Cyanobacteria and cyanotoxins: a long history of studies in Sardinia (Italy)

Antonella Lugliè,[1] Paola Buscarinu,[2] Claudia Crosio,[3] Cecilia Teodora Satta,[4] Silvia Pulina,[1] Paola Casiddu,[1] Cristina Pittalis,[1] Marco Cherchi,[1] Oriana Soru,[2] Tomasa Virdis,[2] Ciro Iaccarino,[3] Paola Sini,[3] Bachisio Mario Padedda[1]

[1]Department of Architecture, Design and Urban Planning, University of Sassari; [2]Ente Acque della Sardegna (ENAS), Cagliari; [3]Department of Biomedical Sciences, University of Sassari; [4]Agenzia Regionale per la Ricerca in Agricoltura (Agris), Sassari, Italy;

ABSTRACT

The aim of this review is to reconstruct the scientific research on cyanobacteria and cyanotoxins carried out in the artificial lakes of Sardinia (Italy), the second largest island in the Mediterranean Sea, over a period of about 50 years, from the 1970s to the present. The information contained in scientific articles in Web of Science and Scopus databases was analysed using specific keywords. In addition, scientific articles not included in the above-mentioned databases were considered if they were published after a peer review process or if they were available on the website of public institutions. The analysis resulted in the identification of 60 scientific papers containing information on cyanobacteria and/or cyanotoxins, leading to a list of 77 taxa signalled and four groups of cyanotoxins ascertained (microcystins, anatoxin, cylindrospermopsin; β-N-Methylamino-L-Alanine, BMAA). In addition to these results, we point out the research we are carrying out and would like to continue in the future, a future in which it will be extremely important to be able to juggle the expected progressive affirmation of cyanobacteria and related issues. We highlight the difficulty of finding scientific articles published before the 2000s in the most widely used databases (Web of Science and Scopus), despite the fact that the production is rich and cutting-edge compared to the period in which the research was carried out. Finally, we signal the importance of close collaboration between institutions to obtain the results reported, the overcoming of disciplinary boundaries and the development of a collaborative rather than competitive environment between different research groups in dealing with such complex topics.

Corresponding author: Cecilia Teodora Satta, Agenzia Regionale per la Ricerca in Agricoltura (Agris), Sassari, Italy.
E-mail: ctsatta@agrisricerca.it

Key words: Mediterranean basin, microcystin, reservoirs, drinking water.

Authors' contributions: all the authors made a substantive intellectual contribution. All the authors have read and approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Received: 20 May 2025.
Accepted: 5 August 2025.

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Advances in Oceanography and Limnology, 2025; 16:14014
DOI: 10.4081/aiol.2025.14014

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Introduction

This review is one of the results of the meeting "Potentially toxic cyanobacterial blooms in the Alpine area: event framework, study methods and risk assessment" organised by the colleagues from the Research and Innovation Centre of the Edmund Mach Foundation in San Michele all'Adige (Italy) on the December 14, 2023. On this occasion, experts from different institutions and stakeholders met with the aim of exchanging knowledge and to presenting the wide variety of experiences in the approaches for studying cyanobacteria and cyanotoxins, their harmful blooms (CyanoHABs) and impacts, their management, the time scales of the investigations, considering a wide geographical area, not strictly limited to the Alpine area as indicated in the title of the meeting, and more.

This review aims to reconstruct the scientific research on cyanobacteria and cyanotoxins carried out in Sardinia, the second largest island in the Mediterranean Sea, located in the centre of its western basin (Figure 1). In this paper, the reader is taken along a narrative path starting from the beginning of data collection on cyanobacteria and cyanotoxins in Sardinian artificial lakes from the end of the 1970s to the end of the 1990s (during the about first 25 years of research; Past), the progress and evolution of research objectives and applied methods during the following two decades of the new millennium (about other 25 years; Present), up to the prospects and the new scientific interests that we hope to satisfy in the future (Future).

Recount this story, which spans around 50 years, follows the important changes in research approaches and, also, in the communication of results. Gather all this information together could contributes to the knowledge on the ecology of Mediterranean artificial lakes, with a particular focus on cyanobacteria and cyanotoxins, and in supporting the careful management of a fundamental and strategic resource such as water is. It is also a story of intense and long-lasting collaboration among researchers from different public institutions, at national and regional levels, demonstrating the importance of unity and coordination of efforts among different entities when dealing with complex scientific, socio-economic and political issues.

Study area

Sardinia, with a surface area of about 24,000 km^2, is characterised by a typically Mediterranean climate and a coastline of about 1,900 km, equal to a quarter of the total length of the Italian coastline. It is rich in transitional ecosystems such as lagoons (Abbiati *et al.*, 2010; Padedda *et al.*, 2019) and hosts only one natural lake, Lake Baratz (Padedda *et al.*, 2022).

The underground water resources are estimated in about 150÷200 Mm^3, of which about 1/3-1/4 is distributed through aqueducts for domestic supply (Barroccu *et al.*, 2004). The average annual runoff is about 6,100 Mm^3 and more than 2,000 Mm^3 can be stored in a system of artificial lakes, which should ensure the satisfaction of most water needs for domestic, agricultural and industrial purposes.

