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Detection and monitoring of MC-LR and MC-RR in the artifical irrigation ponds at Oltu district

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Abstract
Although it is defining as natural organic pollutant, surface water resources which are frequently exposed to Harmful Algae Blooms (HABs) due to the increasing nutrient loading in recent years, the increase in temperature caused by climate change and the increase in surface run-off caused by extreme rainfall as a result of the risk of the increasing concentrations of algal toxins into drinking water. Although HABs have caused the problem of eutrophication in surface waters especially at hot seasons in the past decades due to the water pollution, increasing surface water temperatures with climate change cause this problem to extended periods out of season and to be permanent for the year. Therefore, studies including the detection and monitoring of algal toxins are gaining importance in order to observe HAB events at their source. As global temperature increases, HAB events have spread to regions that have even cold climates. Consequently, Microcystin-LR (MC-LR) and Microcystin-RR (MC-RR), which are the main indicators of HAB events in surface waters were aimed to detect and monitor at the artificial ponds designed for agriculture and animal husbandry purposes in Oltu District of Erzurum Province which has cold climate, for the first time in this study.Microcystins (MCs) concentrations were measured in the samples taken from ponds in four seasons for one year, by LC-MS/MS and; water temperature and pH values were also determined simultaneously. The relationship between the MC-LR and MC-RR distributions and, the pH and temperature were calculated by Pearson Correlation Coefficient (r).

Keywords: Harmful Algal Blooms, Eutrophication, Microcystins, Surface Water Pollution, Climate Change

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INTRODUCTION

In recent years, drinking water treatment plants (DWTPs) have focused on newly emerging micropollutants (MPs) in the water resources rather than classical pollutants. Since they are generally designed for basic parameters such as taste, odor, color, turbidity, dissolved oxygen, and therefore the treatment of MPs that emerge in the water environment in both type and quantity and cause concern for public health cannot be possible in traditional DWTPs. Among these MPs, some toxic compounds caused by HABs have become frequently researched groups due to their hazards to the aquatic ecosystem and public health (Simith et al., Turner et al. 2018; Munoz et al. 2021).

Since these compounds released from algal cells during HAB events cannot be treated in DWTPs, they may directly affect public health and may also be harmful to the aquatic ecosystem (Wu et al. 2011; LaLiberte and Haber, 2014). The main groups known to cause HABs and produce toxins in the freshwater environment are cyanobacteria, also known as blue-green algae. Microcystis group algae, which are the most important species known to produce toxins among cyanobacteria species, are responsible for global eutrophication and have become the biggest problem of DWTPs by causing taste and odor problems in drinking water (Vidal et al. 2021). WHO reports state that 60% of HABs event contain toxins and these toxins are generally called cyanobacterial toxins classified as MCs, cylindrospermopsins, anatoxins, saxitoxins, anatoxin-a (s) and dermatotoxins, which have important effects on human health. These toxins can be produced by more than one species; for example, the MC group cyanobacterial toxin can be produced by any of the Microcystis, Planktothrix, Dolichospermum and Nostoc species. These species are known as the primary MCs producing species in freshwater environments. MCs are the largest and most diverse group of cyanotoxins, consisting of more than 80 species. The most common producers of MCs are known as Microcystis aeruginosa (Žegura et al. 2011; Mishra et al. 2018; Díez-Quijada et al. 2019). As one of the well-known and most researched compounds of MCs, MC-LR should not be exceed 1 µg/L recommended by the World Health Organization (WHO) due to its carcinogenic properties (WHO 1998). In 2010, the International Agency for Research on Cancer (IARC) classified MC-LR as a possible human carcinogen Group 2B (He et al. 2016; Chen et al. 2018). Cyanobacterial toxins are classified as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins/tumour-promoter and irritant toxins (lipopolysaccharides) according to their hazard mechanism on animals and plants (Du et al. 2019), as they have the potential to cause death in aquatic organisms, form tumors or affect nutrition, growth and the immune system (Zhang et al. 2019; Shi et al., 2021).

