

Estimation of Microcystins in the Freshwater Fish *Oreochromis niloticus* in an Egyptian Fish Farm Containing a *Microcystis* Bloom

Zakaria A. Mohamed,¹ Wayne W. Carmichael,² Ahmed A. Hussein³

¹Department of Botany, Faculty of Science, South Valley University, Sohag, Egypt

²Department of Biological Sciences, Wright State University, Dayton, Ohio, USA

³Department of Zoology, Faculty of Science, South Valley University, Sohag, Egypt

Received 8 December 2002; revised 13 January 2003; accepted 13 January 2003

ABSTRACT: Microcystins (MCYSTs) that accumulated in different organs of the freshwater fish *Oreochromis niloticus*, collected from a fish farm in Egypt containing heavy blooms of *Microcystis aeruginosa*, were investigated using an enzyme-linked immunosorbent assay (ELISA). The distribution of MCYSTs in the organs varied significantly. The highest MCYST level was recorded in the guts (821 ng/g fresh weight), followed by the livers (531.8 ng/g) and kidneys (400 ng/g). Smaller amounts of MCYST were detected in muscles (102 ng/g). The present study suggests that fish farms should be monitored for the presence of toxic cyanobacterial blooms to minimize the exposure of fish to potent hepatotoxins. © 2003 Wiley Periodicals, Inc. *Environ Toxicol* 18: 137–141, 2003.

Keywords: *Microcystis*; microcystins; fish; accumulation; Nile perch

INTRODUCTION

Eutrophication of lakes and reservoirs leads to water blooms of cyanobacteria in many countries of the world. The ingestion of intact cells as well as cyclic peptide toxins [microcystins (MCYSTs)] can cause illness and death in wild and domestic animals and humans (Yu, 1989; Penaloza et al., 1990; Andersen et al., 1993; Carmichael and Falconer, 1993; Rodger et al., 1994; Jochimsen et al., 1998; Carmichael et al., 2001; Zimba et al., 2001).

It is well known that species within certain genera of cyanobacteria, including *Anabaena*, *Hapalosiphon*, *Micro-*

cystis, *Nostoc*, and *Oscillatoria*, produce cyclic peptide hepatotoxins called microcystins (Botes et al., 1984; Carmichael and Falconer, 1993; Carmichael, 1997). MCYST causes liver damage in both mammals and fish through inhibition of protein phosphatase types 1 and 2A (Mackintosh et al., 1990; Matsushima et al., 1990; Yoshizawa et al., 1990; Suganuma et al., 1992; Runnegar et al., 1993). Promotion of tumor growth has been shown in animals studies (Ito et al., 1997; Humpage et al., 2000). MCYSTs not only cause damage to aquatic organisms but also can be accumulated in the bodies of these organisms (Eriksson et al., 1989; Kotak et al., 1996; Watanabe et al., 1997; Thostrup and Christofferson, 1999; Mohamed, 2001). Consequently, transfer of these toxins to humans upon eating fish and mussels is possible.

Occurrence of MCYST-producing strains of *Microcystis aeruginosa* in El-Dowyrat fish farm, Sohag, Egypt, has been recorded previously (Mohamed, 1998). These strains were

Correspondence to: Dr. Zakaria A. Mohamed, Department of Botany, Faculty of Science, South Valley University, Sohag, 82524, Egypt; e-mail: mzakaria_99@yahoo.com.

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.10111

TABLE I. Occurrence of algal species in the fish farm and fish gut

Algal Species	In Fish Pond	In Fish Gut		
		Group 1	Group 2	Group 3
Cyanobacteria				
<i>Chroococcus limneticus</i> Lemm.	+	–	+	–
<i>Mersimopedia elagans</i> A. Brawn	++	–	–	–
<i>Microcystis aeruginosa</i> Kuetz.	bloom	++	+++	++++
<i>Oscillatoria limnetica</i> Lemm.	++	–	–	–
Chlorophyta				
<i>Ankistrodesmus falcatus</i> Ralf.	++	+	+	–
<i>Pediastrum simplex</i> Lemm.	+	–	–	–
<i>Scenedesmus quadricauda</i> Turp.	+	+	+	+
Diatoms				
<i>Fragillaria</i> sp.	+	+	–	–
<i>Synedra</i> sp.	+	–	+	+
<i>Nitzschia</i> sp.	+	–	–	+
Dinoflagellates				
<i>Ceratium</i> sp.	+	–	–	–

Group 1, fish containing a low number of cell ($5-10 \times 10^6$ cells/g of gut tissue) of *Microcystis* in the gut.