Sardinia is also among the Italian region with the largest number of artificial lakes (28 with individual maximum potential total volume >5 Mm^3; Table 1). Sardinia has the greatest potential capacity for water accumulation within artificial lakes if the maximum potential volumes of all of them is considered, particularly that of the Lake Cantoniera (Lake Omodeo), the biggest one in Italy. Most of the dams are managed by the Regional Authority through Ente Acque della Sardegna (ENAS) and by Enel Produzione s.p.a. for hydroelectric power production (Table 1).

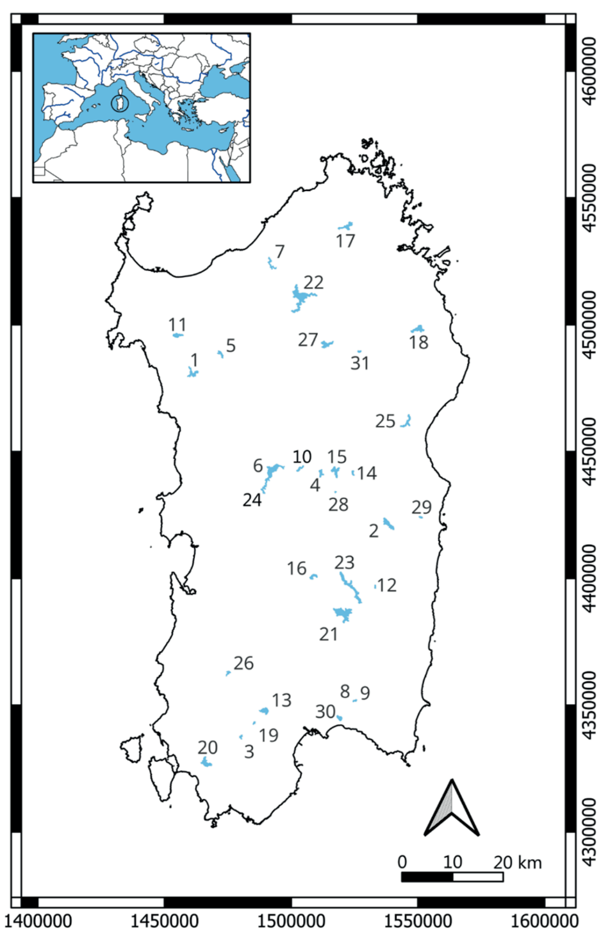

Figure 1. Geographical position of Sardinia in the Mediterranean and location of Sardinian artificial lakes, numbered according to *Supplementary Table 1*.

Materials and Methods

The information found in scientific papers from Web of Science (WoS) and Scopus databases was analysed to reconstruct the history of scientific knowledge on cyanobacteria and cyanotoxins in Sardinia. The following keyword combinations were used: "Sardinia and cyanobacteria", "Sardinia and Cyanophyceae", "Sardinia and cyanotoxins", "Sardinia and phytoplankton", "Sardinia and eutrophication", "Sardinia and trophic status", "Sardinia and reservoirs", "Sardinia and artificial lakes", "Sardinia and each single name of the Sardinian artificial lakes studied". In addition, scientific papers not included in the above cited databases were also considered, if they were published after a peer review process or if they were available on the website of public institutions, such as the Italian National Institute of Health (ISS) (https://www.iss.it/ web/iss-en).

The lists of scientific papers obtained from the databases were refined selecting only those related to our topic. Information on cyanobacterial species reported for each individual lake overtime and data on cyanotoxins, if present, were extrapolated from the selected papers (*Supplementary Tables 1 and 2*). Cyanobacterial species names are also reported in the text and tables as they appeared in the original documents.

The search carried out on WoS and Scopus databases, according to the criteria described above, led to the identification of 36 and 56 papers, respectively, spanning from 1978 to 2024 and from 1987 to 2024 as publication year, respectively. The numbers were considerably reduced by the subsequent verification of the actual correspondence of the papers with the subject of our interest (23 and 18, spanning from 1978 to 2024 and from 1987 to 2024 as publication year, respectively). The information was then organised in *Supplementary Tables 1* (scientific papers from 1978 to 2000) *and 2* (scientific papers from 2001 to 2024). Tables were enriched with peer-reviewed scientific papers and book chapters that were not included in the databases above mentioned, up to, respectively, 31 and 29. Most of them can be downloaded from other websites, particularly, from the "Resources - Proceedingss 1974-2007" section of the website of the Italian Association of Oceanology and Limnology (https://www.aiol.info/atti-volumi-abstract/) and from the ISS website. Figure 2 summarizes the composition of the *Supplementary Tables 1 and 2*.

Table 1. List of Sardinian artificial lakes in operation according to "Registro delle grandi dighe" (https://dgdighe.mit.gov.it:5001/$DatiCmsUtente/registro_dighe/Registro%20dighe%20anno%202023.pdf), their potential total volumes and main use (https://www.dighe.eu/dati/grandi_dighe_italiane.htm).

