

Concentrations of microcystins in tissues of several fish species from freshwater reservoirs and ponds

Radovan Kopp · Miroslava Palíková · Ondřej Adamovský ·
Andrea Ziková · Stanislav Navrátil · Jiří Kohoutek ·
Jan Mareš · Luděk Bláha

Received: 16 January 2013 / Accepted: 30 May 2013
© Springer Science+Business Media Dordrecht 2013

Abstract The aim of this study is to summarise the determination of concentrations of microcystins (MCs) in muscle and liver of freshwater fish species caught in stagnant waters of the Czech Republic. Within the years 2007–2009, 351 muscle samples and 291 liver samples of 16 freshwater fish species derived from four fishponds, and four water reservoirs were analysed. MCs were detected in 53 liver samples. The highest concentrations of microcystins were determined in liver samples of carnivorous fish species; 50.3 ng/g of fresh weight (FW) in perch (*Perca fluviatilis*) and 22.7 ng/g FW in pikeperch (*Sander lucioperca*). MCs in liver were detected in other five fish species; asp (*Aspius aspius*), pike (*Esox lucius*), common carp (*Cyprinus carpio*),

grass carp (*Ctenopharyngodon idella*) and European eel (*Anguilla anguilla*). Concentrations of MCs in liver of nine fish species (European bream, whitefish, tench, silver carp, European catfish, roach, chub, crucian carp and rudd) were below the detection limit of 1.2–5.4 ng/g FW for different MC congeners. However, the concentrations of MCs in all muscle samples were below the detection limit. The assessment of MCs concentrations might be influenced by the detection method used. Due to the concentrations of MCs being below the detection limit in muscle samples of all fish species analysed, it seems that there might be a low potential threat for human health in case of fish muscle consumption.

R. Kopp (✉) · J. Mareš
Department of Fishery and Hydrobiology,
Faculty of Agronomy, Mendel University in Brno,
Zemědělská 1,
CZ61300 Brno, Czech Republic
e-mail: kopp@mendelu.cz

M. Palíková · S. Navrátil
Department of Veterinary Ecology and Environmental
Protection, Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého 1/3,
612-42 Brno, Czech Republic

O. Adamovský · J. Kohoutek · L. Bláha
Research Centre for Toxic Compounds in the Environment
(RECETOX), Faculty of Science, Masaryk University,
Kamenice 753/5,
625-00 Brno, Czech Republic

A. Ziková
Department of Ecophysiology and Aquaculture,
Leibniz-Institute of Freshwater Ecology and Inland Fisheries,
Muggelseedamm 310,
12587 Berlin, Germany

R. Kopp
Institute of Botany ASCR (Centre for
cyanobacteria and their toxins),
Lidická 25/27,
657-20 Brno, Czech Republic

Keywords Fish · Microcystin · Cyanobacteria · Cyanotoxins · Liver · Muscle

Introduction

Microcystins (MCs) are cyclic heptapeptides produced by certain species of cyanobacteria being considered as harmful. MCs are highly organ and cell specific, and in vivo damage is mainly restricted to the liver, whereas cellular in vitro studies showed that mostly hepatocytes are affected by these cyanotoxins (Eriksson et al. 1990). It is suggested that MCs could be taken up via the transport system for bile acid (Eriksson et al. 1990); however, they might be also detected in other organs such as muscle, skin, intestinal epithelium etc. MCs are known to covalently bound to protein serine/threonine phosphatases and cause their inhibition (Mackintosh et al. 1995).

Cyanotoxins have severe negative impacts on fishes depending on the stage of development. They cause malformations of embryos, changes in hatching periods of fish larvae, and differences in haematological and biochemical parameters (Malbrouck and Kestemont 2006). MCs might bioaccumulate in several aquatic organisms, such as fish, molluscs and planktonic invertebrates. The natural way of MCs intake by fishes might be performed differently. Phytoplanktivorous fish species take MCs in via consumption of toxic cyanobacteria. Fish that do not actively feed on toxic cyanobacteria might intake MCs via gill epithelium and/or skin. Another indirect way to take MCs in, known especially for carnivorous fish species, is via the food chain. Oral exposure to cyanotoxins is considered to be one of the most important one concerning natural ecosystems (Ernst et al. 2001).

The highest cyanotoxin concentrations could be expected in fish actively feeding on cyanobacterial blooms; however, MC-LR concentration in phytoplanktivorous fish species seems to be rather low. Under natural conditions, higher concentrations of cyanotoxins are recorded in tissues of carnivorous and omnivorous fish species rather than of herbivorous ones (Xie et al. 2005). Cyanotoxin intake is dependent on the amount of total food intake containing cyanotoxins that is substantially lower in the case of carnivorous fishes compared to herbivorous fishes (Gkelis et al. 2006). Differences in the morphology of digestive tracts among carnivorous, omnivorous and herbivorous fish species play also an important role for the intake and

bioaccumulation of MCs (Fischer and Dietrich 2000). Cyprinids as well as cichlids are known for the longer intestine with larger area for potential MCs absorption and subsequent bioaccumulation. However, several studies in Chinese lakes revealed the highest concentrations of MCs in carnivorous fishes (Xie et al. 2005). The lower cyanotoxin intake in cyprinids is due to neutral to alkaline pH levels in the digestive tract that is not as efficient in disruption of cyanobacterial cell walls as acid environments (Carbis et al. 1997). However, it seems there is no clear and direct relation between the fish species with different feeding strategies and cyanotoxin concentrations measured in their tissues. The feeding behaviour and the way of food digestion of different fish species does not seem to be a concise indicator for cyanotoxin accumulation (Ibelings and Chorus 2007).