Group 2, fish containing a moderate number of cell ($10-15 \times 10^6$ cells/g of gut tissue) of *Microcystis* in the gut.

Group 3, fish containing a high number of cell ($>15 \times 10^6$ cells/g of gut tissue) of *Microcystis* in the gut.

found to produce 4.5 mg of microcystin/g dry weight. Because of the occurrence of this toxic cyanobacterium in El-Dowyrat's pond, one of the fish sources for Sohag City, there is a potential for ingestion and accumulation of MCYSTs by fish. Therefore, the present study was carried out to examine whether MCYSTs are accumulated in fish organs, especially those tissues eaten by humans.

MATERIALS AND METHODS

Location of Fish Farm

El-Dowyrat fish farm is 8750 m² in area and is located 15 km southeast of Sohag city (26° 30' north, and 31° 50' east). Near this pond is a restaurant where fried and smoked fish from the pond are sold.

Collection of *Microcystis* Waterbloom and Fish

Microcystis waterbloom was collected using a phytoplankton net (mesh, 25 μ m in diameter) from El-Dowyrat fish farm in June 2000. *Microcystis* and other associated algal species were identified according to Prescott (1978) and counted using a hemocytometer. The water-bloom material was lyophilized and the dried cell material was stored at -20°C . The fish were kindly provided by fishermen who use this pond.

Toxin Extraction

From *Microcystis* Waterbloom

MCYSTs from the *Microcystis* waterbloom were extracted from the dried cell material by placing a known weight of freeze-dried cells in 100% methanol and stirring overnight at room temperature (25°C). The extract was centrifuged at 10 000 rpm, and the pellet was reextracted in 100% methanol and centrifuged. The supernatants were combined together and mixed 1:1 with distilled water. The organic solvent was removed by placing the extract under an air-stream overnight. The aqueous fraction remaining after removing the organic solvent was applied to C18 silica cartridges using the method of Harada et al. (1988). The toxin was eluted with 80% methanol, and the MCYST content that remained in the aqueous fraction after methanol evaporation was determined by an enzyme-linked immunosorbent assay (ELISA) according to An and Carmichael (1994) and Carmichael and An (1999).

MCYST Extraction from Fish Tissues

To extract toxin from fish organs, fish were sacrificed by a sharp blow to the head. All animals were weighed. A necropsy was performed; livers, kidneys, guts, and tissues (muscles) were excised and then weighed. Liver-to-body-mass ratio was calculated to estimate whether enlargement in this organ had occurred. Algal species within fish guts were investigated and counted. Fish were placed into 3 groups according to the number of *Microcystis* cells within the gut: Group 1 included guts with a low cell number

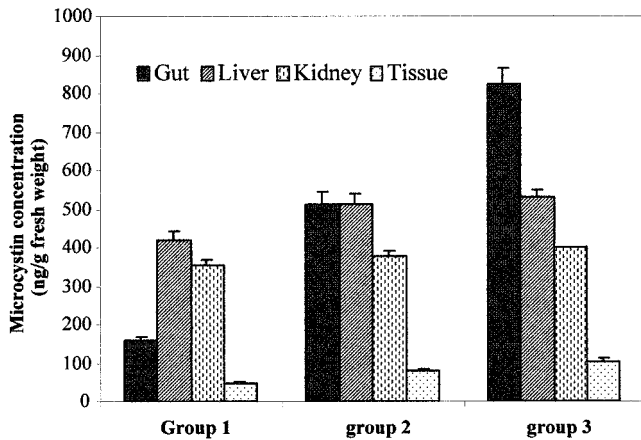


Fig. 1. Microcystin concentrations (ng/g fresh weight) in methanol extracts of gut, liver, and kidney of the freshwater fish *Oreochromis niloticus* collected from El-Dowyrat fish farm.