Name	Main use	Dam management body	Reservoir volume >5 Mm³	Reservoir volume <5 Mm³	Others names in the literature
Alto Temo	I	Enas	91.0		Monteleone Roccadoria
Bau Mandara	H	Enel Produzione S.P.A.		0.3	
Bau Mela	H	Enel Produzione S.P.A.		0.2	
Bau Muggeris	H	Enel Produzione S.P.A.	61.6		Alto Flumendosa
Bau Pressiu	P	Enas	8.5		
Benzone	H	Enel Produzione S.P.A.		2.1	
Bidighinzu	P	Enas	12.6		
Bosa	L	Enas	28.9		Bosa-Monte Crispu
Cantoniera	I	Enas	748.2		Nuovo Omodeo, Tirso
Caprera	P	Marina Militare		0.04	Diga Ferrante
Carru Segau	I	Enas	17.2		
Casteldoria	H	Enel Produzione S.P.A.	8.0		
Corongiu 2	P	Cagliari Municipality		0.4	
Corongiu 3	P	Cagliari Municipality		4.3	
Cucchinadorza	H	Enel Produzione S.P.A.	18.8		
Cuga	I	Enas	34.9		
Donegani	P	Arbus Municipality		0.3	
Flumineddu	I	Enas		1.9	
Genna is Abis	I	Enas	25.4		Cixerri
Govossai	P	Abbanoa S.P.A.		3.1	Fonni
Gusana	H	Enel Produzione S.P.A.	60.3		
Is Barrocus	P	Enas	12.2		
La Maddalena	P	La Maddalena Municipality		0.6	Diga Puzzoni
Liscia	P	Enas	105.1		
Maccheronis	I	Enas	32.3		Posada
Medau Zirimilis	I	Enas	17.2		
Mogoro	L	Enas	10.5		
Monte Pranu	I	Enas	50.0		
Monte su Rei	I	Enas	332.0		Mulargia
Monti di Deu	In	Enas		3.2	
Muzzone	H	Enel Produzione S.P.A.	258.7		Coghinas, Oschiri
Nuraghe Arrubiu	I	Enas	299.3		Medio Flumendosa
Nuraghe Pranu Antoni	I	Enas	9.0		
Pedra e Othoni	L	Enas	20.0		Cedrino
Punta Gennarta	I	Enas	12.6		
Rio Leni	I	Enas	20.0		
Rio Mannu Pattada	I	Enas	76.0		Monte Lerno, Pattada
Rio Olai	P	Abbanoa S.P.A.	16.2		
Rio Perdosu	P	Progetto Esmeralda S.R.L.		0.4	
Rio Torrei	P	Enas		1.0	
Sa Forada de S'acqua	I	Enas		1.4	
Sa Teula	H	Enel Produzione S.P.A.		0.1	
Santa Lucia	I	Enas		3.7	
Santa Vittoria	I	Enas		1.5	
Simbirizzi	I	Enas	30.3		
Sinnai	P	Acqua Vitana S.P.A.		0.2	
Sos Canales	P	Enas		4.3	
Surigheddu	I	Enas		1.9	
Traversa Rio Minore	I	Ittiri Municipality		0.1	Rio Minore
Total potential reservoir volume			*2416.8*	*30.9*	
Number of reservoirs			*21*	*28*	

I, irrigation; H, hydroelectric; P, potabilization; L, lamination; In, industrial.

The past: starting with limnological investigations of Sardinian artificial lakes up to the first cases of toxic cyanobacteria blooms

The number of scientific papers documenting research activities in the "Past" is 31. Among these, five found in Scopus, four in WoS, and 23 from "Other sources" (Figure 2). The selected papers regard 30 artificial lakes (*Supplementary Table 1*).

The first knowledge on the presence of cyanobacteria in Sardinian aquatic environments dates to the second half of the 1970s, when the first studies were carried out on phytoplankton for evaluating the trophic state of artificial lakes and coastal lagoons. This new knowledge was the result of collaboration between the nascent ecology group at the University of Sassari, led by Professor Nicola Sechi, and the Italian Institute of Hydrobiology in Pallanza (now Consiglio Nazionale delle Ricerche – Istituto di Ricerca Sulle Acque, Verbania headquarters), at that time led by Professor Livia Tonolli. Almost all the Sardinian artificial lakes investigated were eutrophic to hypertrophic, *i.e.* in a state that may compromise all potential water uses (Sechi and Lugliè, 1992). The impact on drinking water could be particularly serious, especially due to the dominance of potentially toxic cyanobacterial species and their impact on functioning of potabilization plants. Sechi (1992), analysing data from eight potabilization plants, affirmed that plants that treat eutrophic waters with high phytoplankton cell densities, especially cyanobacteria, do not behave as "safe" systems, because their raw water composition varies, precluding a single, definitive operational set-up. The quality of the raw lake water, such as the type and cell density of phytoplankton, is constantly changing and the setups should be adjusted in real time. However, this is a challenge because the biological, biochemical and chemical process implications of purifying waters with high phytoplankton cell content are numerous, difficult and variable. Sechi (1992) also highlighted that many aspects cannot be addressed because the ecology, physiology and biochemistry of phytoplankton components are not sufficiently known, essential prerequisites for predicting the interference mechanisms at the various stages of purification. In that paper, *Microcystis aeruginosa* (Kützing) Kützing, *Oscillatoria rubescens* De Candolle ex Gomont (=*Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek), *Anabaena* species (=*Dolichospermum* species) and *Aphanizomenon* species were reported as those capable of creating the greatest problems in the water purification plants. In 1985, following blooms of *O. rubescens* (Sechi and Lugliè, 1987 1989), studies started in Lake Mulargia (dam of Monte su Rei) to investigate the phytoplankton, including

