ELSEVIER

Aquatic Toxicology 59 (2002) 201–208

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Accumulation of microcystins by a tropical zooplankton community

Aloysio da S. Ferrão-Filho [a,*], Betina Kozlowsky-Suzuki [b], Sandra M.F.O. Azevedo [c]

[a] *Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-901, Brazil*

[b] *Department of Marine Sciences, University of Kalmar, 39182 Kalmar, Sweden*

[c] *Instituto de Biofisica Carlos Chagas Filho, CCS, Bloco G, Cidade Universitária, Ilha do Fundão, RJ 21949-900, Brazil*

Received 16 July 2001; received in revised form 27 September 2001; accepted 5 October 2001

Abstract

In the current study, the hepatotoxic peptide microcystins, were measured in the zooplankton community of Jacarepaguá Lagoon during a 6-month period. Concurrent phytoplankton and seston samples were obtained. Microcystins were measured in seston and phytoplankton by High Performance Liquid Chromatography (HPLC), and in zooplankton by an Enzyme-Linked Immunosorbant Assay (ELISA). Zooplankton community was comprised mainly by the rotifers *Brachionus angularis* and *B. plicatilis*, the cladocerans *Moina micrura* and *Ceriodaphnia cornuta* and the copepod *Metacyclops mendocinus*. Phytoplankton was dominated by *Microcystis aeruginosa* during all the studied period. Microcystins in zooplankton ranged from 0.3 to 16.4 μg g^{-1} DW, while in the sestonic samples they ranged from undetectable values to 5.8 ng g^{-1} DW. Microcystins in net phytoplankton ranged from 0.3 to 3.9 mg g^{-1} DW. We conclude that zooplankton from Jacarepaguá Lagoon were efficient accumulators of microcystins from seston and that these animals can be potential vectors in the transferring of such toxins to higher trophic levels in the aquatic food chain.

Keywords: Cyanobacteria; *Microcystis aeruginosa*; Microcystins; Zooplankton; Accumulation; Hypereutrophic; Tropical coastal lagoon; Brazil

1. Introduction

Cyanobacterial toxins have been a major concern of aquatic toxicologists lately, regarding their worldwide distribution in aquatic ecosystems and the occurrence of some cases of poisoning of wild and domestic animals (Carmichael, 1992; Sivonen, 1996). The first confirmed case of human death in consequence of exposure to microcystin occurred through the utilization of contaminated water in a hemodialysis center in Caruaru (Pernambuco, Brazil) (Jochimsen et al., 1998).

Microcystins are the most common toxins produced by some cyanobacteria genera such as *Mi-*

* Corresponding author. Tel./fax: +55-16-602-3832

E-mail address: aloysiosff@ffclrp.usp.br (A.S. Ferrão-Filho).

0166-445X/02/$ - see front matter
PII: S0166-445X(01)00253-3

crocystis, *Anabaena*, *Oscillatoria* and *Nostoc*. They are hepatotoxic heptapeptides with a cyclic structure containing five fixed D-amino acids and two variable L-amino acids. There are more than 50 kinds of microcystins known at present, differing in the combinations of the two variable amino acids. Microcystin-LR is the most common, with leucine and arginine. These toxins are potent protein phosphatase 1 and 2A inhibitors, causing tumor promotion in the liver of animals exposed to sublethal doses. In acute exposure, they may cause hepatic hemorrhage and necrosis, killing the animal in minutes or hours (Carmichael, 1994).

Although there have been many studies on the effects of cyanobacterial toxins in aquatic communities (reviewed by Christoffersen, 1996), little is known about the potential for accumulation and transferring of such toxins in food webs. This is an important issue in aquatic toxicology, as it involves the risk of human exposure through the consumption of contaminated fish and other aquatic organisms.

