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# Cyanobacterial blooms and water quality in Greek waterbodies

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*Key words:* associations, biovolume, chlorophyll *a*, *Microcystis*, risk-assessment, toxin-producing

## Abstract

The cyanobacterial species composition of nine Greek waterbodies of different type and trophic status was examined during the warm period of the year (May–October). Cyanobacterial water blooms were observed in all waterbodies. Forty-six cyanobacterial taxa were identified, 11 of which are known to be toxic. Eighteen species are reported for the first time in these waterbodies, 8 of which are known to produce toxins. Toxin producing species were found in all of the waterbodies and were primarily dominant in bloom formations (e.g., *Microcystis aeruginosa*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii*). Cosmopolitan species (e.g., *M. aeruginosa*), pantropic (e.g., *Anabaenopsis tanganyikae*) and holarctic species (e.g., *Anabaena flos-aquae*) were encountered. Shallow, eutrophic waterbodies had blooms dominated by *Microcystis* species and were characterized by phytoplankton association M. *Anabaena* and *Aphanizomenon* species of association H were dominant in waterbodies with low dissolved inorganic nitrogen and thermal stratification in the summer. Total cyanobacterial biovolumes (CBV) ranged from 7 to 9,507 $cm^3 m^{-3}$ and were higher than Alert Level 2 and Guidance Level 2 (10 $cm^3 m^{-3}$; World Health Organization; WHO) in seven of the waterbodies. Chlorophyll *a* concentrations ranged from 6 to 90,000 $mg m^{-3}$ and were higher than Alert Level 2 and Guidance Level 2 (50 $mg m^{-3}$; WHO) in eight of the waterbodies. There is also an elevated risk of acute toxicosis (Guidance Level 3; WHO) in five waterbodies. Water of an undesirable quality, hazardous to humans and animals occurs in several Greek waterbodies.

*Abbreviations*: CBV, Cyanobacterial biovolume; Chl *a*, Chlorophyll *a*; EC, European Community; EU, European Union; PCA, Principal Components Analysis; TCBV, Toxic cyanobacterial biovolume; WFD, Water Framework Directive; WHO, World Health Organization

## Introduction

Water blooms have become a frequent phenomenon in eutrophic waterbodies worldwide as in the Mediterranean region. The warm Mediterranean climate favours the extended duration of the cyanobacterial blooms, which may start in spring and persist until December, or in some cases, may even be continuous throughout the year. Phytoplankton studies in some Greek lakes (Vegoritis, Volvi, Mikri Prespa, Doirani and Kastoria) have shown that prolonged cyanobacterial blooms of up to 8 months can occur, which are dominated by known toxic species (Moustaka-Gouni & Nikolaidis, 1990; Moustaka-Gouni, 1993; Tryfon & Moustaka-Gouni, 1997; Temponeras et al., 2000; Vardaka et al., 2000).

Comprehensive long-term studies of phytoplankton do not exist for the majority of Greek waterbodies.

Mainly, there are isolated reports concerning the floristic data of a sparse number of samples from individual lakes. A comprehensive floristic study exists for L. Amvrakia (Spartinou, 1992), L. Volvi (Moustaka, 1988a), L. Doirani (Temponeras et al., 2000) and L. Mikri Prespa (Tryfon & Moustaka-Gouni, 1997). However, trophic state transitions and changes in water depth have occurred in Greek lakes over the past 20 years which affect species composition and water quality. For example, the maximum depth of L. Doirani has decreased from 8.5 to 5.0 m during the period 1986–1993 (Temponeras et al., 2000).

Knowledge of the cyanobacterial species composition enables the characterisation of waterbodies according to the associations outlined by Reynolds (1997) that are used as major descriptors of phytoplankton at a higher level than that of species and also reflects the characteristics of the waterbody. The species composition also indicates the presence of toxin producing cyanobacterial species and the potential toxicity of a bloom. To date, hepatotoxic cyanobacterial blooms have been reported in several Greek waterbodies (Lanaras et al., 1989; Cook et al., 2004). The presence of cyanobacterial toxins in drinking water supplies and irrigation water is known to pose a potential hazard to human health and agricultural and aquaculture products directed for animal and human consumption (Falconer, 1999; Falconer et al., 1999). Some characteristics of the surface and ground waters used for irrigation are monitored in Greece and it has been acknowledged that there are problems with pollution and decreasing water levels in some waterbodies (Parliament of the Hellenic Republic, 2001).

Good water quality should be maintained in waterbodies particularly since many are used for recreation, aquaculture and drinking water. The European Union Water Framework Directive (EU-WFD; 2000/60 European Community; EC), which requires that the ecological status of bodies of surface water exceeding 0.5 $km^2$ are assessed in addition to physical and chemical properties, is in the process of being implemented in Greece. According to the EU-WFD, ecological status has to be assessed by comparing data of biological quality elements to a reference (undisturbed) condition. However, due to anthropogenic disturbance it is often difficult to find and describe the reference condition. Furthermore, biological quality elements are influenced by natural variation. Data contributing towards the evaluation of ecological status is valuable. Basic information concerning the biological quality element phytoplankton (species composition and abundance, biomass and blooms), necessary for the implementation of the EU-WFD in Greece to date, is limited.

The aims of this study were (1) to examine the cyanobacterial species composition of blooms in waterbodies of different type (size, depth, altitude, mixing and trophic state) and the presence of known toxin producing species; (2) to determine the cyanobacterial biovolumes and chlorophyll concentrations of blooms in order to assess the risks to humans and wildlife; and (3) to examine whether the studied biological parameters can be used as tools in characterising waterbodies and in assessing risk levels.