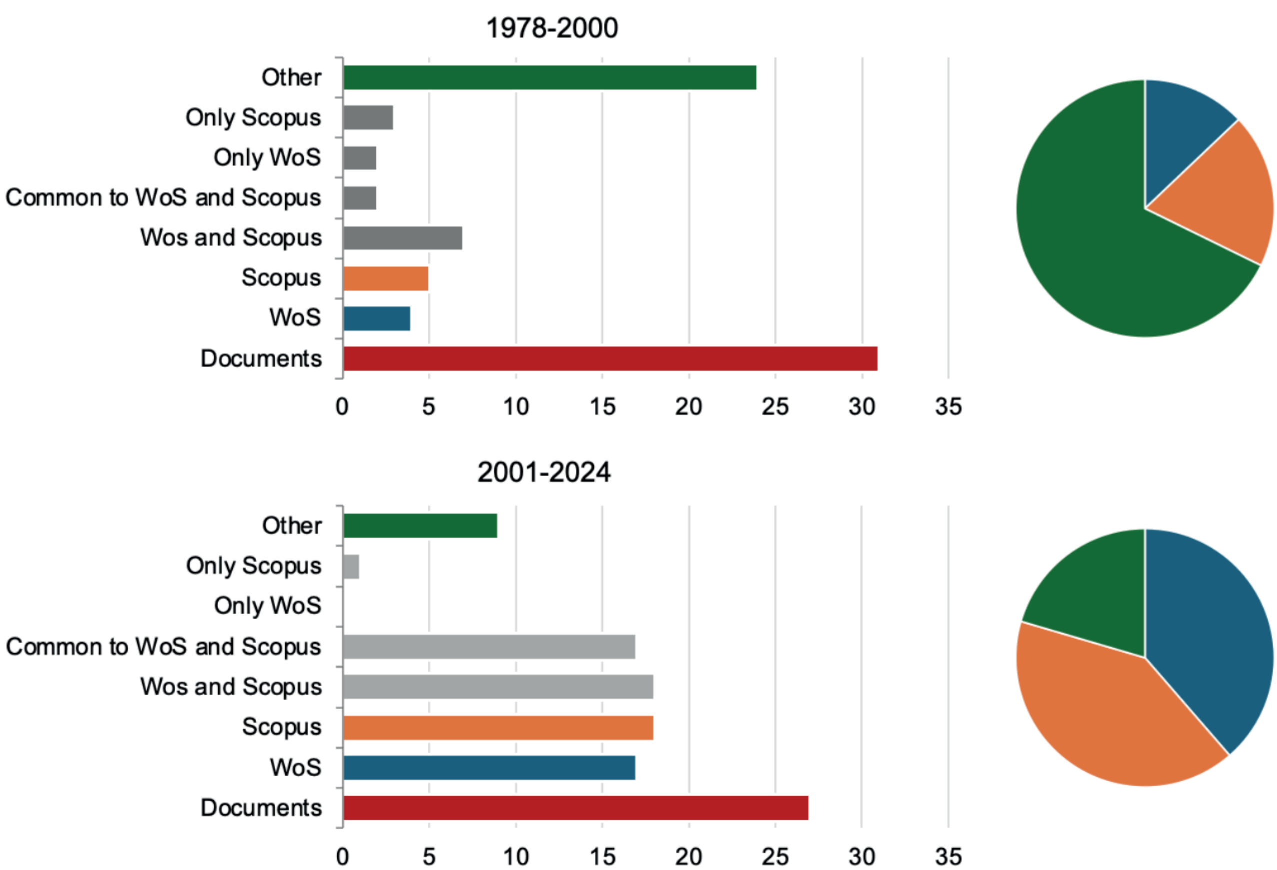

Figure 2. On the left, number of scientific documents present in different online platforms and databases considered in this review in the periods 1978-2000 and 2001-2024. On the right, the aerograms show the different online archives where scientific documents can be found in the two periods considered, respectively.

cyanobacteria, and removal rates at different treatment stages in the plant receiving raw water from this lake, to have a constantly updated picture of the functioning of the plants.

It was because, in the early months of 1985, *O. rubescens* had caused a malfunction of the water treatment plant, resulting in insufficient removal of trichomes, which became visible in the drinking water, causing public alarm (Contu *et al*., 1987; Loizzo *et al*., 1988; Sechi, 1992). On this event, which involved two interconnected artificial lakes, Lake Flumendosa or Medio Flumendosa (dam of Nuraghe Arrubiu) and Lake Mulargia, a toxic cyanobacterium was reported for the first time in Italy (Volterra *et al*., 1986; Loizzo *et al*., 1989) and Oscillatoriaceae other than *Oscillatoria aghardii* Gomont (=*Planktothrix agardhii* (Gomont) Anagnostidis & Komárek) were found to be toxic due to the ascertainment of microcystin-like compounds during their blooms (Bruno *et al*., 1992).