Cyanotoxin concentrations present in fishes are considerably dependent on the length of exposure to cyanotoxins and therefore rather unpredictable. The study using *Tilapia rendalli* showed certain dependency of accumulation of MCs present in the cells of *Microcystis* sp. and the intensity of food intake (Soares et al. 2004).

In general, it is very difficult to compare the results obtained because the studies differ in cyanotoxin concentrations, exposure time, intake or application of cyanotoxins, and biotic and abiotic factors. Despite differences in experimental designs using fish to determine the fate of MCs in their bodies, the bioaccumulation of MCs was recorded (Ibelings and Chorus 2007).

Our study is concerned with the 3-year (2007–2009) monitoring of MCs concentrations in liver and muscle of freshwater fish species cultured in stagnant waters of the Czech Republic. Muscle and liver tissues were selected for analyses because they represent the major target for microcystins (liver) and the main edible part used for human consumption (muscle). In total, 351 muscle samples and 291 liver samples were analysed to determine potential MCs bioaccumulation using 16 fish species.

Materials and methods

Characteristics of the area of interest and fish sampling

Samples were collected at four fishponds and four stratified reservoirs (see Fig. 1 and Tables 1 and 2).

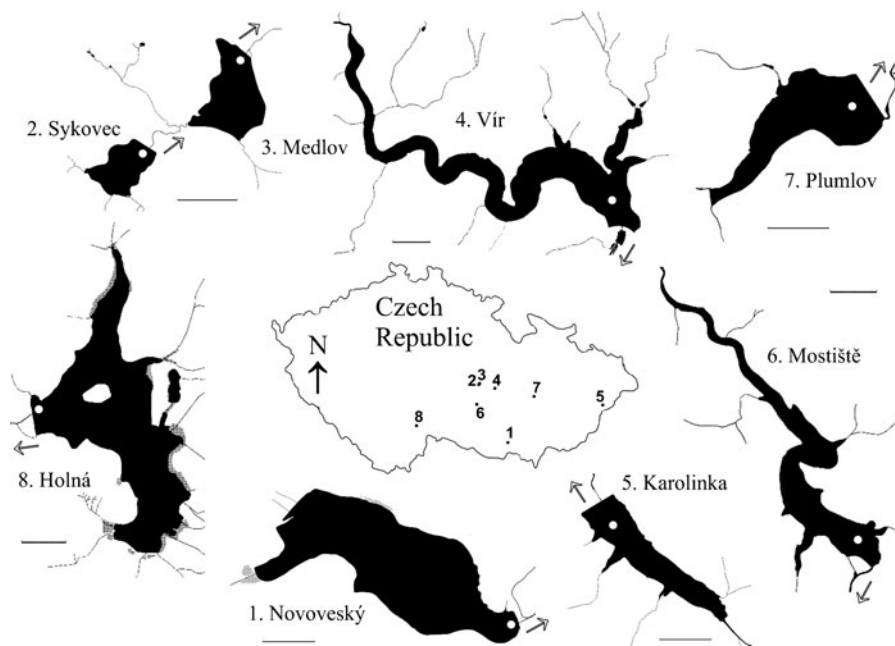


Fig. 1 Map of the Czech republic indicating positions of sampling localities (black dots with no. 1–8 in the central map) with schematic presentations of individual ponds or reservoirs

(white dots indicate sampling points within each reservoirs or pond, arrows water outlet from water bodies, scale bar 500 m)

The Sykovec and Medlov fishponds are eutrophic, used for the semi-intensive fish production, and fishing out takes place every year. The Novoveský and Holná fishponds are designated for intensive fish production being distinguished by hypertrophic level of nutrients and also fished out yearly. Fish for all analyses were taken on the first day of autumn, fishing out from the following fishponds within the dates mentioned: Medlov October 16, 2008, Sykovec October 23, 2008, Novoveský November 20, 2008 and Holná October 24, 2009. Fish from the Novoveský fishpond in 2007 were taken in ca 14-day intervals during the vegetation period until the fishing out took place (10.07., 24.07., 07.08., 20.08., 06.09., 18.09., 02.10., 16.10. and 14.11.).

Selected reservoirs are mostly used as waterworks reservoirs, only the Plumlov reservoir is designed as a flood control and recreational reservoir being eutrophic. The Mostiště and Vír reservoirs are rather mesotrophic to eutrophic, and the Karolinka reservoir is mesotrophic. Trophic state of all reservoirs was evaluated according to Wetzel (1983).

The fish community in reservoirs was sampled by a standard method of electrofishing using BMA PLUS electroshocker (Bednář Inc., Czech Republic) based

on Honda Eu 20i gear (output pulsating DC 240–300 V, 2.5 A, frequency of pulses 50 Hz). The dates of sampling were July 23, 2008 (Mostiště), August 24, 2008 (Karolinka), August 26, 2008 (Plumlov) and September, 2008 (Vír).

Each fish taken for analyses was weighted, and total body length was measured and afterwards killed. All muscle and liver samples were stored at -79°C until analyses. Cyanobacterial biomass was collected by plankton net, with mesh diameter of $40\text{ }\mu\text{m}$ and kept frozen at -20°C until analyses of MCs. Water filtered by the plankton net was collected in the field into glass bottles, transferred into laboratory (cooled at 4°C), filtered with glass fibre filter and then stored at -79°C until further processing and analyses of MCs.

