariophyta, and Chlorophyta (Fig. 3). In both sampling periods, phytoplankton composition in the Seferihisar Reservoir consisted of Ochrophyta, Cryptophyta, and Bacillariophyta, covering ~95% of the phytoplankton community. Similarly, in the Kavakdere Reservoir ~85% of the phytoplankton community was comprised of Bacillariophyta, Charophyta, Ochrophyta, and Miozoa. Cyanobacteria was not detected in both reservoirs. In other water bodies, the detected cyanobacterial genera were *Anabaenopsis, Aphanizomenon, Aphanocapsa, Chrococcus, Dolichospermum, Limnothrix, Merismopedia, Microcystis, Planktolyngbya, Pseudanabaena,* and *Snowella*. Data showed that *Microcystis aeruginosa* was the dominant cyanobacteria species in the studied water bodies in the Küçük Menderes River Basin. Sixteen cyanobacterial species were detected and 10 of them were potentially microcystin-producing species. As a general phytoplankton seasonal succession pattern in freshwater ecosystems, Bacillariophyta dominated in spring and cyanobacteria were relatively high in autumn in the studied basin. The presence of cyanobacteria was generally strongly affected by physicochemical variables such as temperature and nutrient availability (Yang et al., 2019, Huisman et al., 2018). The dominance of cyanobacteria was generally found in eutrophic water bodies in the Küçük Menderes River Basin. Xu et. al (2019) demonstrated that under conditions of medium nutrient availability (30 < TSI < 50), as in the Tahtalı Reservoir (TSI=49.3), cyanobacteria dominated. On the other hand, with the highest TN and TP concentrations, Chlorophyta dominated the phytoplankton community (Wei et al., 2020) and in Çatal Lake, which had the highest nutrient concentrations, Chlorophyta was the dominant group.

Generally, the concentration of microcystin was influenced by both water temperature and the abundance of cyanobacteria (Walls et al., 2018). In autumn, the water temperature varied between 15.3 to 16.7 °C, while in spring it measured between 24.2-26.8 °C (Fig. 4). Cyanobacterial blooms have been reported in temperate zones in summer and autumn, but with global warming, they have also been reported in spring and winter (Ma et al., 2016). Especially in the Tahtalı and Beydağı reservoirs, cyanobacterial biomass was higher in spring (Fig. 4). Apart from the Tahtalı Reservoir and Çatal Lake, microcystin concentrations were generally low, ranging from undetectable to 1.4 µg/L throughout the studied period. The highest microcystin value was measured at 9.1 µg/L in the autumn when the water level reached the lowest level in the Tahtalı Reservoir.

Even when the cyanobacterial population is high, it is important to evaluate the abundance of potentially toxin-producing species. In the Beydağ Reservoir, 61.5% of the phytoplankton community consisted of cyanobacteria which were dominated by *D. mendotae* (81.7%) in spring and *M. wesenbergii* (99.1%) in autumn, however, microcystin was not detected (Fig. 4). *Microcystis* blooms in freshwater ecosystems generally resulted in microcystin production, on the contrary, *M. wesenbergii* blooms generally have no microcystin production or low toxin production (Honma and Park, 2005). Via-Ordorika et al. (2004) could not detect microcystin-producing genes in *M. wesenbergii* colonies (n=21) in their study conducted in 13 water bodies in 9 European countries. Co-existing toxic and non-toxic *Microcystis* genotypes are common around the world and the proportion of microcystin production was affected by diverse factors such as nutrient concentrations, level of eutrophication, and also differences in morphotypes (Via-Ordorika et al. 2004).

In the Tahtalı Reservoir, cyanobacteria constituted 67.6% and 40.3% of the phytoplankton community in autumn and spring, respectively, and *M. aeruginosa* was the only dominant cyanobacteria in the reservoir. In autumn, microcystin concentration was the highest with 9.1 µg/L and in spring it was as low as 0.13 µg/L. This could be due to the seasonal dynamics of potentially toxic genotypes (Joung et al. 2011). *Microcystis*