WHO has determined limit values for both cyanotoxins and cyanobacteria (WHO 2003) for the protection of freshwater resources against HAB events. For this purpose, if water media contains 20,000 cyanobacterial cells in 1 mL, it indicates a low probability of adverse health effects, if it is in between 20,000-100,000 cyanobacterial cells in 1 mL it indicates a moderate probability of harmful health effects, and finally if it is greater than 100,000 cyanobacterial cells in 1 mL then this is a high probability of harmful health effects and long-term effects in humans that will cause many diseases. In a study where cyanobacteria were monitored in Yerevan Lake in Armenia, 695.9×10^3 cells in 1 mL were analyzed and it was determined that they were highly hazardous to public health (Minasyan et al. 2018). First outbreak was recorded at 1996 at a diyalysis center in Caruaru, Brazil. 130 patients had died of acute liver failure, where the center received water from a nearby reservoir and Microcystins produced by cyanobacteria were detected in the water supply (Pouria et al., 1998; Jochimsen et al. 1998). Later In 2013, MC amounts were detected in the range of 1.4 µg/L-3.6 µg/L in treated drinking water in Ohio (USA), and the autorities explicitly warned to public as "do not drink" fort he first time (He et al. 2016) considering the limit value set by WHO (2020).

The occurrence of toxic cyanobacteria in freshwater has been increasing in both frequency and distribution in recent years due to increased global temperatures as well as water water pollution (Eren,2021; Köker et al. 2021), because there is a scientific consensus that ecosystem impacts caused by HAB have increased especially in the last few decades. In addition, increasing water temperatures due to climate change may cause cyanobacterial growth to spread over wider seasons. As a matter of fact, it was stated for the first time that this frequency of HABs was directly linked to climate change in the "Ocean and Cryosphere in a Changing Climate (SROCC)" Special Report of the United Nations (UN) Intergovernmental Panel on Climate Change (IPCC) approved in September 2019 (IPCC 2019). For instance, Lake Taihu as China's third largest lake is an important drinking water source for many major cities including more than 30 million people. In 2007, record temperatures in China caused dramatically HAB event, releasing of toxins into the drinking water (Wu et al. 2011). Recent studies have revealed that 54% of the lakes in the Asia Pacific Region, 53% in Europe, 28% in Africa, and 48% and 41% in North America and South America, respectively, are eutrophic associated with HABs, due to increasing surface water temperatures and pollution loads in aquatic environments (WHO 1998).

In acute contact with water sources containing cyanobacterial toxins may be caused abdominal pain, vomiting, diarrhea, irritation of the eye, nose and throat mucosa, asthma attacks, nausea, tingling in the fingertips and toes, blurred vision, headache, dizziness, fever, hypoxia, resulting in paralysis and even death (Pantelić et al. 2013). Other episodes of cyanobacteria-associated human diseases are included some examples of a statistically significant correlation between drinking water from reservoirs containing Microcystis aeruginosa in Australia and signs of liver damage (Metcalf and Codd, 2004) and a high incidence of primary liver cancer in China, attributed to drinking water contaminated with cyanobacterial toxins (Harada et al.1996).

HAB monitoring and detection studies have gained great importance worldwide. Especially the increased temperature by climate change have led to HAB events not only in hot climate regions but also in cold climate regions. Although Erzurum is a cold climate city but climate temperatures have been increasing in recent years, no monitoring studies have been conducted in this region in terms of algal toxins until now. Therefore, MCs anaylsis method was developed for two important algal toxins (MC-LR and MC-RR) considered as indicators of HAB events and their monitoring in surface water resources was carried out for the first time in this study. In order to contribute to the studies conducted in terms of the increase, distribution and spread of HAB events in our country, the first HAB monitoring study was conducted in our region for protecting water resources becoming increasingly important. This is the first step of the multibarrier approach required to reduce the risk of toxic cyanobacterial growth in water resources. Accordingly, the first priority step is to prevent the contamination of the water source and control processes at the source, then it is important to use treatment technologies that will minimize the release of these toxins and optimize these methods. Therefore, determining and monitoring the amounts of cyanobacterial toxins in water resources is the most important step in eutrophication control (He et al. 2016). For this purpose, MC-LR and MC-RR was aimed to detect and monitor in 8 artificial ponds created for agricultural irrigation and husbandary purposes within the borders of Oltu District of Erzurum City in this study.

Composite water samples were taken from the ponds during three seasons, starting from August 2022, January 2023 and May 2023. The temperature and pH of the ponds were also measured during sampling period and the realtion of these parameters with MCs were investigated as well.

