Cyanobacterial blooms in the Po River basin and the eastern Alps

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ABSTRACT

In this paper, we briefly describe episodes of cyanobacterial blooms that have occurred in lakes of northern Italy since 2000. In addition to listing the species involved in these blooms, we provide information on the trophic and ecological status of the water bodies and the presence of algal toxins. Furthermore, we report an example of a risk assessment effort aimed at developing a quality control system for water intended for human consumption. The use of high-frequency monitoring techniques, integrated with predictive modelling, remote sensing, and molecular analysis for species identification, is becoming increasingly important in the context of the effects of ongoing climate change.

Introduction

In the last twenty years, the frequency of cyanobacterial blooms in northern Italian lakes has increased, affecting all lake types. Climate change and increasing trophic levels of water bodies were shown to favour cyanobacterial blooms (Hilt *et al*., 2017; Sterner *et al*., 2020). In this note, we report and briefly discuss an overview of cyanobacterial blooms recorded in northern Italy since 2000. Specifically, the purpose of this contribution is to highlight the temporal evolution of blooms, and the methods of investigation used for their characterization in relation to anthropogenic pressure factors.

In this study, we define blooms as cell population growth of all potentially toxic cyanobacterial species in surface water exceeding the thresholds provided by the Italian law (100,000 cells/mL). Lakes were grouped according to their altitude, alkalinity, average and maximum depth, and surface area. The geographical area covered by this study includes the whole southern Alpine chain, from Piedmont to Friuli Venezia Giulia. For each lake, we reported the trophic status, the ecological status derived from physical-chemical parameters and the phytoplankton index IPAM (Wolfram *et al*., 2014), and the species list and cell density of potentially toxic cyanobacteria (Chorus and Welker, 2021). Furthermore, we reported the presence of cyanotoxins and their concentration. Data reported have been retrieved from laboratory reports and environmental agencies websites.

Data were collected by regional Environmental Protection Agencies (EPAs) and local authorities as part of: i) monitoring activities aiming at the assessment of the ecological status of water bodies according to the Water Framework Directive (2000/6) (WFD); ii) evaluation of water bodies as suitable for bathing, according to the EU 2020/2184 directive; iii) monitoring of drinking water supplies. The chemical and physical parameters of the lakes were determined using the sampling protocol published by the National Agency for Environmental Protection (ISPRA, 2014) and the manual of analytical methods published by the National Research Council (APAT CNR-IRSA, 2003). The monitoring of cyanobacteria in bathing waters was carried out according to the Italian national legislation and the Italian National Institute of Health report (Funari *et al.*, 2014). The detection of microcystins was carried out using either ELISA tests (concentrations expressed as μg/L MC-LR) (Gurbuz *et al*., 2012) or by LC-MS (concentration expressed as μg/L of each MC variant). MC data reported in the text have been obtained by ELISA test, if not stated otherwise.

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Results

We reported the results of our study presenting the northern Italy lakes grouped by category and from the westernmost to the easternmost (Table 1). According to the Italian legislation, large and deep lakes are categorized as water bodies of type AL-3; lakes with a maximum depth of less than 125 meters are categorized as water bodies of type AL-6; shallow lakes with an average depth equal to or lower than 15 m are categorized as AL-5. All these categories include lakes that provide essential and valuable services to the communities and economic activities located in their surroundings; among these, AL-6 lakes will increasingly be important as major water sources in the near future (Sterner *et al*., 2020; Sterner, 2021).

Lake Maggiore is the most oligotrophic of the Italian deep subalpine lakes, and its phytoplankton community is characterized by low production levels (CNR IRSA, 2024). According to the WFD, its ecological quality status ranks as "Good". Moderate surface algal blooms, dominated by *Dolichospermum lemmermannii* (Richter) P.Wacklin, L.Hoffmann & J.Komárek, were recorded in 2011 (Callieri *et al*., 2014). Another moderate event was recorded in 2023, which was instead dominated by *Microcystis* spp. (Figure 1a). Microcystin concentrations were below the Limits of Quantification (LOQ) in both cases.

