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Toxic Cyanobacteria in Greek Freshwaters, 1987–2000: Occurrence, Toxicity, and Impacts in the Mediterranean Region

In a survey in Greece from 1987 to 2000 hepatotoxic cyanobacterial blooms were observed in 9 out of 33 freshwaters. Microcystins (MCYSTs) were detected by HPLC in 7 of these lakes, and the total MCYST concentration per scum dry weight ranged from 50.3 to 1638 ± 464 μg g^{-1}. Cyanobacterial genera (*Microcystis, Anabaena, Anabaenopsis, Aphanizomenon, Cylindrospermopsis*) with known toxin producing taxa were present in 31 freshwaters. From our data and a review of the literature, it would appear that Mediterranean countries are more likely 1) to have toxic cyanobacterial blooms consisting of *Microcystis* spp. and 2) to have higher intracellular MCYST concentrations. A case study in Lake Kastoria is used to highlight seasonal patterns of cyanobacterial and MCYST-LR occurrence and to assess cyanotoxin risk. Cyanobacterial biovolume was high (> 11 μL L^{-1}) throughout the year and was in excess of Guidance Level 2 (10 μL L^{-1}) proposed by WHO for recreational waters and Alert Level 2 for drinking water. Further, surface water samples from April to November exceeded Guidance Level 3, with the potential for acute cyanobacterial poisoning. Intracellular MCYST-LR concentrations (max 3186 μg L^{-1}) exceeded the WHO guideline for drinking water (1 μg L^{-1}) from September to November with a high risk of adverse health effects. Preliminary evidence indicates that in 3 lakes microcystins are accumulated in some aquatic organisms. Generally, a high risk level can be deduced from the data for the Mediterranean region.

Toxische Cyanobakterien in Süßgewässern Griechenlands 1987–2000: Vorkommen, Toxizität und Beeinflussungen im Mittelmeergebiet

In einer Untersuchung innerhalb Griechenlands von 1987 bis 2000 wurden Wasserblüten Hepatotoxin-bildender Cyanobakterien in 9 von 33 Binnengewässern beobachtet. Microcystine (MCYSTs) wurden mittels HPLC in sieben dieser Seen bestimmt, wobei die Gesamtkonzentration an MCYST in aufgerahmten Algenmassen zwischen 50.3 und 1638 ± 464 μg g^{-1} Trockenmasse lagen. Gattungen (*Microcystis*, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Cylindrospermopsis*) mit bekannt Toxin-bildenden Arten kamen in 31 Binnengewässern vor. Aus den Ergebnissen und einer Literaturübersicht wird offensichtlich, dass in mediterranen Ländern eine Wahrscheinlichkeit für Massenentwicklungen toxischer Cyanobakterien, hauptsächlich von *Microcystis*, und höhere intrazelluläre MCYST-Konzentrationen besteht. Eine Fallstudie im Lake Kastoria dient zur Aufklärung der saisonalen Muster des Auftretens von Cyanobakterien und MCYST-LR und der Einschätzung des Cyanotoxin-Risikos. Das Biovolumen von Cyanobakterien war während des ganzen Jahres hoch (> 11 μL L^{-1}) und überschritt den WHO-Richtwert 2 von 10 μL L^{-1} für Erholungsgewässer und die Warnstufe 2 für Trinkwasser. Weiterhin wurden in Proben aus dem Oberflächenwasser von April bis November die Richtwerte der Stufe 3 für eine potentiell akute Vergiftung durch Cyanobakterien überschritten. Die intrazellulären Konzentrationen von MCYST-LR (maximal 3186 μg L^{-1}) überschritten die WHO-Richtlinie für Trinkwasser (1 μg L^{-1}) von September bis November mit einem hohen Risiko für Gesundheitsbeeinträchtigungen. Vorläufige Ergebnisse weisen darauf hin, dass in drei Seen Microcystine in aquatischen Organismen akkumuliert werden. Generell kann aus den Ergebnissen ein hohes Risiko für die mediterrane Region abgeleitet werden.

Keywords: Microcystin-LR, *Microcystis*, Bioaccumulation, Temporal Variation, Spatial Variation, Health Risk, Water Quality

Schlagwörter: Microcystin-LR, *Microcystis*, Bioakkumulation, Räumliche Variation, Zeitliche Variation, Gesundheitsrisiko, Wasserbeschaffenheit

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DOI 10.1002/aheh.200300523

Review

1 Introduction

Cyanobacteria (blue-green algae) are photosynthetic, prokaryotic organisms which occur primarily in freshwater and saline environments, but also in terrestrial ecosystems. Despite their prokaryotic nature they assimilate CO_2 by typical higher plant type photosynthesis with two photosystems. Their presence in lakes with high nutrient levels can lead to a mass increase in cyanobacterial cell numbers, with the formation of blooms, which results in a depreciation of water quality. Some cyanobacteria can form thick surface accumulations (scums) as the waterbloom develops, due to the possession of buoyancy-conferring gas vacuoles. Waters become unsightly and have an unpleasant odour and are unsuitable either for leisure activities or as drinking water supplies. It became evident that certain cyanobacterial blooms were responsible for the illness and death of wildlife and livestock which had ingested water containing scum or bloom material [1, 2]. Further, allergic reactions, gastroenteritis, liver diseases, and even death in humans were reported following acute exposure to high doses during renal dialysis [3] and the promotion of tumours after chronic exposure to low doses [4].

Subsequent research revealed that several genera of cyanobacteria for example, *Anabaena*, *Aphanizomenon, Cylindrospermopsis, Haphalosiphon, Lyngbya, Microcystis, Nodularia, Nostoc, Oscillatoria, Planktothrix, Schizothrix, Umezakia* [5, 6], *Anabaenopsis* [7], and *Aphanocapsa* [8] produce metabolites which are potent toxins. On the basis of their biological activity three major categories of toxins have been described, hepatotoxins (cyclic peptides and alkaloids), neurotoxins (alkaloids) and dermatotoxins (alkaloids), and also irritant toxins (lipopolysaccharides) [5].

Cyanobacterial hepatotoxins have been purified from several cyanobacteria. Most are structurally related cyclic peptides and heptapeptides are the most common [5]. The general formula for the structure of the cyclic heptapeptides is cyclo(-D-Ala^1-L-X^2-*erythro*-β-methyl-D-iso-Asp^3-L-Z^4-ADDA^5-D-iso-Glu^6-*N*-methyldehydro-Ala^7) [9−12], where ADDA is the β-amino acid (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid [12] and X and Z are certain L-amino acids [11]. The nomenclature for these toxins is microcystin (MCYST) plus the two letter suffix that designates the variant L-amino acids according to the single letter system for the abbreviation of amino acids [11]. The main differences in structure are due to the variant amino acids at X and Z and the demethylation of amino acids 3 and/or 7, and more than 60 structural variants are known to date [6]. MCYST-LR, where X is L-leucine and Z is L-arginine, has been found in most strains of *Microcystis aeruginosa* in the Northern Hemisphere [13]. MCYST-LR has been shown to be a potent inhibitor of protein phosphatases 1 and 2A [14] and this activity is believed to be associated with hepatoxicity [15]. It is now apparent that although toxic cyanobacteria often contain one major toxin, they also contain several other toxins in minor quantities [16, 17].

Microcystins are suspected of causing cancer in humans and can also be taken up by plants, causing the death of leaves and the irreversible inhibition of photosynthesis [1, 18]. In addition to the possibility of internal accumulation of microcystins by plants, irrigation with water containing cyanobacteria may lead to the accumulation of toxins on the external surfaces of edible plant material [19]. The presence of microcystins in drinking water supplies and irrigation water poses a potential hazard to human health and agricultural products directed for animal and human consumption [1, 4]. Farmers are potentially in danger from the inhalation of droplets containing toxin during spray irrigation [20] or through dermatological contact with toxin containing water [19]. However, as novel, potent chemicals cyanobacterial metabolites may find potential uses in medical and pharmaceutical applications.

As part of an awareness campaign in 1980 the European Union supported a survey of waterbodies to examine the extent and seriousness of toxic cyanobacterial blooms in European countries [2]. Water pollution caused by urbanisation, industrialisation, and modern agricultural methods is widespread and long established in Europe [21]. Water pollution combined with surface water temperatures of 15 to 30 °C, temperatures frequently encountered in Mediterranean waterbodies, favour bloom formation [2]. In the 1980's there was no information concerning the toxicity of cyanobacterial blooms in Greece, even though blooms of *Microcystis aeruginosa* were quite common phenomena in surface waters used for recreational and other purposes [22, 23]. The limited information concerning toxic cyanobacteria in Greece and in general in other Mediterranean countries, where climatic conditions are more likely to promote cyanobacterial blooms, and the serious problems encountered elsewhere in northern Europe, led to the necessity of our initial investigation. Since several Greek waterbodies are used for fishing, recreation, and as drinking water supplies, studies were carried out to identify the cyanobacterial species present and investigate the occurrence of toxic cyanobacterial blooms, in order to evaluate the associated risks.