The zooplankton community is one of the most important links between the primary producers and higher consumers such as fish. Thus, zooplankton may be an important vector of cyanobacterial toxins along the food chain. Previous studies have attempted to quantify zooplankton accumulation and transferring of microcystins but have failed to detect these toxins directly in zooplankton (Watanabe et al., 1992; Laurén-Määtä et al., 1995). Only recently, studies have directly measured cyanobacterial toxins in zooplankton (Kotak et al., 1996; Thostrup and Christoffersen, 1999). Watanabe et al. (1992) have developed an equation to estimate microcystin accumulation in zooplankton using fractionated samples from Lake Kasumigaura, a hypereutrophic lake in Japan. Their measurements were, however, very indirect since they were not able to separate animals from phytoplankton to measure toxin directly from zooplankton tissue. Using a simple food chain with *M. aeruginosa*, *Daphnia pulex* and mosquito larvae (Chaoborus), Laurén-Määtä et al. (1995) showed that Chaoborus had higher mortality when preying on *Daphnia* fed with toxic *Microcystis* than fed with non-toxic algae, suggesting that *Daphnia* may have transferred toxins from *Microcystis* to Chaoborus. However, they did not find any microcystin in the midge larvae, and suggested that most of the toxin was metabolized or excreted in this two-step food chain. Both studies used not very sensitive methods (Ion-Exchange Chromatography and High Performance Liquid Chromatography (HPLC)) for low microcystin content detection and this was probably the reason why they failed to detect toxins in zooplankton.

In this study, we used the Enzyme-Linked Immunosorbant Assay (ELISA), a very specific and sensitive method for detection of microcystins (Chu et al., 1990). With this method, we were able to detect microcystins in zooplankton samples from a hypereutrophic coastal lagoon in Brazil.

2. Materials and methods

From January to June 1997, seston and plankton samples were taken in two sampling stations of Jacarepaguá Lagoon, a coastal lagoon located in Rio de Janeiro, Brazil. The cyanobacterium *M. aeruginosa* have been reported to form dense blooms during most of the year in this lagoon (Magalhães and Azevedo, 1998). Plankton samples were taken with a 20 μm mesh-size plankton net by surface hauls and placed in polyethylene bottles kept on ice. At the laboratory, these samples were passed through a 200 μm sieve and zooplankton >200 μm were separated from *Microcystis* colonies, other algae and zooplankton <200 μm. Animals retained in the sieve were vigorously washed with distilled water to remove all *Microcystis* colonies and were placed in a beaker with distilled water for about 15–30 min to allow gut content to be excreted. The animals were then transferred to a glass flask with new distilled water and freeze dried for toxin analysis. Optical microscope observations allowed us to verify that zooplankton samples were free of *Microcystis* colonies, although some cells could be still intact inside the gut. The fraction with *Microcystis* colonies that passed through the sieve were then centrifuged and separated in two other fractions: the supernatant, containing only buoyant *Microcystis* colonies, and the precipitated mate-

rial, containing *Microcystis* cells, small zooplankton and other algae. Both fractions were freeze-dried for toxin analysis.

Phytoplankton and zooplankton samplings for qualitative and quantitative analyses were conducted and water samples were taken for microcystin analysis in the seston. Seston samples were obtained by filtering 2 l of water through glass-fiber filters and keeping them in a freezer until the analysis.

For the analysis of microcystins in zooplankton we used an extraction method adapted from Krishnamurthy et al. (1986). For toxin quantification, we performed a direct competitive ELISA (Chu et al., 1990), using an EnviroLogix Inc. Microcystin Plate Kit with wavelength set at 490 nm. The same extraction protocol was conducted for the toxin analysis of the *Microcystis* fractions (< 200 μm) and the seston samples. However, the detection and quantification of microcystins in these samples were carried out by HPLC. The HPLC consisted of a Shimadzu system with SPD-M10A diode array detector, a LC-10AT pump, a DGU-14A degasser and a SCL-10A system controller. An analytical reverse phase C_{18} column (Lickrospher 100 RP-18, 125×4 mm) was used under isocratic conditions, with a mobile phase of acetonitrile and 20 mM ammonium acetate (28:72% v/v; pH 5.0), at a flow rate of 1 ml min^{-1}. UV-detection was performed at 238 nm and the absorption spectrum of each peak was analyzed over the range of 190–300 nm and compared against a standard of microcystin-LR.

Some chromatograms showed more than one peak with the same absorption spectrum of microcystin-LR, but their chemical characterization were not performed. Therefore, the concentration of microcystins was estimated by the sum of all peak areas presenting an absorption spectrum $\geq 95\%$ of similarity index with the standard microcystin-LR and was expressed as 'concentration equivalents (CE)' of microcystin-LR, as described in Chorus and Bartram (1999).