MATERIALS AND METHODS

Study Area

In order to detect MC-LR and MC-RR, two important MC species that occur as a result of HABs in surface waters, composite water samples were taken during three seasons, starting from August 2022, January 2023 and May 2023 in the 8 artificial ponds located within the borders of Oltu District, Erzurum City and created for agricultural irrigation and livestock purposes. Figure 1 shows the pictures taken in various periods of these ponds namely; Çengelli-1, Çengelli-2, Güzelyayla, İnanmış, Subatık, Ünlükaya, Bahçecik and Çatalsöğüt Ponds, respectively.

Figure 1. The MCs detection studies on 8 irrigation ponds in Oltu District, Erzurum.

Method

MC-LR and MC-RR compounds were analyzed with an LC-MS/MS. Based on literature searches, MC-LR constitutes 46-99.8% of the total MCs observed in HABs event (Sharma et al. 2012), MC-LR and MC-RR compounds were primarily selected to detect and analsis in this study. Analysis of MC-LR and MC-RR compounds was carried out with an LC-MS/MS, since they are found at nano or micro gram levels in surface waters. The LC-MS/MS device is located at Atatürk University Eastern Anatolia High Technology Application and Research Center (DAYTAM) and has an Agilent 6460 Triple Quadropol model system with Agilent G4226A autosampler and Jet Stream electrospray source. The LC-MS/MS device is created by combining liquid chromatography and spectroscopy systems that can provide robust, accurate qualitative and quantitative analysis of target compounds at extremely low levels. In order to perform analysis on the device, method validation was first carried out. Validation parameters for MC-LR and MC-RR compounds considering the literature studies; were determined as precision, accuracy, sensitivity, specificity and linearity. For method validation, samples were analyzed in 6 replicates (n>5). The LOD value was determined for a signal/noise ratio of 3. The LOQ value is determined as the value at which the signal/noise ratio is 10. The calibration curve was drawn to include the LOQ value in the range where the curve is linear (0.05, 0.10, 0.25, 0.5, 1, 5 and 10 µg/L) (Vashist and Luong, 2018). System suitability was achieved by 6 repeated injections of standard MC-RR at a concentration of 0.75 µg/L and standard MC-LR solution at a concentration of 1 µg/L (Pekar et al. 2016; Beversdrof et al. 2018; Aparicio-Muriana et al. 2022). The samples to be analyzed were taken as 10 mL, filtered through a 0.22 µm cellulosic filter and transferred directly to 2 mL autosampler vials (Figure 2). Table 1 includes the device operating conditions.

Table 1. LC-MS/MS Conditions

Colon	Agilent InfinityLab Poroshell SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)	
Colon Temp.	50 ºC	
Enjeksiyon hacmi	20 µL	
Mobile phase A (%80) + B (%20)	A) 1 mM ammonium fluoride (HPLC grade) + %0.1 formic acid (%100 su V.03) B) 20% IPA + %0.1 formic acid (LC/MS grade) (%100 Acetonitryl V.03)	
Auto sampler temp.	5 ºC	
Flow rate	0.6 mL/min	
Gradient	Time, min	B, %
	3.00	30
	5.00	50
	6.00	100
Triple quadrupole MS Koşulları		
Ionization mode	ESI with Agilent Jet Stream Technology	
Drying gas temp. °C	350	
Drying gas flow, L/min	12	
Nebulizer pressure	40 psig	
Sheath gas temp.	400 °C	
Sheath gas flow	11 L/min	
Capillary voltage	4,000 V	
Nozzle voltage	1,000 V	
EMV	400 V	

Figure 2. The water samples to be analyzed in LC-MS/MS

RESULTS AND DISCUSSION

The concentrations of MC-LR and MC-RR in the water samples taken 3 times from each irrigation pond were analyzed 3 times on the LC-MS/MS and the results were obtained as in Figures 3-4 to better understand the MC-LR and MR-RR occurance during three seasons. Each season were selected to monitor the HAB event for the representative of summer, winter and spring on August 2022, January 2023 and May 2023, respectively. The samples collected from the ponds were stored at +4 ºC at dark conatiner and delivered to the laboratory, made ready for analysis and analyzed within 48 hours. The temperature of ponds and pH were also analyzed during the sampling time.