The purpose and objectives of this paper are

1. to give a review of the published information on toxic cyanobacterial populations in Greece, as compared to the available information in the rest of the Mediterranean region,

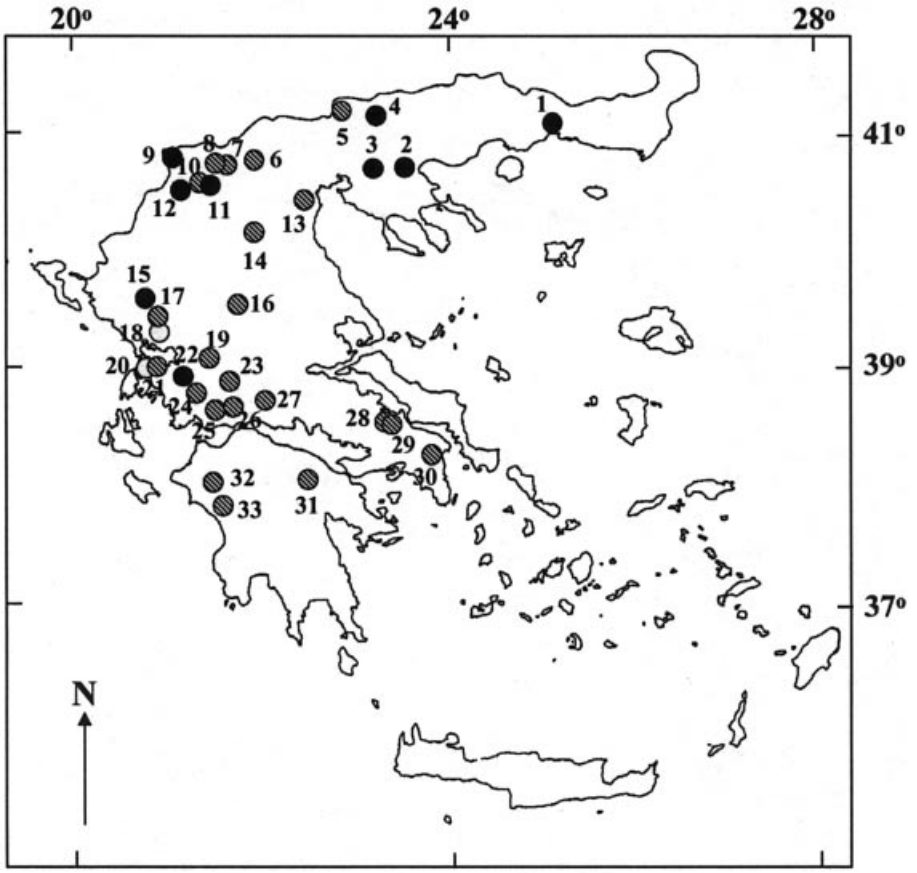

Fig. 1: Map of Greece showing the location of freshwaters in which a) toxic cyanobacterial blooms (black circles), b) potentially toxic cyanobacterial genera (striated circles), and c) non-toxic cyanobacterial genera (white circles) have been observed. See Table 1 for details corresponding to each number.

Karte Griechenlands mit Binnengewässern, in denen a) Wasserblüten von toxischen Cyanobakterien (ausgefüllte Kreise), b) Wasserblüten potentiell-toxischer Cyanobakterien (schraffierte Kreise) und c) nicht-toxische Cyanobakterien-Gattungen (weiße Kreise) beobachtet wurden. Details in Tabelle 1.

2. to use original and published data from a case study in Greece to highlight seasonal patterns of cyanobacterial and cyanotoxin occurrence, and
3. to deduce a first assessment of the cyanotoxin risk for waterbody-users in Greece and also the necessary management action, as an exemplary case for other Mediterranean regions.

2 The occurrence of toxic cyanobacteria and microcystins

2.1 Greece

Although cyanobacterial blooms in some lakes have become common occurrences due to urbanisation and farming practices, the presence of toxic cyanobacterial blooms was first established in Greek freshwaters in 1987 [24]. In a survey of four lakes in Northern Greece, L. Vistonis, L. Volvi, L. Koronia, and L. Kastoria, the dominant species of cyanobacteria in the samples were *Anabaena viguieri*, *Anabaenopsis milleri*, *Microcystis aeruginosa* and *Oscillatoria* sp. (Fig. 1; Table 1). The highest cyanobacterial scum densities (dry weight, DW, per volume of scum) were encountered in L. Koronia with values of up to 57 g L^{-1}. Lethal doses (LD_{50}), cyanobacterial biomass per mouse body weight, administered by the intraperitoneal route (i.p.), ranged from 40 to 1500 mg kg^{-1} and gross pathological signs of poisoning were characteristic of cyanobacterial hepatotoxins [25]. Post-mortem revealed enlarged and congested livers. Samples containing *M. aeruginosa* from L. Kastoria were the most toxic in mice with i.p. LD_{50}'s from 40 to 60 mg kg^{-1} (Table 1).

It is of practical importance to know not only if a cyanobacterial scum is toxic, but also the actual amount of toxin in a given volume of water, since the lakes are used for watering livestock. This depends on the scum density and the LD_{50}. Richard et al. [26] have made some calculations on bovine oral toxicity based on the assumption that a 500 kg cow drinks about 50 L of water a day if unrestricted. In the survey in 1987, three samples from L. Kastoria containing *Microcystis aeruginosa* would have been potentially lethal to animals drinking from the water, with LD_{50} bovine oral of 23 L, 73 L, and 83 L, respectively. Lethal bovine oral volumes as low as 6 L have been reported [26]. It is noteworthy that

Table 1: Cyanobacterial genera, scum density (dry weight per volume of scum), and biovolume (volume of cyanobacteria per volume of lake water), toxicity in mice administered by the intraperitoneal (i.p.) route (LD_{50} in scum dry weight per mouse body weight), and total microcystin (MCYST) concentrations (MCYST per scum dry weight) determined by HPLC, of samples collected from 33 freshwaters during the warm period of the years 1987 to 2000. Locations are shown in Figure 1. Black circles indicate that one or more species of the genera were dominant, i.e. constituted > 10 % (v/v) of the total cyanobacterial biovolume. White circles indicate the presence of species of the genera which were not dominant.

Cyanobakterien-Gattungen, Biomasse (Trockenmasse L^{-1}) und Biovolumen ($\mu L\ L^{-1}$), Toxizität für Mäuse bei intraperitonealer Gabe (LD_{50} in mg Trockenmasse je kg Lebendgewicht) und Gesamtkonzentration an Microcystin (bestimmt mittels HPLC) in Proben von 33 Binnengewässern, gesammelt während der warmen Jahreszeit der Jahre 1987 bis 2000. Untersuchte Gewässer aus Bild 1. • Eine oder mehrere Arten des Genus sind dominant [> 10 % (v/v) der Gesamtbiomasse der Cyanobakterien]. ○ Vorkommen als nicht dominante Art innerhalb der Gattung.

Freshwater	Cyanobacteria										Toxicity in mice	MCYST concentration
	Genera								Scum density	Biovolume	i.p. LD_{50}	
	Aphano-capsa	*Micro-cystis*	*Oscilla-toria*	*Ana-baena*	*Anabae-nopsis*	*Aphanizo-menon*	*Cylindro-spermopsis*	Other genera	in g L^{-1}	in $\mu L\ L^{-1}$	in mg kg^{-1}	in $\mu g\ g^{-1}$
1. Vistonis		●	○	○	●				4.32...14.97	200	1130...1500	317.2
2. Volvi	○	●		●	○	●	○	●		7...14	1500	
3. Koronia					●				57.20		600	
4. Kerkini		●		●		○		○		104...1116		68.7 ± 24.8
5. Doirani		●		○	○	●		●		255		
6. Agras	○			○				○		< 0.001		
7. Vegoritis				○		●				0.898		
8. Petron		○				○		○		0.512		
9. Mikri Prespa	○	●	○	○				○		8		1091
10. Zazari		●		○		○	○	○		349		50.3
11. Cheimaditis		○	○	○						0.07		
12. Kastoria		●		●			●	●	4.17...51.40	11...7585	40...1500	1638 ± 464
13. Asomaton			○			○	○	○		0.013		
14. Polyphyton		○		●		○	○			0.269		
15. Pamvotis	○	●	○	●		○		○		184...9507		958 ± 75
16. Tavropos			○	○						< 0.001		
17. Louros		○	○	○						< 0.001		
18. Pournariou								○		< 0.001		
19. Kremaston		○								0.028		
20. Saltini								○		< 0.001		
21. Voulkaria		○	○			○		○		0.051		
22. Amvrakia		●		●				○		629...6124		84 ± 41
23. Kastrakiou				○						< 0.001		
24. Ozeros			○	○		○				0.330		
25. Lysimachia			○	○		○		○		0.003		
26. Trichonis			○	○		○				0.003		
27. Mornos				○				○		0.044		
28. Yliki	○		○	○	○	○	○	○		0.020		
29. Paralimni				○						0.019		
30. Marathonas		○					○	○		< 0.001		
31. Stymfalia				○		○				0.004		
32. Pinios		○		○			○	○		< 0.001		
33. Floka		○	○	○						< 0.001		

some samples with the same cyanobacterial species composition collected on the same day at different locations in L. Kastoria had different i.p. LD_{50}'s [24]. These samples consisted of 99% (v/v) cyanobacterial cells [24].