It was also the beginning of the collaboration between the ecology group of the University of Sassari, the regional entity that managed artificial lakes interested by *O. rubescens* blooms (Ente Alto Fumendosa – EAF; after the approval of the Regional Law n. 19/2006, ENAS) and the Italian National Institute of Health (ISS). These collaborations have been strengthened in the following decades. Throughout the second half of the 1980s and the following decade, toxicities in natural samples from Sardinian lakes were reported (Loizzo *et al*., 1989; Bruno *et al*., 1992; Bruno *et al*., 1994) with new information on toxins produced by different species: *M. aeruginosa* in Lake Liscia, *Oscillatoria tenuis* C.Agardh ex Gomont and *O. rubescens* in Lake Simbirizzi, *O. tenuis*, *Oscillatoria mougeotii* Bory (=*Planktothrix mougeotii* Anagnostidis & Komárek) and *O. rubescens* in Lake Medio Flumendosa. Further, Bruno *et al.* (1994) identified for the first time Anatoxin-a (ANA-a) in natural samples dominated by *Anabaena planctonica* Brunnthaler (=*Dolichospermum planctonicum* (Brunnthaler) Wacklin, L.Hoffmann & Komárek) from lakes Mulargia and Flumineddu.

The present: enlarging insights on cyanobacteria and cyanotoxins adding innovative study approaches

The number of scientific papers, documents and research activities is slightly smaller in the "Present" than in the "Past". Of the 27 recognized scientific papers, 18 are reported in Scopus and 17 in WoS. The other nine scientific papers came from "Other sources" (Figure 2). Papers regard 20 artificial lakes (*Supplementary Table 2*).

From 2001 to the present day, innovative study approaches have accompanied the traditional ones and further public institutions joined to the former in research activities (e.g. Istituto Zooprofilattico Sperimentale della Sardegna - Experimental Zootechnic Institute of Sardinia in Stefanelli *et al*., 2017; Istituto per il Rilevamento Elettromagnetico dell'Ambiente, CNR-IREA, in Bresciani *et al*., 2019). Examples of innovative studies include the integration of limnological standard methods and remote sensing techniques for developing efficient approaches for monitoring lake water quality, including CyanoHabs (Bresciani *et al*., 2012; Bresciani *et al*., 2019); the development of mathematical models to define synthetic indices for classifying water quality providing tools for evaluating the impact of cyanobacteria on water-allocation policy for different water uses (Sulis *et al*., 2014); the determination of cyanotoxins using the rapid Enzyme-Linked Immunosorbent Assay (ELISA) technique and more complex Liquid Chromatography/Mass Spectrometry (LC/MS) methods (Messineo *et al*., 2009; Mariani *et al*., 2015b; Lugliè *et al*., 2017; Stefanelli *et al*., 2017); the study of the molecular interaction between exposure to cyanotoxins and the onset of Neurodegenerative Diseases (NDs), using *in vitro* and *in vivo* models (Sini *et al*., 2021; Sini *et al*., 2024).

Messineo *et al*. (2009) showed a widespread occurrence of a broad spectrum of cyanotoxins in 28 Italian lakes, 15 of which were Sardinian. Microcystins (MC and its variants, MCs) were the most abundant in the Sardinian samples. The authors also reported the first detection of Cylindrospermopsin (CYN) and confirmed the presence of ANA-a in Sardinian lakes, coinciding with the observation of *Aphanizomenon ovalisporum* Forti (= *Chrysosporum ovalisporum* (Forti) Zapomelová, Skácelová, Pumann, Kopp & Janeček = *Umezakia natans* M.Watanabe) in Lake Monteleone (dam of Alto Temo) and *Aphanizomenon* spp. in Lake Mulargia, respectively. *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya & Subba Raju (= *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera *et al*.), one of the best-known potential CYN-producing species, had already been reported in the Lake Cedrino (dam of Pedra e Othoni) since 2004 (Manti *et al*., 2005), but without the simultaneous detection of CYN.

Stefanelli *et al*. (2017) investigating Lake Alto Flumendosa (dam of Bau Muggeris), reported highly significant linear regressions between *P. rubescens* and the sum of the demethylated MC variants, and between *Microcystis botrys* Teiling and the sum of MC-LR and MC-LA, also when co-occurring. In this study, MCs were always below the 1 $\mu g\ L^{-1}$ MC-LR threshold set by the World Health Organization (WHO) (2004) for drinking water. Instead, Mariani *et al*. (2015b), evaluating the abundance of cyanobacteria and MCs in four Sardinian artificial lakes characterised by different trophic status, detected MCs in 71% of the analysed samples and concentration >1 $\mu g\ L^{-1}$ MC-LR was found in almost half of the samples, up to about the 90% in samples from Lake Pattada (dam of Rio Mannu Pattada; eutrophic) but only once in Lake Torrei (oligo-mesotrophic). They found the most important species (*Planktothrix agardhii–rubescens* group, *Aphanizomenon flos-aquae* (L.) and *D. planctonicum*) were accompanied by others potential MCs producers not yet reported in the floristic lists of cyanobacteria in Sardinian artificial lakes (*Aphanizomenon klebahnii* (Elenkin) Pechar and Kalina, *Dolichospermum macrosporum* (Klebahn) Wacklin, Hoffmann and Komárek, and *Dolichospermum viguieri* (Denis and Frémy) Wacklin, Hoffmann and Komárek), emphasizing the importance of long-term studies for the early detection of biological community changes, which is fondamental when the changes involve harmful species.