($5-10 \times 10^6$); Group 2, those with a moderate cell number ($10-15 \times 10^6$); and Group 3, those with a high cell number ($>15 \times 10^6$; Table I). Each group consisted of 6 fish.

Livers, kidneys, guts, and tissues of each group were separately homogenized in 100% methanol, stirred overnight at room temperature, and then centrifuged at 10 000 rpm. The pellets were reextracted in 100% methanol. The supernatants of each organ were combined and mixed with a known volume of distilled water. The aqueous fractions remaining after methanol evaporation were applied to C18 cartridges and eluted with 80% methanol. MCYSTs that remained in the aqueous fractions after methanol evaporation were determined by ELISA.

RESULTS

El-Dowyrat fish farm is covered during the warm season (April–December) with a heavy bloom of *Microcystis aeruginosa* every year. The microcystin content of this bloom as estimated by ELISA was 1.12 mg/g dry weight. The algal biomass in this farm was about 6.7 g dry weight/L.

Figure 1 presents the distribution of MCYSTs in the organs of the fish. The highest level of MCYST was found in the guts, followed by the livers and kidneys. Small amounts of MCYST were detected in the muscles (45.7–102 ng/g fresh weight).

MCYST concentrations in the kidney and muscle had a strong correlation with the concentration in the liver ($r = 0.92$ and 0.96 , respectively). We also found that the liver-to-body-mass ratio (Group 1, 0.74 ± 0.10 ; Group 2, 0.82 ± 0.08 ; Group 3, 1.23 ± 0.18) had a positive correlation with toxin concentration in the liver ($r = 0.74$).

DISCUSSION

Cyanobacteria can contribute to the diet of several species of fish (Bowen, 1982). Beveridge et al. (1993) reported that tilapia, *Oreochromis niloticus* L., and silver carp, *Hypophthalmichthys molitrix*, showed a marked difference in grazing response to toxic *Microcystis aeruginosa*. Our study recorded the presence of high numbers of toxic *Microcystis* cells in fish guts, confirming that grazing by this kind of fish on toxic cyanobacteria does occur.

Toxic blooms of cyanobacteria are also associated with fish mortality (Zimba et al., 2001). This harmful effect may be caused by either direct toxicosis or changes in the water chemistry as a consequence of the bloom. For example, oxygen depletion or hydrogen sulfide (H_2S) production can occur when the bloom collapses (Prescott, 1948; Eriksson et al., 1986; Tornazo et al., 1990; Rodger et al., 1994).

Intraperitoneal (i.p.) exposure of fish to microcystins causes tissue damage in liver (Phillips et al., 1985; Rabergh et al., 1991; Andersen et al., 1993), kidneys (Rabergh et al., 1991; Kotak et al., 1996), cerebellar and optic neurons (Phillips et al., 1985), and gill epithelial (Eriksson et al., 1986; Rodger et al., 1994) and ionic imbalance and reduced growth (Bury et al., 1995). Our study found an increase in liver-to-body-weight ratio with increasing MCYST concentration in this organ, showing the presence of liver enlargement induced by MCYSTs.

Even though the target organ for MCYST is the liver, in our study to find MCYSTs were found in the kidney and muscles as well, which was surprising. 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. However, when this process (presystematic hepatic elimination) is overwhelmed or bypassed by exposure to toxins such as MCYST, it may allow MCYST to circulate to these other organs (Klaassen and Watkins, 1984).

The ability of aquatic invertebrates, which are a food source for many kinds of fish, to accumulate microcystins in their body has been addressed by several authors (Eriksson et al., 1989; Kotak et al., 1996; Watanabe et al., 1997; Thostrup and Christoffersen, 1999; Mohamed 2001). The accumulation of MCYSTs by tilapia in a natural environment has also been reported by deMagalhaes et al. (2001). Our study supports this finding: that MCYSTs can accumulate in fish to levels that, although not toxic to the fish, may lead to levels consumed by humans that are above the recommended levels of MCYST consumption for drinking water. The WHO guideline for drinking water is $1 \mu\text{g/L}$ based on water consumption of 2 L/day (Chorus and Bartram, 1999). The average portion of fish eaten by a person is about 100–200 g. At the levels found in tissue in our study (100 ng/g), a 100-g serving would contain $10 \mu\text{g}$ of MCYST, or about 5 times the recommended daily MCYST intake from drinking water. Because MCYSTs are heat

stable, they are not broken down by cooking (Harada et al., 1996). For these reasons we recommend the monitoring of MCYST-producing water blooms in fish farms and a system of fish tissue monitoring to allow alerts to be issued designed to protect humans from the possible toxicity associated with MCYST exposure.