The concentration factor (CF) of microcystins in zooplankton was estimated for the two sampling stations, based on the ratio between the concentration of microcystins (in μg g^{-1}) in zooplankton and seston. In order to calculate the CF, microcystin concentrations in seston (in μg l^{-1}) were divided by the total seston concentration (in g l^{-1}) to obtain values in μg g^{-1} for each station. Since we have not performed the zooplankton and seston samplings at exactly the same sites, we may consider the CF as an inferential measure of the potential for accumulation of microcystins in zooplankton.

Spearman rank correlation was used to detect relations among the measured variables, such as toxin content in the zooplankton, in the supernatant and precipitated fractions and in sestonic samples.

3. Results

The zooplankton community in Jacarepaguá Lagoon was composed of two small cladocerans, *Moina micrura* and *Ceriodaphnia cornuta*, rotifers (mostly *Brachionus angularis* and *B. plicatilis*) and the copepod *Metacyclops mendocinus*. During most of the sampling period, rotifers were the most numerous zooplankters and reached high densities in January (station 1) and February (station 2). Cladocerans reached their highest densities in January and March, in both stations, having a breakdown in February and collapsing after May 1997. Copepods presented their highest numbers in May (station 1) and April 1997 (station 2).

A bloom of *M. aeruginosa* started in November 1996 and reached peak densities in early January 1997 (7.4×10^5 cells per ml in station 1 and 9.4×10^5 cells per ml in station 2). The bloom lasted until the end of the sampling period and *M. aeruginosa* biomass comprised about 80–90% of the phytoplankton community during this period.

The results of microcystin analysis in the zooplankton, *Microcystis* fractions and seston are presented in Table 1. Zooplankton contained conspicuous amounts of microcystins, with higher contents at the beginning and at the end of the sampling period. Higher values of toxin accumulation by zooplankton occurred together with higher concentrations (in μg l^{-1}) of microcystin in the seston. Although there was not a significant correlation between these two variables at 5%

level for station 1 ($n = 10$, $r = 0.067$, $P = 0.85$), there was a marginal positive correlation for station 2 ($n = 11$, $r = 0.56$, $P = 0.076$). No significant correlation was verified between zooplankton and supernatant fraction ($n = 5$, $r = -0.30$, $P = 0.68$) and the precipitated fraction ($n = 5$, $r = 0.21$, $P = 0.78$). The supernatant fraction, in which *Microcystis* colonies were almost the only component of phytoplankton, showed the higher contents of microcystins. The precipitated fraction showed lower amounts of microcystins than the supernatant, which was probably due to the contribution of non-toxic phytoplankton cells and small zooplankton.

4. Discussion

The microcystin contents found in *Microcystis* samples from the Jacarepaguá Lagoon were comparable to that of three *Microcystis* strains (NPLJ-2, NPLJ-3 and NPLJ-6) isolated from this lagoon, which proved to be toxic to several cladoceran species in previous studies (Ferrão-Filho et al., 2000). As dry weight, microcystin contents ranged from 0.3 to 16.4 μg g^{-1} in zooplankton while in the seston they ranged from undetectable values to 1.1 ng g^{-1} in station 1 and up to 5.8 ng g^{-1} in station 2. The estimated CF of microcystins in zooplankton ranged from 615 to 14 393 in station 1 and from 380 to 28 932 in station 2, showing that zooplankton was an efficient accumulator of such toxins. The fact that microcystins could be detected in zooplankton when these toxins were under the HPLC detectable level in seston (28 January), shows that these animals were able to concentrate microcystins from seston even though these toxins were in very low concentration in the water. Since ELISA has a lower detection level than HPLC (Chu et al., 1990), seston samples in late January should contain small amounts of microcystins that could not be detected by HPLC. However, Kuiper-Goodman et al. (2000) found a good correlation between ELISA and HPLC analyses of algal food products and thus our results with seston samples should not be regarded as underestimates.

The small number of samples and high variability of the data made it difficult to detect significant correlations between microcystin contents in the zooplankton and seston. Also, zooplankton for microcystin analysis was collected in different sampling sites than seston, together with net phytoplankton samples, which can explain in part the

Table 1
Microcystin concentrations in zooplankton (>200 μm), *Microcystis* fractions (supernatant and precipitate) and seston in Jacarepaguá Lagoon

Date	Zooplankton (μg g^{-1})	Microcystis		Seston		Concentration Factor (CF)	
		Supernatant (mg g^{-1})	Precipitate (mg g^{-1})	Station 1 (μg l^{-1})	Station 2 (μg l^{-1})	Station 1	Station 2
01/08/97	3.0	–	–	41.0	307.8	2744	517
01/28/97	1.1	–	–	ND	ND	–	–
02/06/97	1.4	–	–	5.1	1.2	6458	28 932
02/20/97	0.3	2.4	0.3	14.0	ND	755	–
03/06/97	0.3	–	–	1.9	21.9	4303	380
03/20/97	0.6	4.2	1.6	13.8	2.4	2598	14 938
04/03/97	0.5	3.9	3.1	20.3	16.1	616	1235
04/17/97	1.6	3.6	3.1	15.9	8.0	2254	4760
05/15/97	16.4	2.0	0.7	ND	54.0	–	21 867
05/28/97	6.0	2.4*	–	17.3	34.5	14 393	8035
06/12/97	1.4	2.3*	–	–	120.1	–	909

CF is the estimated concentration factor of microcystins in zooplankton; (–) missing data; ND = non-detected value; *, not centrifuged.

lack of correlation. In spite of this, the marginal and positive correlation in station 2 showed that higher values of microcystins in zooplankton were more likely to have been associated with higher values in seston.

Microcystin contents in the supernatant and precipitated fractions of *Microcystis* also did not show any correlation with the toxin content in zooplankton. The lack of significant correlations, however, does not mean that these variables are unrelated. Spatial heterogeneity in distribution pattern, both horizontally and vertically, of *Microcystis* colonies in the water column and also the big colony size of *Microcystis* may have prevented zooplankton from ingesting most part of the colonies, which may be in part responsible for the lack of significant correlations.

Some studies have attempted to show microcystin accumulation by zooplankton in experimental approaches (Laurén-Määtä et al., 1995; Thostrup and Christoffersen, 1999), while others have measured toxin accumulation, directly or indirectly, by naturally occurring freshwater zooplankton (Watanabe et al., 1992; Kotak et al., 1996). To our knowledge, the current study presents the first data on direct accumulation of cyanobacterial toxins by naturally occurring zooplankton in the tropical region.

Watanabe et al. (1992) showed that a community dominated by the cladoceran *Bosmina fatalis* was able to accumulate microcystins from a *Microcystis* bloom in a Japanese lake. Their data, however, were based in mathematical extrapolations of toxin analyses from fractionated plankton samples containing both zooplankton and phytoplankton, mostly *Microcystis* ($>99\%$). They calculated that zooplankton in Lake Kasumigaura accumulated up to 1387 μg microcysting DW^{-1} (202% higher than *Microcystis* toxin content), which is very high compared with this and other studies. On the other hand, the fact that they did not separate the zooplankton from the *Microcystis* cells implies that the animals could have high toxin contents inside the gut as intact cells but not assimilated in the tissues, leading to an overestimation of their measurements. In our study, although we cannot guarantee that all gut content was excreted during the separation procedure, it is likely that this source comprised a small percentage of total toxin content in zooplankton.

Kotak et al. (1996) found microcystin-LR in three food web compartments (phytoplankton, zooplankton and gastropods) in Canadian lakes varying in trophic status. These authors presented the first data on microcystin-LR accumulation in temperate zooplankton communities. They found values up to 67 μg g^{-1} DW of zooplankton, which is about four times the upper limit detected in our study.

Recently, Thostrup and Christoffersen (1999) showed that *D. magna* accumulated microcystins from indigenous and cultured *Microcystis*. Using the same method as we did for the detection of microcystins, they found a maximum accumulation of 24.5 μg g^{-1} DW of *D. magna* in laboratory experiments with the *M. aeruginosa* strain CYA 228. In enclosure experiments with lake water containing indigenous *Microcystis*, these authors found a maximum accumulation of 2.7 μg g^{-1} DW of microcystins, which is within the range found in the zooplankton from Jacarepaguá Lagoon.

Microcystin accumulation by zooplankton showed in this study falls in the same range to other studies with zooplankton and other invertebrate and vertebrates (Table 2). Although molluscs, especially mussels, are considered efficient filter-feeding organisms, their potential for microcystin accumulation is not so high, falling in the range of some zooplankters. This means that zooplankton can transfer microcystin to higher trophic levels as well as or even more efficiently than molluscs. The primary difference, and maybe the more important one, relays on the fact that molluscs, as well as fish, are part of human diet and may be a potential risk when consumed routinely. Zooplankton, however, may act as a link between cyanobacteria and planktivorous fish and, therefore, be an important vector in the transfer of cyanobacterial toxins to higher trophic levels.

The potential for accumulation of microcystins in aquatic organisms vary up to six orders of magnitude (from about 10^0–10^6 ng g^{-1} DW or WW), according to data in Table 2. However, these estimates must be viewed with care, since

Table 2
Microcystin accumulation by several freshwater, brackish and estuarine animals

Animal	Habitat	MCYST-LR eq. units (μg g^{-1}DW or WW)	Detection method	Reference
Zooplankton				
Entire community	Freshwater (Kasumigaura Lake, Japan)	75.0–1387	I-EC	Watanabe et al. (1992)
Entire community	Freshwater (four lakes in Central Alberta, Canada)	Up to 67.0	HPLC PPase	Kotak et al. (1996)
Daphnia magna	Freshwater†	Up to 24.5 ($\pm$ 1.9)	ELISA	Thostrup and Christoffersen (1999)
Entire community	Brackish (Jacarepaguá Lagoon)	0.3–16.4	ELISA	This study
Other invertebrates				
Anodonta cygnea	Freshwater†	Up to 130	HPLC	Erickson et al. (1989)
Marine mussels	Marine (Northeastern Pacific Ocean, Eastern Canada)	0.002–0.6	LC-linked PPase	Chen et al. (1993)
Mytilus galloprovincialis	Estuarine†	Up to 10.5	HPLC	Vasconcelos (1995)
Helisoma trivolvis; *Lymnaea stagnalis*; *Physa gyrina*	Freshwater (four lakes in Central Alberta, Canada)	Up to 121	HPLC	Kotak et al. (1996)
Anodonta grandis simpsoniana	Freshwater† (three lakes in Central Alberta, Canada)	Up to 0.776 ($\pm$ 0.569)	Ppase	Prepas et al. (1997)
Unio douglasiae, *Anadonta woodiana*	Freshwater (Suwa Lake, Japan)	Up to 2.7	HPLC–MS	Watanabe et al. (1997)
Mytilus edulis	Marine†	Up to 336.9 $\pm$ 45.8 0.014–0.204	GC–MS PPase	Williams et al. (1997)
Pacifastacus leniusculus	Freshwater†	–	HPLC	Lirås et al. (1998)
Mytilus galloprovincialis	Marine †	Up to 16.0	ELISA	Amorim and Vasconcelos (1999)
Fish				
Tilapia rendalli	Brackish (Jacarepaguá Lagoon)			Magalhães et al. (2001)
Liver		Up to 31.1	HPLC	
Vicera		Up to 67.8	HPLC	
Muscles		2.9–26.4	ELISA	

MCYST-LR Eq. Units, microcystin-LR equivalent units; I-EC, Ion-Exchange Chromatography; Ppase, protein phosphatase bioassay; LC, liquid chromatography; MS, mass spectrometry; GC–MS, gas chromatography–mass spectrometry; (†) laboratory and/or field experiments; (–) not accurately quantified according to the authors.

the different studies used different detection methods, which may have under- or overestimated the results. For instance, some of the microcystin may be covalently bounded to proteins phosphatases 1 and 2A (Lambert et al., 1994), being more or less extractable by the different analytical methods. Williams et al. (1997), comparing the results obtained with the Lemieux oxidation Gas Chromatography–Mass Spectrometry (GC–MS) analysis, with the results of the Protein Phosphatase Assay (PPase assay), showed that less than 0.1% of the total microcystin in the tissues of the mussel *Mytilus edulis* was extractable by methanol. According to the authors, the Lemieux oxidation GC–MS procedure provides a comparison between the amount of microcystin in tissue which is released by methanol extraction and detected by the PPase assay and the total micro-

cystin burden in the tissue, since this oxidation of microcystin-LR gives 2-methyl-3-methoxy-4-phenylbutanoic acid in high yield, which represents a unique marker for the presence of microcystin. Therefore, Lemieux oxidation of tissues exposed to microcystin should liberate the butanoic acid from both free and covalently linked microcystin (Williams et al., 1997). It can be one of the possible explanations for the lower values of microcystin accumulation found in this and other studies, when compared with the values obtained with the Lemieux oxidation GC–MS procedure (Williams et al., 1997).

There are few planktivorous fish species in Jacarepaguá Lagoon, with *Tilapia rendalli* being the most conspicuous one. This species, which was introduced in the lagoon, is mainly phytoplanktivorous, although zooplankton is also preyed but as a minor dietary item (Arcifa and Meschiatti, 1996). Therefore, it is likely that the biomass of zooplankton in Jacarepaguá Lagoon is only a small part of *Tilapia* diet, since this fish is able to filter and ingest large amounts of *Microcystis* colonies directly from water. Magalhães et al. (2001) also observed that *T. rendalli* collected from this water body presented microcystins in its liver, viscera and muscles (Table 2).

In the present study we measured the content of microcystin only in the large fraction (>200 μm) of zooplankton, which was composed mostly by the cladocerans *M. micrura* and *C. cornuta* and by copepods and rotifers. These two cladocerans showed very different tolerances to toxic *Microcystis* in acute toxicity experiments carried out in the lab by Ferrão-Filho et al. (2000), with *Moina* being much more sensitive than *Ceriodaphnia*. This means that *Ceriodaphnia* could be considered a better vector in the transferring of microcystins throughout the food chain. However, besides being a larger cladoceran, *Moina* occurred at much higher densities than *Ceriodaphnia* in the lagoon, which means that its biomass was probably more important in the microcystins accumulation.

Small zooplankton such as rotifers and protozoa are likely to ingest high amounts of toxic *Microcystis*, when they are as single cells or small colonies, and should also accumulate microcystin. These small organisms are important links in food webs and should be considered in future studies on the accumulation and transferring of toxins to higher trophic levels. Unfortunately, most of the work done on the cyanobacteria-zooplankton interactions deals with large cladocerans such as *Daphnia*, especially the temperate species. Further studies, including field work and experiments, should be conducted especially in tropical lakes, which are composed primarily by smaller zooplankton species.

In conclusion, the ELISA technique showed to be a reliable and sensitive method for detecting microcystins in field samples of zooplankton. Also, the high estimated CF for microcystins in zooplankton showed that these organisms may efficiently accumulate these toxins and that they can be potential vectors in the transferring of microcystins to higher trophic levels.

Acknowledgements

The authors would like to thank the colleagues of the Laboratory of Cyanobacterial Ecophysiology and Toxicology, IBCCF, UFRJ, for their assistance in the field and laboratory activities. We thank Dr William R. DeMott for his reviews on the article. We also thank CNPq for the fellowships and financial assistance.

References

Amorim, A., Vasconcelos, V., 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. Toxicon 37, 1041–1052.

Arcifa, M.S., Meschiatti, A.J., 1996. *Tilapia rendalli* in the Lake Monte Alegre, a case of planktivory. Acta Limnol. Brasil 8, 221–229.

Carmichael, W.W., 1992. Cyanobacteria secondary metabolites: the cyanotoxins. Appl. Bacteriol. 72, 445–459.

Carmichael, W.W., 1994. The toxins of cyanobacteria. Scientific Am. 270, 78–86.

Chen, D.Z.X., Boland, M.P., Smillie, M.A., Klix, H., Ptak, C., Andersen, R.J., Holmes, C.F.B., 1993. Identification of protein phosphatase inhibitors of the mycrocystin class in the marine environment. Toxicon 31, 1407–1414.

Chorus, I., Bartram, J., 1999. Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring, and Management. E&FN Spon, London/New York.

Christoffersen, K., 1996. Ecological implications of cyanobacterial toxins in aquatic food webs. Phycologia 35, 42–50.

Chu, F.S., Huang, X., Wei, R.D.J., 1990. Enzime-linked imunoabsorbant assay for microcystins in blue-green algal blooms. Assoc. Anal. Chem. 73, 451–456.

Erickson, J.E., Meriluoto, J.A.O., Lindholm, T., 1989. Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anodonta cygnea*. Hydrobiologia 183, 211–216.

Ferrão-Filho, A.S., Azevedo, S.M.F.O., DeMott, W.R., 2000. Effects of toxic and non-toxic cyanobacteria on the life-history of tropical and temperate cladocerans. Freshwater Biol. 45, 1–19.

Jochimsen, E.M., Carmichael, W.W., An, J., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antunes, B.C., Filho, D.A.M., Lyra, T.M., Barreto, V.S.T., Azevedo, S.M.F.O., Jarvis, W.R., 1998. Liver failure and death after exposure to mycrocystins at a hemodialysis center in Brazil. New Engl. J. Med. 338, 873–878.

Kotak, B.G., Zurawell, R.W., Prepas, E.E., Holmes, C.F.B., 1996. Microcystin-LR concentrations in aquatic food web compartments from lakes of varying trophic status. Can. J. Fish. Aquat. Sci. 53, 1974–1985.

Krishnamurthy, T., Carmichael, W.W., Sarver, E.W., 1986. Toxic peptides from freshwater cyanobacteria (blue–green algae). I. Isolation, purification and characterization of peptides from *Microcystis aeruginosa* and *Anabaena flos-aquae*. Toxicon 24, 873–895.

Kuiper-Goodman, T., Lawrence, J.F., Morrisey, M., 2000. Risk assessment of microcystins in blue-green algal health food products. Proceedings of the X International Symposium on Mycotoxins and Phycotoxins, Guarujá-SP, Brazil, p. 65.

Lambert, T.W., Holmes, C.F.B., Hrudey, S.E., 1994. Microcystin class of toxins: health effects and safety of drinking water supplies. Environ. Rev. 2, 167–186.

Laurén-Määtä, C., Hietala, J., Reinikainen, M., Walls, M., 1995. Do *Microcystis aeruginosa* toxins accumulate in the food web: a laboratory study. Hydrobiologia 304, 23–27.

Lirås, V., Lindberg, M., Nyström, P., Annadotter, H., Lawton, L.A., Graf, B., 1998. Can ingested cyanobacteria be harmful to the signal crayfish (*Pacifastacus leniusculus*)? Freshwater Biol. 39, 233–242.

Magalhães, V.F., Azevedo, S.M.F.O., 1998. Ecological implications of hepatotoxic *Microcystis aeruginosa* in Jacarepaguá Lagoon, RJ-Brazil. In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), Proceedings of the VIII International Conference on Harmful Algae, 25–29 June 1997, Vigo, Spain, pp. 26–28.

Magalhães, V.F., Soares, R.M., Azevedo, S.M.F.O., 2001. Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39, 1077–1085.

Prepas, E.E., Kotak, B.G., Campbell, L.M., Evans, J.C., Hrudey, S.E., Holmes, C.F.B., 1997. Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam *Anodonta grandis simpsoniana*. Can. J. Fish. Aquat. Sci. 54, 41–46.

Sivonen, K., 1996. Cyanobacterial toxins and toxin production. Phycologia 35, 12–24.

Thostrup, L., Christoffersen, K., 1999. Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*. Arch. Hydrobiol. 145, 447–467.

Vasconcelos, V.M., 1995. Uptake and depuration of the hepatopeptide toxin microcystin-LR in *Mytilus galloprovincialis*. Aquat. Toxicol. 32, 227–237.

Watanabe, M.M., Kaya, K., Takamura, N.J., 1992. Fate of the toxic cyclic hepatopeptides, the microcystins, from blooms of *Microcystis* (Cyanobacteria) in a hypereutrophic lake. Phycology 28, 761–767.

Watanabe, M.F., Park, H.D., Kondo, F., Harada, K.I., Hayashi, H., Okino, T., 1997. Identification and estimation of microcystins in freshwater mussels. Nat. Toxins 5, 31–35.

Williams, D.E., Dawe, S.C., Kent, M.L., Andersen, R.J., Craig, M., Holmes, C.F.B., 1997. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and in vitro evidence for covalently bound microcystins in mussel tissues. Toxicon 35, 1617–1625.