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Analysis, levels and seasonal variation of cyanotoxins in freshwater ecosystems

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ABSTRACT

Nutrient over-enrichment in freshwater environments, together with the on-going climate change, favour the toxin-producing cyanobacteria bloom. Human health hazard may arise from drinking contaminated water. Additionally, cyanobacterial blooms affect other economic areas such as tourism, recreation, commercial fishery, water management and monitoring. Nowadays there is a scarcity of information on seasonal variations of cyanotoxins in various regions. Understanding of historical trends and seasonal variation patters is a foundation for forecasting and will help to develop effective water management strategies.

This review gives an overview of cyanotoxins' analysis and levels in freshwater environments with particular emphasis on seasonal variations in Europe. Recent analytical approaches are discussed and the seasonal patterns for three major European climate zones (Mediterranean, continental, and Atlantic) were distinguished. Additionally, data from multi-year studies showed a tendency of increasing cyanotoxins' levels.

1. Introduction

Climate change, eutrophication associated with anthropogenic activities causing oxygen depletion and nutrient over-enrichment (such as nitrogen and phosphorus) are leading the increasing number of episodes of harmful cyanobacteria bloom (CyanoHAB) [1,2], even in geographical areas where it had not occurred before [3,4].

Cyanobacteria are prokaryotes and components of regular periphyton formation. They are photosynthetic microorganisms (except for the uncultured nitrogen-fixing cyanobacterium, UCYN-A) that are ubiquitous in marine and freshwater environments. Cyanobacteria produce cyanotoxins as a secondary metabolite, which vary in structure and harmful properties, among them: hepatotoxins, dermatoxins, neurotoxins and cytotoxins [5]. Cyanobacteria can be adapted to extreme environmental conditions thanks to their capacity of nutrient storage, nitrogen fixation, buoyancy, and the formation of resting cells known as akinetes. Commonly, phosphorus is stored as polyphosphate and nitrogen as cyanophycin or phycobilin pigments [6]. Irrespective of the fact that N-fixation from N_2 is energetically expensive, it provides a competitive advantage in N-deficient conditions [7]. Formation of akinete by some cyanobacteria species ensures their survival during unfavourable conditions such as lack of light, nutrients scarcity, changes in temperatures and desiccation. The akinete outlives in the bottom sediments due to their metabolism and germinate when conditions are favourable [7]. While buoyancy allows the developed bacteria access to well-lit surface waters via the presence of gas vesicles [7].

The most well-studied cyanotoxins are microcystins (MCs) and nodularins (NODs). These are cyclic peptides with hepatotoxic activity [5,8]. Nowadays, more than 246 isoforms of MCs have been detected [9]. MCs are produced by different cyanobacteria genera, such as *Microcystis*, *Anabaena*, *Plankthotrix*, *Aphanizomenon*, *Anabaenopsis*, *Nostoc*, *Rivularia* and *Fisherella* [8,10]. NOD occurs in several variants: two demethylated variants, one with D-Asp instead of D-MeAsp, and the second one with DMAdda instead of Adda. NOD is synthesized by cyanobacterium *Nodularia spumigena*, which is found in brackish waters [8]. Cylindrospermopsin (CYN) is a tricyclic alkaloid, possessing a guanidine moiety combined with hydroxymethyluracil, which has been demonstrated to be hepatotoxic, cytotoxic, dermatotoxic and possibly carcinogenic [11]. At first, CYN production was associated exclusively with Cylindrospermopsis raciborskii, however, the list of potential CYN

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producing species is expanding and includes: *Umezakia natans*, *Anabaena bergii*, *Anabaena lapponica*, *Anabaena planctonica*, *Aphanizomenon flos-aquae*, *Aphanizomenon gracile*, *Aphanizomenon ovalisporum*, *Lyngbya wollei*, *Raphidiopsis curvata*, and *Raphidiopsis mediterranea* [8,11]. Finally, anatoxins are neurotoxins, which can be classified into three groups: anatoxin-a (ANA-a), its structural homologue homoanatoxin-a (homoANA-a), and the unrelated anatoxin-a(s) [12]. ANA-a and homoANA-a are bicyclic secondary amines [13]. ANA-a is produced by different species of *Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Microcystis*, *Oscillatoria*, *Planktothrix* and *Raphidiopsis* genera. And homoanatoxin-a is synthesized by some members of *Oscillatoria*, *Anabaena*, *Raphidiopsis*, and *Phormidium* genera [13].

Main routes of human exposure to cyanotoxins are drinking water, recreational water use, and consumption of food in which toxin may have accumulated [11]. Due to their dangerous biological activity, several measures have been performed to regulate their amount in the environment and drinking water. World Health Organization (WHO) has thus appointed a provisional guideline value for total MC-LR in drinking water of 1 μg/L, and this standard is accepted in most of the countries in the world [14]. US National Centre for Environmental Assessment suggested lowering drinking water guideline value to 0.1 μg/L [15]. Combined with human health impact, cyanobacterial blooms affect other economic areas such as tourism, recreation, commercial fishery, water management and monitoring, causing synergic implications [16].