## Study area and methods

### *Description of waterbodies*

According to the EU-WFD (2000/60/EC) surface water body typology the types and some other characteristics of the waterbodies examined are given in Table 1. Lakes Doirani, Kastoria, Mikri Prespa, Pamvotis, Vistonis, Zazari, and Kerkini Reservoir are shallow, while L. Volvi is relatively shallow and L. Amvrakia is deep. The surface areas of the waterbodies range from 1.9 to 74.7 $km^2$ (see Table 1 for references). L. Amvrakia and L. Volvi are warm monomictic, while L. Kastoria and L. Mikri Prespa are polymictic. L. Doirani is warm monomictic and potentially polymictic, depending on the inter-annual climate variability (see Table 1 for references). The waterbodies examined experience a Mediterranean climate. The lakes are eutrophic (Lakes Doirani, Mikri Prespa, Pamvotis, Vistonis, Volvi and Zazari), eutrophic-hypertrophic (L. Kastoria) or oligo-mesotrophic (L. Amvrakia). The trophic state of Kerkini Reservoir has not yet been described (Table 1). Lakes Kastoria and Pamvotis are urban lakes and are used for recreational activities.

### *Sample collection*

Water samples were collected from the surface layer (0–40 cm) of the waterbody margins during the period May to October of 1994, 1995, 1999 and 2000.

### *Chlorophyll* a *and biovolume determination*

Water samples were filtered through Whatman GF/C glass-fibre filters on site, within 1 h of collection. Chlorophyll *a* (Chl *a*) of the filtered samples was estimated using standard methods (Jeffrey & Humphrey,

*Table 1.* Characteristics and types of the waterbodies studied.

| Waterbody | Geographic coordinates (N) | (E) | Area (km$^2$) | Maximum depth (m) | Mean depth (m) | Eco -region[j] | Size typology[j] (km$^2$) | Depth typology[j] (m) | Altitude typology[j] | Mixing type | Trophic state |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kerkini Reservoir | 41°08′ | 23°13′ | 51.5–74.7[a] | 4.0–8.7[a] | 3–5[g] | 7[o] | 10–100 | 3–15 | L | – | – |
| Lake Amvrakia | 38°45′ | 21°11′ | 14.5[b] | 53.0[b] | 19.2[b] | 6[n] | 10–100 | >15 | L[g] | Mm[k] | O-Mt[k] |
| Lake Doirani | 41°11′ | 22°45′ | 28.0[c] | 5.0[c] | 3[g] | 7[o] | 10–100 | 3–15 | L[g] | Mm or P[c] | E[c] |
| Lake Kastoria | 40°31′ | 21°18′ | 24.0[d] | 8.5[d] | 5[d] | 6[n] | 10–100 | 3–15 | M[g] | P[d] | E-H[d] |
| Lake Mikri Prespa | 40°45′ | 21°07′ | 48.5[e] | 7.0[e] | 5[g] | 6[n] | 10–100 | 3–15 | H[g] | P[e] | E[e] |
| Lake Pamvotis | 39°40′ | 20°51′ | 22.8[f] | 10.0[f] | 4.5[g] | 6[n] | 10–100 | 3–15 | M[g] | – | E[f] |
| Lake Vistonis | 41°01′ | 25°08′ | 45.0[g] | 4.7[g] | 3.0[g] | 7[o] | 10–100 | 3–15 | L[g] | – | E[l] |
| Lake Volvi | 40°41′ | 23°25′ | 68.6[h] | 19.0[h] | 13.5[h] | 7[o] | 10–100 | 3–15 | L[g] | Mm[h] | E[h] |
| Lake Zazari | 40°37′ | 21°33′ | 1.9[g] | 8.0[i] | 4.6[g] | 6[n] | 1–10 | 3–15 | M[g] | – | E[m] |

*Note.* L: lowland (<200 m); M: mid-altitude (200–800 m); H: high altitude (>800 m); Mm: Monomictic; P: Polymictic; O-Mt: Oligotrophic-Mesotrophic; E: Eutrophic; E-H: Eutrophic-Hypertrophic; -: not available.
[a]Albanakis et al. (1993).
[b]Albanakis et al. (1995).
[c]Temponeras et al. (2000).
[d]Vardaka et al. (2000).
[e]Tryfon et al. (1997).
[f]Anagnostidis and Economou-Amilli (1980).
[g]Albanakis (personal communication).
[h]Moustaka-Gouni (1993).
[i]National Statistical Service of Greece (1994).
[j]European Community (EC) (2000).
[k]Spartinou (1992).
[l]Moustaka-Gouni (unpublished data).
[m]Stanković (1931).
[n]Hellenic (Greek) Western Balkan.
[o]Eastern Balkan.

1975; Barnes et al., 1992).The identification of cyanobacterial species and cyanobacterial biovolume determination were carried out as previously described (Vardaka et al., 2000). Biovolumes were expressed either, as the total cyanobacterial biovolume (CBV), the sum of the biovolumes of the individual cyanobacterial species in a sample or, as the known toxin producing cyanobacterial biovolume (TCBV) the sum of the biovolumes of known toxin producing cyanobacterial species in a sample. Known toxin producing cyanobacteria were those which have been reported in the literature (Lanaras et al., 1989; Otsuka et al., 1999; Sivonen & Jones, 1999).

### *Associations*

Cyanobacterial species comprising more than 10% (v/v) of the total cyanobacterial biovolume were considered to be dominant. The dominant cyanobacteria were classified according to the associations (functional groups) outlined by Reynolds (1997) and Reynolds et al. (2002). According to Reynolds, associations are groups of species with similar morphological and physiological traits and with similar ecologies. Associations are described by a code letter (e.g., M, H, $S_N$, S), habitat properties, typical representatives (genera or species) and tolerances and sensitivities to environmental conditions, for example to nutrient concentrations, light intensities, water mixing, etc.

The associations (Reynolds, 1997; Reynolds et al., 2002) encountered in this study were

1. Association M. Typical representatives: *Microcystis* and *Sphaerocavum*; habitat properties: dielly mixed layers of small eutrophic, low latitude lakes; tolerances: high light duration and intensity; sensitivities: flushing and low total light.
2. Association H. Typical representatives: *Anabaena*, *Aphanizomenon* and *Gloeotrichia*; habitat properties: relatively large mesotrophic lakes; tolerances: low nitrogen; sensitivities: mixing, low light intensities and low P concentrations.