Table 1. List of lakes and cyanobacterial species that caused the blooms described in the text.

Lake	Lake type	Trophic status	Ecological status	Species	Surface bloom/ cellular density (cells mL^{-1})	Toxins (MC, µg L^{-1})
Maggiore	AL-3	oligotrophic	Good	*D. lemmermannii* *Microcystis* spp.	Yes	<LOQ
Lugano	AL-3	Meso-Eutrophic	Moderate	*Microcystis* spp.	Yes	>30
Como	AL-3	Mesotrophic	Moderate	*M. aeruginosa* *D. lemmermannii*	Yes	<20
Iseo	AL-3	Eutrophic	Moderate	*P. rubescens*	Yes	N.A.
Garda	AL-3	Oligotrophic	Good	*D. lemmermannii*	Yes	<20
Idro	AL-6	Eutrophic	Moderate	*P. rubescens* *A. flos aquae*	Yes	N.A.
Grande d'Avigliana	AL-6	Meso-eutrophic	Moderate	*Microcystis* spp.	177,358	<20
Sirio	AL-6	Meso-eutrophic	Moderate	*Aphanothece/Aphanocapsa*	129,000	<20
Varese	AL-5	Eutrophic	Moderate	*P. rubescens* *Microcystis* spp. *L. robusta* *W. Naegeliana*	Yes	16.7
Alserio	AL-5	Eutrophic	Poor	*A. flos-aquae*	Yes	N.A.
Annone est	AL-5	Eutrophic	Moderate	*W. naegeliana*	Yes	N.D.
Annone ovest	AL-5	Eutrophic	Moderate	*W. naegeliana*	Yes	N.D.
Montorfano	AL-5	Eutrophic	Moderate	*Microcystis spp.*	Yes	N.A.
Piano	AL-5	Eutrophic	Moderate	*A. flos-aquae*	Yes	N.A.
Pusiano	AL-5	Meso-eutrophic	Moderate	*Microcystis* spp. *D. lemmermannii* *L. robusta*	Yes	12.8
Segrino	AL-5	Meso- oligotrophic	Good	*Microcystis* spp.	Yes	N.D.
Candia	AL-5	Eutrophic	Moderate	*Microcystis* spp. *Planktolyngbya* sp. *Anabaena* sp.	Yes	4.45
Del Frassino	AL-5	Eutrophic	Poor	*D. planctonicum* *C. raciborskii* *A. gracile*	Yes	N.D.
Ragogna	AL-4	Mesotrophic	Moderate	*D. planctonicum**	No	N.D.
Sartirana	AL-4	Hypereutrophic	Bad	*D. crassum*	Yes	N.D.
Caldaro	AL-4	Oligo-mesotrophic	Moderate	*Aphanocapsa* sp. *Aphanothece* sp. *Microcystis* sp.*	117,495	0.609
Fiè	AL-4	Eutrophic	N.A.	*Aphanocapsa* sp. *Aphanothece* sp. *Cyanodiction* sp.	220,663	1.5
Costalovara	AL-4	Mesotrophic	N.A.	*Aphanocapsa* sp. *Cyanodiction* sp.	93,314	0.204

N.A., not available; N.D., not determined; LOQ, limits of quantification.

The northern basin of Lake Lugano is eutrophic and meromictic (Lepori *et al*., 2022), and its ecological quality status ranks as "Moderate." An extensive bloom of *Microcystis* spp. lasting two months was recorded in 2023. Microcystin LR concentration was as high as 30 µg L^{-1}.

Lake Como is mesotrophic (Rogora *et al*., 2018) and, since 2000, has been affected by several large-scale surface blooms covering up to tens of square kilometers. The species causing the blooms were *Microcystis aeruginosa* (Kützing) Kützing and *D. lemmermannii* (Figure 1b). However, concentrations of algal toxins were lower than LOQ.

Lake Iseo is eutrophic and meromictic (Leoni *et al*., 2019). In 2013, a significant population of *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek developed in the metalimnetic area, giving rise to a bloom in April 2013. There is no information on the presence of cyanotoxins in this episode.