Nowadays important piece of research is done to study the presence of cyanotoxins in the environment, and in the development of analytical methods and approaches to assess them at ultra-trace levels. Nevertheless, little quantitative information is available on temporal variations in Europe. However, understanding historical trends is crucial as it reduces uncertainty and provides a solid foundation for forecasting. Distinguishing seasonal trends of cyanotoxins will promote the development of effective water management strategies for resource distribution and establishing objectives for different seasons and climatic zones [4]. The main goal of this manuscript is to review the current analytical methods for the determination of cyanotoxins in the aquatic environment, as well as the temporal variations of cyanotoxins in freshwater systems by distinguishing patterns, peaking periods and levels in the three main European climatic zones: the Mediterranean, continental, and Atlantic. Additionally, levels of cyanotoxins within several years were considered in order to evidence the influence of climate change.

2. Analytical methods

The evaluation of the occurrence and the risks of exposure to cyanotoxins requires robust, straightforward, and sensitive analytical approaches for their identification and quantitation in the aquatic environment, in particular in drinking water reservoirs. Moreover, to face extensive monitoring studies, these methods should be cost-effective and rapid. The main analytical approaches used for the evaluation of cyanotoxins in water can be divided into two major groups, the biological methods and those based on analytical chemistry.

Biological approaches such as enzyme-linked immunoassays (ELISAs) allow high-throughput, fast, and moderately costly analysis. The reported limits of detection (LODs) applying ELISA are in general between 0.04−0.1 μg/L [17–19]. Moreover, different ELISA kits are commercially available from different companies such as Abraxis and Beacon [20]. Another essay format is the antibody-based test strips which provide a robust, cheap, and simple method for initial risk assessment [21]. Nevertheless, the sensitivity of this type of assays is in general poor in comparison with conventional ELISA with LOD for example for MC about 10 mg/L, which is the regulatory level for recreational waters [21], but not enough for the drinking water regulation. ELISA approaches have been very much used for environmental monitoring studies during the last decade. However, suffer from matrix effects and cross-reactivity, which can lead to overestimation [14,22]. For class-antibodies, the correspondent assays cannot differentiate between structurally related compounds but with different affinities for the different variants. For example, Birbeck et al. [23] noticed that results obtained with ELISA show higher MCs concentrations in comparison to high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). It was due to cross-reactivity between different MCs variants and detection of MCs degradation products. Additionally, the calibration was non-linear. In another example, Gurbuz et al. [24] observed a false positive result of MCs in fish obtained by ELISA, which could be because of cross-reactivity of MCs and their detoxification products formed in animal and plant tissues. These findings indicate that results obtained by ELISA should be interpreted carefully due to possible false-positives and the cross-reactivity. Therefore, even they are excellent analytical tools for screening, the results should be confirmed using other methods.

Another important group of techniques for the fast and sensitive detection of target genes present in toxigenic algal species is the quantitative polymerase chain reaction (qPCR), but subsequent forecasting of cyanotoxin production in the water is in general problematic [14].

Other receptors that have been used to develops biological analytical tools to assess cyantoxins contamination are the assays based on the protein phosphatase enzyme inhibition (PPI). This group of assays are can reach LOD of 0.16 mg/L for MCs [3]. But, the major drawbacks are the variations in enzyme purity and the instability of enzyme dimers in solution [26]. Therefore, these assays are primarily used for research purposes instead for regular environmental and water reservoir monitoring studies or for drinking water control [25].

An advanced group of techniques is biosensors [14,20]. Biosensors are based on biological receptors immobilised onto transducers to convert a biological signal in a primary signal. In spite of the research reported during the last decades in this field using as central receptors antibodies (immunoassays) [26], enzyme inhibition, or aptamers [27]. These techniques present as main limitations [20]: (i) the lack of long term stability of biomolecular receptors; (ii) for the immunosensors, the cross-reactivity and the matrix effects that affects immunoassays in general ; (iii) the lack of specificity as in enzyme biosensors; (iv) the lack of commercially available sensors for cyanotoxin analysis, and; (v) the lack of multi-class assays able to specific quantitation of the different congeners.

2.1. Physico-chemical approaches

Among the separation techniques, liquid chromatography (LC) is the technique of choice due to characteristics of cyanotoxins. In spite of some methods that used gas chromatography coupled to mass spectrometry (GC—MS). But, as it can be seen in this application, the oxidation of the Adda fragment contained in the MCs is required as the previous step. LC facilitates the analysis since the oxidation is not required, and it has been used with different detectors. For example, different authors reported the use of LC and diode-array detection (DAD) [24,28–31]. But, during the last decade due to the superior sensitivity and selectivity of mass spectrometry (MS), this is the most common approach. However, these approaches require a sample preparation step, for the extraction and purification of cyanotoxins that is commonly

Table 1
Reported levels of cyanotoxins in surface freshwater.