3. Association S. Typical representatives: *Planktothrix agardhii*, *Limnothrix redekei* and *Pseudanabaena*; habitat properties: turbid mixed layers; tolerances: extremely low light intensities; sensitivities: flushing.
4. Association $S_N$. Typical representatives: *Cylindrospermopsis* and *Anabaena minutissima*; habitat properties: warm mixed layers; tolerances: low light and nitrogen concentrations; sensitivities: flushing.

In this study *Jaaginema subtilissimum* and *Planktolyngbya limnetica* were assigned to association S on the basis of similar morphological traits and similar ecologies.

*Health risk assessment*

The WHO Alert Levels and Guidance Levels Framework model (Bartram et al., 1999) for the monitoring and management of cyanobacteria in drinking and recreational waters were used to assess the potential health risks associated with the potentially toxic cyanobacterial blooms in the waterbodies studied.

*Statistical analysis*

Principal Components Analysis (PCA; Legendre & Legendre, 1998) of log-transformed data was used to group the waterbodies on the basis of the contribution of the relative dominance of each of the associations (parameters) in each waterbody. The higher the correlation between the parameter and the axis of the PCA, the higher is the contribution of the parameter to axis formation. Therefore, parameters with correlation values $>0.7$ (absolute value) were the major contributors to axis formation. The significance of the correlations between the parameters and axes cannot be tested using a standard statistical test for Pearson correlation coefficients (Legendre & Legendre, 1998).

The relationship between the ratio of TCBV:CBV and CBV was analysed using non-linear regression analysis (Legendre & Legendre, 1998).

## Results

Cyanobacteria comprised more than 90% (v/v) of the total phytoplankton biovolume in all the samples collected. A total of 46 cyanobacterial taxa were identified, 11 of which are known to be toxic (Table 2). The number of newly reported species (this study) and previously reported species for the waterbodies examined are given in Table 3. Eleven cyanobacterial species are reported for the first time in L. Zazari (*Microcystis aeruginosa*, *Microcystis flos-aquae*, *Microcystis ichthyoblabe*, *Microcystis novacekii*, *Microcystis viridis*, *Microcystis wesenbergii*, *Pseudanabaena* sp., *Anabaena flos-aquae*, *Anabaena spiroides*, *Aphanizomenon issatschenkoi*, *Cylindrospermopsis raciborskii*), ten species in Kerkini Reservoir (*M. aeruginosa*, *M. flos-aquae*, *M. ichthyoblabe*, *M. novacekii*, *M. wesenbergii*, *Pseudanabaena* sp., *Anabaena flos-aquae*, *Anabaena viguieri*, *A. spiroides*, *A. issatschenkoi*), five species in L. Amvrakia (*M. flos-aquae*, *M. novacekii*, *M. wesenbergii*, *Anabaena perturbata*, *A. viguieri*), four species in L. Pamvotis (*M. flos-aquae*, *M. novacekii*, *M. viridis*, *M. wesenbergii*), three species in L. Vistonis (*M. ichthyoblabe*, *M. wesenbergii*, *Anabaena* sp.), two species in L. Doirani (*Jaaginema subtilissimum*, *Anabaena aphanizomenoides*) and one species in L. Volvi (*Anabaenopsis cunningtonii*) and one in L. Kastoria (*Anabaena affinis*) (Table 2). Eight (*M. aeruginosa*, *M. ichthyoblabe*, *M. novacekii*, *M. viridis*, *M. wesenbergii*, *Anabaena flos-aquae*, *A. viguieri* and *C. raciborskii*) of the 18 species reported for the first time in the waterbodies examined are known to produce toxins (Table 2).

*Microcystis aeruginosa* was the most commonly dominant species (Table 2). Association(s) were assigned to each sample according to the dominant cyanobacteria present. Association M (*Microcystis* species) was the most common association encountered in the waterbodies, followed in decreasing frequency of occurrence by association H (*Anabaena* and *Aphanizomenon* species), association S (*Jaaginema subtilissimum*, *Limnothrix redekei* and *Planktolyngbya limnetica*) and association $S_N$ (*Cylindrospermopsis raciborskii*).

PCA analysis grouped the nine waterbodies according to the relative dominance of the associations found in the respective samples. Axis I, which accounts for 59% of the variance, is highly correlated with the associations M, S and H ($r = 0.977$, $-0.834$ and $-0.738$, respectively; Figure 1). Lakes Kastoria, Mikri Prespa, Vistonis, Zazari and Kerkini Reservoir are differentiated along this axis and are characterized by a relative dominance of association M. Lakes Doirani, Volvi and Amvrakia are characterized by the dominance of associations S and H. Lake Pamvotis is ordinated in the middle of Axis I. Axis II, which accounts for a further 27% of the variance, is highly correlated with the association $S_N$ ($r = 0.833$) in L. Kastoria (Figure 1).