Lake Garda is oligotrophic, and its ecological quality status ranks as "Good". Four surface blooms caused by *D. lemmermannii* were observed in 2011, 2012, 2017, and 2020 (Figure 1c). However, blooms observed in this lake were less widespread in comparison with the blooms of *D. lemmermannii* observed in Lake Como. In each bloom, cyanotoxins concentrations were lower than the LOQ, regardless of the most recent technological improvements adopted by EPA Veneto (implementation of a LC-MS analysis). The appearance, ecology, and toxicity of *D. lemmermannii* populations in Lake Garda were investigated by Salmaso *et al*. (2015a, 2015b, 2024a) and Capelli *et al*. (2017).

Regarding the AL-6 typology, Lake Idro is meromictic and eutrophic (Viaroli *et al*., 2018). Its ecological quality status is "Moderate." In winter 2004, a surface bloom of *P. rubescens* developed, whereas, in 2010 and 2023, blooms were dominated by *Microcystis* spp. and *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, respectively. No algal toxins were detected on all these occasions.

The Piedmontese water bodies type AL-6, Lake Grande of Avigliana, which was subjected to hypolimnetic aeration for over ten years, and Lake Sirio, developed surface blooms in June 2023. In the first case, blooms were dominated by *Microcystis* spp., whereas in the second case by *Aphanothece* spp. and *Aphanocapsa* spp. During these blooms, algal toxins were not detected. Both lakes have "Moderate" ecological status.

Among the AL-5 lakes typology, Lombardy has many morainic lakes in the eutrophic state, which favoured the development of several blooms of potentially toxic cyanobacteria. Lake Varese, which has a "Moderate" ecological quality status, has the highest Total Phosphorus (TP) concentration (60 µg L^{-1}). This lake developed intense blooms of *A. flos-aquae*, *P. rubescens*, and *Microcystis* spp. More recently, it developed blooms of *Woronichinia naegeliana* (Unger) Elenkin and *Limnoraphis robusta* (Paracutty) J.Komárek, E.Zapomelová, J.Smarda, J.Kopecký, E.Rejmánková, J.Woodhouse, B.A.Neilan & J.Komárková (Figure 1d). In 2022, *Microcystis* spp. produced microcystins at concentrations as high as 16.7 µg L^{-1}.

The Lakes Alserio, East Annone (Figure 1e), West Annone (Figure 1f), Montorfano, Piano, and Segrino are between oligotrophic and eutrophic state with spring TP concentrations ranging between 15 and 45 µg L^{-1}. Their ecological quality status is reported in Table 1, with cyanobacteria blooms that have affected them.

Among the Piedmont water bodies AL-5, Lake Candia is eutrophic and has "Moderate" ecological quality status. In 2023, it developed a surface bloom of "*Anabaena"* sp. (Figure 1g); during this episode, the microcystins concentration reached 4.45 µg L^{-1}.

Lake Frassino is a small eutrophic lake of glacial origin in the province of Verona. The lake, which has "Poor" ecological quality status, occasionally developed summer blooms of *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L.Hoffmann & Komárek and *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya & Subba Raju. The presence of cyanotoxins was not investigated.

Among the Friulian water bodies type AL-5, Lake Ragogna developed relatively high summer-autumn abundances of *D. planctonicum*. Abundances were within the range expected for mesotrophic lakes during a seasonal ecological succession, from 100 to 1000 cells/mL.

The small and polymictic Lake Sartirana showed the highest trophic level (Table 1). In this water body, blooms of *Dolichospermum crassum* (Lemmermann) P.Wacklin, L.Hoffmann & J.Komárek were associated with fish die-off following a very strong heatwave and a storm that stirred up sediments and contributed to oxygen consumption.

Among the Sudtyrol water bodies of type AL-5, the polymictic lakes Caldaro, Fiè, and Costalovara showed high cell densities of *Aphanothece* sp., *Aphanocapsa* sp., and *Cyanodiction* sp., which did not produce surface blooms. Consequently, algal toxins were detected but at very low concentrations.