More recently, in the warm periods of 1999 and 2000, a comprehensive survey of the occurrence of toxic cyanobacteria in Greek freshwaters was carried out [27, 28]. The lakes and reservoirs examined, natural and manmade, are used for irrigation, drinking water, recreation, fishing and aquaculture, and as wildlife refuges. Cyanobacterial species composition, biovolume, and the presence of microcystins were examined.

The data collected from a total of 33 freshwaters (Fig. 1) from 1987 to 2000 has been compiled in Table 1 [24, 27–29]. Cyanobacterial biovolume per volume of lake water ranged from 0.001 to 1 $\mu L\ L^{-1}$ in lakes without blooms, and from 7 to 9507 $\mu L\ L^{-1}$ in lakes with blooms (9 lakes) (Table 1). Hepatotoxic cyanobacterial blooms were observed in 9 lakes L. Amvrakia, L. Kastoria, L. Kerkini, L. Koronia, L. Mikri Prespa, L. Pamvotis, L. Vistonis, L. Volvi, and L. Zazari (Fig. 1, Table 1). Lakes Pamvotis, Kastoria, and Amvrakia had the heaviest blooms with maximum biovolumes of 9507 $\mu L\ L^{-1}$, 7585 $\mu L\ L^{-1}$, and 6124 $\mu L\ L^{-1}$, respectively. Genera (*Anabaena, Anabaenopsis, Aphanocapsa, Aphanizomenon, Cylindrospermopsis, Microcystis, Oscillatoria*) with toxin producing taxa were present in 31 of the 33 freshwaters examined (Fig. 1, Table 1). Taxa of *Microcystis*, *Anabaena,* and *Aphanizomenon* were present in almost all of the freshwaters. *Microcystis* spp. were present in 18 of the 33 freshwaters and were dominant in 9 of them. In the freshwaters with hepatotoxic cyanobacterial blooms *Microcystis* spp. were dominant in 8 of the 9. *Anabaena* spp. were present in 25 of the 33 freshwaters and were dominant in 6 of the freshwaters which had hepatotoxic blooms. *Aphanizomenon* spp. were present in 15 of the 33 freshwaters and were dominant in 3.

The two most abundant toxin producing species were *Microcystis aeruginosa* and *Anabaena flos-aquae* [28]. *M. aeruginosa* was identified in 8 lakes and in 6 of them formed dense blooms with biovolumes > 100 $\mu L\ L^{-1}$ (maximum biovolume: 6090 $\mu L\ L^{-1}$; L. Kastoria). *A. flos-aquae* was identified in 5 lakes and in 4 of them formed dense blooms with biovolumes > 100 $\mu L\ L^{-1}$ (maximum biovolume: 4500 $\mu L\ L^{-1}$; L. Pamvotis) [28]. *Microcystis* species, most frequently *M. aeruginosa*, are a major cause of hepatotoxic blooms worldwide as reported to date [6]. *A. flos-aquae* has been reported to produce microcystins and anatoxin-a [6]. Despite this there is no evidence to date for the occurrence of neurotoxic cyanobacterial blooms in Greece. *Anabaena lemmermannii*, a common bloom-forming species in Scandinavian freshwaters which produces microcystins and anatoxin-a (S), was identified in one lake, Mikri Prespa, but was not dominant (Vardaka, Moustaka and Lanaras, unpublished). Toxic species of *Aphanizomenon* producing anatoxin-a have been reported in Finland [30] and Germany [31]. *Cylindrospermopsis raciborskii* was identified in 8 of the 33 freshwaters, but was only dominant in L. Kastoria. The only record of *C. raciborskii* in Europe before 1970 was in L. Kastoria [32]. It is regarded to be a pan-tropic species and a successful invader, with increasing occurrences in Europe and the USA [33]. *C. raciborskii* produces cylindrospermopsin (Australia [34]) and saxitoxins (Brazil [35]), but its toxicity in Greece has not yet been examined.

It was of particular interest that toxic cyanobacterial blooms dominated by *Anabaenopsis milleri* were encountered in Greek lakes [24], since this represents the first report worldwide. Although the toxicity was fairly low (i.p. LD_{50} 600...1500 mg kg^{-1}) symptoms and gross pathological signs of poisoning were typical of hepatotoxins. The toxicities encountered were in the same range as those reported in Scottish freshwaters for *Gloeotrichia echinulata* [36] and *Aphanizomenon flos-aquae* [26]. Subsequent research demonstrated the presence of MCYST-LR in purified extracts of a natural bloom of *A. milleri* from L. Vistonis (L. Porto Lagos) [7].

Microcystins were detected by HPLC in all of the samples from L. Amvrakia, L. Kastoria, L. Kerkini, L. Mikri Prespa, L. Pamvotis, L. Vistonis and L. Zazari, where cyanobacterial blooms were observed in 1999 and 2000 (Table 1) [28, 29]. Lakes Kastoria and Pamvotis had the heaviest and most toxic blooms and the total microcystin concentrations (MCYST per scum dry weight) encountered were 1638 $\pm$ 464 $\mu g\ g^{-1}$ in L. Kastoria and 958 $\pm$ 75 $\mu g\ g^{-1}$ in L. Pamvotis (Table 1). MCYST concentrations ranged from 50.3 to 1638 $\pm$ 464 $\mu g\ g^{-1}$ in the 7 lakes (Table 1). Several MCYST variants were detected and identified by HPLC and HPLC-MS. The most abundant variants were MCYST-LR and MCYST-RR, while MCYST-LA, MCYST-YR and demethylated derivatives of MCYST-LR and MCYST-RR were also found [29].

2.2 Mediterranean region

Although cyanobacterial blooms have been reported worldwide, and in some detail in European countries, results of such surveys are often influenced by the sampling strategy and the trophic state of the lakes in a given country. *Anabaena* is generally a competitive genus under low nutrient conditions, while *Microcystis* and some *Aphanizomenon* species require more nutrient rich waters [37]. Surveys of cyanobacterial blooms in the Mediterranean region have only recently been carried out. However, such studies are often restricted to the investigation of individual lakes or waterbodies which may not be representative of the country as a whole (Table 2). For example, in Lake Kinneret, Israel, *Aphanizomenon*

Table 2: Toxic cyanobacterial blooms in countries of the Mediterranean region: Country, year(s), freshwater, dominant species, cyanotoxins and toxicities and/or toxin concentrations and method of determination. *: isolated strain material. DW: dry weight, FW: fresh weight, ELISA: enzyme-linked immunoabsorbent assay, PP2A: protein phosphatase 2A inhibition assay.

Toxische Wasserblüten von Cyanobakterien in mediterranen Ländern. *: Daten von isolierten Stämmen; DW: Trockenmasse; FW: Frischmasse; ELISA: Immunoassay; PP2A: Protein-Phosphatase-2A-Hemmung.