REFERENCES

- An J, Carmichael WW. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon* 32:1495–1507.
- Andersen RJ, Luu HA, Chen DZX, Holmes CFB, Kent ML, LeBlanc M, Taylor FJRM, Williams DE. 1993. Chemical and biological evidence links microcystins to salmon netpen liver disease. *Toxicon* 31:1315–1323.
- Beveridge MCM, Baird DJ, Rahmatullah SM, Lawton LA, Beattie KA, Codd GA. 1993. Grazing rates on toxic and non-toxic strains of cyanobacteria by *Hypophthalmichthys molitrix* and *Oreochromis niloticus*. *J Fish Biol* 43:901–907.
- Botes DP, Tuinam AA, Wessels PL, Viljoen CC, Kruger H, Williams DH, Santikarn S, Smith RJ, Hammond SJ. 1984. The structure of cyanoginosin-LA, a cyclic peptide from the cyanobacterium *Microcystis aeruginosa*. *J Chem Soc Perkin Trans 1*:2311–2318.
- Bowen SH. 1982. Feeding and digestion and growth-qualitative considerations. In: Pullin RSV, Lowe-McConnell RH, editors. *The biology and culture of tilapias*; Manila, the Philippines: p 141–156.
- Bury NR, Eddy FB, Codd GA. 1995. The effects of the cyanobacterium *Microcystis aeruginosa*, the cyanobacterial hepatotoxin microcystin-LR, and ammonia on growth rate and ionic regulation of brown trout. *J Fish Dis* 46:1042–1054.
- Carmichael WW. 1997. The cyanotoxins. In: *Advances in botanical research*; London: Academic Press London; p 211–240.
- Carmichael WW, Falconer IR. 1993. Diseases related to freshwater blue green algal toxins, and control measures. In: Falconer IR, editor. *Algal toxins in seafood and drinking water*. London: Academic Press; p 187–209.
- Carmichael WW, An J-S. 1999. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PIIA) for the detection of microcystins and nodularins. *Nat Toxins* 7:377–385.
- Chorus I, Bartram J. 1999. *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*; World Health Organization; London: E&FN Spon, Routledge.
- deMagalhaes VR, Soares RM, Azevedo SMFO. 2001. Microcystin contamination in fish from the Jacarepagu? Lagoon (Rio de Janeiro, Brazil): Ecological implication and human health risk. *Toxicon* 39:1079–1085.
- Eriksson JE, Meriluoto JAO, Lindholm T. 1986. Can cyanobacterial toxins accumulate in aquatic food chains. In: Megusar F, Gantar M, editors. *Perspectives in microbial ecology*; Proceedings of the 4th International Symposium on Microbial Ecology. Solvne Society for Microbiology, Ljubljana; p 655–658.
- Eriksson JE, Meriluto JAO, Lindholm T. 1989. Accumulation of peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anadonta cyagnea*. *Hydrobiol* 183:211–216.
- Harada K, Matsuura K, Suzuki M. 1988. Analysis and purification of toxic peptides from cyanobacteria by reversed-phase high-performance liquid chromatography. *J Chromatogr* 488:275–283.
- Harada KI, Tsuji K, Watanabe MF, Kondo F. 1996. Stability of microcystins from cyanobacteria. III. Effect of pH and temperature. *Phycologia* 35:83–88.
- Humpage AR, Hardy SJ, Moore EJ, Frosco SM, Falconer IR. 2000. Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon. *J Toxicol Environ Health A* 61(3):155–165.
- Ito E, Kondo F, Terao K, Harada KI. 1997. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. *Toxicon* 35(9):1453–1457.
- Jochimsen EM, Carmichael WW, An J, Cardo DM, Cookson ST, Holmes CEM, Antunes MBC, de Melo Filho DA, Lyra TM, Barreto VST, Azevedo SMFO, Jarvis WR. 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *New Engl J Med* 338:873–878.
- Klaassen CD, Watkins JB. 1984. Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol Rev* 36:1–67.
- Kotak BG, Zurawell RW, Prepas EE, Holmes CFB. 1996. Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Can J Fish Aquat Sci* 53:1974–1985.
- Mackintosh C, Beattie KA, Klumpp S, Cohen P, Codd GA. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *FEBS Lett* 264:187–192.
- Matsushima R, Yoshizawa S, Watanabe MF, Harada KI, Furusawa M, Carmichael WW, Fujiki H. 1990. *In vitro* and *in vivo* effects of protein phosphatase inhibitors, microcystins and nodularin, on mouse skin and fibroblasts. *Biochem Biophys Res Com* 171:867–874.
- Mohamed ZA. 1998. Studies on Egyptian toxic freshwater cyanobacteria (blue-green algae): Identification, production and removal of microcystins [Ph.D. thesis]. Egypt: South Valley University, in collaboration with Wright State University, OH. 182 pp.
- Mohamed ZA. 2001. Accumulation of cyanobacterial hepatotoxins by *Daphnia* in some Egyptian irrigation canals. *Ecotoxicol Environ Saf* 50(1):4–8.
- Penaloza R, Rojas M, Vila I, Zambrano F. 1990. Toxicity of a soluble peptide from *Microcystis aeruginosa* to zooplankton and fish. *Freshwater Biol* 24:133–240.
- Phillips MJ, Roberts RJ, Stewart JA, Codd GA. 1985. The toxicity of the cyanobacterium *Microcystis aeruginosa* to rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 8:339–343.
- Prescott GW. 1948. Objectionable algae with reference to the killing of fish and other animals. *Hydrobiol* 1:1–13.
- Prescott GW. 1978. *How to know the freshwater phytoplankton*. 3rd ed. Cambridge, UK: Cambridge University Press.