*Table 2.* Cyanobacterial species identified in water bloom samples collected during the study period.

| Species | Association | Waterbody | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Amvrakia | Doirani | Kastoria | Kerkini | Mikri Prespa | Pamvotis | Vistonis | Volvi | Zazari |
| Chroococcales | | | | | | | | | | |
| *Aphanocapsa* sp. | | | | | | ○ | ○ | | ○ | |
| *Aphanothece nidulans* P. Richter | | | | | | | | | ○ | |
| *Chroococcus limneticus* Lemmermann | | | | | | ○ | | | ○ | |
| *Coelosphaerium* sp. | | | | | | ○ | | | | |
| *Cyanodictyon imperfectum* Cronberg et Weibull | | | | | | ○ | | | ○ | |
| *Merismopedia minima* G. Beck | | | | | | ○ | | | ○ | |
| *M. punctata* Meyen | | | | | | ○ | | | ○ | |
| *M. tenuissima* Lemmermann | | | | ○ | | | | | ○ | |
| **Microcystis aeruginosa* (Kützing) Kützing | M | ● | ○ | ● | ■ | ○ | ● | ● | ● | ■ |
| *Microcystis* cf. *holsatica* Lemmermann | M | | | | | | | | ○ | |
| *M. flos-aquae* (Wittrock) Kirchner | M | □ | | ○ | ■ | | □ | | | □ |
| **M. ichthyoblabe* Kützing | M | | | ○ | □ | | | □ | | □ |
| **M. novacekii* (Komárek) Compére | M | □ | | ● | □ | | □ | | ○ | □ |
| **M. viridis* (A. Braun) Lemmermann | M | | | | | | □ | | | □ |
| **M. wesenbergii* (Komárek) Komárek in Kondratjeva | M | □ | | ● | ■ | ● | □ | □ | | □ |
| *Microcystis* sp. | M | ● | | | | | ● | | | |
| *Radiocystis geminate* Skuja | | | | | | ○ | | | ○ | |
| *Snowella lacustris* (Chodat) Komárek et Hindák | | | | | | ○ | | | ○ | |
| *Woronichinia* cf. naegeliana (Unger) Elenkin | | | | | | ○ | | | | |
| Oscillatoriales | | | | | | | | | | |
| *Arthrospira platensis* (Nordstedt) Gomont | S | ○ | | | | | | | | |
| *Jaaginema subtilissimum* (Kützing ex De Toni) Anagnostidis et Komárek | S | | ■ | | | | | | ● | |
| *Limnothrix redekei* (Van Goor) Meffert | S | | | ● | | | | | | |
| *Lyngbya* sp. | S | | | | | | ○ | | | |
| *Oscillatoria* sp. | S | | | | | ○ | ○ | | ○ | |
| *Planktolyngbya circumcreta* (G.S. West) Anagnostidis et Komárek | S | | ○ | | | ○ | | | ○ | |
| *P. limnetica* (Anagnostidis et Komárek) Komárková-Legnerová et Cronberg | S | | | | | ○ | | | ● | |
| *Pseudanabaena* sp. | S | | | | □ | | ○ | | | □ |

(*Continued on next page*)

*Table 2.* (*Continued*)

| | | Waterbody | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Association | Amvrakia | Doirani | Kastoria | Kerkini | Mikri Prespa | Pamvotis | Vistonis | Volvi | Zazari |
| Nostocales | | | | | | | | | | |
| *Anabaena affinis* Lemmermann | H | | | □ | | | | | | |
| *A. aphanizomenoides* Forti | H | | □ | | | | | | ○ | |
| *A.* cf. *circinalis* Rabenhorst ex Bornet et Flahault | H | | ○ | | | | | | | |
| *A.* cf. *scheremetievi* Elenkin | H | | | | | | ○ | | | |
| *A.* cf. *solitaria* Klebahn | H | | | | | ○ | | | | |
| **A. circinalis* Rabenhorst ex Bornet et Flahault | H | | | | | | | | ○ | |
| **A. flos-aquae* Brébisson ex Bornet et Flahault | H | | ○ | ○ | □ | | ● | | | □ |
| **A. lemmermannii* P. Richter | H | | | | | ○ | | | | |
| *A. perturbata* Hill | H | ■ | | | | | | | ● | |
| *A. spiroides* Klebahn | H | | | | ■ | | | | | |
| **A. viguieri* Denis et Frémy | H | ■ | | ○ | □ | | | | | □ |
| *Anabaena* sp. | H | | | ○ | | | | □ | | |
| *Anabaenopsis cunningtonii* R. Taylor | H | | | | | | | | □ | |
| *A. elenkinii* Miller | H | | ○ | | | | | | | |
| *A. tanganyikae* (G.S. West) Miller | H | | | | | | | | ○ | |
| *Aphanizomenon* cf. *flos-aquae* Ralfs ex Bornet et Flahault | H | | | | | | | | ● | |
| **A. flos-aquae* Ralfs ex Bornet et Flahault | H | | ● | | | | ○ | | | |
| *A. issatschenkoi* (Usačev) Proškina-Lavrenko | H | | ○ | ○ | □ | | | | ○ | □ |
| **Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya et Subba Raju | $S_N$ | | | ● | | | | | ○ | □ |

*Note.* Description of associations is given in Study Area and Methods; *: species known to produce toxins (see Lanaras et al., 1989; Otsuka et al., 1999; Sivonen & Jones, 1999); ○: species present; □ : first record of the species in the respective waterbody; solid symbol (●, ■): dominant.

*Table 3.* Number of new species reported in this study and the number of reported taxa prior to this study.

| Waterbody | Number of new species | Number of reported taxa prior to this study | Prior study periods | Reference |
|---|---|---|---|---|
| Kerkini Reservoir | 10 | 0 | | |
| Lake Amvrakia | 5 | 23 | March 1978, August 1978, October 1979, and September 1981 | Overbeck et al. (1982) |
| | | | November 1984 to September 1989 | Spartinou (1992) |
| Lake Doirani | 2 | 28 | March to October 1996, and February 1997 | Temponeras et al. (2000) |
| Lake Kastoria | 1 | 22 | August 1929 | Stanković (1931) |
| | | | Autumn 1961 | Ocevski et al. (1975) |
| | | | August 1986 | Hindák (1992) |
| | | | August to October 1987 | Lanaras et al. (1989), Vardaka et al. (2000) |
| | | | April 1994 to July 1997 | Cook et al. (2004) |
| Lake Mikri Prespa | 0 | 33 | May 1990 to September 1992 | Tryfon et al. (1997) |
| Lake Pamvotis | 4 | 17 | August 1967 and June 1970 | Anagnostidis and Economou-Amilli (1980) |
| Lake Vistonis | 3 | 3 | August 1987 | Lanaras et al. (1989) |
| Lake Volvi | 1 | 36 | April 1983 to March 1986 | Hindák & Moustaka (1988); Moustaka-Gouni (1988a,b) |
| Lake Zazari | 11 | 0 | | |

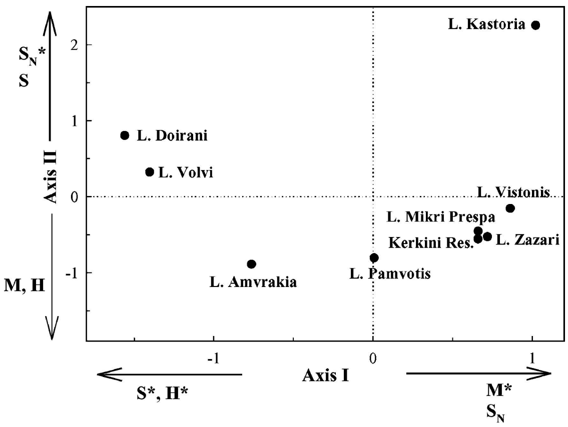


*Figure 1.* Ordination of the Principal Components Analysis (PCA) for the nine waterbodies (Lakes: Amvrakia, Doirani, Kastoria, Mikri Prespa, Pamvotis, Vistonis, Volvi, Zazari and Kerkini Reservoir) based on the relative dominance of each of the associations (M, H, S and $S_N$) in each waterbody. Correlations between the associations and the two axes exceeding 0.7 (absolute value) are indicated by (*).

The CBV ranged from 7 to 9,507 $cm^3\ m^{-3}$ in the waterbodies examined (Figure 2a). The highest CBV value (9,507 $cm^3\ m^{-3}$) was measured in a sample from L. Pamvotis which consisted of *Microcystis* species. The lowest CBV (7 $cm^3\ m^{-3}$) was measured in a sample from L. Volvi which consisted of *Planktolyngbya limnetica*, *Anabaena perturbata* and *Jaaginema subtilissimum*. The values of CBV were higher than the recommended threshold (10 $cm^3\ m^{-3}$) of Alert Level 2 for drinking water and Guidance Level 2 for recreational waters (Bartram et al., 1999) in all of the waterbodies examined, with the exception of L. Mikri Prespa and L. Volvi which were borderline (Figure 2a).

Chl *a* concentrations (Figure 2b) ranged from 6 $mg\ m^{-3}$ in L. Volvi, in which *Planktolyngbya limnetica*, *Microcystis aeruginosa* and *Anabaena perturbata* were dominant to 90,000 $mg\ m^{-3}$ in a sample from L. Kastoria, in which *M. aeruginosa* was dominant. Chl *a* concentrations were higher than the recommended threshold (50 $mg\ m^{-3}$) of Alert Level 2 for drinking water and Guidance Level 2 for recreational waters (Bartram et al., 1999) in all of the waterbodies examined, with the exception of L. Volvi (Figure 2b).

The contribution of TCBV to CBV increased as the CBV of the collected samples increased, such that when heavy blooms were encountered these were more likely to consist of toxic cyanobacteria. In the majority of the samples collected with a CBV $>1{,}000\ cm^3\ m^{-3}$ known

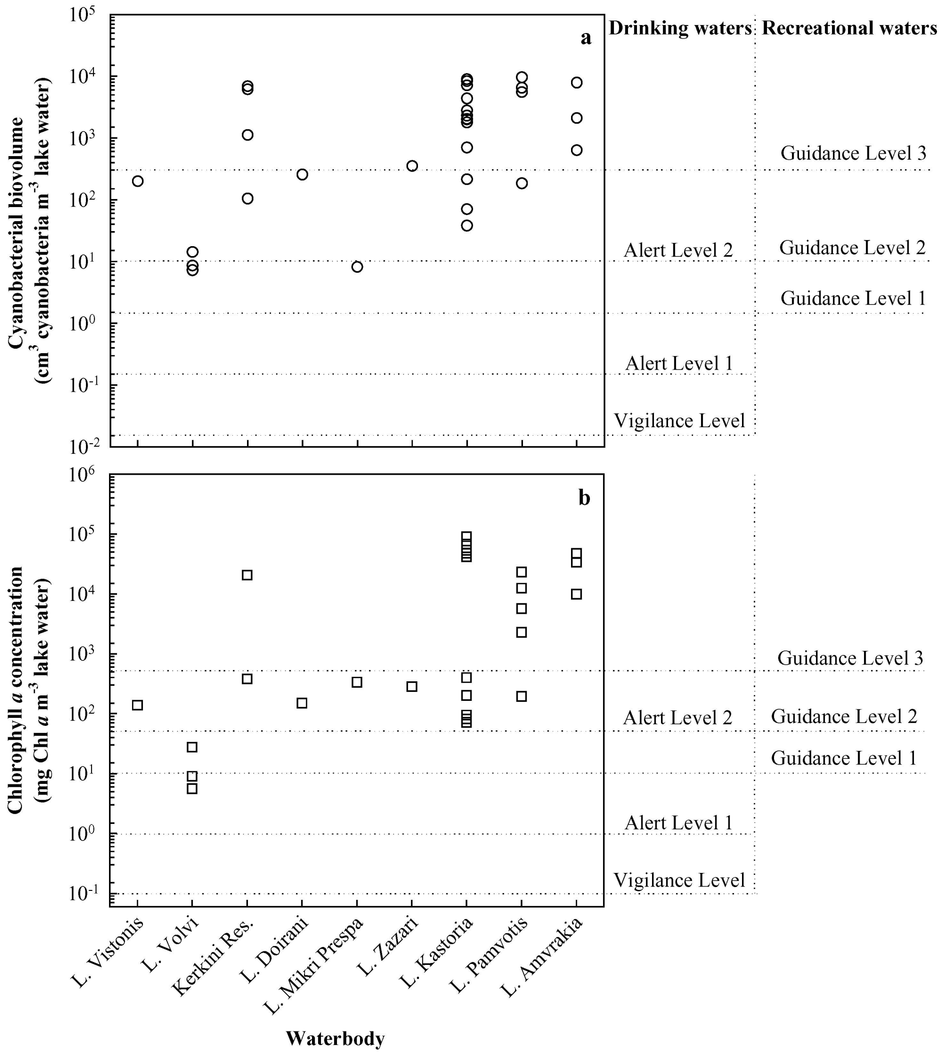


*Figure 2*. Cyanobacterial biovolume (a) and chlorophyll *a* concentration (b) in the waterbodies in relation to the Vigilance and Alert Levels for drinking water supplies and Guidance Levels for recreational waters proposed by the World Health Organization (Bartram et al., 1999).

toxin producing species accounted for more than 90% of the CBV. A significant relationship was found between the ratio TCBV:CBV and CBV using non-linear regression analysis (Figure 3).

## Discussion

Water blooms were observed in all of the eutrophic, eutrophic–hypertrophic and oligo-mesotrophic waterbodies examined in this study, during the warm period of the year (May–October). Water blooms in these Mediterranean waterbodies start in spring and persist sometimes until December (Moustaka-Gouni, 1993; Tryfon & Moustaka-Gouni, 1997; Vardaka et al., 2000). In northern temperate waterbodies cyanobacterial blooms are usually observed in the summer and autumn (Reynolds & Petersen, 2000). The Mediterranean climate, with a higher irradiance, higher temperature and lower rainfall than northern, temperate climates, promotes cyanobacterial growth over a longer period of the year and increases the possibility of water bloom development (Moustaka, 1988a).

The floristic data (Table 2) indicate that apart from species with a wide geographical distribution (e.g., *Microcystis aeruginosa*, *Microcystis wesenbergii*), species with pantropical (e.g., *Anabaenopsis tanganyikae*) and holarctic distribution (e.g., *Anabaena*

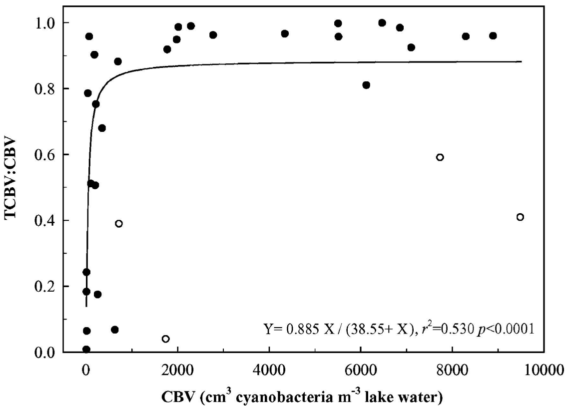


*Figure 3.* The relationship between the ratio of the known toxin producing cyanobacterial biovolume and the total cyanobacterial biovolume (TCBV:CBV) and CBV in all the samples collected from the nine waterbodies. In the samples indicated by white circles not all of the *Anabaena* or *Microcystis* could be identified to species level and therefore, it was not possible to assign to a toxic or non-toxic grouping. For this reason they were excluded from the non-linear regression analysis (hyperbolic model).

*flos-aquae*, *Anabaena lemmermannii*, *Aphanizomenon flos-aquae*, *Aphanizomenon issatschenkoi*) (Hoffmann, 1996) are encountered in these waterbodies of the Mediterranean region. The presence of a total of 18 species has been reported for the first time in the waterbodies examined in this study (Table 2). Comprehensive floristic lists do not exist for some of the waterbodies examined (see Table 3). Therefore, taxa recorded for the first time cannot be attributed to a "sudden" appearance. It is probable that many, if not all, of the newly recorded species will prove to have a widespread occurrence in the region. A comprehensive floristic study exists for L. Amvrakia (Overbeck et al., 1982; Spartinou, 1992) which was at that time an oligo-mesotrophic system. However, the high values of CBV and Chl *a* concentration measured in this study, indicate a highly eutrophic system. The new species recorded in L. Amvrakia may be a result of an increase in cyanobacterial abundance, above the detection limits of the methods used for phytoplankton studies, during the trophic state transition of the lake.

Known toxin producing species were found in each of the waterbodies and in many cases were dominant (e.g., *Microcystis aeruginosa*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii*). These species have been reported to be commonly dominant in toxic water blooms in other Mediterranean waterbodies (see Cook et al., 2004; Albay et al., 2003; Nasri et al., 2004).

Association M was found in five waterbodies of similar habitat properties (water depth, mixing type and trophic state). These five waterbodies (Kerkini Reservoir and Lakes Kastoria, Mikri Prespa, Vistonis and Zazari) (Figure 1) are shallow, polymictic, eutrophic waterbodies (Table 1). Association H was dominant in warm monomictic lakes (Lakes Amvrakia, Doirani and Volvi) (Figure 1) with low dissolved inorganic nitrogen (Spartinou, 1992; Moustaka-Gouni, 1993; Temponeras et al., 2000). Similar results have also been found in other phytoplankton studies (e.g., Huszar et al., 2000; Stoyneva, 2003). It appears that these (functional) associations capture much of the phytoplankton ecology and associations are potentially predictable from the habitat properties. Therefore, based on our study, water bloom associations M, and H are potential descriptors of the habitat properties (kind of waterbody). Association S was found in the same three waterbodies as association H and this indicates the mixing conditions during sampling. *Cylindrospermopsis raciborskii*, the $S_N$ association, was dominant in two different types of lakes, a polymictic eutrophic to hypertrophic lake and a eutrophic warm, monomictic lake (L. Kastoria and L. Volvi, respectively) (Figure 1) and this may indicate that *C. raciborskii* has wider habitat preferences than those described by Reynolds et al. (2002).

The CBV and Chl *a* concentration measured in the waterbodies examined are generally high. Extremely high values of CBV were recorded for the urban lakes, Pamvotis (CBV: 9507 cm$^3$ m$^{-3}$) and Kastoria (CBV: 7585 cm$^3$ m$^{-3}$). These CBV values are higher than those reported for some hypertrophic lakes (18–560 cm$^3$ m$^{-3}$; Alvarez-Cobelas & Jacobsen, 1992). To the best of our knowledge, the highest values of CBV which have been determined worldwide are those from a hypertrophic South African lake, Hartbeespoort Dam, with a dense population of *Microcystis aeruginosa* (41,000–116,000 cm$^3$ m$^{-3}$; Zohary, 1985). The Chl *a* concentration of the scum formed by *M. aeruginosa* in L. Kastoria (Chl *a*: 90,000 mg m$^{-3}$) is amongst the highest recorded for cyanobacterial blooms in waterbodies and is comparable to those recorded in hyperscums (Zohary, 1985; Ibelings & Mur, 1992; Chorus & Fastner, 2001). The high CBV and Chl *a* concentration values measured (Figure 2), in combination with the presence of known toxin producing cyanobacteria species in all of the waterbodies examined (Table 2), indicate the possibility of adverse human health effects even after short-term exposure (Alert Level 2 and Guidance Level 2; Bartram et al., 1999). There is also an elevated risk of acute toxicosis (Guidance Level 3; Bartram et al., 1999) in Lakes Kastoria, Zazari, Pamvotis, Amvrakia and Kerkini Reservoir. Cyanotoxin risk has been assessed in L. Kastoria after studying seasonal patterns of cyanobacterial and microcystin-LR occurrence (Cook et al., 2004).

In accordance with the EU-WFD (2000/60 EC), the data from this study in terms of species composition (taxa richness, indicator species of trophic state, toxicity, habitat properties), phytoplankton biomass (biovolume, Chl *a*) and bloom frequency/intensity (presence of phytoplankton blooms, occurrence of cyanobacterial blooms) even in the absence of reference conditions, can contribute to the present evaluation of waterbody ecological status and as a point of future comparison. Results indicate a deviation from high and good ecological status as outlined by the EU-WFD (2000/60 EC).

It was observed that when a cyanobacterial bloom occurs during the warm season in the nine waterbodies examined it is most likely to consist of known toxin producing species. In fact the heavier the bloom the higher was the contribution of TCBV to CBV. Toxic cyanobacterial species are generally well defended against grazing and subsequently this can lead to the accumulation of cyanobacterial biomass consisting predominantly of toxic species. CBV can be used as an indicator of the *in situ* microcystin-LR concentration in L. Kastoria since microcystin-LR concentrations $>1$ mg m$^{-3}$ were observed when *Microcystis* species (mainly *M. aeruginosa*) constituted $>50$ % (v/v) of the CBV (Cook et al., 2004).

In conclusion, water blooms were observed in all the types of Mediterranean waterbodies examined. The M, H and $S_N$ association characterization of water blooms can be used as descriptors of waterbody type. Known toxin producing species were found in all of the waterbodies and in many cases these were dominant and were primarily responsible for bloom formation. In addition, there is a significant relationship between the ratio of TCBV:CBV and CBV in the water blooms which may be useful as a tool in water management. The CBV and Chl *a* concentrations in the waterbodies are in excess of the recommended WHO guidelines for waters used as drinking water sources or for recreational activities. Therefore, in accordance with WHO guidelines (Bartram et al., 1999) there is an undesirable water quality hazardous to humans and animals in several Greek waterbodies. It follows that these waterbodies should be monitored for the presence of cyanotoxins, and that management policies should be implemented.


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## References


Albanakis K, Psilovikos A, Papaphilipou-Penou E (1993) Evolution of the deltaic sedimentation in the artificial lake Kerkini since its construction. Proceedings of the 4th National Symposium, Oceanography and Fisheries, 276–279. Rhodes, Greece, April 26–29 (in Greek with English summary).

Albanakis K, Psilovikos A, Vouvalidis K, Palikaridis X (1995) Anoxic conditions in the karstic cryptolake Amvrakia. Proceedings of the 4th National Geographical Congress, Athens, Greece, October 12–14 (in Greek with English summary).

Albay M, Akcaalan R, Tufekci H, Metcalf JS, Beattie KA, Codd GA (2003) Depth profiles of cyanobacterial hepatotoxins (microcystins) in three Turkish freshwater lakes. Hydrobiologia 505: 89–95.

Alvarez-Cobelas M, Jacobsen BA (1992) Hypertrophic phytoplankton: An overview. Freshwater Forum 2: 184–199.

Anagnostidis K, Economou-Amilli A (1980) Limnological studies on Lake Pamvotis (Ioannina), Greece I. Hydroclimatology, phytoplankton-periphyton with special reference to the valency of some microorganisms from sulphureta as bioindicators. Arch. Hydrobiol. 89: 313–342.

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. Environ. Exp. Bot. 32: 85–100.

Bartram J, Burch M, Falconer IR, Jones G, Kuiper-Goodman T (1999) Situation assessment, planning and management. In Chorus I, Bartram J (eds), Toxic Cyanobacteria in Water. E & FN Spon, London, New York. pp. 179–209.

Chorus I, Fastner J (2001) Recreational exposure to cyanotoxins. In Chorus I (ed), Cyanotoxins-occurrence, causes, consequences. Springer-Verlag, Berlin Heidelberg New York, pp. 190–199.

Cook CM, Vardaka E, Lanaras T (2004) Toxic cyanobacteria in Greek freshwaters, 1987–2000: Occurrence, toxicity, and impacts in the Mediterranean region. Acta Hydrochim. Hydrobiol. 32: 107–124.

European Community (EC) (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the Community action in the field of water policy. Off. J. Eur. Commun., 22-12-2000, L 327/1-72.

Falconer IR (1999) An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. Environ. Toxicol. 14: 5–12.

Falconer IR, Bartram J, Chorus I, Kuiper-Goodman T, Utkilen H, Burch M, Codd GA (1999) Safe levels and safe practices. In Chorus I, Bartram J (eds), Toxic Cyanobacteria in Water. E & FN Spon, London, pp. 155–178.

Hindák F (1992) Several interesting planktic cyanophytes. Algol. Stud. 66: 1–15.

Hindák F, Moustaka M (1988) Planktic cyanophytes of Lake Volvi, Greece. Arch. Hydrobiol. Suppl. 80: 497–528.

Hoffmann L (1996) Geographic distribution of freshwater blue-green algae. Hydrobiologia 336: 33–40.

Huszar VLM, Silva LHS, Marinho M, Domingos P, Sant' Anna CL (2000) Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. Hydrobiologia 424: 67–77.

Ibelings BW, Mur LR (1992) Microprofiles of photosynthesis and oxygen concentration in *Microcystis* sp. scums. FEMS Microbiol. Ecol. 86: 195–203.

Jeffrey GH, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls $a$, $b$, $c_1$ and $c_2$ in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen. 167: 191–194.

Lanaras T, Tsitsamis S, Chlichlia C, Cook CM (1989) Toxic cyanobacteria in Greek freshwaters. J. Appl. Phycol. 1: 67–73.

Legendre P, Legendre L (1998) Numerical ecology, 2nd English Edition. Developments in Environmental Modelling 20, Elsevier, Amsterdam, 870 pp.

Moustaka M (1988a) Seasonal variations, annual periodicity and spatial distribution of phytoplankton in Lake Volvi. PhD Thesis, Aristotle University of Thessaloniki, Thessaloniki, Greece (in Greek with English summary).

Moustaka M (1988b) The structure and dynamics of the phytoplankton assemblages in Lake Volvi, Greece. I. Phytoplankton composition and abundance during the period March 1984–March 1985. Arch. Hydrobiol. 112: 251–264.

Moustaka-Gouni M (1993) Phytoplankton succession and diversity in a warm monomictic, relatively shallow lake: Lake Volvi, Macedonia, Greece. Hydrobiologia 249: 33–42.

Moustaka-Gouni M, Nikolaidis G (1990) Phytoplankton of a warm monomictic lake - Lake Vegoritis, Greece. Arch. Hydrobiol. 119: 299–313.

Nasri AB, Bouaucha N, Fastner J (2004) First report of a microcystin-containing bloom of the cyanobacteria *Microcystis* spp. in Lake Oubeira, eastern Algeria. Arch. Environ. Con. Tox. 46: 197–202.

National Statistical Service of Greece (1994) Statistical Yearbook of Greece 1990–1991. National Statistical Service of Greece, Athens (in Greek).

Ocevski B, Kozarov G and Serafimova-Hadžišče J (1975) Distribution and characteristics of bacteria, phytoplankton and zooplankton in Lake Castoria. Symp. Biol. Hung. 15: 233–245.

Otsuka S, Suda S, Li R, Watanabe M, Oyaizu H, Matsumoto S, Watanabe MM (1999) Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. FEMS Microbiol. Lett. 172: 15–21.

Overbeck J, Anagnostidis K, Economou-Amilli A (1982) A limnological survey of three Greek lakes: Trichonis, Lyssimachia and Amvrakia. Arch. Hydrobiol. 95: 365–394.

Parliament of the Hellenic Republic (2001) Reply by the Minister for Agriculture to written question 1344/10-9-2001 (in Greek).

Reynolds CS (1997) Vegetation Processes in the Pelagic: A Model for Ecosystem Theory. Excellence in Ecology Books, Ecology Institute, Oldendorf/Luhe. 371 pp.

Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S (2002) Towards a functional classification of the freshwater phytoplankton. J. Plankton Res. 24: 417–428.

Reynolds CS, Petersen AC (2000) The distribution of planktonic cyanobacteria in Irish lakes in relation to their trophic states. Hydrobiologia 424: 91–99.

Sivonen K, Jones G (1999) Cyanobacterial toxins. In Chorus I, Bartram J (eds), Toxic Cyanobacteria in Water. E & FN Spon, London, New York, pp. 41–110.

Spartinou MG (1992). The microflora of Lake Amvrakia, Western Greece. A systematic and ecological approach. PhD Thesis, The National and Kapodistrian University of Athens, Athens, Greece (in Greek with English summary).

Stanković S (1931) Sur les particularités limnologiques de lacs égéens. Verh. Intern. Verein. Limnol. 5: 158–196.

Stoyneva MP (2003) Steady-state phytoplankton assemblages in shallow Bulgarian wetlands. Hydrobiologia 502: 169–176.

Temponeras M, Kristiansen J, Moustaka-Gouni M (2000) Seasonal variation in phytoplankton composition and physical-chemical features of the shallow Lake Doirani, Macedonia, Greece. Hydrobiologia 424: 109–122.

Tryfon E, Moustaka-Gouni M (1997) Species composition and seasonal cycles of phytoplankton with special reference to the nanoplankton of Lake Mikri Prespa. Hydrobiologia 351: 61–75.

Tryfon E, Moustaka-Gouni M, Nikolaidis G (1997) Planktic cyanophytes and their ecology in the shallow Lake Mikri Prespa, Greece. Nord. J. Bot. 17: 439–448.

Vardaka E, Moustaka-Gouni M, Lanaras T (2000) Temporal and spatial distribution of planktic cyanobacteria in Lake Kastoria, Greece, a shallow, urban lake. Nord. J. Bot. 20: 501–511.

Zohary T (1985) Hyperscums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic lake (Hartbeespoort Dam, South Africa). J. Plankton Res. 7: 399–409.