Analysis of long-term cyanobacteria dynamics performed by Mariani *et al*. (2015a) and Pulina *et al*. (2016; 2019) indicates significant changes at the order level (Chroococcales, Nostocales and Oscillatoriales) and in specific seasons (particularly summer) in lakes Bidighinzu (DEIMS-ID https://deims.org/3707cf71-7e04-41e3-8afc-518b293f6c07) and Temo (DEIMS-ID https://deims.org/5bd7ec0b-8215-4764-8f4a-9b1d42c95e24), both belonging to the Italian (LTER-Italy), European (LTER-Europe) and International (ILTER) Long Term Ecological Research networks. Further, beyond the taxonomic changes, trends highlight that small-sized species (<5 µm) have significantly increased. Toxicity of small-sized cyanobacteria remains poorly studied, in contrast to the larger-sized (>5 µm) cyanobacteria, while the number of reports concerning their presence in ecosystems is increasing. Their presence can be highly hazardous to people and other organisms representing an emerging issue (Jakubowska and Szeląg-Wasielewska, 2015). Stefanelli *et al.* (2017), based on the molecular analysis of the mcyB+ gene in *P. rubescens*, indicated the presence of a persistent toxic population in Lake Alto Flumendosa. Luglìè *et al*. (2017) reported the first data on β-N-Methylamino-L-Alanine (BMAA), which has not yet been thoroughly investigated in Mediterranean artificial lakes. BMAA was positively assessed by ELISA in natural samples from Lake Bidighinzu and in cell cultures of *Microcystis aeruginosa* (Kützing) Kützing and *Dolichospermum flos-aquae* (Brébisson ex Bornet and Flahault) P. Wacklin, L. Hoffmann and J. Komárek. Furthermore, Sini *et al*. (2024) to explore the complex interplay between genetic and environmental factors in NDs including Parkinson's disease and Amyotrophic Lateral Sclerosis, exposed cell systems to crude extracts of a BMAA positive strain of *M. aeruginosa* isolated from the Lake Coghinas, highlighting a significant increase in specific stress pathways and an impairment in autophagic processes.

The future: needs for knowledge and scientific curiosity to be satisfied

Our experience in the study of cyanobacteria and cyanotoxins in Sardinia helps us to better understand which research objectives we consider useful to support future management decisions on a precious resource as water. Indeed, although since the end of the 19th century Sardinia has gradually been equipped with a complex infrastructure system to cope with the water scarcity typical of the Mediterranean climate (García-Ruiz *et al*., 2011; Noto *et al*., 2023) and with the geological, geomorphological and hydrological characteristics of the island, in recent decades the reduction in the availability of water resources has become increasingly evident (Montaldo *et al*., 2024). The increase in temperature and the decrease in rainfall, both clear effects of the ongoing climate change, will continue to intensify and will make the crises in the system more frequent and more severe, making it harder to provide adequate responses to the various demands, from agriculture to drinking water supply to the development of tourism (García-Ruiz *et al*., 2011). If we add the purely quantitative aspect to the qualitative one, the picture becomes dramatic. Among the qualitative aspects, cyanobacteria and their cyanotoxins are of primary importance. Their widespread presence has been well documented in Sardinia since the 1970s (as referenced in this article) and urgently requires the development of tools to enable appropriate management approaches. It is important to understand that the problem is not purely technical, in terms of the amount of water to be provided, but it also requires a holistic view and an understanding of its ecological complexity. Despite the great progress, we still do not know what environmental thresholds favour one species over another, when and how toxin production is stimulated, which species produce which toxins, what the consequences are for other living organisms and, inevitably, for humans (Huisman *et al*., 2018). We have clear evidence of the important role played by certain environmental factors and processes, such as nutrients, eutrophication, water temperature, light, and the hydrodynamics of water masses (Huisman *et al*., 2018; Zepernick *et al*., 2022). However, considering all these factors together, as well as their continuous and unpredictable changes, remains a challenge. The impact of the emergence of problems related to cyanobacteria and cyanotoxins, which until a few years ago were of interest only for freshwater ecosystems, is also beginning to be felt across the continuum of continental, transitional and marine ecosystems. For example, it is affecting animal production and food chains due to the possibility of toxins accumulation with the onset of human health problems and ecosystems in general (Luglìè *et al*., 2015; Zepernick *et al*., 2022). Therefore, at least some aspects are of particular interest to us, and we want to continue with what we have already started and with future research based on the ecosystem approach (Secretariat of the Convention on Biological Diversity, 2004) and the One Health approach, which are in any case closely linked. The One Health High Level Expert Group (OHHLEP) formulated the following synthetic definition in 2022: "One Health is an integrated and unifying approach that aims to sustainably balance and optimise the health of people, animals and ecosystems. It recognises that the health of humans, domestic and wild animals, plants and the environment as a whole (including ecosystems) are intimately linked and interdependent". Accordingly, Messineo *et al*. (2024) reported that "In a One Health approach, information about harmful algal bloom exposures and health effects support efforts to detect these events and mitigate and prevent associated illnesses".