- Rabergh CMI, Bylund G, Eriksson JE. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa*, on common carp. *Aquat Toxicol* 20:131–146.
- Rodger HD, Turnbull T, Edwards C, Codd GA. 1994. Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, *Salmo trutta* L., in Loch Leven, Scotland. *J Fish Dis* 17:177–181.
- Runnegar MT, Kong S, Berndt N. 1993. Protein phosphatase inhibition and *in vivo* hepatotoxicity of microcystins. *Am J Physiol* 265:G224–G230.
- Suganuma M, Fujiki H, Okabe S, Nishiwaki S, Brautigan D, Ingebritsen TS, Rosner M. 1992. Structurally different members of the okadaic acid class selectively inhibit protein serine/threonine but not tyrosine phosphatase activity. *Toxicon* 30:873–878.
- Thostrup L, Christoffersen K. 1999. Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*. *Arch Hydrobiol* 145:447–467.
- Tornazo AE, Nieto F, Barja JL. 1990. Mortality associated with cyanobacterial bloom in farmed rainbow trout in Galicia (north-western Spain). *Bull Europ Assoc Fish Pathol* 10:106–107.
- Watanabe MF, Park HD, Kondo F, Harada KI, Hayashi H, Okino T. 1997. Identification and estimation of microcystins in freshwater mussels. *Nat Toxins* 5:31–35.
- Yoshizawa S, Matsushima R, Watanabe MF, Harada KI, Carmichael WW, Fujiki H. 1990. Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity. *J Cancer Res Clin Oncology* 116:609–614.
- Yu SZ. 1989. Drinking water and primary liver cancer. In: Tang ZY, Wu MC, Xia SS, editors. *Primary liver cancer*. New York: China Academic Publishers; p 30–37.
- Zimba PV, Khoo L, Gaunt P, Carmichael WW, Brittain S. 2001. Confirmation of catfish mortality from *Microcystis* toxins. *J Fish Diseases* 24:41–47.