Country, year(s), freshwater	Dominant species	Cyanotoxin	Toxicity and/or toxin concentration in natural samples or isolated strain material	Reference
Algeria 2000 (Lekhal reservoir)	*Microcystis aeruginosa*			[49]
Egypt 1993	*M. aeruginosa*	MCYSTs	18.2...40 mg kg^{-1}, LD_{50} mouse bioassay*	[40]
1995 (River Nile)	*Oscillatoria tenuis*	MCYSTs	300 μg g^{-1} DW, ELISA*	[41]
France 1994 (Brittany)	*M. aeruginosa* *Anabaena* sp. *Anabaena circinalis* *Aphanizomenon flos-aquae* *Oscillatoria* sp.	MCYSTs	70...3970 μg g^{-1} DW, HPLC	[52]
1994 (Lake Grand-Lieu)	*M. aeruginosa* *A. circinalis*	MCYSTs	up to 230 μg g^{-1} DW, HPLC up to 5060 μg g^{-1} DW, HPLC*	[53]
1998–1999 (Saint-Caprais Reservoir, Toulouse)	*Aph. flos-aquae*	MCYSTs	0.270 μg g^{-1} FW, HPLC 0.014...0.074 μg L^{-1}, PP2A	[56]
2000 (Adour-Garonne, Rhin-Meuse, Loire-Bretagne, Rhône-Méditerranée-Corse, Artois-Picardie)	*Planktothrix agardhii* *Microcystis* spp. *Anabaena* sp.	MCYSTs	1.5...10.7 μg L^{-1}, HPLC	[54]
1999–2000 (Viry–Châtillon)	*P. agardhii*	MCYSTs	0.05...4.1 μg L^{-1}, PP2A 0.2...5.2 μg L^{-1}, HPLC	[55]
Greece (see Table 1)				
Israel 1994 (Lake Kinneret)	*Aphanizomenon ovalisporum*	Cylindrospermopsin	465 mg kg^{-1}, LD_{50} mouse bioassay* 2000 μg g^{-1} DW, HPLC*	[38] [39]
Italy 1994 (Lake Mulargia)	*Anabaena planctonica*	Anatoxin-a		[42]
1997 (Lake Varese)	*Aphanizomenon* sp. *Oscillatoria* sp. *Anabaena* spp. *Microcystis* spp.	Saxitoxin		[43]
1997 (Lake Varese)	*Planktothrix* spp. *Planktothrix* sp. FP1	Saxitoxin Saxitoxin*		[44]

Table 2 (continued).

Country, year(s), freshwater	Dominant species	Cyanotoxin	Toxicity and/or toxin concentration in natural samples or isolated strain material	Reference
Morocco				
1994–1998 (Lalla Takerkoust Reservoir)	*M. aeruginosa*	MCYSTs	700...8800 μg g^{-1} DW, ELISA 1.99...83 mg kg^{-1}, LD_{50} mouse bioassay	[46]
1999 (Oued Mellah Lake)	*Microcystis ichthyoblabe*	MCYSTs	0.1...0.78 μg g^{-1} DW, ELISA 502...1924 mg kg^{-1}, LD_{50} mouse bioassay	[47]
1994–1999 (seven freshwaters)	*M. aeruginosa* *M. ichthyoblabe* *Microcystis wesenbergii* *Pseudanabaena mucicola* *Synechocystis* sp.	MCYSTs	2.2...944 μg g^{-1} DW, ELISA* 26.8...1844 μg L^{-1}, HPLC* 28...350 mg kg^{-1}, LD_{50} mouse bioassay*	[48]
Portugal				
1989–1998	*M. aeruginosa* *Anabaena flos-aquae* *Phormidium mucicola* *Aph. flos-aquae*	MCYSTs Paralytic Shellfish Poisoning toxins	0.2...31 μg L^{-1}, ELISA up to 7100 μg g^{-1} DW, HPLC 20...700 mg kg^{-1}, LD_{50} mouse bioassay	[51]
Spain				
1997–1999 (Madrid reservoir)	*Microcystis flos-aquae* *M. wesenbergii* *Anabaena spiroides* *Aph. flos-aquae*	MCYSTs	1...150 μg L^{-1}, HPLC	[50]

ovalisporum formed a bloom containing the hepatotoxin cylindrospermopsin [38, 39]. In Egypt, microcystins were detected in strains of *Microcystis aeruginosa* [40] and *Oscillatoria tenuis* isolated from the River Nile [41]. In studies in Italy, anatoxin-a was detected in blooms of *Anabaena planctonica* in Lake Mulargia [42], while most of the toxic blooms in many Italian regions were found to be dominated by species of *Oscillatoria* and *Microcystis* (cited in [43]). Cyanobacterial blooms in Lake Varese were dominated by species belonging to the genera *Aphanizomenon, Anabaena,* and *Oscillatoria* (which was later classified as *Planktothrix* [44]) and saxitoxin was detected [43, 44]. In addition, saxitoxin was detected in strain *Planktothrix* sp. FP1 isolated from Lake Varese [44]. However, the identity of this strain has been questioned [45].

Blooms of *Microcystis aeruginosa* and *Microcystis ichthyoblabe* have been reported in two Moroccan freshwaters and microcystins were detected [46, 47]. In addition, the more widespread occurrence of toxic cyanobacterial blooms in Morocco has been reported, which consist mainly of *Microcystis* species [48]. The presence of *M. aeruginosa* has also been reported in Lekhal Reservoir, Algeria [49]. Cyanobacterial blooms occur recurrently in a Madrid reservoir, Spain, and a toxic microcystin containing bloom of *Microcystis flos-aquae* occurred in 1997 [50]. In surveys of toxic cyanobacteria in Portugal [51] and Brittany, France [52, 53], the most frequently dominant species was *M. aeruginosa*. In five French river systems the dominant species were *Planktothrix agardhii*, *Microcystis* spp. and *Anabaena* sp. [54]. In addition in France, a microcystin containing bloom of *P. agardhii* in Lake Viry-Châtillon [55], and a mono-specific bloom of *Aphanizomenon flos-aquae* in the Saint-Caprais Reservoir, Toulouse [56] have been reported.

In temperate zones, cyanobacterial blooms are most prominent during the late summer and early autumn and may last 2 to 4 months. In regions with more Mediterranean (mild, wet winter and warm, dry summer) or subtropical climates, the bloom season may start earlier and persist longer. Ecological studies of phytoplankton populations in some Greek lakes (Volvi, Mikri Prespa and Kastoria) have shown that prolonged cyanobacterial blooms occur, which last up to 8 months and are dominated by potentially toxic species

[57–59]. Generally, cyanobacterial water blooms in Mediterranean countries may be expected to be of extended or even continuous duration throughout the year, particularly in freshwaters experiencing high temperatures and irradiance, stratification of the water column, high phosphorus concentrations, and low zooplankton grazing. *Microcystis aeruginosa* was the most widespread and most frequently dominant cyanobacteria in water blooms in Greece [28] and apart from isolated incidences where other cyanobacteria are dominant (see Table 2), would appear to date, to be the most frequently occurring toxic cyanobacterial species in the Mediterranean region as a whole.

Toxin concentrations in cyanobacterial blooms in the Mediterranean region have been determined by several methods (mouse bioassay, HPLC, ELISA, and protein phosphatase inhibition assay) and the values are compiled in Table 2. MCYST concentrations determined in Greek freshwaters by HPLC (50.3...1638 ± 464 μg g^{-1}; Table 1), were similar to those reported in France (up to 3970 μg g^{-1}; [52]), but were lower than those in Portugal (up to 7100 μg g^{-1}; [51]). The highest MCYST concentration reported in the region was 8800 μg g^{-1} (ELISA) in Morocco [46].

3 A case study: toxic cyanobacteria in Lake Kastoria

The presence of toxic cyanobacterial blooms in L. Kastoria was established in 1987 [24]. Although cyanobacterial blooms are common occurrences in the lake, data on the composition and population dynamics of the phytoplankton were lacking, with published reports referring only to the floristic composition of isolated phytoplankton samples [22, 24, 32, 60, 61]. Until recently, the lake was exposed to nutrient loading from agricultural run-off and sewage discharge, which have contributed to its eutrophic status. A sewage treatment plant for the town of Kastoria became operational in 1994. L. Kastoria has a surface area of 24 km^2, a maximum length of 7.6 km, a maximum width of 5.0 km, a maximum depth (in a very limited area) of 8.5 m, an average depth of 5 m and an altitude of 620 m [62]. The town of Kastoria is situated on a peninsula that divides the lake into two main basins.

Subsequently, the temporal and spatial variation of planktic cyanobacteria, MCYST-LR concentration and some environmental parameters were studied in L. Kastoria from April to November 1994, June to November of 1995, and June 1996 to June 1997 [27, 59]. Over these periods the water temperature varied from 5.5...33.1 °C, pH from 7.25...9.50, dissolved O_2 concentration from 0.1...15 mg L^{-1} and conductivity from 170...460 μS cm^{-1}.

In the following subsections some previously published data for the period June 1996 – June 1997 [59] is supplemented with original data from the case study

1. to highlight seasonal patterns (and annual trends) of cyanobacterial and MCYST-LR occurrence and
2. to investigate differences in the biovolumes of individual cyanobacterial species between years using a discriminant analysis of original data, in order to get a contemporary picture of the situation in L. Kastoria.

Sampling procedures, identification of cyanobacterial species and determination of cyanobacterial biovolume (total and of individual species) were carried out according to procedures described in [59]. Intracellular (cell-bound) MCYST-LR concentrations were measured using standard HPLC techniques [63].