In this spirit, we would like to achieve the following objectives.

To improve technological monitoring

The integration of satellite monitoring techniques with the standard *in situ* approaches is a fundamental way to predict the occurrence of cyanobacterial blooms, to have early warning signals, to follow their evolution and to have the best management options. This objective is supported by the presence in 18 lakes in Sardinia (the only region in Italy) of automatic systems consisting in a hydrological measuring station equipped with a multiparameter probe with sensors for the daily acquisition along the vertical profile of data of temperature, pH, dissolved O_2, conductivity, chlorophyll *a* at predetermined depths. The flexibility of the system allows both temporal and spatial sampling to be planned. The data acquired in this way are transmitted to a local centre and from there, via a satellite system, to the general acquisition and processing centre at the ENAS headquarters. The information obtained can be visualised in real time whenever necessary. The data obtained in this way are verified and validated by periodic *in situ* sampling and laboratory analysis using standard methods. Satellite Remote Sensing (SRS) has become a fundamental tool for monitoring phytoplankton blooms in lakes and lagoons, as already demonstrated also in Sardinia (Buscarinu *et al*., 2003; Bresciani *et al*., 2012; Bresciani *et al*., 2019). This is due to SRS ability to provide large-scale, repeated and synoptic information. Satellite imagery allows the estimation of bio-optical parameters such as chlorophyll *a* concentration, turbidity and the presence of dissolved coloured organic matter. These parameters serve as indicators of phytoplankton biomass and water quality (Topp *et al*., 2020). A key challenge in SRS is the ability to distinguish between different phytoplankton groups, particularly cyanobacteria. This is crucial for managing the risk of CyanoHABs and implementing appropriate management measures (Shi *et al*., 2019; Igwaran *et al*., 2024). In recent years, several approaches have been developed to address this challenge. One is based on spectral analysis of satellite images, which allows the identification of unique "spectral signatures" for different phytoplankton species or functional groups (Nair *et al*., 2008). However, this approach requires careful calibration with field data and can be significantly influenced by environmental factors such as the presence of other substances in the water. Another promising strategy is the use of high-resolution multispectral data, which provide more detailed information on the spectral composition of the water signal. These data, combined with bio-optical models, can improve the ability to distinguish phytoplankton groups (Xi *et al*., 2020). In addition to spectral and multispectral techniques, specific algorithms have been developed to detect cyanobacteria based on their ability to produce characteristic pigments such as phycocyanin. These algorithms can be effectively applied to satellite imagery to map the distribution and abundance of cyanobacteria in water bodies (Lyu *et al*., 2016). Through national and international collaborations, such as the one active between the University of Sassari and the Remote Sensing and Geographic Information Systems Unit of the Asian Institute of Technology, the integration of satellite data with other sources of information is being developed in Sardinia. These sources include *in situ* data, hydrodynamic models and meteorological data for a more complete understanding of the processes governing the development of microalgal blooms. The use of artificial intelligence and machine learning techniques to analyse satellite data will allow the identification of complex patterns and improve the ability to predict blooms.

To improve knowledge of cyanobacteria biodiversity and of their ecological niches

We aim to contribute to the knowledge of cyanobacterial biodiversity in Mediterranean artificial lakes using innovative methods, such as molecular tools (Humbert *et al*., 2010), combined with the standard microscopic methods. Analyses can be carried out on freshly collected natural samples, on fixed water samples and preserved in our archived phytoplankton collections, and on sediment samples collected in lakes. Indeed, both archived samples and sediments can be considered as a biodiversity treasure trove for cyanobacteria (Salmaso *et al*., 2015; Legrand *et al*. 2019). The study of sediments, using the mentioned methods and with germination experiments, can reveal the presence of undetected species in the water column by microscopic observation alone, allowing to erase boundary between domains that are in continuity but considered and studied as separate (Satta *et al*., 2010, 2013, 2014; Boero, 2022). Further, the establishment of cellular cultures from isolates from natural samples, both collected from the water column and from the sediments, may allow to study in detail their taxonomy, toxicity and ecology. Cell cultures can also enable setting up laboratory manipulative experiments to understand the intriguing ecological success of cyanobacterial species in relation to their biological characteristics compared to those of other phytoplankton components, and the abiotic factors that may favour one species over another (niches), including in relation to toxin production (Zepernick *et al*., 2022). For example, the detection of CYN still requires further investigation (Antunes *et al*., 2015). Indeed, over the years, a potential CYN-producing species such as *C. raciborskii* has shown its typical ability to spread (Antunes *et al.*, 2015; Burford *et al.*, 2016), gradually appearing also in several Sardinian lakes (personal communication, P. Buscarinu and O. Soru) in addition to Lake Cedrino (Manti *et al*., 2004). On these occasions, the simultaneous presence of CYN was detected in water samples by ELISA (personal communication, P. Buscarinu and O. Soru), on the contrary to what was previously observed. The data supports the need for comparative studies on ecological conditions and on phylogenetic relationships among European wild populations of this species to explain their difference in toxins production (Manti *et al*., 2005; Meriluoto *et al*., 2017).