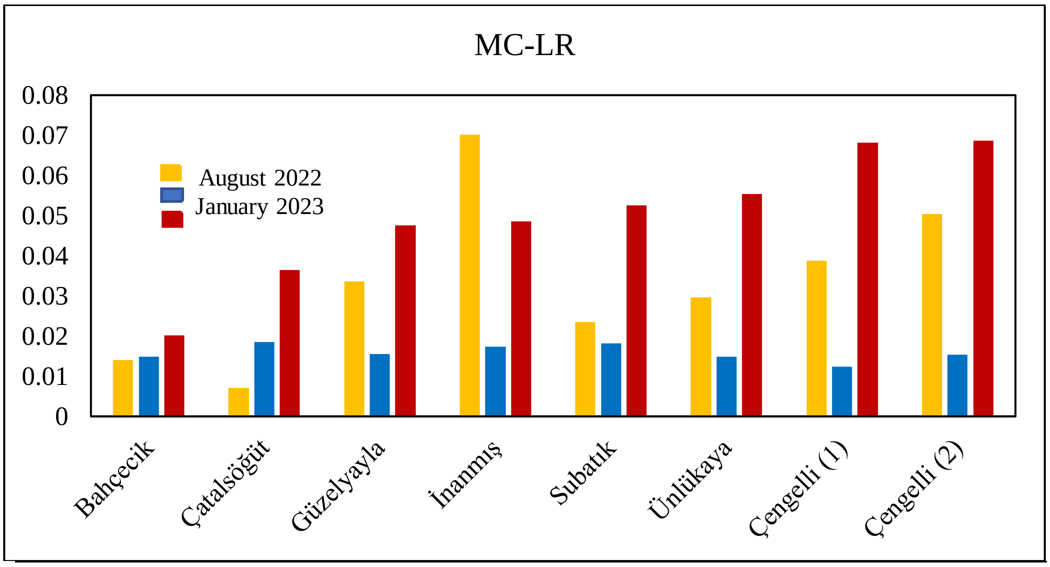

Figure 3. The Comparision of MC-LR concentration of ponds.

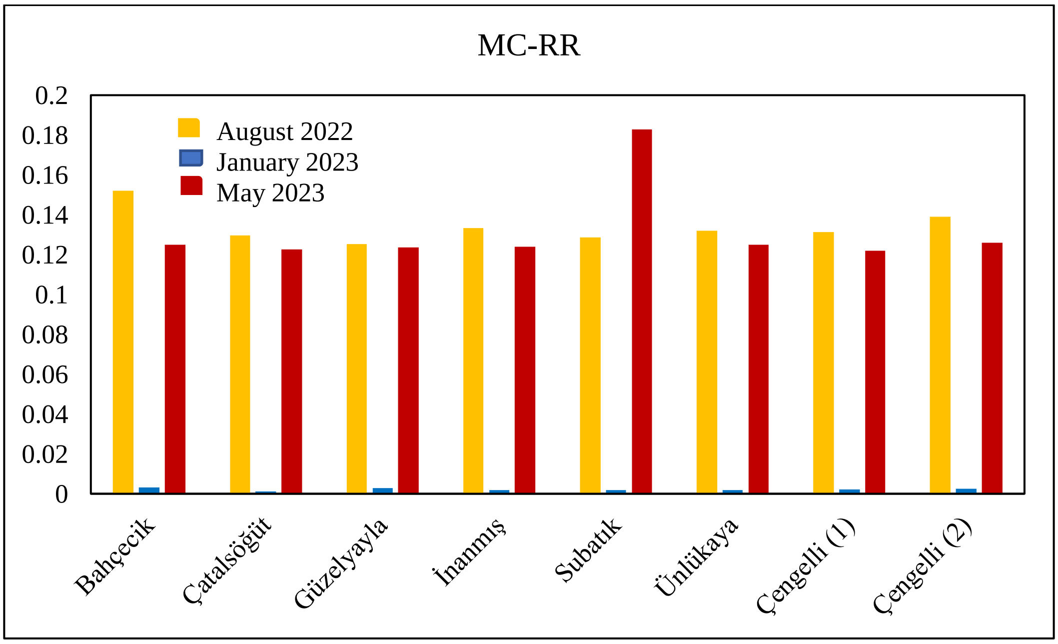

Figure 4. The Comparision of MC-RR concentration of ponds.