Country	Sampling point	Sampling period	Toxins	Max levels, μg/L	Peaking period	Reference
Mediterranean						
Italy	Lake Vico	February 2009 – December 2010	MCs	2009: 3.4 2010: 5.205	2009: May and October 2010: February and November	[35]
Italy	Lake Alto Flumendosa	October 2011 – May 2013	MCs	100	May, October	[57]
Spain	Reservoirs Ojos and Cenajo	October 2000 – September 2001	MCs	Ojos: 0.17 Cenajo: 0.085	Ojos: spring, summer Cenajo: summer, autumn	[48]
Spain	Reservoir Rosarito	June – October 2013	MCs, ANA, STXs	MCs: 18.6; ANA 2.1; STXs 0.12	MCs and ANA: September, STXs: July	[43]
Portugal	Reservoirs Alvito, Enxoé, Odivelas and Roxo	May – December2005, April – July 2006	MCs	Alvito: 2.58 Enxoé: 0.63 Odivelas: 0.5 Roxo: 7.2	Enxoé: April Alvito: July, September Odivelas: July, September Roxo: September	[49]
Portugal	Reservoirs Alqueva and Beliche	February, April, June, July, September, November 2011	MCs	0.776	September	[56]
Greece	Lake Pamvotis	October 2007, June 2008, Desember 2008	MCs	Water: 0.0034 Scum: 0.0036	October	[50]
Greece	Lake Pamvotis	January 2008 – February 2009	MCs, STXs	MCs: 19 STXs: 2.1	March, September	[51]
Greece	Lake Marathonas	July 2007 – December 2010	MCs	0.717	February, September-October	[36]
Turkey	Lake Egirdir	April – December 2013	MCs	20.5	April, August	[24]
Turkey	Lake Sapanca	September 2012 – October 2013	MCs	1.522	March	[33]
Continental						
Italy	Lakes Occhito, Pusiano, Lerdo, Garda	April 2009 – December 2012	MCs	Occhio: 7.5 Pusiano: 4.6 Ledro: 1.15 Garda: 0.26	Occhio: April Pusiano and Ledro: November Garda: August	[53]
Italy	Lake Garda	February 2014 – October 2015	ANA	2.2	May	[44]
Italy	Lake Garda	September 2008 – September 2013	MCs	0.23	September	[55]
Poland	Lakes Mytycze and Tomaszne	May – September 2010 and 2011	MCs	Mytycze: 30.68 Tomaszne: 23.62	Mytycze: mid-August - September Tomaszne: July – mid-August	[28]
Poland	Dam reservoir Zemborzycki	May – September 2005 –2011	MCs, ANA	MCs: 22.2 ANA: 14.4	August, September	[29]
Poland	Lake Lubosinskie	July 2006 – March 2008	MCs	71.2	October	[54]
Poland	Lakes Niegocin, Piłwąg and Rekąty	July – September 2007	MCs	0.03	September	[72]
Czech Republic	94 water reservoirs	July – September 2004	MCs	37.0	August, September	[52]
Germany	Lakes Langer See and Melangsee	June – September 2004, April-October 2005	CYN	1.8	Langer See and Melangsee: June and September	[42]
Germany	Lakes Klostersee, Bergknappweiher	May – October 2015 (Klostersee), August – October (Bergknappweiher)	MCs	6.7	Klostersee: October, Bergknappweiher: September	[73]
Russia	Lakes Suzdal and Sestroretskij Razliv	June – October 2010, June – September 2011, May – September 2012	MCs, ANA	MCs: 41.37 ANA: 0.54	August-September	[45]
Oceanic						
France	Lake Aydat	September – October, 2011 – 2013	MCs, ANA	MCs: 0.077 ANA < LOD	September	[46]
France	Reservoir Pen Mur	May 2016 – April 2018	MCs	60	June, September	[59]
Netherlands	Lakes Nuldernauw, Wolderwijd, Zoetermeerse Plas, De Put, De Grote Plas	August 2013	MCs, ANA	MCs: 0.31 ANA: 0.074		[47]
Spain	Reservoir Trasona	January 2006 – December 2010	Cyanotoxins (predicted)	>7000 (predicted)	October	[58]

achieved by solid-phase extraction (SPE) with octadecyl silica (C_{18}) and copolymeric sorbents. Zervou et al., [32] used a combination of polar and less polar cartridges in tandem to extract cyanotoxins from different groups dissolved in water. In this work, the authors used pH > 10.5 to neutralise the charge of the polar toxins such as CYL, ANA-a, and domoic acid (DA). Liquid chromatography coupled to tandem mass spectrometry (MS/MS) has been widely used [23,33,34] because of the high sensitivity and specificity. In general, using these approaches, the LODs are at the low ng/L range. For instance, in different studies of cyanotoxin seasonal variations [33,35,36], the LOD was between 0.3–5.6 ng/L. In general, electrospray ionisation (ESI) is used [14], but some

applications have been based on laser diode thermal desorption-atmospheric pressure chemical ionisation (APCI). For example, Zhang et al. [37], also used the cleavage of the Adda fragment and the subsequent quantification of the 2-methyl-3-methoxy-4-phenyl butyric acid as the oxidation product obtained by ozonolysis. More recently, the techniques based on high-resolution mass spectrometry (HRMS) are increasingly used, because provide excellent selectivity, specificity, sensitivity, and quantitation thanks to the high linear dynamic range. Also, non-target analysis can be performed to evaluate degradation products or to assess the presence of non-targeted toxins. Among these techniques, LC-HRMS using Orbitrap instruments and Matrix assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF-MS) are the most common [38,39]. For example, to determine intracellularly and dissolved in water MCs, the performance of both platforms were compared by Flores and Caixach [40]. Both positive and negative ionisation modes were applied to obtain an extended amount of data used for the identification and confirmation of MCs. Nevertheless, matrix effects expressed as ion-suppression is one of the main limitations of these techniques [41].

3. Levels and seasonal variation of cyanotoxins

3.1. Cyanotoxins in European freshwaters

The most detected group of cyanotoxins, the maximum levels, and the months of the peak season in different European countries grouped by the clime frames are presented in Table 1. However, it is very difficult to compare the results obtained by the different studies because a variety of analytical techniques and sampling protocols were applied. Nevertheless, the available literature provides an overview of the occurrence and the most abundant groups of cyanotoxins in European blooms. As it can be seen in Table 1, the most detected group was MCs, and only some studies CYN [42], ANA [29,43–47] were identified. Even though when different groups of cyanotoxins were analysed, MCs was the dominant group. For analysis of MCs, the methods based on enzyme-linked immunosorbent assays (ELISA) were often chosen because of immunoassays' sensitivity, short analysis time and simpler sample manipulation in comparison with application of chromatography coupled to mass spectrometry [43,48–52]. However, in general, the immunoassays are not selective for the different cyanotoxins species. Among the different MCs, MC—RR, -LR, -YR, and demethylated forms of MCR—R, and -LR were detected the most frequently [24,33,36,47,48,53–58]. For example, a study in the lake Vico showed that the most abundant MC variant was demethylated form of MC—RR that represented more than 95 % of total MCs content [35]. In another study, in the lakes, Occhito, Pusiano, Lerdo and Garda, both demethylated forms of MC—RR and -LR were found the most abundant ones; MC—YR, RR, and LR were also present but in a lower concentration [53,55]. Several studies showed that MC—LR, –RR and -YR were the prevalent toxins [36,48,53,59]. However, the abundances are variable even in the same clime frame, for example, MC—LR ranged from 2.6–74%, MCRR from 3 to 75 %, and of MCYR from 1 to 53 ——% in lakes Ojos and Cenajo. MC—RR was dominating in lake Ojos during autumn and in lake Cenajo during spring which was probably due to the predominating oscilatoriales (Oscillatoria, Lyngbya and Phormidium) [48]. Variation of toxins within the season and differences in their proportions could be associated with a variety of environmental parameters and cyanobacterial biomass.

Based on the data reported in about the last 30 years in Europe, the cyanotoxins were predominant in lakes and MCs were the most frequent toxin in freshwater ecosystems [1]. The second group most frequently found was CYN, in particular in the northern part of Germany, Poland, the Czech Republic, and Serbia, but also some punctual blooms were reported in the southwest part of Spain and Portugal. STXs and ANAs were less represented.

In many cases, reported levels of MCs were higher than the guideline value for total MC-LR in drinking water appointed by WHO. Thus, monitoring during peaking seasons is crucial for prevention of human health hazard.

3.2. Relationship between toxin concentration, environmental parameters and cyanobacterial biomass

Environmental parameters such as temperature [19,51], pH [51], light intensity [42], and nutrient availability [17,30,31,42,51,60,61] influence both the structure and distribution of phytoplankton and the levels of cyanotoxins in freshwater environments [62]. Recently, a European Multi Lake Survey was conducted, where the effects of temperature and nutrients on the variability of cyanotoxins at a continental scale were studied [63]. The results showed that direct and indirect effects of temperature have a higher influence on cyanotoxins' distribution and the toxic potential of the lakes. Furthermore, the excess of nutrients may have a synergic interaction with increased water temperatures and enhance cyanobacterial growth. On the other hand, in another interesting work in freshwater bodies of central Europe [64], the interactions between cyanotoxin producers and degraders have been demonstrated. The positive correlations between the capacity of a community to degrade MC-LR and temperature, pH, chlorophyll-a concentration and the abundance of MC-producers, was established. These facts make it more challenging to develop models, and more comprehensive analyses of the existing correlations are needed to understand the natural mechanisms of MC elimination. Therefore, this is also another factor contributing to the variation between studies. The relationship between cyanotoxins' concentrations, cyanobacteria and the environmental parameters were varying among lake studies. Most frequently, cyanotoxins' levels were positively correlated with water temperature, total nitrogen, total phosphorus and pH [30,31,42,51,61,65]. As reported in Table 1, Italian lakes Garda, Occhito, Ledro, Pusiano and Vico were under a sampling campaign in April and June 2009, April 2010, and between February and May 2011 reported higher values of MCs for Occhito lake. After online search, the authors discovered a report from 1985 in which the nitrogen/phosphorous (N/P) rate (= 49.3) of the entire lake is higher than the normal values, this aimed to a lower sedimentation a phosphorous availability were limitation factors to the lake trophy [66]. However, in 2014 it was found a high concentration of *Plantothrix rubescens* in lakes Occhito, Pusiano and Ledro associated to higher availability of nutrients, particularly phosphorus [53]. Hydrogeological relieves from the previous report confirmed also the high apportion of phosphorous into the Occhito lake that can potentially modify the N/P rate to positive tropism levels. At the same time in several cases, no correlation with nutrients' levels was recorded [35,53]. There is a scarcity of data in some seasonal variation studies, as not always nutrient variations were monitored [23,24,33,49].

In another example, Walls et al. [19] focused their study on the effect of temperature (without confounding influence of nutrients' variation). It was demonstrated that the amount of intracellular toxins released by *Planktothrix agardhii* significantly rises at temperatures which are higher than that of the optimal growing conditions. Intracellular MC levels were reaching maximum when cyanobacterial biomass and cell density were reduced. This study demonstrates that elevated temperatures cause higher levels of cyanotoxins. It should be remarked that in general, the optimum growth temperature for cyanobacteria is higher comparing with most algae. H. Paerl [67] reported that the optima is higher than 25 °C (arriving at circa 33 °C) [68], overlapping with optima for green

algae (27–32.8 °C) but clearly differing from with the one for dinoflagellates (17–27 °C) and diatoms (17–22 °C). The optimal temperature varies for different toxin-producing cyanobacteria species [19,69].

Cyanobacterial biomass was often correlating with levels of cyanotoxins [17,30,31,51,53,60,61]. For instance, dominating cyanotoxins (MC—LF > MCLY > MCLA > MCL———R) in Zemborzycki dam reservoir had high positive correlation biomass of toxin-producing *Anabaena planctonica, Anabaena affinis* and *Microcystis spp*. [29]. However, in some cases, no correlation was observed [35,65]. Several authors highlight that toxins cannot be always directly connected with the total amount of cyanobacterial cells, and that variation in the ratio between toxic and non-toxic genotype should be considered [35,51,53]. Manganelli et al. [35] additionally mentioned two more parameters: shifts in toxins' production rate and potential utilisation of toxin inside the cell, which should be taken into account for the determination of toxins' variation. Moreover, the potential of the occurrence of degrader heterotrophic groups of bacteria can also decrease the final amounts of MCs. Nowadays, there is a scarcity of deep understanding which factors influence the production of cyanotoxins. One of the reasons is the heterogeneity of data from different field studies. In order to overcome this problem and to built a robust tool for both monitoring and prediction of seasonal patterns of cyanotoxins' variation, we can suggest several steps that could be implemented. First, standardisation of sampling, sample treatment and analysis should be performed. This will lead to comparable results from different locations. Second, multidisciplinary studies such as measurement of environmental parameters (including nutrients), cyanobacterial abundance and amount of toxic species, and cyanotoxins determination are also needed. This step will provide data needed for the determination of both toxins' and cyanobacterial abundance drivers. Combination of these steps will contribute to effective lake management and consequently minimization of human health hazard.

4. Seasonal variations of cyanotoxins in different climate zones of Europe

Considering that CyanoHAB is spread globally in different climate zones, distinguishing cyanotoxins variations patterns in different climatic conditions is needed. To tackle this problem, we applied the Köppen system used by Peel et al., that created a comprehensive map of the different climate regions around the globe [70]. In Fig. 1, the three main climatic zones of Europe.

Mediterranean climate is defined by weather characteristics in the sub-region around the Mediterranean sea, however, similar climate can be found also in the west coast of the United States and part of Australia [70]. It is generally characterized by dry and hot summers, rainy and cool winters and located between about 30° and 45° latitude north and south of the Equator and on the western sides of the continents. The temperature within the year varies between 10 and 35 °C, while precipitation are between 0 and 120 mm. The graphs of both temperature and precipitation around the year for to Mediterranean cities Rome and Athens are presented in Figure S1 of the *supplementary information*.

For ***humid continental climate*** temperature change along the year is severe, hot summers and cold winters characterize this climatic zone extending from 30° and 60 °N in central and eastern North America and Asia. Precipitations are high but they vary along the different zones with snow from one to four months in several parts of the region, especially in the north, where mean temperatures are around 0 °C for more than 4 months per year with no frost layer for 150–200 days. Annual precipitation ranges from 40

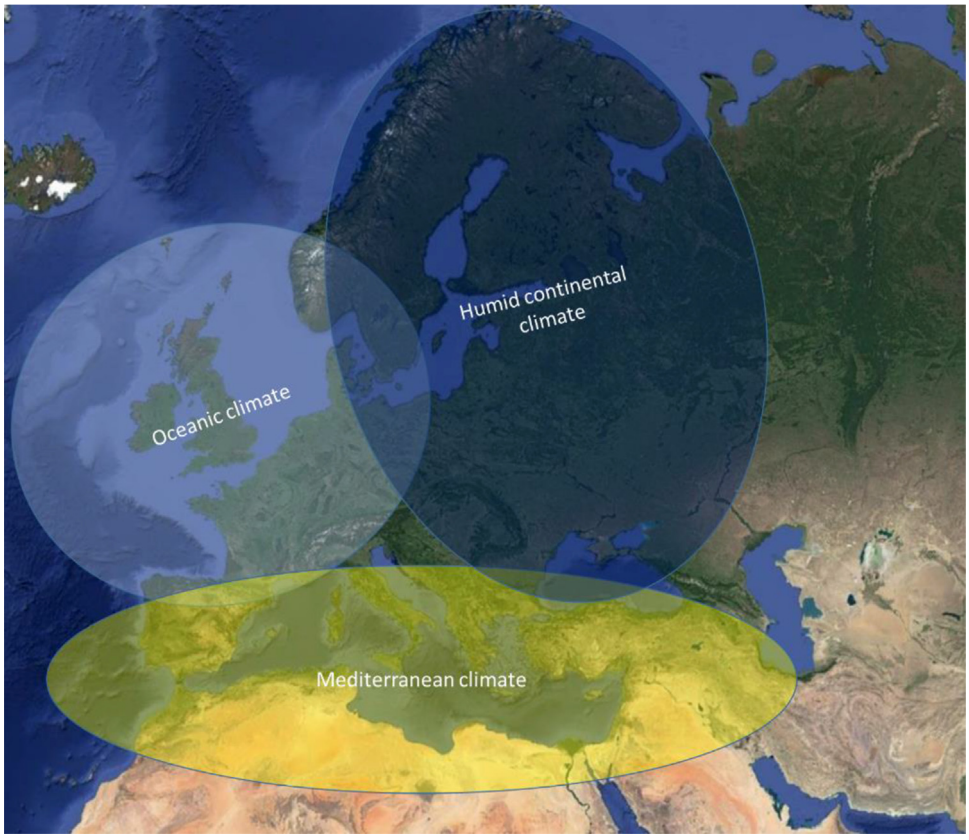

Fig. 1. Climatic zones in Europe.

to 125 mm. Both temperature and precipitation variations around the year for Munich and Trento are shown in the Figure S1 (*supplementary information*).

The Atlantic climate (oceanic climate) is characterized by extended precipitation in all months. It is located in the north of the Mediterranean climate region on the western sides of the continents, between 35° and 60 °N and S latitude. Total precipitations vary but the annual range is between 50–250 mm that can last for more than 150 days per year. Mean annual temperatures are usually 7–13 °C with mild winters the summers with monthly temperatures above 20 °C. Mountain regions are generally characterized by oceanic climate (North America and South America, Asia, Australia, and New Zealand). By contrast, in Europe Alps and Pyrenees permit oceanic climates to arrive until the eastern Germany and Poland. Graphs of temperature and precipitation for Gijon (North of Spain) and Amsterdam are shown in the Figure S1 (supplementary information). Considering that each climate zone has characteristic variations in temperature and precipitation, dynamic of CyanoHABs and levels of released cyanotoxins are expected to have differences.

Despite that monitoring of cyanotoxins in freshwater is performed more and more often, there remains a scarcity of data to establish seasonal variations. In some studies, it was impossible to observe the toxins' changes during a whole year due to the established sampling periods, covering only several months [23,42,46,52,71]. In these cases, authors were only focusing on a blooming period, which was usually not enough to determine the moment at which toxins' levels start to grow. In order to distinguish peaking seasons in different European climate zones, collected data from various sampling campaigns is presented in

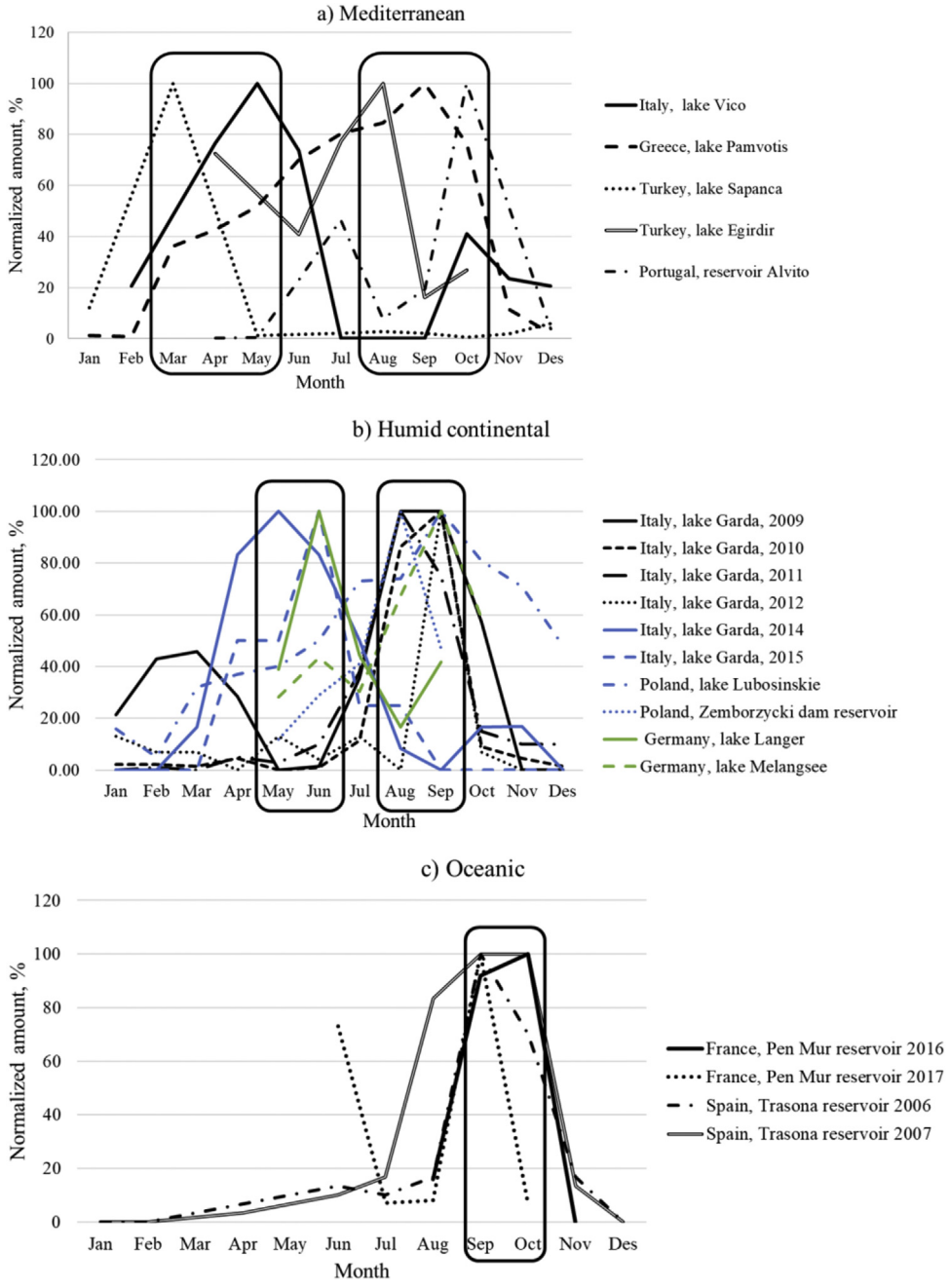

Fig. 2. Seasonal variations of cyanotoxins in a) Mediterranean, b) humid continental, c) Atlantic climate zones in Europe.

Fig. 2. Data were normalised to have a maximum level at 100 %, and to obtain the patterns for the three European climate zones. The main goal of these graph charts is to observe peaking seasons.

The Fig. 2a illustrates the seasonal variations of cyanotoxins in four different Mediterranean countries (Italy, Greece, Turkey, and Portugal), which were monitored during the whole year [24,33,35,49,51]. Overall, two peaking areas can be observed. The first peaking period was from March to May and the second one from August to October. However, Portugal's seasonal variation does not fit the first peaking sector. This can be related to the sampling period, which had started only in April [49]. Such trends also match with peaking months for shorter sampling campaigns. For instance, in the lakes, Odivelas, Roxo [49], Marathonas [36], Alto Flumendosa [57] and reservoir Rosarito [43] maximum toxins levels were reached in September and October. In the Mediterranean region the blooms are persistent along the year compared with another two climate zones, as it was expected because the summers are hotter and drier, that creates favourable conditions for the proliferation of cyanobacteria. In Fig. 2b, the variation of cyanotoxins' concentrations during the year in humid continental climate [29,42,44,53,54] is shown. Similarly, as in the Mediterranean region, two peaking seasons were distinguished. The first one was in May and June, and the second was during August and September. Several studies with shorter sampling periods from the same climate zone match this pattern. The second peaking area can be confirmed by levels of cyanotoxins in Zemborzycki dam reservoir in Poland [29], where August and October were usually the peaking months for MCs and ANA. Furthermore, higher levels of cyanotoxins were reached within August to September in the lakes Mytycze [28], Niegocin, Piłwąg, Rekąty [72] and Bergknappweiher [73]. Higher precipitations and lower temperatures can explain shorter blooming periods. Additionally, in the Alpine region, the rainfalls contribute to higher turbidity and the dilution in freshwater reservoirs. While, in the Atlantic climate region, only one peaking period (see Fig. 2c), was distinguished [58,59]. It might be due to two reasons: weather conditions and scarcity of available seasonal sampling campaigns. The slow growth of cyanotoxins concentrations until July because the slope of the temperature growth is very slow in comparison with other regions. Moreover, the Atlantic region is characterized with rains during spring and summer which also contributes to the lower concentrations of cyanotoxins. Nevertheless, the presence of cyanotoxins was reported in England during April and May [74]. All things considered, obtained seasonal variation patterns in different climatic zones demonstrate that climate conditions (such as temperature and precipitation regime) are the driving forces for cyanotoxins variation. Furthermore, multiple-year studies on cyanotoxins levels in surface freshwater can also be found in the literature. As a general trend, these studies show increases in cyanotoxins concentrations within the study periods. For example, Taranu et al. [75] reported a study by examining about 200 years of sedimentary records, showing that cyanobacteria have significantly increased since 1800, and more rapidly during the last decades (since 1945) in north temperate-subarctic lakes. Manganelli et al. [35] monitored the levels of cyanotoxins in the lake Vico (Italy) from February 2009 to December 2010. In this occasion, the maximum concentration of MCs at the surface water increased from around 3.4 μg/L in 2009 to 4.5 μg/L in 2010. In lake Marathonas (Greece) MCs were analysed during three-and-a-half-years, from July 2007 to December 2010 [36], by dividing the sampling period into four stages (from July 2007 to June 2008; from July 2008 to June 2009; from July 2009 to June 2010; and from July 2010 to December 2010). During the first three periods the concentrations of MCs was slightly rising, with reported maximum concentrations for MC-RR, MC-LR, and MC-YR of 62, 25 and 4 ng/L for the three first sampling periods, respectively. In contrast, during the fourth reported sampling period, MC-RR, MC-LR, and MC-YR concentrations increased considerably up to 451, 174, and 717 ng/L, respectively. Authors assume that seasonal variation of toxins could be due to influence of environmental parameters, intracellular MCs and physiological status of cyanobacterial cells. Another study found in the literature also showed a substantial increase on MCs' concentrations in Zemborzycki dam reservoir during a six year sampling period (2005–2011 from May to June) [29]. That being said, while ANA-a was fluctuating during the whole study period, MCs' concentration increased from 0.8 μg/L in 2005 to 22.2 μg/L at the peaking year (2010). In the last sampling period, the maximal concentration of MCs dropped to 12.4 μg/L. Results obtained by Gkelis et al. [51] in combination with 25-year period data suggest that both water temperature and nutrient enrichment may have a synergetic effect and promote cyanobacterial blooms in Lake Pamvotis. Despite the small amount of multi-year studies, a general pattern of rising cyanotoxins levels can be clearly observed, that can be related to global climate change, showing the importance of performing seasonal variation studies.

5. Conclusions and future work

Regarding the new analytical approaches, the use of HRMS techniques in full-scan to perform non-target and suspected screening will offer a powerful tool to assess degradation products and non-targeted toxins in environmental samples. This will facilitate a more rigorous risk assessment and will approach the understanding of the bacterial behaviour producing toxins. However, the efficient application of these techniques still requires the development of specifically designed libraries.

The influence of climate conditions and climate change on cyanotoxins' concentrations in different European regions was observed. Seasonal variation patterns for the Mediterranean, humid continental and Atlantic climate areas were distinguished based on literature review. Provided seasonal variation graphs contribute to understanding the cyanotoxins seasonal variations on a continental scale, which can be used for the improvement of water management strategies. Multiple-year studies demonstrate that cyanotoxins levels are rising and it can be related to global climate change. As there is on-going climate change, obtained patters might shift as well. Thus, regular revision (our suggestion each 5 years) of cyanotoxins' seasonal variation will contribute greatly to dynamic of climate change.

Above mentioned facts demonstrate the importance of performing seasonal variation studies. For homogeneous datasets and effective lake management, standardization of monitoring strategies is needed. What is more, for monitoring of cyanotoxins in the global scale, patterns for other regions should be distinguished and compared with those obtained in this review. Our hypothesis for future work is that similar patterns could be obtained for other parts of the world as parts of Australia, west coast of the United States and parts of Africa have similar climate to Mediterranean one; while Asia, central and eastern North America also have humid continental; and Atlantic is characteristic for North America and South America, but some trends characteristics in general of oceanic climate are influencing some regions in Asia, Australia, and New Zealand.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.teac.2020.e00091.

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