Integrating conventional and innovative approaches in the study of algal blooms

The increase in frequency of cyanobacterial blooms requires the use of integrated methods employing modern technologies to characterize and possibly predict the events with high accuracy. High-frequency data acquisition enables the implementation of tools for bloom prediction (meteorological index, Ndong, 2014; machine learning, Rousso, 2019) and of "early warning" systems to signal potential bloom initiations, associated with the use of remote sensing (Bresciani *et al*., 2017; Giardino *et al*., 2019). Data collected from buoys deployed in the middle of the lakes are also crucial for calibrating process-based models (QWET) used to assess changes in nutrient loads in relation to climate change (Fenocchi *et al*., 2019; Fenocchi *et al*., 2020). In Lakes Maggiore, Como, and Varese, buoys equipped with sensors for both meteorological and continuous measurement of chemical, physical, and biological parameters in surface layers have already been deployed (SIMILE Interreg Italy Switzerland Project; AQST Action program for the protection of Lake Varese financed by Regione Lombardia). Thermistor chains extend up to 50 meters in deep lakes, while in Lake Varese, they reach the bottom. Continuous temperature data acquisition allows the characterization of temperature evolution profiles and the study of internal lake waves. A simple example of an early warning system is the one implemented in Lake Varese, which uses a combination of three threshold levels, one for dissolved oxygen, one for pH, and the last for phycocyanin concentration. In case two out of three thresholds are exceeded, the system sends an alert to the health authorities.

In Piedmont and Lombardy, measures for reducing internal loads through hypolimnetic withdrawal during stratification were adopted in some small and medium-sized eutrophic lakes, namely lakes Alserio, Annone Est, Varese, Grande d'Avigliana, and Sirio.