It can be evaluated that MC-LR and MC-RR concentrations in artificial irrigation ponds have reached visible levels from Figure 4 and Figure 5. MC-LR concentrations were detected in the range of 0.0071-0.0701 µg/L, while the concentration of MC-RR was analyzed in the range of 0.1253-0.1522 µg/L, during first anaylsis period of August 2022. Considering the 1 µg/L limit value determined by WHO for MC-LR, the ponds monitored in this study appear to pose a low risk for humans and animals which are drinking water from those areas. However, it is important to monitor MC concentrations in different seasons and long periods for the observation of HAB events.

For winter season, the amount of MC-LR in the ponds was analyzed in the range of 0.0124-0.0185 µg/L and MC-RR was analyzed in the range of 0.0013-0.0033 µg/L. While MC-RR concentrations were observed to decrease, MC-LR was observed to increase compared to the summer period that may indicate a possible accumulation due to their resistance to degradation. Finally, MC-LR and MC-RR concentration were analyzed in the ranges of 0.0202-0.0687 µg/L and 0.1251-01827 µg/L during spring season of May 2023.

When comparing the literature studies with our results; the concentration of MC-LR dissolved in water was 0.04 µg/L and the concentration of MC-RR was 1.50 µg/L were detected in Los Padres Lake in Argentina in 2007 (Amé et al. 2010). In a study conducted in Yanghe Reservoir in China in 2010, dissolved MC-RR was determined as 1.56 µg/L, while MC-LR was determined as 0.544 µg/L (Li et al. 2010). Analyzes conducted in 14 regions in Northern Ireland show that MC-LR varies between 0.004–0.014 µg/L and MC-RR varies between 0.005–0.060 µg/L (Mooney et al. 2011). Analyzes conducted in Europe in the Jeziorsko reservoir in Poland found as 0.38 µg/L for MC-LR and 0.23 µg/L for MC-RR (Mankiewicz-Boczek et al. 2011). In analyzes conducted in clean water sources in Uganda, MC-LR was found to be 3.3 and MC-RR was found to be 23.0, indicating major contamination (Okello et al. 2010). In a study conducted in two different water reservoirs in Portugal, MC-LR was detected in the range of 15–344 ng/L and MC-RR 14–212 ng/L (Rodrigues et al. 2013). As a result of the analysis made on samples taken from water bodies in six different countries (France, Italy, Germany, Bulgaria, Ireland), including Turkey, MC-LR was found to be in the range of 0.1–132.8 µg/L and MC-RR was 0.07–103.2 µg/L (Pavlova et al., 2015).

Pearson correlation measures the strength of the linear relationship between two variables. It has a value between -1 and 1, - value means total negative linear correlation, while + value means total positive correlation. 0 means no correlation (Berman 2016). Additionally, $0 < r \leq 0.19$ suggests very low correlation; $0.2 \leq r \leq 0.39$ is low correlation; $0.4 \leq r \leq 0.59$ is moderate correlation; $0.6 \leq r \leq 0.79$ is high correlation and $0.8 \leq r \leq 1.0$ is very high correlation (Selvanathan et al. 2022). For this purpose, the relationship between the measured MC-LR and MC-RR concentrations and, the pH of the ponds and water temperature of the ponds was investigated with Pearson Correlation and, the Pearson Correlation Coefficient (r) values were calculated and shown in Tables 2-4 with rRR for MC-RR and rLR for MC-LR, together with the total measurement parameters. The pH value of the ponds was between 8.2-9.7; the temperatures vary between 18.1-18.4 ºC during August 2022 season, water temperatures in the ponds were measured in the range of 0.7-2.4 ºC and pH in the range of 8.4-9.5 for winter 2023 season and, finally pond temperatures varied between 12.3-21 ºC, pH varied between 6.1-8.9 and DO values varied between 5.1-8 mg/L during spring 2023 season.

Table 2. Detection of MC-LR and MC-RR concentrations in ponds at August 2022.