3.1. Cyanobacterial biovolume

Data from June 1996 until June 1997 [59] is here supplemented with original data from 1994 and 1995 to examine annual and seasonal trends. Total cyanobacterial biovolume in lake water ranged from 12...7585 μL L^{-1} at inshore and from 11...238 μL L^{-1} at offshore stations and constituted 90 % (v/v) or more of the total phytoplankton biovolume. Cyanobacterial biovolume varied temporally between months and years and spatially between stations and depths (Fig. 2). Non-uniform, vertical variation of cyanobacterial biovolume was observed mainly from June to November and coincided with the formation of temperature, pH, and O_2 gradients in the water column. Differences in the cyanobacterial biovolumes at 0...0.2 m and the other depths were significant (ANOVA, $P < 0.05$) in November 1995 and September, October and November 1996. Differences in the biovolumes between the depths of 1.5 m, 3.0 m, and 4.5 m were not significant (ANOVA, $P > 0.05$) in any year.

The cyanobacterial biovolumes at the offshore stations in 1996 were significantly higher (ANOVA, $P < 0.05$) than those in 1994 and 1995, while the biovolumes at the inshore stations were not significantly different between the years. The mean annual (June 1996 to June 1997) cyanobacterial biovolume for the inshore stations (716 ± 235 μL L^{-1}) was 16 times higher than the mean annual biovolume at the offshore stations (46 ± 3 μL L^{-1}). Cyanobacterial scum was observed at inshore stations from April to November and the biovolumes were from 133 to 250 times higher than those of the offshore stations. Typically, cyanobacterial biovolumes at the inshore stations increased significantly from July onwards attaining a maximum in October to November, decreasing thereafter, but maintaining a low presence throughout the remainder of the year (Fig. 2). At offshore stations cyanobacterial biovolume increased in the period June to August,

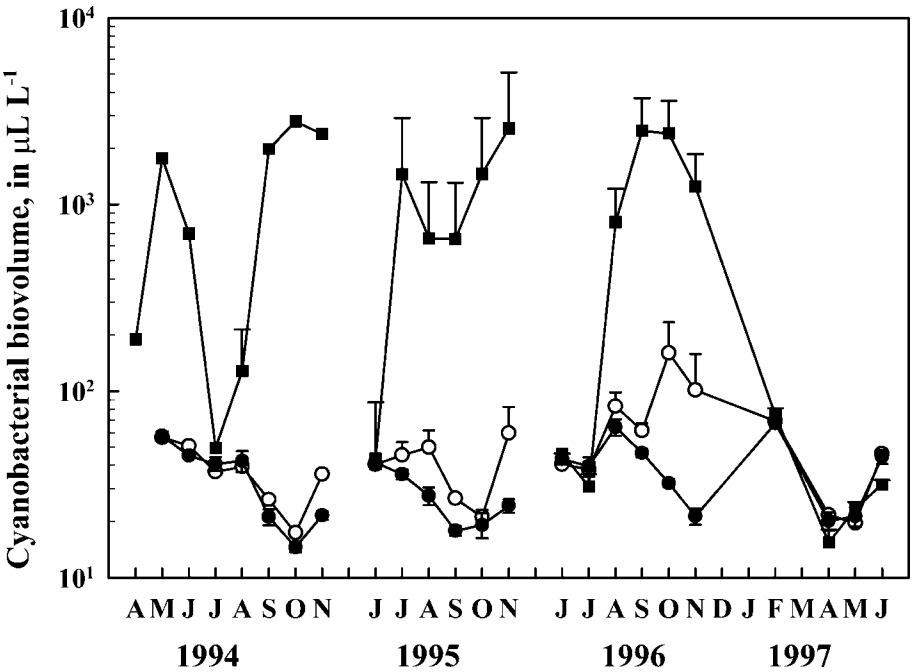

Fig. 2: Mean monthly total cyanobacterial biovolume (volume of cyanobacteria per volume of water sample) in samples collected in surface water (0...0.2 m) at inshore stations (■) and offshore stations (○) and the mean value of samples taken at different depths in the water column (1.5 m, 3.0 m, and 4.5 m) at offshore stations (●), in Lake Kastoria from April to November 1994, June to November 1995, and June 1996 to June 1997. Bars represent SE.

Mittleres monatliches Gesamt-Biovolumen von Cyanobakterien ($\mu L\ L^{-1}$) in Proben aus dem Oberflächenwasser (0...0.2 m) des Litorals (■), des freien Wassers (○) und Mittelwerte aus 1.5 m, 3.0 m und 4.5 m Tiefe (●) im Lake Kastoria von April bis November 1994, Juni bis November 1995 und Juni 1996 bis Juni 1997. Die Balken repräsentieren die Standardabweichung.

and tended to decrease as the inshore biovolumes began to increase, but maintained a continual presence in the water column throughout the year (Fig. 2).

3.1.1. Temporal variation

A discriminant analysis [64] was used to examine how the biovolumes of individual cyanobacterial species varied during the warm period (June to November) of 1994, 1995, and 1996 in L. Kastoria, and the extent to which they were influenced by climatic conditions and the physical and chemical properties of the water. The data was grouped a priori according to the year (1994, 1995, 1996) in which the measurements were made. The variables used in the analysis were the biovolume of *Microcystis aeruginosa*, *Microcystis ichthyoblabe*, *Microcystis flos-aquae*, *Microcystis novacekii*, *Microcystis wesenbergii*, *Anabaena flos-aquae*, *Anabaena viguieri*, *Anabaena* sp., *Limnothrix redekei*, *Cylindrospermopsis raciborskii* and *Raphidiopsis mediterranea*, the water temperature, pH, dissolved O_2 concentration, conductivity, the precipitation in mm, and the number of days with precipitation per month. Samples were collected monthly from the surface layer (0...0.2 m) of three inshore stations and from four depths (0...0.2 m, 1.5 m, 3.0 m and 4.5 m) at three offshore stations.

The spatial positions of the 3 groups (1994, 1995, 1996), with respect to the two discriminant functions (Axis I and Axis II) arising from the analysis, are shown in Figure 3. Axis I and Axis II accounted for 82% and 18% of the total variation, respectively. Differences between the years were evident from the minimal overlapping of the groups. The higher mean water temperature in L. Kastoria for the period June to November 1995, compared to the same period in 1994 and 1996, appeared to favour the growth of *Cylindrospermopsis raciborskii*. In addition, the increased number of storms in 1995 may have prevented the growth of *Microcystis* species. In contrast in 1996, although the precipitation was similar to that in 1995, there were periods with storms, or episodes of mixing, at low frequency intervals (> 10 days, [65]) which permitted the increased growth of *Microcystis aeruginosa* and *Anabaena* sp. in comparison to the previous years.

3.1.2 Spatial variation

More than 90% (v/v) of the cyanobacterial biovolume at the inshore stations consisted of *Microcystis* species (predomi-

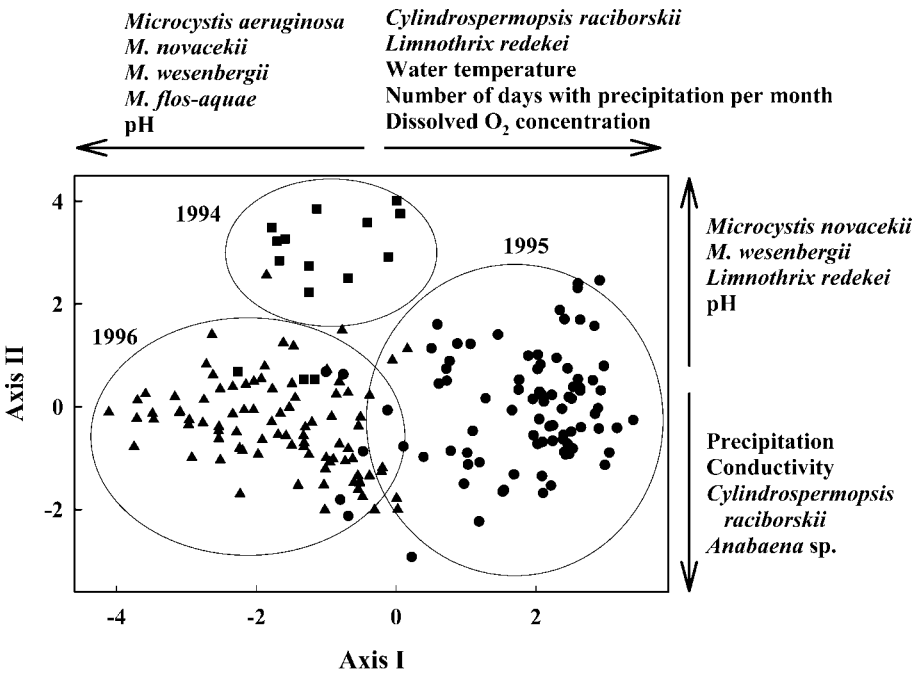

Fig. 3: Discriminant analysis of the inter-correlations between the determined biovolumes of the cyanobacterial species (volume of cyanobacteria per volume of lake water), the values of some physical and chemical parameters of the water, and the climatic factors, with groups based on each year, for data collected monthly from Lake Kastoria, during the warm period (June to November) of 1994 (■), 1995 (●), and 1996 (▲). See text for details.

