

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

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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; Penalzoza 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

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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

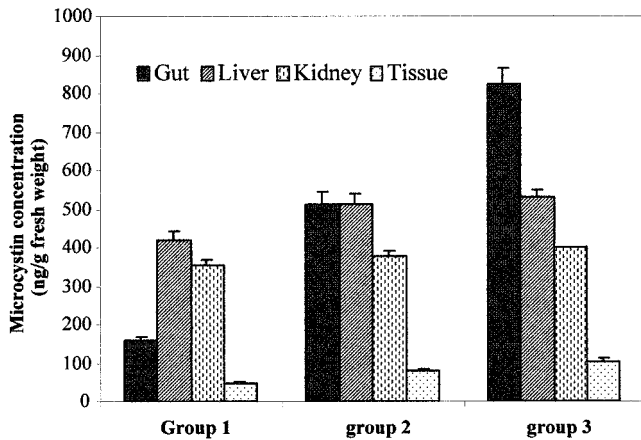


Fig. 1. Microcystin concentrations (ng/g fresh weight) in methanol extracts of gut, liver, and kidney of the freshwater fish *Oreochromis niloticus* collected from El-Dowyrat fish farm.

($5-10 \times 10^6$); 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 deMagathaes 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.

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