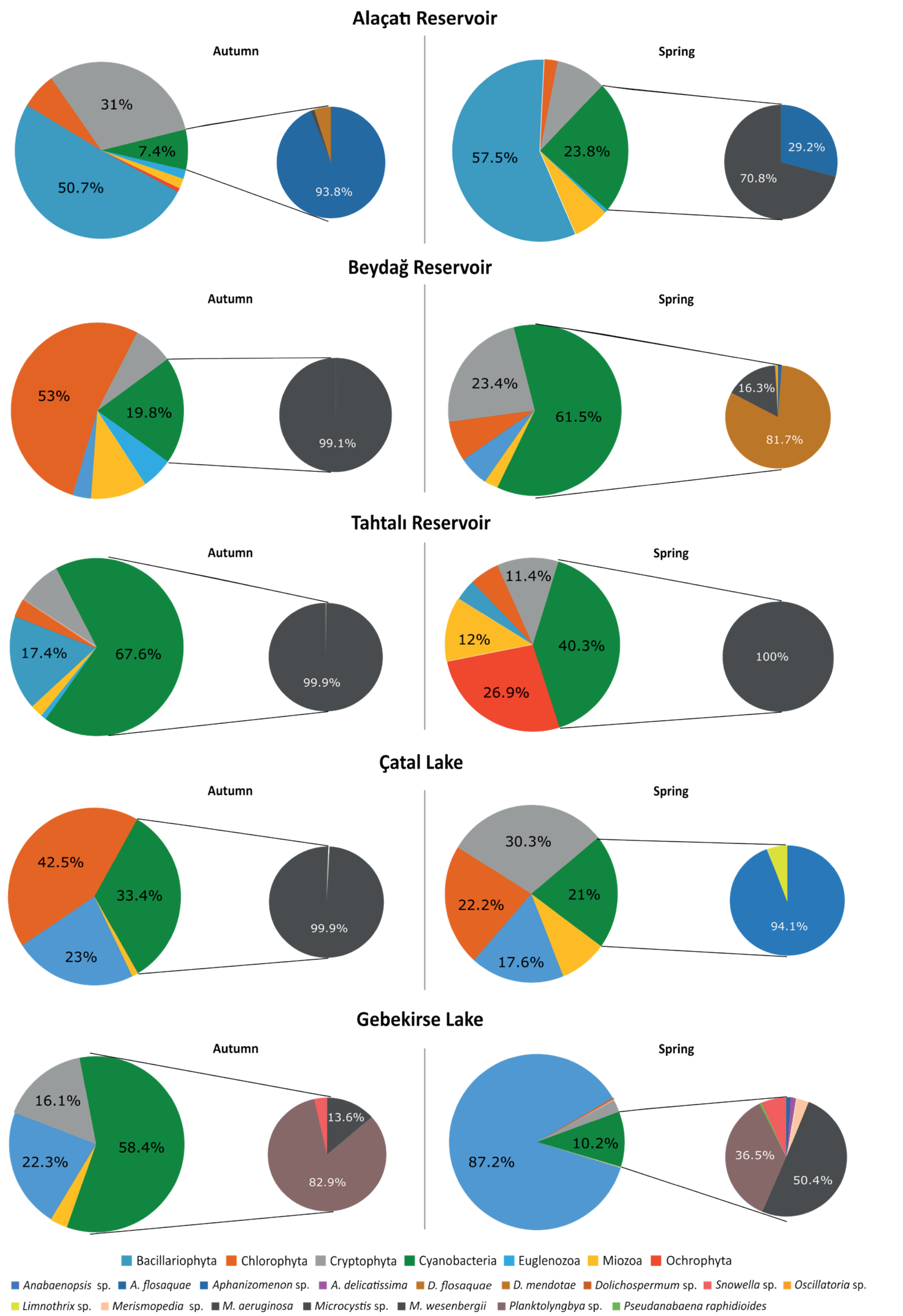

Figure 3. Biomass of phytoplankton community and cyanobacteria species in the studied water bodies.

species are dominant during the summer season with favorable water temperatures which could last through autumn (Carrasco et al., 2006; Koker et al., 2017). However, in 2006-2007, Ispirli (2009) did not detect microcystin above 1 µg/L in the Tahtalı Reservoir.

In the Alaçatı Reservoir, the phytoplankton communities were dominated by Bacillariophyta (more than 50%) in both sampling periods (Fig.2). The cyanobacteria biomass consisted of *A. flosaquae* and *Microcystis* sp. In spring, *Microcystis* sp. comprised 70.8% of the total cyanobacteria biomass which in turn composed 23.8% of the total phytoplankton biomass. *A. flosaquae* decreased and the abundance of *Microcystis* sp. increased in spring. Together, they contributed >93.8% of cyanobacteria biomass and formed a bloom. Microcystin production was detected 0.14 µg/L in autumn and 0.1 µg/L in spring. Generally, in the Alaçatı Reservoir, the microcystin production was low and co-occurrence with *Microcystis* sp. in both sampling periods may have been the reason for microcystin production (Lyon-Colbert et al. 2018).