Water quality analyses

Water samples for chemical analyses were taken into plastic bottles from the depth of 0–35 cm. One hundred milliliter of water sample was cooled down to 4°C and transported to the laboratory, where the taxonomic determination of cyanobacteria and algae using light microscopy was performed. Further 1,000 ml of the water sample were also kept at 4°C

Table 1 Basic physicochemical parameters of waters measured two times per month in the period from May to October at selected water bodies. Results are given as mean values, min–max (the Holná fishpond was not measured)

Year Locality	2007 Novoveský (fishpond)	2008 Sykovec (fishpond)	2008 Medlov (fishpond)	2008 Novoveský (fishpond)	2008 Vír (reservoir)	2008 Karolinka (reservoir)	2008 Mostišťe (reservoir)	2008 Plumlov (reservoir)
Temperature (°C)	18.8 11.3–24.1	18.9 13.5–23.5	18.9 12.4–23.5	19.2 12.0–27.0	15.0 9.3–20.8	15.0 9.1–21.3	17.8 13.4–21.2	17.3 14.0–19.7
Dissolved oxygen (%)	91 43–144	92 61–117	90 70–102	118 82–167	103 61–145	104 95–117	102 73–142	89 49–110
pH	8.8 7.9–9.8	7.4 6.8–8.1	7.2 6.4–8.6	9.0 8.3–10.0	8.1 7.2–9.8	8.3 8.1–8.5	8.3 7.3–9.9	8.0 7.9–8.4
Conductivity (mS/m)	78.4 66.4–93.7	8.4 7.5–9.4	10.6 9.4–11.8	70.2 63.5–76.3	17.5 13.4–22.5	29.1 19.0–29.1	32.4 28.7–39.7	35.1 28.9–49.2
Transparency (cm)	19 0–25	105 65–170	102 70–150	28 20–40	253 100–650	442 210–600	178 10–430	93 30–280
N-NH ₄ (mg/L)	0.26 0.02–1.02	0.02 0–0.04	0.14 0.01–0.50	0.06 0.04–0.08	0.08 0.04–0.18	0.06 0.02–0.22	0.10 0.03–0.25	0.48 0.16–1.29
N-NO ₂ (mg/L)	0.02 0.01–0.03	0.01 0–0.02	0.01 0–0.02	0.07 0.06–0.07	0.02 0.01–0.03	0.01 0–0.01	0.07 0.01–0.14	0.08 0.02–0.41
N-NO ₃ (mg/L)	0.83 0.16–2.30	0.28 0–1.20	0.35 0–1.0	2.35 1.70–3.00	2.04 0.94–3.09	0.60 0.33–1.00	4.57 1.81–8.01	1.14 0.24–2.74
COD _{Cr} (mg/L)	115.6 65.0–311.0	37.8 26.0–52.5	43.5 33.6–50.8	86.8 71.0–103.0	24.6 14.1–38.8	6.6 5.0–16.8	25.3 15.2–54.7	24.5 11.3–48.5
Chlorophyll a (mg/L)	629.4 275–2,308	27.9 16–47	85.2 40–149	388.5 352–424	35.0 3–113	1.9 0–4	42.3 5.9–80.0	35.0 2.5–195.0
Total Nitrogen (mg/L)	5.6 1.2–7.4	1.1 0.7–1.6	1.8 1.0–3.1	6.2 4.6–7.8	2.7 1.7–3.1	0.7 0.5–1.0	6.3 2.7–9.0	2.8 1.6–5.0
Total Phosphorus (mg/L)	0.84 0.45–4.11	0.07 0.04–0.10	0.09 0.05–0.11	0.28 0.24–0.32	0.09 0.02–0.19	0.03 0.01–0.14	0.14 0.02–0.45	0.23 0.03–0.67

Table 2 Characteristics of the water bodies including dominant cyanobacterial species, concentrations of microcystins (MCs) in water and phytoplankton biomass (dry weight) during the vegetation period (May–October, 2007–2009)

Locality	Area (ha)	GPS localization	Year	MCs in water $\mu\text{g/L}$	MCs in biomass ($\mu\text{g/g DW}$)	Dominant species of cyanobacteria	Subdominant species of cyanobacteria	% cyanobacteria from total biomass of phytoplankton (range during the year)
NOVOVESKÝ (fishpond)	138.7	48°56.042'N 16°31.787'E	2007	0–9.51	20.2–180.9	<i>Planktothrix agardhii</i>	<i>Pseudanabaena limnetica</i> , <i>Limnothrix redekei</i>	91–100
SYKOVEC (fishpond)	17.2	49°36.601'N 16°02.495'E	2008	0–5.27	0–49.3	<i>Planktothrix agardhii</i>	<i>Pseudanabaena limnetica</i>	98–100
MEDLOV (fishpond)	28.5	49°37.019'N 16°03.165'E	2008	0–1.36	Not measured	<i>Woronichinia naegeliana</i>	<i>Microcystis</i> sp.	84–99
VÍR (reservoir)	223.5	49°34.030'N 16°18.520'E	2008	0–1.99	0–39.5	<i>Woronichinia naegeliana</i>	<i>Microcystis</i> sp.	49–85
KAROLINKA (reservoir)	50.5	49°20.663'N 18°14.256'E	2008	0–1.20	1,779–3,312	<i>Microcystis aeruginosa</i>	<i>Dolichospermum</i> sp. <i>Woronichinia naegeliana</i>	63–99
MOSTIŠTĚ (reservoir)	93.0	49°23.777'N 16°0.792'E	2008	0	Not measured	<i>Aphanizomenon</i> sp.		<1
PLUMLOV (reservoir)	68.0	49°28.182'N 17°2.237'E	2008	0–0.53	0–979	<i>Microcystis aeruginosa</i>	<i>Dolichospermum</i> sp., <i>Aphanizomenon</i> sp.	56–100
			2008	0.21–0.51	Not measured	<i>Planktothrix agardhii</i>	<i>Microcystis</i> sp., <i>Dolichospermum</i> sp., <i>Aphanizomenon</i> sp.	50–99
HOLNÁ (fishpond)	230.0	49°8.030'N 14°52.020'E	2009	0.18–5.59	Not measured	<i>Limnothrix redekei</i>	<i>Microcystis</i> sp. <i>Planktothrix agardhii</i>	94–99