Diskriminantanalyse der Beziehungen zwischen dem Biovolumen der Cyanobakterien-Arten (μL L^{-1}), einigen physikalisch-chemischen Parametern des Wassers und klimatischen Faktoren. Gruppen monatlicher Daten vom Lake Kastoria während der Monate Juni bis November 1994 (■), 1995 (●), 1996 (▲). Details s. Text.

nantly *M. aeruginosa,* and also *M. flos-aquae, M. ichthyoblabe, M. novacekii, M. wesenbergii*). At offshore stations the principal cyanobacteria in the water column were *Limnothrix redekei* (59%, v/v), *Cylindrospermopsis raciborskii* (18%, v/v)*, Microcystis* species (17%, v/v), and *Anabaena* species (6%, v/v) of the total annual cyanobacterial biovolume.

3.1.3 Dominant cyanobacterial species

Microcystis aeruginosa, Microcystis flos-aquae, Microcystis novacekii, Microcystis wesenbergii, Limnothrix redekei (formerly *Oscillatoria redekei*, [66]), *Anabaena viguieri, Anabaena* sp., and *Cylindrospermopsis raciborskii* were dominant (biovolume of an individual taxa > 10% (v/v) of the total cyanobacterial biovolume) at some time during the study period in L. Kastoria [27, 59]. These taxa are characteristic of eutrophic and hypertrophic waters [33, 67, 68]. The dominant cyanobacterial species in L. Kastoria could be grouped on the basis of their temporal and spatial variation in the water column:

1) *Microcystis* species: maximum biovolume occurred in late summer after the formation of temperature, pH, and O_2 gradients, at pH > 8, temperature 12...33 °C, depth < 0.2 m. Species of *Microcystis* were dominant during the formation of water blooms or scum in surface waters [27, 59]. *Microcystis aeruginosa* had the highest biovolumes of any of the cyanobacteria and was present almost continuously in the phytoplankton. Even in the cold period (< 10 °C) a small number of colonies were observed in the lower levels of the water column, probably as a result of overwintering on the sediment [27, 59]. *Microcystis* is considered to be a late entrant into the summer phytoplankton community of stratified lakes, since stimulatory conditions for growth of overwintering colonies occur only after the lake has become stratified [67]. The marked increase in the biovolume of *Microcystis* species in L. Kastoria in October 1996 may be related to the almost anoxic conditions (< 0.7 mg L^{-1} dissolved O_2) at a depth of 4.5 m in the preceding month, a phenomenon which has been observed in other lakes [67].

2) *Anabaena* sp. and *Cylindrospermopsis raciborskii*: maximum biovolumes occurred in mid-summer (July to August) at temperatures 23...26 °C. *Anabaena* sp. generally had a non-uniform vertical distribution with maximum biovolumes at a depth < 0.2 m, while *C. raciborskii* had a uniform distribution [27, 59].

3) *Limnothrix redekei*: had a continuous presence throughout the year and maximum biovolumes occurred over a tem-

perature range of 6...33 °C [27, 59]. It was dominant during the cold period (January to February) with a uniform distribution and during the warm period (July to September) had a tendency to accumulate at deeper depths during stratification of the water column. *L. redekei* was considered to be a key lake organism since it was dominant at all stations and all depths almost throughout the whole study period, constituting up to 59 % (v/v) of the total annual cyanobacterial biovolume [27].

Limnothrix redekei may exhibit a competitive advantage over other bloom-forming species through its ability to control buoyancy by gas vacuoles [66], its mixing tolerance [69], its shade and temperature tolerance and grazing resistance [67]. *Cylindrospermopsis raciborskii* is also tolerant to mixing [69] and grazing by zooplankton [33] and dominated together with *L. redekei* in L. Kastoria when water temperatures exceeded 20 °C [27]. Stable thermal stratification is known to give a competitive advantage to the growth of the genus *Microcystis* [67], but the mixing observed in L. Kastoria did not appear to limit biovolume increase [27, 59]. *Microcystis aeruginosa*, *Anabaena* sp., and *C. raciborskii* belong to genera that include toxin-producing species [6].

3.2 Microcystin-LR concentrations and water quality

Studies worldwide on the temporal and spatial distribution of MCYSTs are relatively few and recent [70–76], but essential for assessing associated risks. The temporal and spatial variation of intracellular MCYST-LR concentration (MCYST-LR per volume of lake water, $\mu g\ L^{-1}$) was studied in L. Kastoria [27]. MCYST-LR was detected by HPLC in all samples collected from June to November of 1994, 1995 and from June 1996 to June 1997. MCYST-LR concentrations varied between years and between months within a year. The concentration of MCYST-LR ranged from 0.08...58.16 $\mu g\ L^{-1}$ at offshore stations and from 0.09...3186 $\mu g\ L^{-1}$ at inshore stations. The offshore values are higher than those from some Canadian (up to 6 $\mu g\ L^{-1}$ [71]), German (up to 3 $\mu g\ L^{-1}$ [74]) and Korean (up to 0.2 $\mu g\ L^{-1}$ [75]) freshwaters. The highest inshore MCYST-LR concentrations which have been determined worldwide are those along the shore of the Havel River, Germany (>100 to 24000 $\mu g\ L^{-1}$ [73]).

MCYST-LR was detected in all the samples collected from L. Kastoria (1994 to 1997) and MCYST-LR concentration was positively and significantly correlated with the total cyanobacterial biovolume (r^2 = 0.724, $P < 0.05$, n = 277) [27]. Similar relationships have been observed for German lakes (r^2 = 0.48 to 0.67 [74, 77]) and *Microcystis* species in Canadian lakes (r^2 = 0.21 to 0.41 [70, 71]). Therefore, cyanobacterial biovolume can be used as an indicator of the in situ MCYST-LR concentration in L. Kastoria. MCYST-LR concentrations > 1 $\mu g\ L^{-1}$ were observed when *Microcystis* species (mainly *Microcystis aeruginosa*) constituted > 50 % (v/v) of the cyanobacterial biovolume [27]. The indications are that *M. aeruginosa* is the major MCYST-LR producing cyanobacterium in L. Kastoria [27]. *M. aeruginosa* is widely known to produce MCYST-LR [6] and several lake studies have shown that MCYST-LR concentrations correlate with the biovolume of *M. aeruginosa* [53, 70, 71]. *Microcystis* strains isolated from L. Kastoria had total MCYST concentrations per dry weight cyanobacterial cells, of 90 to 1200 $\mu g\ g^{-1}$ and structural variants -LR, -RR, and -LA were detected [29].

The World Health Organisation (WHO) provisional guideline value of 1 $\mu g\ L^{-1}$ MCYST-LR equivalent in drinking water is considered to be safe for lifelong consumption. A series of guidelines associated with incremental severity and probability of adverse effects from cyanobacteria in recreational waters has been defined on three levels [19]. Relatively mild and/or low probabilities of adverse health effects deal mainly with the irritant or allergenic effects of cyanobacterial compounds, other than cyanobacterial toxins, and that even though MCYSTs may range from 1...10 $\mu g\ L^{-1}$ accidental ingestion would be unlikely to cause adverse health effects. 'Moderate probability' and 'High risk' of adverse health effects concern MCYST concentrations in recreational waters in the range of 10...40 $\mu g\ L^{-1}$ and >40 $\mu g\ L^{-1}$, respectively, which would potentially, adversely affect health following involuntary ingestion. High risk can be characterised by cyanobacterial scum formation in which extremely high MCYST concentrations (24 mg L^{-1}) may be encountered in localised areas over a short time period, with the potential for acute poisoning, animal fatalities and liver damage in humans following oral ingestion of scum.

The MCYST-LR concentrations determined in L. Kastoria from April 1994 to June 1997 [27] are grouped on a monthly basis and a typical annual trend appears in Figure 4. Low levels (mean values < 1 $\mu g\ L^{-1}$) with the exception of the individual samples indicated, are observed from February to June with no associated health risks. In July and August MCYST-LR levels tend to increase, with about 14 % of the samples posing a moderate to high risk of adverse health effects. MCYST-LR concentrations are maximal in September, October, and November, with 14 % of the samples indicating the potential for a high risk of adverse health effects. From August to November MCYST-LR concentrations are higher than 2 $\mu g\ L^{-1}$, the threshold recommended by WHO for recreational waters [19]. In L. Kastoria 41 % of the samples from the surface water (0...0.2 m) had MCYST-LR concentrations higher than 1 $\mu g\ L^{-1}$, the WHO provisional guideline value for drinking water.

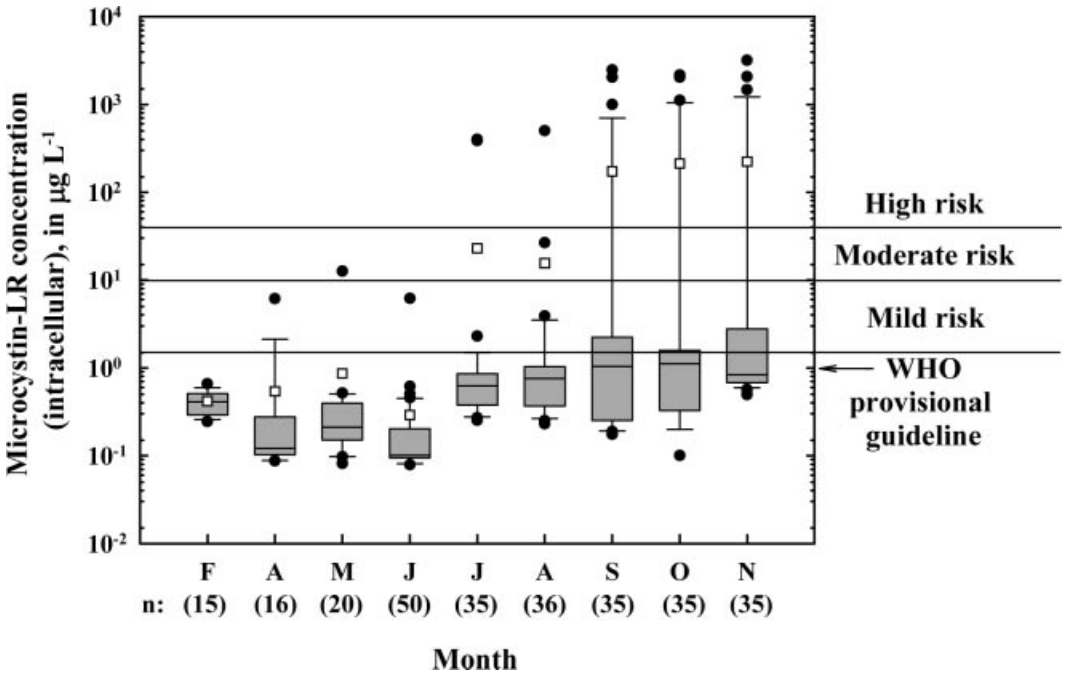

Fig. 4: Intracellular Microcystin-LR concentrations (MCYST-LR per volume of lake water) in Lake Kastoria compared with guidelines for assessing adverse health risks. The relative distributions of Microcystin-LR concentrations are expressed on a monthly basis independent of the year and were determined from April 1994 to June 1997, at three inshore stations (depth: 0...0.2 m) and three offshore stations (depths: 0...0.2 m, 1.5 m, 3.0 m, and 4.5 m) in L. Kastoria. The relative distribution of the values is indicated by box and whisker plots. The box represents the 25th...75th percentiles and the median value, the bars the 10th and 90th percentiles. Outlying values (●) and the mean value (□) for each month independent of year are given, where *n* is the total number of samples collected for a particular month.

Intrazelluläre Konzentrationen von Microcystin-LR (μg L^{-1}) im Lake Kastoria im Vergleich zu den Richtwerten zur Einschätzung des Risikos von Gesundheitsbeeinträchtigungen. Die relative Verteilung der Konzentrationen ist dargestellt als Monatswerte von April 1994 bis Juni 1996 von je drei Litoralstationen (0...0.2 m) und Freiwasserstationen (0...0.2 m, 1.5 m, 3.0 m und 4.5 m). Im Box-Whisker-Plot entspricht die Box dem Median und dem 25- bis 75-Percentil, die Balken entsprechen den Werten für 10- und 90-Percentil. ● Ausreißer, □ arithmetisches Mittel. *n*: Anzahl der Proben je Monat über alle Jahre.

4 The presence of microcystins in aquatic organisms

Cyanobacterial toxins are primarily a problem when they are ingested and concentrated by fish and shellfish, which are in turn eaten by humans or animal foragers [78, 79]. Cyanobacteria have been implicated in fish poisonings and fish die after intraperitoneal injection of toxic cyanobacteria [6]. Whether blooms of toxic cyanobacteria are a hazard to fish farming and aquaculture in Greece requires further investigation to estimate the dangers to consumer health and to avert economic damage to the industry.

The presence of MCYSTs in some aquatic fauna has been investigated in three lakes, L. Kastoria, L. Kerkini, and L. Pamvotis, where toxic cyanobacterial blooms frequently occur. Muscle tissue and viscera of seven species of fish, a frog, a mollusc, and a gastropod collected in the warm period of 1999 and 2000 were examined [80]. MCYSTs were detected in all of the samples by 2 methods, protein phosphatase 1 (PP1) inhibition assay and immunoassay using ELISA [81, 82]. The MCYST concentrations (MCYST-LR equivalents per dry weight tissue, determined by ELISA) in the viscera were on average higher than those of the muscle tissue and were in the order of 200...600 ng g^{-1}. The average MCYST concentration for fish and frog muscle tissue was 225 ng g^{-1} and 125 ng g^{-1}, respectively [80].

A Tolerable Daily Intake (TDI) value for MCYSTs of 0.04 μg kg^{-1} body weight per day has been proposed by the WHO [25]. If an adult human (60 kg) was to consume 300 g

of fish or frog from the lakes examined, the levels of MCYSTs ingested, the Estimated Daily Intake, would exceed the TDI by 28 and 15 times, respectively [80]. Therefore, lake products targeted for human consumption should be monitored for MCYST content, and further research is necessary in order to implement lake management policies.

The accumulation of MCYSTs in the edible parts (i.e. muscle) of wild fish species living in the eutrophic waters of rivers of central and southern Portugal has been reported with concentrations of up to 0.3 $\mu g\ g^{-1}$ [51]. However, concentrations in mussels (*Mytilus galloprovincialis*) and crayfish (*Procambarus clarkii*) were much higher, 16.0 $\mu g\ g^{-1}$ and 2.7 $\mu g\ g^{-1}$, respectively, and posed a threat to populations such as fishermen that feed frequently and in large amounts on these products. In Italy, saxitoxin was detected in extracts of shellfish (*Unio anadonta*; 0.215 nmol g^{-1}) and fish tissues following blooms of *Oscillatoria* sp. (reclassified as *Planktothrix* sp. [44]) and *Aphanizomenon* sp. [43, 44].

5 Impacts of cyanobacterial blooms and toxins: particular consequences in the Mediterranean region

The occurrence of toxic cyanobacterial blooms in lakes presents a potential hazard for human health and wildlife, particularly in the absence of legislation concerning lake usage and products. Research to date in Greece suggests that the potential hazards of toxic cyanobacterial blooms are heightened compared to countries with more temperate climates. More blooms can be expected due to the warmer, drier, and sunnier Mediterranean climate and the increasing eutrophication of freshwaters. In addition, the blooms will be of greater intensity and of extended or even continuous duration.

The widespread occurrence of the genus *Microcystis* in Greek lakes and its propensity to form toxic blooms in the summer and autumn is a potential threat, since it is the most frequently cited organism to date in incidents of poisonings of humans and livestock by cyanobacteria or their toxins [25]. In addition, a wider range of toxic cyanobacterial species than in temperate climates may be encountered, resulting from the different growth conditions. Already toxic blooms of *Anabaenopsis milleri* have been reported in Greece [7, 24], which have not been observed elsewhere in the world, and furthermore there are indications that *Anabaena viguieri* may also be toxic [24].

The occurrence of toxic cyanobacterial blooms in Greek freshwaters will have serious consequences on drinking water resources, due to the reliance on surface waters for domestic drinking supplies and the limited number of available lakes and reservoirs for such purposes. Although most of the smaller towns and cities in Greece use groundwater for drinking, the drinking water in Athens originates from three surface water sources. Due to increased demand it is also projected that within the next 3 years Thessaloniki, the second largest city in Greece, will also be using surface water. The inhabitants of Athens and Thessaloniki together constitute more than 50% of the national population. Although the freshwaters serving Athens and the prospective source for Thessaloniki are oligotrophic, the presence of species of *Microcystis*, *Anabaena,* and *Cylindrospermopsis* has been observed.

High cyanobacterial biovolumes are observed mainly in late summer and autumn in temperate lakes. In contrast, the cyanobacterial biovolumes were high throughout the duration of the study in L. Kastoria (> 11 $\mu L\ L^{-1}$) (Fig. 5). Cyanobacterial biovolumes were highest from August to November (Fig. 5) and correlated well with the MCYST-LR concentrations (Fig. 4), which presented a high risk of adverse health effects following ingestion or contact with lake water.

Only hepatotoxic cyanobacterial blooms have been recorded in Greece to date, but investigations on the presence of neurotoxins by mouse bioassays have been limited. The presence of neurotoxins appears less likely though, based on the potentially toxic cyanobacterial species encountered so far (Table 1). Results of similar surveys in other European countries have shown that although hepatotoxic blooms are found more frequently (> 60%), up to 25% of the cases investigated had neurotoxic blooms in Denmark, Germany, Norway, and Portugal [83]. In the Czech Republic MCYSTs were recorded in 90% of all samples investigated [84].

Survey results to date also suggest significant geographic differences in the dominance of microcystin-producing cyanobacterial taxa. In southern Norway about 62% of the hepatotoxic blooms were due to filamentous cyanobacteria and most frequently the genus *Anabaena* and to a lesser extent *Planktothrix*, and about 25% were due to *Microcystis* spp. [85]. *Anabaena* spp. also frequently form hepatotoxic blooms in Finland [30]. In Germany, *Planktothrix* spp. were the dominant microcystin-producing cyanobacteria in blooms, but were closely followed by *Microcystis* spp. [83]. Further south in the Mediterranean region, *Microcystis* spp. were dominant in hepatotoxic cyanobacterial blooms in Egypt, France, Morocco, Portugal, Spain, and Greece (see Table 1, Table 2).

It has also been noted that characteristic ranges of MCYST concentrations within the cellular biomass can be established for common cyanobacterial taxa, with some geographic differences in absolute concentrations [83]. For example, samples dominated by *Microcystis* spp. had MCYST concentrations in relation to dry weight which were similar, mean 770 to 870 $\mu g\ g^{-1}$ and maxima 1500 to 5800 $\mu g\ g^{-1}$,

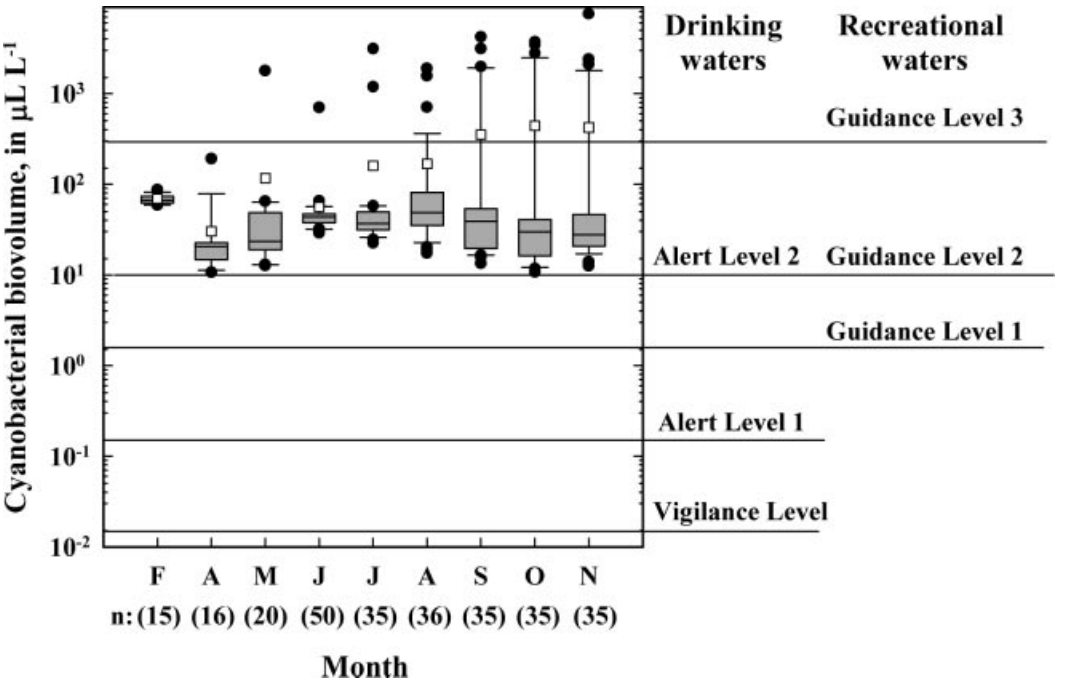

Fig. 5: Cyanobacterial biovolumes (volume of cyanobacteria per volume of lake water) in Lake Kastoria and Alert and Guidance levels proposed by the World Health Organisation for drinking water supplies and recreational waters, respectively. The relative distributions of cyanobacterial biovolumes are presented on a monthly basis independent of year and were determined from April 1994 to June 1997, at three inshore stations (depth: 0...0.2 m) and three offshore stations (depths: 0...0.2 m, 1.5 m, 3.0 m, and 4.5 m) in L. Kastoria. The distributions are represented by box and whisker plots. The box represents the 25th...75th percentiles and the median value, the bars the 10th and 90th percentiles. Outlying values (●) and the mean value (□) for each month independent of year are given, where *n* is the total number of samples collected for a particular month.

Biovolumen der Cyanobakterien (µL L^{-1}) im Lake Kastoria und Sicherheits- und Richtwerte der WHO für Trinkwasserversorgung bzw. Erholungsgewässer. Die relative Verteilung der Biovolumina ist dargestellt als Monatswerte von April 1994 bis Juni 1996 an je drei Litoralstationen (0...0.2 m) und Freiwasserstationen (0...0.2 m, 1.5 m, 3.0 m und 4.5 m). Im Box-Whisker-Plot entspricht die Box dem Median und dem 25- bis 75-Percentil, die Balken entsprechen den Werten für 10- und 90-Percentil. ● Ausreißer, □ arithmetisches Mittel. *n*: Anzahl der Proben je Monat über alle Jahre.

in independent data from Germany [72], the Czech Republic [84], and Korea [86]. Values were lower for Denmark (mean 160 µg g^{-1}; max 1280 µg g^{-1}) [87] and higher for Greece (mean 1638 µg g^{-1}) and Portugal (mean 4100 µg g^{-1}; max 7100 µg g^{-1}) [51]. It would appear that southern European countries are more likely firstly, to have toxic cyanobacterial blooms consisting of *Microcystis* spp. and secondly, to have higher MCYST concentrations within the cellular biomass. Survey data from Germany show that MCYST toxin quotas (expressed as per biovolume) differ between taxa, but that variation within most of the samples dominated by the same taxon is only moderate (two to five fold) [72].

An Alert Levels Framework outlining a monitoring and management action sequence that water treatment plant operators and managers can use to provide a graduated response to the onset and progress of a potentially toxic, cyanobacterial bloom has been described [88]. The sequence of response levels is based upon the initial detection of cyanobacteria at the 'Vigilance Level', the presence of moderate to high cyanobacterial numbers and the detection of toxins above the WHO guideline concentrations for MCYST-LR, at 'Alert Level 1', and the presence of very high cyanobacterial biomass levels with the confirmed presence of toxins and the possibility of acute poisoning at 'Alert Level 2', which requires immediate action either in the implementation of effective water treatment systems or the use of alternative water supplies.

All of the samples from L. Kastoria have biovolumes in excess of the Guidance Level 2 (10 µL L^{-1}) (Fig. 5) proposed by WHO for waters used for recreational purposes and Alert

Level 2 for drinking water [88]. Furthermore, surface water samples from April to November exceeded Guidance Level 3, with the potential for acute cyanobacterial poisoning. The highest concentration of MCYST-LR encountered in the surface waters of L. Kastoria was 3186 $\mu g\ L^{-1}$, far surpassing the recommended level for drinking water (1 $\mu g\ L^{-1}$), as did 41 % of the total number of surface water samples collected [27]. Based on toxicological data the involuntary ingestion of 2 mg MCYST-LR during swimming could cause liver disease in a 10 kg child [19]. The situation in L. Kastoria is representative of other Greek lakes located in different geographic regions, which also have high cyanobacterial biovolumes and microcystin concentrations in the summer months (Table 1). It is also evident that MCYSTs have entered the food chain in Greece, with their presence in detectible quantities in fish, frogs, molluscs, and gastropods (see section 4). A similar situation has been reported in Portugal [51].

The combination of high cyanobacterial biovolumes and MCYST concentrations in water samples and the presence of MCYSTs in the food chain indicate elevated risks of acute toxicosis and adverse human health effects in several Greek lakes and in general many other countries of the Mediterranean region. The absence of management policies for the lakes where toxic blooms occur presents a potential hazard for human health and wildlife concerning the consumption of lake products and water. The instigation of monitoring programs for the presence of MCYSTs in freshwaters and the quality control of lake products are required.

Acknowledgements

This work was supported by the General Secretariat of Research and Technology, Greece (91 EΔ 756) and by the EU CYANOTOX program (IC18-CT98-0293). This support is gratefully acknowledged. We would also like to thank the anonymous reviewer of the manuscript for constructive, thorough, and valuable comments.

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[Received: 28 April 2003; accepted: 12 February 2004]