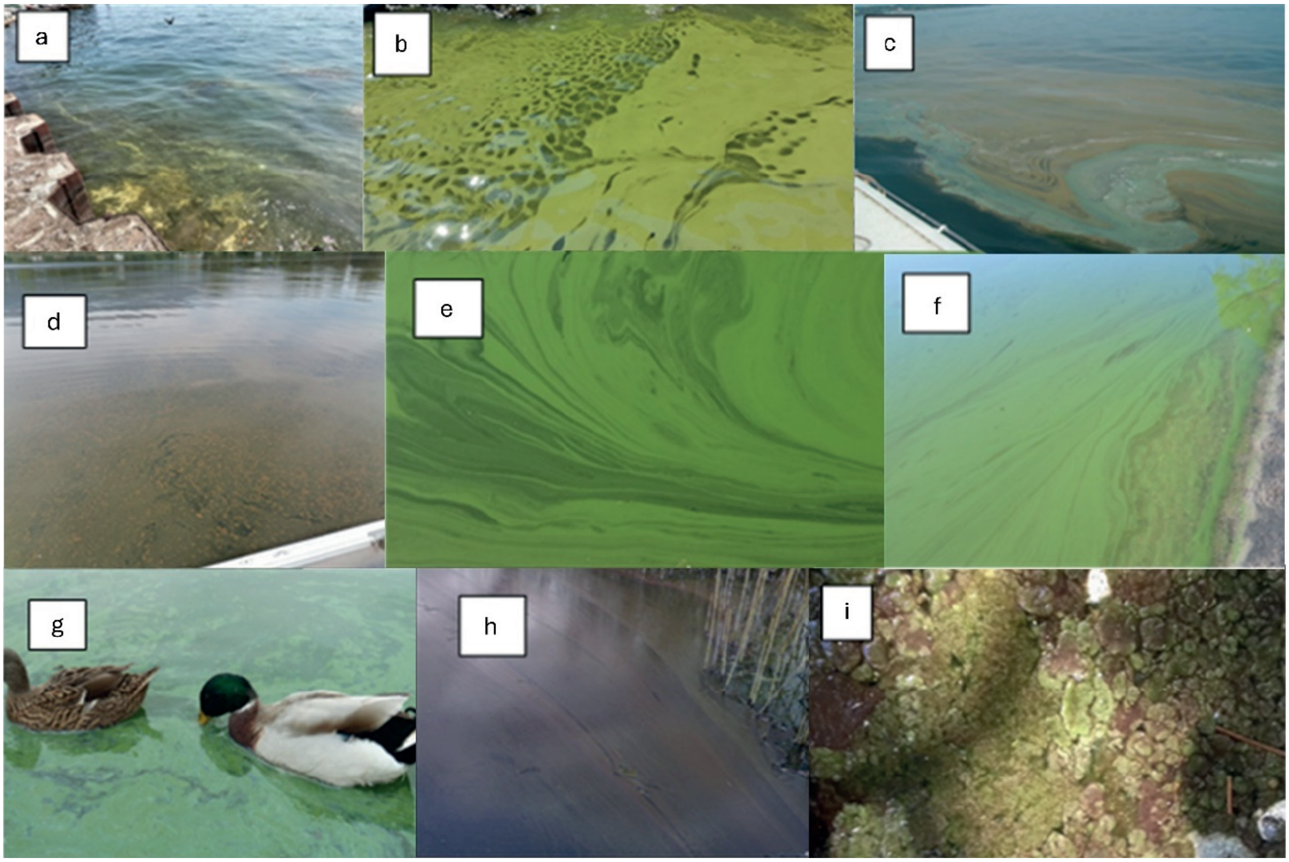

Figure 1. Cyanobacterial blooms recorded in northern Italy during the last 20 years. **a)** Lake Maggiore during a bloom of *Microcystis* sp. **b)** Surface bloom of *D. lemmermannii* in Lake Como. **c)** Summer bloom of *D. lemmermannii* in Lake Garda. **d)** *L. robusta* in Lake Varese. **e)** *W. naegeliana* in Lake Annone-eastern basin. **f)** *A. flos-aquae* in Lake Annone -western basin. **g)** Surface accumulation of "*Anabaena*" sp. in Lake Candia. **h)** *P. rubescens* in Lake Pusiano. **i)** Accumulation of *O. limosa* mats along the shore in Lake Montorfano.

Moreover, washout of dissolved nutrients will be adopted for nutrient dilution in Lake Sartirana.

Some lakes monitored by the EPA are used as drinking water supply and connected to aquifers and wells. In some of these cases (e.g., Lake Pusiano; Figure 1h), in compliance with the drinking water directive, the health authority of Brianza has started assessments aimed at the identification of potentially toxic cyanobacteria and their toxins (specifically microcystins) in some of the water intake points surrounding the lake.

Benthic cyanobacteria are pioneer organisms adapted to extreme conditions. In the Alpine lakes and rivers, these microorganisms are important components of periphyton in benthic substrates and submerged vegetation (Salmaso *et al*., 2024b) and have also been found in high-altitude melting lakes (Metz *et al*., 1998). In our investigations, low nutrient levels and high concentrations of suspended solids were found. For example, floating mats consisting of a mixture of *Spirogyra* sp. and *Oscillatoria limosa* C.Agardh ex Gomont were detected in Lake Montorfano (Figure 1i). ELISA tests conducted by the local health authority detected concentrations of microcystin LR as high as 2.8 $\mu g\ L^{-1}$. In perspective, also considering the high toxic potential of cyanobacterial populations that form mats on a variety of substrates, this component should be closely monitored in lakes and rivers.

Conclusions

The increase in temperature observed in recent years has been accompanied by an increase in the frequency and duration of cyanobacterial blooms. Trophic state paradigms have shifted, due to the warming effect, more prominently in average and small-sized water bodies. Molecular techniques, aimed at the identification of certain species and the detection of genes encoding for cyanotoxins, would be of great help for a more precise bloom characterization. For surveillance and bloom episode characterization, the adoption of multidisciplinary approaches that use high-frequency monitoring (cytometry), remote sensing, and predictive modelling approaches would be also particularly useful, especially in the case of extreme events. In terms of prevention, it is essential to determine nutrient loads and implement measures for their reduction to the lowest possible level, while remediation efforts could be facilitated by the complementary use of predictive modelling.

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