until the transportation to the laboratory, where the chemical analyses of water was carried out. Water–oxygen saturation, temperature, pH, conductivity and water transparency were measured immediately on the locality by CyberScan PCD 650 (Eutech Instruments, USA). The transparency of water was assessed using a Secchi disc. Ammonium ions (N-NH_4) were determined by the indophenols method, nitrite nitrogen (N-NO_2) by the method using $\text{N-(1-naphthyl)-ethylenediamine}$, nitrate nitrogen (N-NO_3) by the method using sodium salicylate, total nitrogen was measured with dimethylphenol after transformation of all nitrogen compounds into nitrate by Koroleff's method, total phosphorus by a method using ascorbic acid and ammonium molybdenate and chemical oxygen demand by the method using potassium dichromate. All the chemical parameters described were measured using the standards methods according to APHA (1998). Cyanobacterial and algal biomass was evaluated by chlorophyll-*a* concentrations using a heated ethanol extraction (Lorenzen 1967).

Analyses of microcystins in water and cyanobacterial biomass

Microcystins in cyanobacterial biomass and water (Table 2) were analysed by HPLC (high-performance liquid chromatography) as originally described by Lawton et al. (1994). Analytical method was optimised according to Babica et al. (2006). Extracts of lyophilized biomass (50 % v/v methanol) or concentrated water samples by SPE (SepPack C18 cartridges (Waters, Millford, MA, USA)) were analysed with a HPLC Agilent 1100 Series (Agilent Technologies, Waldbronn, Germany) on a Supelcosil ABZ+Plus (length, 150 mm; inner diameter, 4.6 mm; film thickness, 5 μm ; Supelco, Bellefonte, PA, USA) at 30 °C. Mobile phases consisted of $\text{H}_2\text{O}+0.1$ % trifluoroacetic acid and acetonitrile+0.1 % trifluoroacetic acid (linear increase during 0–30 min from 20–59 % of acetonitrile, flow rate 1 mL/min). Chromatograms were recorded by an Agilent 1100 Series photodiode-array detector. MCs were identified by the retention time and characteristic absorption spectra (200–300 nm, maximum at 238 nm). Quantification was based on external calibrations of three MC congeners (MC-LR, -RR and -YR). The presence of other MC congeners was also monitored qualitatively (presence of characteristic UV spectra). However, other variants than MC-LR, -RR and -YR

were present only as minor fraction (up to max 7 % of total MC concentration in sample from Vír reservoir based on the calibration curve for MC-LR). Concentrations of minor MC variants are not present.

Fish tissue extractions and analyses of microcystins

The method of tissue extractions were performed by methods described previously (Adamovský et al. 2007). Half a gramme of the fresh tissue was homogenised three times with 3 ml of methanol, sonicated in an ultrasonic bath for 30 min and centrifuged at $4,000\times g$ for 10 min. Supernatants were pooled and three times extracted with 1 mL of hexane to remove lipids (hexane layers were discarded). The methanol extract was evaporated at 50 °C and the residue dissolved in 300 μL of 50 % aqueous methanol (v/v). LC-MS/MS analyses were performed according to Kohoutek et al. (2010), with an Agilent (Waldbronn, Germany) 1200 series HPLC (C18 Supelcosil ABZ+Plus column at 30 °C). The mobile phase consisted of 5 mM ammonium acetate in water, pH 4 (solvent A) and acetonitrile (solvent B). The binary pump gradient was as follows: 0–12.00 min, 32–40 % solvent B, linear increase; 12.01–20.00 min, 40–42 % solvent B, linear increase; 20.01–30.00 min, 90 % solvent B. The flow rate was 0.4 mL/min. Twenty microlitres of each sample was injected for the analysis. The mass spectrometer was an Agilent Technologies (Waldbronn, Germany) 6410 triple–quadrupole mass spectrometer with electrospray ionisation. Ions were detected in the positive mode. The ionisation parameters were as follows: capillary voltage, 5.5 kV; desolvation temperature, 350 °C; desolvation gas flow, 11 L/min. In SIM mode, the following mass-to-charge ratio (m/z) were monitored: MC-RR $[\text{M}+2\text{H}]^2+$ 519.8, MC-YR $[\text{M}+\text{H}]^+$ 1,045.5, MC-LR $[\text{M}+\text{H}]^+$ 995.5, MCRR–GSH $[\text{M}+2\text{H}]^2+$ 673.8, MC-LR–GSH $[\text{M}+\text{H}]^+$ 1,302.5, MC-RR–Cys $[\text{M}+2\text{H}]^2+$ 580.8, MC-LR–Cys $[\text{M}+\text{H}]^+$ 1,116.5. The transitions from the protonated molecular ion to a fragment of the amino acid (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (an unusual amino acid present only in microcystins and related nodularins) at m/z 135.2 and a fragment at m/z 127.1 were monitored in MRM mode. The collision energies used for fragmentation were as follows: 50 V for MC-RR and respective conjugates; 40 V for MC-YR and MC-LR and respective conjugates). Quantification of analytes was based on external standards of MC-RR, MC-YR and MC-LR in a matrix (final extract of

microcystin-free fish tissue). The mixture of microcystins was added to the extract prior to analysis. The method detection limit (MDL; per gramme of tissue, fresh weight) was 1.2 ng/g (standard deviation (SD) of 5 %) for MC-RR and 5.4 ng/g (SD=10 %) for MC-YR and MC-LR in MRM mode. In SIM mode, the MDL was 3.0 ng/g (SD=5 %) for MC-RR and 2.7 ng/g (SD=7 %) for MC-YR and MC-LR, respectively.