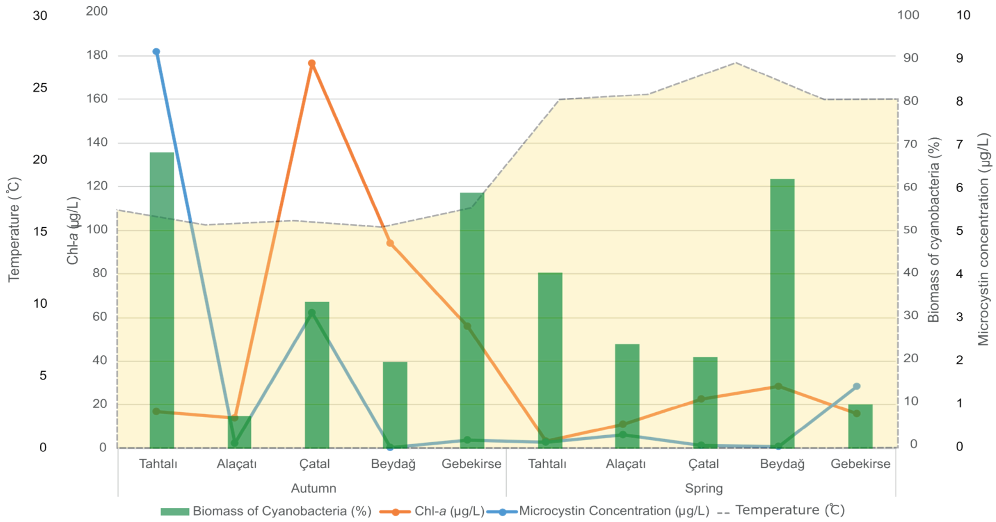

Figure 4. The relationship between water temperature, cyanobacterial biomass, chl-*a*, and microcystin concentrations in the water bodies.

The second highest microcystin concentration was detected in Çatal Lake (3 µg/L) in autumn. The potential microcystin-producing taxa *M. aeruginosa* was the dominant cyanobacterial species, accounting for more than 90% of cyanobacterial biomass in autumn.

In Gebekirse Lake, while the cyanobacteria biomass was 10.2% in spring with 50.4% of *M. aeruginosa*, the microcystin concentration was 1.4 µg/L. In autumn, the cyanobacteria biomass was 58.4% of the total phytoplankton community and 82.9% of the cyanobacteria biomass was dominated by *Planktolyngbya* sp. and 13.5% was *Microcystis aeruginosa*. Although microcystin production is directly related to the presence of cyanobacteria, the presence and abundance of potential microcystin-producing species have crucial importance (Sinang et al., 2013).

In many sites in the Mediterranean basin, cyanobacterial blooms start to occur in the spring-summer periods and last through autumn depending on water temperature (Mariani et al. 2015). Carrasco et al. (2006) demonstrated that from September to November, potentially toxic cyanobacteria were present in seven reservoirs in Madrid which is in line with our results. Furthermore, *Microcystis* spp. was dominant in Mediterranean countries including Greece, Spain, Portugal, France, and Turkiye (Cook et al., 2004; Koker et al., 2017). Although previous studies focused on the temperature effects on cyanobacterial abundances, multiple environmental factors, synergistic interaction between increased nutrients and temperature also promotes cyanobacterial dominance and persistence in water bodies (Carrasco et al., 2006; Gkelis, et al. 2015).

CONCLUSION

This study revealed that potentially toxin-producing cyanobacteria species were dominant in the Küçük Menderes River Basin, although the microcystin production was not high. Most of the harmful cyanobacteria blooms were dominated by the *Microcystis aeruginosa, M. wesenbergii, Anabaenopsis* sp., and other cyanobacterial taxa (*Dolichospermum* and *Aphanizomenon*). However, further data are needed for detection of other cyanotoxins such as cylindrospermopsin, saxitoxin, or anatoxins. Increased input of nutrients and global warming could have stimulated cyanobacterial blooms and cyanotoxin production in the Küçük Menderes River Basin.

Conflict of Interest: The author has no conflicts of interest to declare.

Ethics committee approval: Ethics committee approval is not required.

Acknowledgements: This study was supported by the Ministry of Agriculture and Forestry, General Directorate of Water Management (Project on Management Plan of the Küçük Menderes River Basin).

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