	Pond	**pH**	**Temp. ºC**	**MC-LR µg/L**	**MC-RR µg/L**	**r**
1	Bahçecik	8.3	18.2	0.0141	0.1522	
2	Çatalsöğüt	8.2	18.3	0.0071	0.1298	
3	Güzelyayla	9.4	18.1	0.0336	0.1253	
4	İnanmış	9.3	18.4	0.0701	0.1333	
5	Subatık	8.6	18.4	0.0235	0.1287	
6	Ünlükaya	9.1	18.2	0.0296	0.1319	
7	Çengelli (1)	9.4	18.1	0.0388	0.1312	
8	Çengelli (2)	9.7	18.0	0.0504	0.1390	
		0.80 (P<0.05)	-0.06			$\mathbf{r_{LR}}$
		-0.27	-0.17			$\mathbf{r_{RR}}$

Table 3. Monitoring of MC-LR and MC-RR concentrations in ponds at January 2023.

	Pond	**pH**	**Temp. ºC**	**MC-LR µg/L**	**MC-RR µg/L**	**r**
1	Bahçecik	8.9	2.4	0.0149	0.0033	
2	Çatalsöğüt	8.4	1	0.0185	0.0013	
3	Güzelyayla	9.4	0.7	0.0155	0.0027	
4	İnanmış	9	1	0.0174	0.0017	
5	Subatık	8.4	7.5	0.0182	0.0019	
6	Ünlükaya	9	2.3	0.0148	0.0020	
7	Çengelli (1)	9.5	2.3	0.0124	0.0021	
8	Çengelli (2)	9.5	1	0.0154	0.0024	
		-0.79 (P<0.05)	0.25			$\mathbf{r_{LR}}$
		0.45	-0.07			$\mathbf{r_{RR}}$

Table 4. Monitoring of MC-LR and MC-RR concentrations in ponds at May 2023.

	Pond	pH	Temp. °C	MC-LR µg/L	MC-RR µg/L	r
1	Bahçecik	8.9	15.2	0.0526	0.1827	
2	Çatalsöğüt	7.45	20.7	0.0687	0.1260	
3	Güzelyayla	8.9	21	0.0364	0.1226	
4	İnanmış	7.43	15.1	0.0553	0.1249	
5	Subatık	7.45	20.2	0.0202	0.1251	
6	Ünlükaya	7.2	20	0.0486	0.1239	
7	Çengelli (1)	8.5	12.3	0.0475	0.1235	
8	Çengelli (2)	6.12	21	0.0682	0.1220	
		-0.39	-0.03			r_{LR}
		0.49	-0.36			r_{RR}

According to the results shown in Tables 2-4, although the significance levels could not be determined at a sufficient level since the number of data was small, a high significance level relationship (0.80) was detected between water pH and MC-LR concentration in samples taken during August 2022 ($P<0.05$). It is thought that MC-LR concentrations in water are responsible for the pH changes in the summer period. A similar situation was detected in samples taken in January 2023, which is the winter period. Accordingly, a high significance level negative relationship (-0.79) was detected between water pH and MC-LR concentration ($P<0.05$). MC-LR concentration decreases in January 2023 negatively affected pH changes. When the relationship of MC-RR with pH in ponds was examined; a low negative relationship (rRR=-0.27) was determined in August 2022, and a moderate positive relationship was determined in January 2023 and May 2023 (rRR values 0.45 and 0.49, respectively). Finally, a low relationship was observed between water temperature and MC concentrations in samples taken in each period.

CONCLUSION

In this study, eight irrigation ponds in Oltu District of Erzurum City were selected to detect and monitor of MC-RR and MC-LR during summer, winter and spring seasons 2022-2023. Although MCs were not found in high concentrations based on WHO limits in the pons, the indication of HAB events can cause a significant threat to both the environment and public health. Therefore, risk analysis and management tools as well as sustainable and cost-effective treatment processes need to be developed. The presence of cyanotoxins in freshwater is confirmed in many countries around the world, but limited information is still available and further monitoring is required. In addition, monitoring, analysis, toxicology, treatment methods are carried out only on certain types of MCs. In addition, it is also necessary to develop preventive strategies such as preventing pollution in irrigation water supply systems, especially for drinking and use or agriculture-livestock purposes, developing strategies to reduce nutrient loads, and early detection of algal blooms with remote sensing systems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Mevra Emeç contributed to Sample Collection, Data Obtaining, Writing—Review & Editing.

Zeynep Eren contributed to Conceptualization, Methodology, Data analysis, Visualization, Writing—Original Draft, and Review & Editing.

Data availability

Data will be made availabale on request.

Consent to participate

The authors consent to participate.

Consent for publication

The authors consent to publication.

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