Results

Within the years 2007–2009, there were 351 muscle and 291 liver samples analysed from 16 fish species derived from four fishponds and four reservoirs. The MCs concentration in all analysed muscle samples was below the detection limit of 1.2–5.4 ng/g for different MC congeners. MCs were detected in 53 liver samples (Table 3).

MCs were found only in fish from three monitored reservoirs (Plumlov, Mostiště and Vír) and from the Novoveský pond in 2008. Correspondingly to our expectation, no MCs were detected in fishes from the mesotrophic Karolinka reservoir. Fish tissue samples derived from the fishponds showed no detectable MCs concentration above the detection limit, with the exception of fish liver from the Novoveský fishpond in 2008.

The highest concentrations of MCs were determined in liver samples of carnivorous fish species such as perch

(*Perca fluviatilis*) and pikeperch (*Sander lucioperca*). Almost a half ($N=14$) out of 30 liver samples of pikeperches derived from two fishponds, and two reservoirs had the concentration of MCs above the detection limit. MCs were detected in 14 out of 41 liver samples from perches caught in three fishponds and two reservoirs (Table 3). Ten liver samples of 14 asp (*Aspius aspius*) taken from one fishpond and three reservoirs contained MCs. Surprisingly, only five liver samples of common carp (*Cyprinus carpio*) obtained from the Novoveský fishpond in 2008 (out of 115 analysed in total) exceeded the detection limit for MCs. No MCs in liver samples being above the detection limit were found in nine fish species: European bream (*Abramis brama*) $N=35$, whitefish (*Coregonus maraena*), $N=20$, tench (*Tinca tinca*), $N=16$, silver carp (*Hypophthalmichthys molitrix*), $N=10$, European catfish (*Silurus glanis*), $N=10$, roach (*Rutilus rutilus*), $N=7$, chub (*Leuciscus cephalus*), $N=4$, crucian carp (*Carassius carassius*), $N=2$ and rudd (*Scardinius erythrophthalmus*), $N=2$.

Discussion

In general, MCs are the most studied cyanotoxins, and their concentrations detected in different fish tissues have often been reported (Malbrouck and Kestemont 2006; Ibelings and Chorus 2007). Based on the results published, it seems that the highest concentrations of

Table 3 Mean values of concentrations of microcystins (MCs) and the range of min–max values expressed in ng/g of fresh weight. The n/N ratio shows number of fish, whose liver

contained MCs above the detection limit of 1.2–5.4 ng/g, in all fish analysed from given locality and year 2008 (Within the years 2007 and 2009, no MCs were detected)

Fish species mean values±SD (weight and total length)	(n/N)	Concentrations of MCs in liver	Locality and the year of sampling
pikeperch (<i>Sander lucioperca</i>) (C) 938±537 g, 455±80 mm	10/10	15.81 (9.0–22.7)	NOVOVESKÝ (fishpond) 2008
pikeperch (<i>Sander lucioperca</i>) (C) 900 g, 485 mm	1/1	5.85	MOSTIŠTĚ (reservoir) 2008
pikeperch (<i>Sander lucioperca</i>) (C) 377±59 g, 385±25 mm	3/4	9.79 (6.2–16.2)	PLUMLOV (reservoir) 2008
perch (<i>Perca fluviatilis</i>) (C) 252±51 g, 271±24 mm	10/10	22.69 (5.5–50.3)	VÍR (reservoir) 2008
perch (<i>Perca fluviatilis</i>) (C) 293±24 g, 284±6 mm	4/4	8.57 (5.2–15.5)	MOSTIŠTĚ (reservoir) 2008
pike (<i>Esox lucius</i>) (C) 720 g, 445 mm	1/1	6.48	PLUMLOV (reservoir) 2008
asp (<i>Aspius aspius</i>) (C) 538±103 g, 389±23 mm	9/10	4.60 (1.1–9.5)	NOVOVESKÝ (fishpond) 2008
asp (<i>Aspius aspius</i>) (C) 850 g, 485 mm	1/1	11.68	MOSTIŠTĚ (reservoir) 2008
European eel (<i>Anguilla anguilla</i>) (C) 707 g, 737 mm	1/1	2.4	NOVOVESKÝ (fishpond) 2008
common carp (<i>Cyprinus carpio</i>) (O) 2974±225 g, 545±15 mm	5/10	1.14 (0.5–1.8)	NOVOVESKÝ (fishpond) 2008
grass carp (<i>Ctenopharyngodon idella</i>) (H) 1603±361 g, 520±33 mm	8/10	2.53 (1.0–6.1)	NOVOVESKÝ (fishpond) 2008

Food habits of individual fish species are indicated. C carnivorous, O omnivorous, H herbivorous

MCs occur in liver and intestinal epithelium, followed by kidneys and muscle. Cyanobacterial toxins have been also found in other tissues such as gonads, spleen and gall bladder (Mohamed et al. 2003; Xie et al. 2005; Chen et al. 2005, 2006; Zhang et al. 2007).

The highest cyanotoxin concentrations have been detected in the tissues of freshwater fish species that are not intended to be used for consumption such as intestinal epithelium. MCs in fish liver reached levels of 71.6 and 32.1 µg/g FW in tilapia (*T. rendalli*) and 87.4 µg/g FW in smelt (*Osmerus eperlanus*), respectively, as indicated by Magalhaes et al. (2001) and Ibelings et al. (2005). Attention concerning cyanotoxins should be especially paid to fish muscle that is aimed for market and human consumption. The highest values of MCs of 2.86 (Zhang et al. 2007) and 0.89 µg/g FW (Chen et al. 2007) were detected in the muscle samples of silver carp (*H. molitrix*). In crucian carp (*C. carassius*), concentrations reached up to 3.26 µg/g DW (Xie et al. 2005) and in common carp (*C. carpio*) up to 0.27 µg/g DW (Xie et al. 2007). Lower concentrations of MCs were detected in the muscle of carnivorous fish species, where the maximum detected values were 0.035 µg/g FW for rainbow trout (Wood et al. 2006) or 0.11 µg/g DW for catfish (Gkelis et al. 2006). However, these concentrations are the highest values so far published, and in several cases, MCs are more than one order of magnitude lower or even below the detection limits of the methods used in the present study. The values of MCs detected in liver were shown to be approximately tenfold higher than concentrations in muscle (Kozłowski-Suzuki et al. 2012). The highest concentrations of MCs reported in fish so far were found in smelt (*Osmerus eperlanus*) (Ibelings et al. 2005), tilapia (*Oreochromis niloticus*), silver carp (Zhang et al. 2007; Xie et al. 2005) and common carp (Xie et al. 2005).

The results concerning MCs determination in different tissues of fish aquacultured in the Czech Republic did not confirm such high concentrations as reported previously by other authors. The highest MCs concentrations measured in our previous study using ELISA in liver of silver carp (0.226 µg/g) and common carp (0.132 µg/g) after 9 weeks of exposure to cyanobacterial blooms were tenfold higher than those found in fish muscle (0.029 and 0.019 µg/g, respectively) (Adamovský et al. 2007). In comparison to the results obtained by ELISA, concentrations of MCs determined by LC-MS/MS were one magnitude

lower. These differences might be due to a method of MCs quantification used, as it was previously discussed (Kohoutek et al. 2010). However, in the study of Adamovský et al. (2007), fish were kept in cages during the experiment, having no chance to escape from cyanobacterial water bloom, whereas the present study addressed fish freely swimming in lake and ponds, which could lead to partial avoidance of the exposure to dense biomass of cyanobacteria.

Measurement of MCs was performed during the autumn fishing out, during which the densities of cyanobacteria were already lower than during the high season summer time, and MCs could have been potentially depurated from fish tissues. Adamovský et al. (2007) showed relatively fast elimination of MCs from fish tissues within 2 weeks after transfer of fish into clear water without toxic cyanobacteria, with half-life of MCs in silver carp being 0.7 day for muscle and 3.5 days for liver.

The highest concentrations of MCs reported previously were determined in tissue samples of fish species derived from water bodies containing *Microcystis* sp. (Magalhaes et al. 2001; Chen et al. 2006). This cyanobacterial genus has optimum living conditions in stratified water bodies, with longer water retention. Another cyanobacteria that have similar ecological strategies such as the genus *Microcystis* and produce MCs that resulted in MCs accumulation in fish tissues is *Dolichospermum* (formerly *Anabaena*) (Xie et al. 2005; Kagalou et al. 2008). Accumulation of hepatotoxins was also measured in fish species originating from shallow waters with filamentous cyanobacterium *Planktothrix aghardii* (formerly *Oscillatoria aghardii*) (Ibelings and Chorus 2007; Amé et al. 2010). Published information is scarce, so far reporting examples of accumulation of MCs in fish tissues derived from water bodies, where cyanobacteria of genera *Aphanizomenon*, *Limnospira*, *Anabaenopsis* or *Woronichinia* were present.

It is interesting to compare the data of the Novoveský fishpond between the years 2007 and 2008. Although there was similar density and composition of fishes within both years, and phytoplankton species composition did not vary either, the concentration of MCs in water and cyanobacterial biomass was higher in 2007. However, in all 130 liver samples of 6 fish species, the MCs levels were below the detection limit. Additionally, part of these samples

was taken within the whole vegetation period of 2007, meaning the levels of MCs in water were higher than those ones in autumn time of fishing out. On the other hand, 33 out of 51 liver samples obtained in the year of 2008 exceeded the detection limit of MCs. These samples were taken during autumn, thus the fish were exposed long enough to cyanotoxins, although the concentrations of MCs in water have already been lower than during the summer time of vegetation period, and therefore no clear explanation exists why MCs were detected only in 2008, despite the conditions and exposure time to MCs were more or less similar. The accumulation of MCs in fish tissues seems to be rather unpredictable and depends on several biotic as well as abiotic factors (Landsberg 2002; Ibelings and Chorus 2007).

Apart from carnivorous fish species derived from different localities, the concentrations of MCs exceeded the limit of detection in common carp and silver carp derived from the Novoveský fish pond in 2008. These findings are in agreement with Xie et al. (2005) because they detected also higher concentration of cyanotoxins in tissues of carnivorous fish species than in herbivorous ones living in same habitat. A possible explanation for a lower uptake of cyanotoxins by cyprinids might be neutral to alkaline pH level in their digestive tract (Carbis et al. 1997). Our results show higher concentration of MCs in carnivorous fish species such as pikeperch, perch, asp and pike in comparison with herbivorous ones. Interestingly, MCs were below the detection limit in our single sample of European catfish (*S. glanis*). The only case where MC-LR bioaccumulation in tissue of European catfish so far was detected was in samples derived from the lakes in Greece (Gkelis et al. 2006). Low concentrations of MCs might be due to biology of this fish species to live on the bottom of water bodies, where the cyanobacterial biomass does not reach as high concentrations as in the upper layers of the water column. However, in our study, we focused on fish from shallow fishponds, where cyanobacterial biomass was abundant and more or less homogeneously distributed within the water column, putting forward a hypothesis that an effective biotransformation of MCs in European catfish might take place, and therefore bioaccumulation of MCs was not detected.

According to our results and based on the literature published (Geis-Asteggianti et al. 2011), concentrations

of MCs in fish tissues are apparently dependent on the analytical method used. The enzyme-linked immunosorbent assay (ELISA) method seems to be less suitable for quantification of MCs in animal tissues. Geis-Asteggianti et al. (2011) and other authors (Ernst et al. 2005; Orr et al. 2003) suggested that unspecific matrix effects could occur. The most precise measurement of MCs in fish tissue are likely obtained using LC-MS/MS (Kohoutek et al. 2010), and the results derived from several studies using ELISA for quantification of MCs (Adamovský et al. 2007; Magalhaes et al. 2001, 2003; Soares et al. 2004; Zhao et al. 2006; Gkelis et al. 2006; Kagalou et al. 2008; Poste et al. 2011; Romo et al. 2012) have to be considered as semiquantitative due to possible cross-reactivities of antibodies with MCs and their metabolites. Confirmation of the results by LC-MS/MS is highly recommended.

Despite the potential accumulation of cyanotoxins in different fish organs and tissues, our study indicated that microcystin concentrations mostly do not reach tolerable daily intake set by the World Health Organisation. Therefore, risk by fish consumption especially the most commonly used edible part, i.e. muscle tissue seems to be low in the Czech Republic. Concentrations of MCs found in tissues of freshwater fish species derived from natural eutrophied water bodies of the Czech Republic are at least one order of magnitude lower compared to other values reported from Chinese hypertrophic lakes (Xie et al. 2004; Chen et al. 2006; Zhang et al. 2007). The highest freshwater fish consumption in central Europe is within the Christmas time, when fish from local intensive aquacultural systems are consumed. These fish are after fishing out placed for a couple of weeks into clean water free of cyanobacterial blooms, where the depuration of MCs from fish tissues takes place (Adamovský et al. 2007). The most endangered group of consumers of fishes derived from the waters with cyanobacterial blooms producing cyanotoxins are probably anglers. According to studies from China, the anglers may be chronically exposed to fish containing cyanotoxins causing liver damage and changes of biochemical parameters in their blood (Chen et al. 2009). However, our results showed only slight elevation of MCs detected entirely in liver tissue of several fish species, and due to a lack of preference for liver consumption, it seems to be only a low risk, even for anglers in central Europe.

Acknowledgments This study was supported by a research project funded by the ministry of education, youth and sports of the Czech Republic “Veterinary Aspects of Food Safety and Quality” (MSM 62 15712402) and by the National Agency for Agricultural Research” (grant no. QH 71015). Infrastructure is supported by a project CETOCOEN (no. CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

References

- Adamovský, O., Kopp, R., Hilscherová, K., Babica, P., Palíková, M., Pašková, V., et al. (2007). Microcystin kinetics (bioaccumulation, elimination) and biochemical responses in common carp and silver carp exposed to toxic cyanobacterial blooms. *Environ Toxicol Chem*, 26(12), 2687–2693.
- Amé, M. V., Galanti, L. N., Menone, M. L., Gerpe, M. S., Moreno, V. J., & Wunderlin, D. A. (2010). Microcystin-LR, -RR, -YR and -LA in water samples and fishes from a shallow lake in Argentina. *Harmful Algae*, 9(1), 66–73.
- APHA (1998). *Standard methods for the examination of water and wastewater*. American Public Health Association Inc., Washington D.C.
- Babica, P., Kohoutek, J., Blaha, L., Adamovsky, O., & Marsalek, B. (2006). Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic material contents. *Anal Bioanal Chem*, 385, 1545–1551.
- Carbis, C. R., Rawlin, G. T., Grant, P., Mitchell, G. F., Anderson, J. W., & Mccauley, I. (1997). A study of feral carp, *Cyprinus carpio* L., exposed to *Microcystis aeruginosa* at Lake Mokoan, Australia and possible implications for fish health. *J Fish Dis*, 20(2), 81–91.
- Chen, J., Xie, P., Guo, L., Zheng, L., & Ni, L. (2005). Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large shallow, eutrophic lake of the subtropical China. *Environ Pollut*, 134(3), 423–430.
- Chen, J., Xie, P., Zhang, D., Ke, Z., & Yang, H. (2006). In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* blooms. *Aquaculture*, 261, 1026–1038.
- Chen, J., Xie, P., Zhang, D., & Lei, H. (2007). In situ studies on the distribution patterns and dynamics of microcystins in a biomanipulation fish—bighead carp (*Aristichthys nobilis*). *Environ Pollut*, 147, 150–157.
- Chen, J., Xie, P., Li, L., & Xu, J. (2009). First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. *Toxicol Sci*, 108(1), 81–89.
- Eriksson, J. E., Grönberg, L., Nygird, S., Slotte, J. P., & Meriluoto, J. (1990). Hepatocellular uptake of 3H-dihydromicrocystin-LR a cyclic peptide toxin. *Biochim Biophys Acta*, 1025, 60–66.
- Ernst, B., Hitzfeld, B., & Dietrich, B. (2001). Presence of *Planktothrix* sp. and cyanobacterial toxins in Lake Ammersee, Germany and their impact on white-fish (*Coregonus lavaretus* L.). *Environ Toxicol*, 16, 483–488.
- Ernst, B., Dietz, L., Hoeger, S. J., & Dietrich, D. R. (2005). Recovery of MC-LR in fish liver tissues. *Environ Toxicol*, 20, 449–458.
- Fischer, W. J., & Dietrich, D. R. (2000). Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). *Toxicol Appl Pharmacol*, 164(1), 73–81.
- Geis-Asteggiante, L., Lehotay, S. J., Fortis, L. L., Paoli, G., Wijey, C., & Heinzen, H. (2011). Development and validation of a rapid method for microcystins in fish and comparing LC-MS/MS results with ELISA. *Anal Bioanal Chem*, 401(8), 2617–2630.
- Gkelis, S., Lanaras, T., & Sivonen, K. (2006). The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. *Aquat Toxicol*, 78(1), 32–41.
- Ibelings, B. W., Bruning, K., Jonge, J., Wolfstein, K., Dionisio Pires, L. M., Postma, J., et al. (2005). Distribution of microcystins in a Lake food web: no evidence for biomagnification. *Microb Ecol*, 49, 487–500.
- Ibelings, B. W., & Chorus, I. (2007). Accumulation of cyanobacterial toxins in freshwater seafood and its consequences for public health: a review. *Environ Pollut*, 150(1), 177–192.
- Kagalou, I., Papadimitriou, T., Bacopoulos, V., & Leonardos, I. (2008). Assessment of microcystins in lake water and the omnivorous fish (*Carassius gibelio*, Bloch) in Lake Pamvotis (Greece) containing dense cyanobacterial bloom. *Environ Monit Assess*, 137(1–3), 185–195.
- Kohoutek, J., Adamovský, O., Oravec, M., Šimek, Z., Palíková, M., Kopp, R., et al. (2010). LC-MS analyses of microcystins in fish tissues overestimate toxin levels—critical comparison with LC-MS/MS. *Anal Bioanal Chem*, 398, 1231–1237.
- Kozłowski-Suzuki, B., Wilson, A. E., & Ferrao-Filho, A. S. (2012). Biomagnification or biodilution of microcystins in aquatic food webs? Meta-analyses of laboratory and field studies. *Harmful Algae*, 18, 47–55.
- Landsberg, J. H. (2002). The effects of harmful algal blooms on aquatic organisms. *Rev Fish Sci*, 10(2), 113–390.
- Lawton, L. A., Edwards, C., & Codd, G. A. (1994). Extraction and high-performance liquid chromatographic method for determination of microcystins in raw and treated waters. *Analyst*, 119, 1525–1530.
- Lorenzen, C. J. (1967). Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnol Oceanogr*, 12, 343–346.
- Mackintosh, R. W., Dalby, K. N., Campbell, D. G., Cohen, P. T. W., Cohen, P., & Mackintosh, C. (1995). The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase-1. *FEBS Lett*, 371(3), 236–240.
- Magalhaes, 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.
- Magalhaes, V. F., Marinho, M. M., Domingos, P., Oliveira, A. C., Costa, S. M., Azevedo, L. O., et al. (2003). Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (RJ, Brazil). *Toxicon*, 42, 289–295.

- Malbrouck, C., & Kestemont, P. (2006). Effects of microcystin on fish. *Environ Toxicol Chem*, 25(1), 72–86.
- Mohamed, Z. A., Carmichael, W. W., & Hussein, A. A. (2003). Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. *Environ Toxicol*, 18(2), 137–141.
- Orr, P. T., Jones, J. G., Hunter, A. R., & Berger, K. (2003). Exposure of beef cattle to sub-clinical doses of *Microcystis aeruginosa*: toxin bioaccumulation, physiological effects and human health risk assessment. *Toxicon*, 41, 613–620.
- Poste, E. A., Hecky, E. R., & Guildford, J. S. (2011). Evaluating microcystin exposure risk through fish consumption. *Environmental Science & Technology*, 45(13), 5806–5811.
- Romo, S., Fernández, F., Ouahid, Y., & Báron-Sola, Á. (2012). Assessment of microcystins in lake water and fish (Mugilidae, *Liza* sp.) in the largest Spanish coastal lake. *Environmental Monitoring and Assessment*, 184, 939–949.
- Soares, R. M., Magalhaes, V. F., & Azevedo, S. M. F. O. (2004). Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (Cichlidae) under laboratory conditions. *Aquat Toxicol*, 70, 1–10.
- Wetzel, G. R. (1983). *Limnology*. Orlando USA: Saunders College Publishing. 766 p.
- Wood, S. A., Briggs, L. R., Sprosen, J., Ruck, J. G., Wear, R. G., Holland, P. T., et al. (2006). Changes in concentrations of microcystins in rainbow trout, freshwater mussels and cyanobacteria in Lakes Rotoiti and Rotoehu. *Environ Toxicol*, 21(3), 205–222.
- Xie, L., Xie, P., Ozawa, K., Honma, T., Yokoyama, A., & Park, H. (2004). Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. *Environ Pollut*, 127, 431–439.
- Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y., & Park, H. (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu China. *Environ Toxicol*, 20, 293–300.
- Xie, L., Yokoyama, A., Nakamura, K., & Park, H. (2007). Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan. *Toxicon*, 49(5), 646–652.
- Zhang, H. J., Zhang, J. Y., Hong, Y., & Chen, Y. X. (2007). Evaluation of organ distribution of microcystins in the freshwater phytoplanktivorous fish *Hypophthalmichthys molitrix*. *J Zhejiang Univ Sci B*, 8(2), 116–120.
- Zhao, M., Xie, S., Zhu, X., Yang, Y., Gan, N., & Song, L. (2006). Effect of dietary cyanobacteria on growth and accumulation of microcystins in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 261, 960–966.