Effects of the microcystin profile of a cyanobacterial bloom on growth and toxin accumulation in common carp

_Cyprinus carpio_ larvae

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A 12 day growth trial was conducted to compare the effect of the variation in microcystins (MC) composition of two bloom samples of _Microcystis aeruginosa_ on the growth performance and microcystin accumulation in common carp _Cyprinus carpio_ larvae. Two _M. aeruginosa_ natural bloom samples with different MC profiles were collected and larvae were exposed to cyanobacterial cells through their diet. Three diets, a basal control diet and two diets prepared from the basal diet plus the same toxins content (60 ng MC g⁻¹ diet) of each cyanobacterial bloom, were given at the same ration level to three groups of larvae during the experimental period. Larval mass and standard length from day 9 were significantly different between cyanobacterial treatments and in both cases lower than that of the control. The MC accumulation by larvae, inversely correlated with the growth performance, was also significantly different between cyanobacterial treatments (26·96 v. 17·32 ng g⁻¹ at the end of the experimental period). These results indicate that MC variants profile may have effects on the toxin uptake and toxicity. To date, this is the first laboratory study to show that fish accumulate MC depending on the toxin profile of the cyanobacterial bloom.

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Key words: carp larvae; cyanobacterial blooms; fish growth; microcystins; microcystins accumulation.

INTRODUCTION

The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs and recreational waters has become a worldwide problem (Paerl _et al._, 2001). Several cyanobacteria species, specifically _Microcystis aeruginosa_, may produce a variety of potent toxins, including a group of hepatotoxins called microcystins (MC), which have strong cytotoxic activity (Codd _et al._, 1997; de Figueiredo _et al._, 2004). These
molecules are cyclic heptapeptides, which present >76 variants generally differing in the nature of the two L-amino acids and in the degree of methyl substitution (Fig. 1) (Dittmann & Wiegand, 2006). The toxicity of MC has been attributed to the highly specific inhibition of serine and threonine phosphatases (PP1–PP2A) (MacKintosh et al., 1990; Fischer & Dietrich, 2000a, b) and to the increased formation of reactive oxygen species (ROS) (Ding et al., 1998, 2001; Li et al., 2003). It has been suggested that either of the above mechanisms could induce cytoskeletal damage leading to loss of cell morphology (Toivola & Eriksson, 1999; Ding & Ong, 2003).

Aquatic organisms can be exposed to MC via the consumption of toxic cyanobacteria (Li et al., 2004; Xie et al., 2004) or aquatic organisms that had previously accumulated MC in their tissues. Although negligible amounts of toxins enter the system through the gills or epithelium (Tencalla et al., 1994; Kent et al., 1996), it is generally believed that the oral route is the most important (Ernst et al., 2001). Several studies have been performed to assess the effect of MC on fishes (Bury et al., 1996; Kotak et al., 1996; Tencalla & Dietrich, 1997; Fischer & Dietrich, 2000a; Fischer et al., 2000; Jacquet et al., 2004), although most of them focused on the histopathological changes after the acute administration of MC by intraperitoneal route. Nevertheless, others focused on the study of different effects on fish orally exposed to MC or to extracts of toxic cyanobacteria (Oberemm et al., 1999; Wiegand et al., 1999; Mohamed et al., 2003; Soares et al., 2004; Prieto et al., 2007). Toxic effects have been observed in various fish species, including Salmoniformes, Siluriformes, Cypriniformes as well as Perciformes, and clear differences in susceptibility to toxic cyanobacteria in general (as cell suspensions or bloom material) and microcystins [primarily microcystin-LR (MC-LR)] have been found (Malbrouck & