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Assessment of microcystins in lake water and the omnivorous fish (*Carassius gibelio*, Bloch) in Lake Pamvotis (Greece) containing dense cyanobacterial bloom

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Abstract Toxin-producing cyanobacteria in lakes and reservoirs form a threat to humans as well as various forms of aquatic life. This study examined the occurrence and distribution of Microcystins (MCYST) in the shallow eutrophic lake Pamvotis (Greece). MCYST concentrations in the tissues (liver, kidneys, intestine, gonads, brain and muscle) of the fish species *Carassius gibelio* were also examined. Tests were performed with an enzyme-linked immunosorbent assay (ELISA). MCYST concentration in water and in the scum of Lake Pamvotis were highest during the warm period (April–October, 2005). Phytoplankton samples were dominated by the genera *Microcystis* and *Anabaena* during the same period. MCYST values were always below the WHO Guide level for recreational waters but much higher than the WHO Guide level for drinking water. It was found that MCYST can accumulate in the fish tissues of *C. gibelio*. Even though the target organ for MCYST is the liver, in our study MCYST were found also in the rest of *C. gibelio* tissues in the following order: intestine> kidney> > brain>gonads> muscle. Muscle tissue contained concentrations of microcystins that correspond to 0.096 μg/kg/day well above the recommended limit for human consumption (0.04 μg/Kg/day).

Keywords Cyanobacteria · Microcystins · Fish · *Carassius gibelio* · Accumulation

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Introduction

Toxic blooms of cyanobacteria occur in eutrophicated lakes, ponds and rivers all over the world. Cyanobacteria can produce a wide range of toxins such as neurotoxins, hepatotoxins, cytotoxins and skin irritants (Carmichael 1992; Codd 1995; Chorus and Bartram 1999; Harada et al. 2001; Albay et al. 2003) which can form a threat to humans (Carmichael 1992; Chorus 2001), birds, fish and invertebrates (Lampert 1981; Kotak et al. 1996; Mohamed et al. 2003). Among these are a group of potent cyclic peptide hepatotoxins, the microcystins and nodularins. A wide range of bloom-forming cyanobacteria in fresh, brackish and marine waters includes microcystin producing species such as *Microcystis, Anabaena, Oscillatoria*, and *Nostoc*, or nodularins producing species such as *Nodularia spunigena* strains (Metcalf et al. 2000). MCYST cause liver damage in both mammals and fish through inhibition of protein

phosphatase types 1 and 2A (MacKintosh et al. 1990). Most of the studies on MCYST have focused on terrestrial mammals, and relatively few studies have been conducted on the accumulation of MCYST on aquatic organisms. Since toxic effects of MCYST on aquatic organisms have been assessed thus the transfer of these toxins to humans through the food-chain is possible (Kotak et al. 1996; Fisher and Dietrich 2000; Li et al. 2003; Jacket et al. 2004). Fish, standing at the top of the aquatic food chain, are likely to be most affected by exposure to toxic cyanobacteria, and thus their consumption may pose a great risk to humans. Fish can be exposed to these toxins either during feeding or passively when the toxins pass through gills during breathing (Malbrouck and Kestemont 2006). The mechanisms of uptake and the level of accumulation in different fish species and moreover the distribution of MCYST in different body compartments of fish are scarce. To our knowledge, there are no previous studies concerning the accumulation of MCYST in different tissues of a widespread species of freshwater ecosystems, *C. gibelio*. However, there are only few studies regarding the MCYST content in other freshwater species as *Cyprinus carpio* in Germany (Fisher and Dietrich 2000) *Tilapia vendalli* in Brazil (Magalhães et al. 2001), *Oreochromis niloticus* in Egypt (Mohamed et al. 2003), *Hypophthalmichtys molitrixon*, in China (Zhang et al. 2006; Xie et al. 2005), and cyprinids in Greece (Gkelis et al. 2005) As in many other countries cyanobacterial blooms have become a major water quality problem in Greek lakes. A widespread occurrence of cyanotoxins in Greek freshwaters was reported by Gkelis et al. (2005).

This study focuses on: (a) the occurrence and the distribution of microcystins in Lake Pamvotis from early spring to middle autumn (b) the examination of the accumulation tendency of MCYST in fish tissues and organs of the *C. gibelio*. The potential risk from animal or human consumption of *C. gibelio* is also discussed.

Materials and methods

Description of the study area

The study was conducted in Lake Pamvotis, NW Greece (39° 40′ N, 20° 53′ E,). It is a Mediterranean, shallow lake (mean depth, 4.3 m, maximum depth of 7.5 m) and occupies an area of 22.8 km^2. Lake Pamvotis is about 470.25 m above sea level. The basin has no naturally surface outflows and is recharged by karstic springs. During the last three decades, Pamvotis lake was exposed to many activities such as irrigation, discharge of domestic sewages and sediment deposit, affecting its trophic status (Stalikas et al. 1994; Kagalou et al. 2001, 2003b).The lake is continuously enriched with allochthonous organic matter particularly fertilizers, entering the lake through the leaching process from the watershed. Detailed description of the trophic status of lake Pamvotis is reported by Kagalou et al. (2003a,b; 2007).

Water samples collection, preparation and chemical analysis

Water samples were collected, by filling 1 l polyethylene bottles 10–20 cm below the water surface, from two sampling stations S1 and S2 with depths of 6 m and 2 m correspondingly (Fig. 1). Sampling was taken place during the period March 2005–October 2005. Stations were chosen due to their ecological and recreational importance. Station (S1) is located at the northern area of the lake, characterized by important ecotype sites (NATURA 2000 network priority habitats) but also a well known area of pollutant deposits. Station (S2) is close to the rowing-navigation channel of the lake. Sampling was carried out every month. Water quality parameters (i.e temperature, pH, dissolved oxygen,) were recorded in-situ using electrode probes (YSI type).

Plankton samples were taken using a plankton net (55 μm mesh size) Phytoplankton identification and enumeration was performed according Prescott (1978); Anagnostidis and Economou (1980), using an inverted microscope (Uterhmole 1958).

Phytoplankton biomass was estimated as chlorophyll-a (chl-a, mg/m^3). Sub-samples (500 ml of volume when clear water state was present and 200–300 ml of volume for turbid water state) were filtered through Whatman GF/C filters and 1 ml of magnesium carbonate added to the filter. Chl-a was extracted from the filter with 95% acetone sol. and quantified spectrophotometrically as outlined in A.P.H.A. (1989).

For MCYST analysis sub-samples of 100 ml were filtered onto fiberglass filters. Filter papers and filtrate were stored at −20°C for subsequent total MCYST analysis.

Toxin extraction

MCYST were extracted from the filter papers by placing in 100% methanol and stirring overnight at room temperature (20–22°C) followed by centrifugation at 1,300 g for 15 min. Extraction procedure was repeated three times. The organic solvent was removed by placing the extract under nitrogen-stream. The aqueous fraction remaining after removing of the organic solvent was subjected to ELISA assay. Results are expressed as nanograms of cellular MCYST equivalents per liter (Kotak et al. 1995, 1996). Spearman's rank correlation coefficient (r) was used to identify any significant correlation between the MCYST concentration in water and bloom, and physicochemical parameters of water, abundance of phytoplankton cells and chl-a concentrations in each sampling station.

Fish samples and MCYST extraction from fish tissues

Ten female, 4–5 years old specimens of *C. gibelio* with a weight range of 716.5–1,078.9 g were collected from lake Pamvotis, during October 2005 using trammel net having 60 mm inner and 300 mm outer mesh size. Six specimens of *C. gibelio* originated from artificial propagation were kept for 16 moths in aquariums wih pure recyled water and used as control sample.

To extract the toxin from the fish organs, fish were sacrificed and liver, kidneys, intestine, gonads, brain and muscle tissues were excised, weighed and immediately frozen. All the tissues were separately homogenized and extracted in 100% methanol (Magalhães et al. 2001) stirred overnight at room temperature and then centrifuged at 1,300 g for 15 min in a Universal 32 centrifuge. The supernatant was collected and stored overnight at 4°C. A 5 ml aliquot of the supernatant was concentrated under nitrogen stream, in order to remove the organic solvent, to 350 μl. A 100-μl amount of the concentrated sample extract was diluted to 900 μl distilled water according to Sipia et al. (2002). The final sample was clarified using membrane filters (pore size, 0.45 μm, diameter 4 mm). Sample solutions were analyzed immediately.

For each ELISA run, the negative control and four calibrates were assayed at least in duplicate. The ELISA assay was performed using an Abraxis Microcystins kit (520011, USA) according to the instructions of the manufacturer. This ELISA test, using the β-amino acid 6E-ADDA as the epitope for antibody recognition, has a limit of quantitation of 0.02–0.07 ng/ml, lower than the WHO-proposed guideline (1 ng/ml) for drinking water, irrespective of the sample matrix (Fischer et al. 2001).

The recovery of the method was determined by analyzing real samples before and after the addition of pure microcystin and then subtracting the concentration of microcystin present in the sample prior to spiking. The results are expressed in nanograms MCYST equivalents per gram of fish tissue. The MCYST concentration in each tissue was divided by the body weight of each specimen in order to estimate the tissue concentration to body mass ratio. The concentrations of MCYST in the tissues of the fish were compared using ANOVA (Zar 1999). The Spearman's rank correlation coefficient (r) was used to identify any significant correlation between the MCYST concentration in the various tissues of the fish. All statistical analysis was performed with SPSS vers14.0 for windows.

Results

Water quality

Limnological features during the monitoring period are presented in Table 1. Heavy surface blooms of planktonic cyanobacteria were observed in late-spring in Pamvotis lake. The microscopic examination revealed that during August the water bloom dominated of *Microcystis* sp. reaching in density of 12×10^3 cells/ml and 9×10^3 cells/ml for S1 and S2 station, respectively. July was characterized by *Anabaena* sp. dominance (9×10^4 and 6×10^4 cells/ml for both S1 and S2 stations) (Table 2). It is important to stress out that during the monitoring period, amounts of *Microcystis* and *Anabaena* sp. blooms appeared in June and persisted until autumn. From the rest of phytoplankton genera the most frequently observed were the *Chroococcus, Oscillatoria, Ceratium, Asterionella, Melosira, Pediastrum, Synedra, Scenedesmus and Closterium*.

Microcystins in lake water and surface scum

Aqueous MCYST concentrations in lake water were detected in all monthly samples collected from both stations (Fig. 2). Concentrations ranged between 310 and 2,388 ng/l in the S1 station and 398 and 1,792 ng/

Table 1 Limnological features of Lake Pamvotis during the monitoring period

Month	T (°C)		pH		D.O (mg/l)	
	Station S1	Station S2	Station S1	Station S2	Station S1	Station S2
March	6.5	4.0	8.00	7.90	11.70	12.80
April	14.4	14.2	8.26	8.16	16.10	13.50
May	18.6	20.1	8.19	8.34	9.72	10.02
June	23.3	24.7	8.00	8.00	9.50	8.80
July	27.2	27.3	8.20	9.08	10.20	12.40
August	26.9	27.7	8.75	9.28	10.20	12.40
September	20.5	21.2	8.85	8.97	8.73	8.10
October	14.0	13.6	8.04	8.58	11.04	14.70

l in the S2 station. Aqueous MCYST increased over the summer, peaking in August in both stations.

MCYST concentrations in cyanobacterial blooms ranged between 3,109 and 11,585 ng/l for the S1 station and 3,236 and 11,286 ng/l for the S2 station.

Generally MCYST values in cyanobacterial bloom followed the seasonal pattern observed in dissolved fraction, showing peaking values during the warm months when the lake surface is characterized by the presence of cyanobacterial blooms. There were no statistical differences between the sampling stations whilst regarding the evolution of the concentrations of both dissolved and scum microcystins, a significant correlation was found between Chl-a and MCYST concentration for the shallow station, S2 (r=0.738; P=0.038) although no statistical correlation could be detected for the S1 station (r=0.5; P=0.185). Concerning the relationships between MCYST concentration in the bloom and water, a significant correlation was found between MCYST concentration in the cyanobacterial blooms and lake water for station S1 (r=0.649; P=0.033), although no statistical correlation could be detected for the S2 station (r=0.339; P=0.185). Furthermore significant positive correlations were found between MCYST concentration in cyanobacterial bloom and *Microcystis* sp. abundance in both stations (for station S1, r=0.962; P=0.039 and for S2, r=0.927; P=0.003). No correlations were found between MCYST values in cyanobacterial bloom and *Anabaena* sp. abundance in both stations (for station S1, r=0.342; P=0.637 and for S2, r=0.156; P=0.883).

Microcystins in fish tissues

Weight mean values for the excised fish tissues were: liver: 0.52 g, kidneys: 0.79 g, intestine: 3.21 g, gonads: 4.8 g, brain: 0.44 g, muscle: 5.91 g whereas the corresponding values for the control specimen were: liver: 0.8 g, kidneys: 0.72 g, intestine: 2.58 g, gonads: 3.46 g, brain: 0.42 g, muscle: 4.88 g. The fish samples of *C. gibelio* examined during the present study contained MCYST (Fig. 3). The control samples did not contain any detectable concentration of MCYST. The recovery of MCYST that we obtained from the spiked samples was 54%.

Table 2 Abundance of the species *Microcystis sp*. and *Anabaena sp*. during the period March–August, 2005

Month	Station S1		Station S2	
	Microcystis (cells/ml)	Anabaena (cells/ml)	Microcystis (cells/ml)	Anabaena (cells/ml)
March	1,012	8	814	10
April	610	15	678	18
May	720	25	530	21
June	2,134	8,904	1,574	10,070
July	4,240	94,870	3,180	67,734
August	12,402	9,328	9,752	6,890

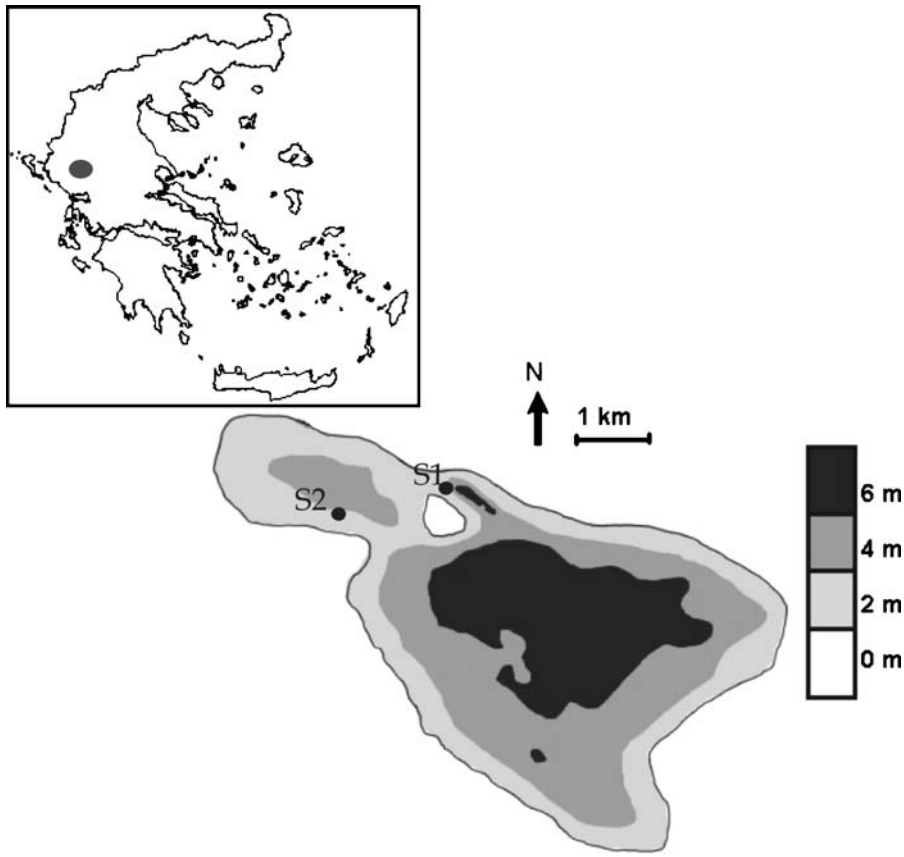

Fig. 1 Map of Pamvotis Lake. S1 and S2 are the sampling stations

The highest level of MCYST was found in liver (mean: 275.1±84.5 ng/g), followed by the intestine (mean: 233.51±196.5 ng/g), kidney (155.8±35.8 ng/g), and brain (mean: 38.5±26.9 ng/g). The lowest concentrations were detected in the gonads (mean: 21.02±9.16 ng/g) and muscle (mean: 16.05±11.97 ng/g) (Fig. 3). MCYST concentrations in the liver had a strong correlation with the concentration in the kidney (r=0.68), respectively the liver to body mass ratio had a positive correlation with kidney to body mass ratio (r=0.66).Moreover, significant statistical differences were found between the concentrations of MCYST in the tissues (ANOVA : F=4.451; P=0.002) (Fig. 3).

Discussion

Environmental conditions

Lake Pamvotis is a shallow lake appearing the typical temperature pattern of the Mediterranean region. High summer water temperatures and the almost complete absence of precipitation lead in enhanced evaporation and strong water level fluctuation in the most of Mediterranean aquatic systems (Naselli-Flores and Barone 2005). This is accompanied by the drop of dissolved oxygen concentration during the warm, dry period. pH was found to be generally, high coupling with photosynthesis in shallow lake ecosystems (Moss 1980). Phytoplankton biomass, as estimated by Chl-a values, reflect the eutrophic to hypertrophic status of the lake. Regarding at the two sampling stations the shallow S2 station, as expected, appeared relatively higher values than the deeper S1 station. The shallow lake Pamvotis has a long eutrophication history (Anagnostidis and Economou 1980; Albanis et al. 1986; Stalikas et al. 1994; Kagalou et al. 2001, 2003a,b) due to the heavy point and non-point loading of nutrients, with cyanophyte blooms occurring since 1978 (Anagnostidis and Economou 1980). As reported by Kagalou et al. (2003a,b) the in-lake nitrogen/phosphorous concentrations ratio is less than the threshold of 10:1 which is considered as indicator for strong nitrogen-limiting conditions, thus favouring the

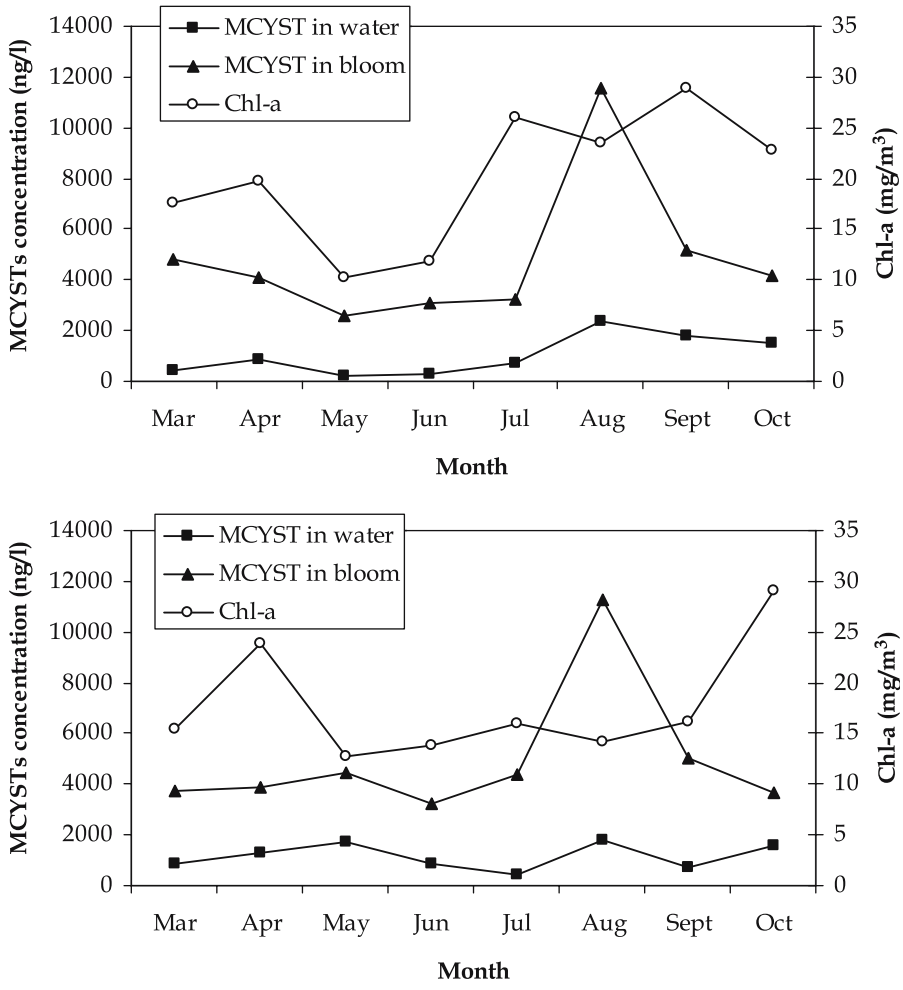

Fig. 2 Variation of the of MCYST concentrations in the water and bloom in relation to the concentration of Chl-a in the water at the sampling stations S1 and S2 (**a** and **b** correspondingly)

growth of N_2-fixing cyanobacteria (Havens et al. 2003). In the present study, massive surface blooms of cyanobacteria were recorded from early summer to late autumn although cyanobacteria were almost always present during the study period. During this study it was found that the dominant species were *Microcystis* sp. and *Anabaena* sp. respectively. Gkelis et al. (2005) reported also blooms of *Microcystis*

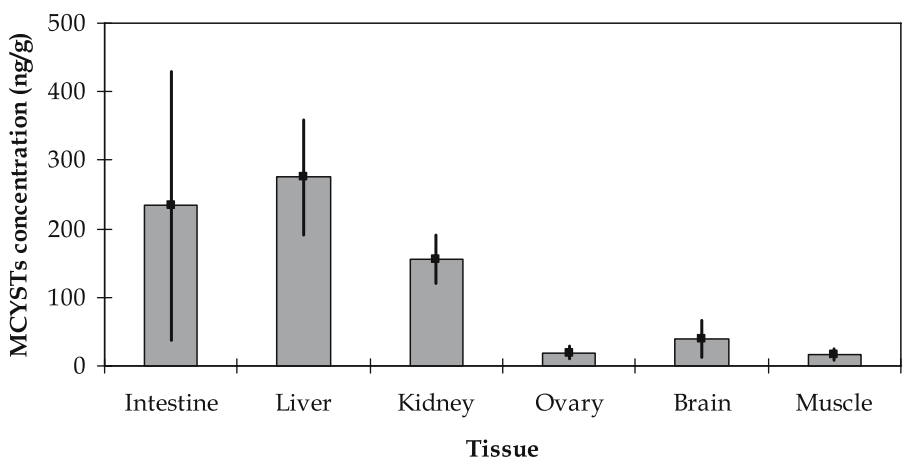

Fig. 3 MCYST concentrations in the tissues of *C. gibelio* collected from Lake Pamvotis during October 2005

aeruginosa, Microcystis sp. and *Anabaena* in Lake Pamvotis during the warm period of a sampling survey, producing the MC-LR, MC-RR and also unidentified MCYST variants. Extremely high values of cyanobacterial volume has been recently reported for the Lake Pamvotis comparable to those reported for hypertrophic lakes (Vardaka et al. 2005). There is evidence that, in the temperate zone, cyanobacteria blooms are most prominent during the late summer (Kotak et al. 1996; Lindholm et al. 2003). In the Mediterranean region cyanobacterial blooms may start earlier and persist longer, although in many Mediterranean lakes they have a continuous duration through the year (Cook et al. 2004). Abiotic factors such as high temperatures, water stratification and high phosphorous concentrations are enhancing their presence and persistence (Briand et al. 2003). In Lake Pamvotis a suitable combination of these factors for cyanobacteria growth is seen (Kagalou et al. 2007). The fact that Lake Pamvotis has a polymictic regime, and is only weakly stratified from July to September (Romero et al. 2002; Kagalou et al. 2003a), prevents the continuous duration of cyanobacterial blooms through the whole year. Ecological studies of phytoplankton population in other Greek lakes have shown that prolonged cyanobacterial blooms occur which last up to 8–9 months (Moustaka-Gouni 1993; Tryfon et al. 1994; Temponeras et al. 2000; Mitraki et al. 2004).

Microcystins in water and surface scum

MCYST concentration in water and cyanobacterial bloom of Lake Pamvotis were highest during the warm period. This study is the first evidence concerning the monthly fluctuation of MCYST in Lake Pamvotis and thus there are no other comparable data. The observed temporal fluctuation may be the result of the dominance of *Microcystis* sp. and *Anabaena* sp. during the warm months. Chorus (2001) argues that spatial and temporal variation of MCYST usually is attributed to changes in the strains or species dominating the bloom. This needs to be further investigated for Lake Pamvotis.

Scum samples contained higher MCYST concentrations than the aqueous fraction. Microcystins are considered endotoxins because the majority of the toxin is found within cells (Kotak et al. 1996). MCYST are released into the water when the cell wall of the cyanobacterium is compromised in the presence of chemicals, that inhibit new cell synthesis, enzymatic reactions or photosynthesis (Lam et al. 1995), or by natural senescence (Lam et al. 1995; Kotak et al. 1996). The total MCYST concentrations found in Lake Pamvotis are similar to those reported for other Mediterranean lakes in Turkey (Albay et al. 2003), in Portugal (Vasconcelos et al. 1996) but generally, lower to those of the temperate lakes in Canada (Kotak et al. 1996) or in Finnish lakes (Lindholm et al. 2003) and also lower than those reported for Brazil lagoons (Magalhães et al. 2001). Regarding the Greek freshwater systems, MCYST were detected in seven eutrophicated lakes, including Lake Pamvotis (Cook et al. 2004). In the present study MCYST concentrations were higher than those reported during a survey from1997 to 2000 by Cook et al. (2004). Gkelis et al. (2005) reported total toxin concentrations in cyanobacterial bloom of Pamvotis Lake exceeded 1,000 μg/g whilst Vardaka et al. (2005) demonstrated an elevated risk of acute toxicosis.

A MCYST level of 20 μg/l represents a guideline value for a moderate health alert in recreational waters (WHO 1998) This level (which is equivalent to 100,000 cyanobacterial cells per ml or approximately 50 μg/l chlorophyll-a if cyanobacteria, and especially *Microcystis* sp. dominate) is 20 times the WHO provisional Guideline Value concentration for microcystin-LR drinking water of 1 μg/l (WHO 1998). MCYST values for Lake Pamvotis are below the WHO Guide level for recreational waters but much higher than the WHO Guide level for drinking water. Lake Pamvotis is used for recreation activities (angling, rowing, water-skiing) and for irrigation purposes but not as drinking water supply.

Regarding the relationship between phytoplankton biomass (as estimated by chl-a) and MCYST concentrations, only a significant correlation was recorded for the shallow station. In many studies (Kotak et al. 1996; Lindholm et al. 2003) MCYST concentrations did not always coupled with the phytoplankton biomass (estimated as chl-a) even though the biomass, mostly, attributed to cyanobacteria. Kardinaal et al. (2005) studying the dynamics of toxic cyanobacteria in Dutch lakes, observed an increase in cyanochlorophyll-a followed by a decrease in MCYST concentrations. The relationships founded between MCYST in lake water and bloom supports the opinion that the function of MCYST in cyanobacterial cells is still unclear depending, mainly, on biochem-

ical processes (Lam et al. 1995). The problem why non-toxic peptides such as aeruginopeptins and cyanopeptolins are produced together with microcystins by toxic cyanobacteria is also a subject of recent genetic studies (Fujii et al. 2002; Janse et al. 2003; Gkelis et al. 2005; Zurawell et al. 2005). Many lakes were negative in the MCYST test with rich occurrence of cyanobacteria indicating that many cyanobacterial blooms were either non-toxic or produced little MCYST (Lindholm et al. 2003). For this reason, Chorus and Bartram (1999) pointed out that although the quantification of cyanobacteria can be used to estimate the toxic risk, it cannot replace toxin monitoring, mainly due to the fact that toxin concentrations per cell show a wide variation among individual strains of the same species.

Microcystins in fish tissues

The knowledge about the effects of MCYST on fish and its response to cyanotoxins in sub-lethal or chronic exposure is very limited (Soares et al. 2004; Magalhães et al. 2001; Fisher and Dietrich 2000). According to Northcott et al. (1991) cyanobacteria are regular components of the cyprinid diet and it is known to feed on non-toxic strains of *M. aeruginosa* in field conditions. However, toxins can limit fish feeding on cyanobacteria. Beveridge et al. (1993) showed suppression in filtration rate and growth of two species, *H. molitrix* and *O. niloticus*, in the presence of toxic *M. aeruginosa*. Carps, also can reduce their grazing activity when the percentage of toxic cyanobacteria increases (Beveridge et al. 1993; Keshavanath et al. 1994). Nevertheless, many fish species are not able to avoid ingestion of these toxic organisms in small eutrophicated lakes and aquaculture ponds. In the present study, we examined the benthopelagic omnivorous fish species *C. gibelio* which mainly feeding on detritus (Specziar et al. 1998) and zoobenthos (Specziar et al. 1997), although in his digestive tract large quantities of filamentous and colonial cyanobacteria often occur due to the filtration of the water in order to obtain its main food source (Kolmakov and Gladyshev 2003). The accumulation of MCYST in *C. gibelio* in a natural environment has not been reported whilst there are other studies indicating MCYST accumulation in other freshwater fish species (Kotak et al. 1996; Magalhães et al. 2001; Zimba et al. 2001; Mohamed et al. 2003). According to Tencalla et al. (1994) the main route for MCYST uptake by fish is the digestive tract.

Our results revealed the presence of MCYST in all fish tissues at various concentrations. The highest MCYST concentrations were found in liver (284.7± 140.1 ng/g). MCYST is thought to be taken up by a bile-acid transporter in intestinal and liver cells with part of the toxin being extracted by faeces and another part accumulated in liver and muscle tissue (Falconer 1993; Vasconcelos 1995; Magalhães et al. 2001; Ernst et al. 2005).

Even though the target organ for MCYST is to be the liver, in our study MCYST were also found in the rest of *C. gibelio* tissues in the following order: intestine> kidney> brain>gonads> muscles. It seems that *C. gibelio* accumulates preferentially in liver but also in other tissues. This finding may be explained by the process known as presystematic hepatic elimination, which prevents, or at least minimizes, the distribution of foreign chemicals to other parts of the body. Mohamed et al. (2003) attributed the presence of MCYST in the kidney of *O. niloticus* to the presystematic hepatic elimination. The overwhelming of this process as a result of the fish exposure to toxins may be the explanation for the MCYST circulation to the other organs. According to Klaassen and Watkins (1984) when the presystematic hepatic elimination is overwhelmed or bypassed by exposure to toxins such as MCYST, it may allow MCYST to circulate to these other organs. The intestine of *C. gibelio* accumulates a part of MCYST. A possible pathway for this it can be the reintroduction from the liver into the intestine by the entero-hepatic recirculation (Falconer et al. 1992). Despite the fact that the organ distribution studies reported to date are somewhat controversial, due to the variant application models and the use of different MCYST congeners, many studies have clearly confirmed the presence of MCYST in gastrointestinal tract, kidneys and brain (Fisher and Dietrich 2000; Dietrich and Hoeger 2005; Kankaanpaa et al. 2005). The most reliable explanation for this at present, is that the tissues and organ distribution of MCYST is governed by the presence/absence, type and expression level of the organic anion transporters since they are responsible for the active passive of MCYST across organs even across the blood-brain barrier (Kusuhara et al. 1998; Fisher and Dietrich 2000; Williams et al. 1997a,b). We add to this knowledge the presence of

MCYST into the brain of *C. gibelio* suggesting their crossing of the blood-brain barrier.

C. gibelio, a non wide commercial fish, is considered as food item in many regions of Greece. A Tolerable MCYST Daily Intake (TDI) value of 0.04 μg/Kg of body weight per day was proposed as a guideline value by WHO (Chorus and Bartram 1999). At the levels found in muscle tissue in our study the mean value of 16.05 ng/gr represented a daily intake of 0.096 μg/kg of body weight, more than two times of the advisable TDI value. It is worth to point out that all present guideline values are based on an adult weight of 60 kg.

Children may be a target group, often, at higher risk than healthy adults. Falconer (2001) suggested the determination of the Interim Maximum Acceptable Concentration (IMAC) in edible fish, applying the following equation: $\mathrm{IMAC} = \mathrm{TDI} \times \mathrm{BW} \times \mathrm{POT}/\mathrm{AFC}$, where BW is the body weight, POT the percentage of uptake of toxin via fish consumption and AFC is the average fish consumption. We suggest that it is highly risk to evaluate the IMAC value because of the different freshwater fish eating habits and local customs among Greek regions.

Monitoring of MCYST in other edible fish species from eutrophicated freshwaters is recommended as well as in fish samples coming from fish farms. Further research is also needed concerning the effects of MCYST in specific organs as stomach, digestive track or the possible distribution mechanism in other organs and tissues as brain. Specific guidelines for the TDI values need to be developed taking into account the climatic conditions in each country in order to protect freshwater ecosystems and the public health from the possible toxicity associated with cyanobacteria toxins.

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