To investigate the link between human health issues and cyanotoxins

In this respect, we aim to continue the multidisciplinary experience started recently, based on the promising results obtained (Sini *et al*., 2024), and further investigate the link between the documented genetic predisposition of the Sardinian human population to the onset of NDs (Borghero *et al*., 2014) and the use of drinking water from eutrophic lakes dominated by toxic cyanobacteria. Thanks to the cyanobacterial cell cultures established as suggested in the previous point, we intend to continue biomolecular experiments by expanding the range of cyanotoxins tested, to determine their possible interactions.

Again, cyanobacteria may harbor Antibiotic Resistance Genes (ARGs) and could promote the spread of ARGs in bacteria due to the significant contribution of mobile genetic elements located in genera such as Microcystis, Planktothrix and Aphanizomenon (Wang *et al*., 2020; Volk and Lee, 2023; Manganelli *et al*., 2024), which are among the most abundant genera also in Sardinia. In addition, rising temperatures may favour the transfer of genetic information among bacteria, increasing horizontal gene transfer rates for some bacterial taxa, which in turn may contribute to the spread of Antibiotic Resistance (AR) in the environment, a major environmental and public health problem. The first data on AR have been collected in Lake Bidighinzu thanks to the project of "a warmer Future world: effects on plankton commUnities and paThogens in Mediterranean vUlneRable Ecosystems (FUTURE)" (PRIN 2022), and the processing of the results may contribute to the assessment of antibiotic resistance in Sardinia and possible links with cyanobacteria.

To continue to study changes over long-time scales (LTER) and make the data available to different users

The presence in Sardinia of the site IT10-Lake Ecosystems of Sardinia - Italy (Padedda *et al*., 2021), consisting of six artificial lakes (Bidighinzu, Cedrino, Cuga, Monte Lerno, Sos Canales, Temo), allows the use of "privileged observation sites" to monitor natural and human-induced ecological changes. LTER sites are a valuable source of ecological knowledge thanks to the collection of long-time data series, and allow the comparison and collaboration between researchers, the training of future ecologists, and the sharing of research findings to the public (https://www.lteritalia.it/wordpress/?page_id=1267). In this case, given the artificial nature of our lakes, the added complexity of their management is implicit. LTER lakes can be considered as sentinels of changes in biological communities, including the composition of cyanobacterial species and the risks posed by their toxicity. ENAS has been regularly collecting data on cyanobacteria and cyanotoxins, especially MCs and CYN, in several lakes in Sardinia for about 15 years. In addition, trends in other features, such as cell size and/or morphology (trait-based approaches; Rosati *et al*., 2017), could be studied to reveal adaptation strategies of cyanobacteria and phytoplankton, in general, to local (e.g. nutrients) and global (e.g. global warming and climate change) scenarios.

Therefore, it is a priority to make the best use of existing and future data. Good data management is a performing way for maximizing knowledge discovery and innovation, and the subsequent data and knowledge integration and reuse by the community after the data publication process (Wilkinson *et al*., 2016). Nowadays, processing data into scientific articles and organizing them according to the FAIR principles (Findability, Accessibility, Interoperability, and Reuse of digital assets) (Wilkinson *et al*., 2016) is of paramount importance.

Conclusions

This review has made it possible to reconstruct the history of the studies carried out on cyanobacteria and cyanotoxins in Sardinia, with a list of 77 taxa signaled and four groups of cyanotoxins ascertained (MCs, ANA-a, CYL, BMAA), and to define the current framework of knowledge acquired over a period of about half a century. We have also focused on topics that we would like to include in our scientific activities, without abandoning the ones we already have, topics that represent novelties and challenges for the future. A future in which it will be extremely important to be able to juggle the expected progressive affirmation of cyanobacteria and related issues.

In addition to these results, the reading and processing of the scientific articles considered, also allows us to reflect on other aspects that we believe deserve to be considered, among which: i) the difficulty of finding scientific articles published before 2000 in the most widely used databases (WoS and Scopus), even though the production is rich and cutting-edge compared to the period in which the research was carried out; ii) the importance of close collaboration between institutions and between apparently very distant research groups, with a transdisciplinary approach (Lawrence and Després, 2004) - the involvement of other research groups, institutions and stakeholders is, in our opinion, the basis for approaching old and new topics of interest in cyanobacteria and cyanotoxins with renewed curiosity and innovation; and iii) the creation of collaborative relationships that transcend the boundaries of highly competitive and disciplinary contexts fosters the indispensable enrichment of expertise in addressing such complex issues.

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Online supplementary material:

Supplementary Table 1. List of Sardinian artificial lakes with their identification number for Figure 1, name, study periods and lake names as reported in the scientific documents, cyanobacteria genera or species as reported in the scientific documents, presence of cyanotoxins data, and their references along the period 1978-2000.

Supplementary Table 2. List of Sardinian artificial lakes with their identification number for Figure 1, name, study periods and lake names as reported in the scientific documents, cyanobacteria genera or species as reported in the scientific documents, presence of cyanotoxins data, and their references along the period 2001-2024.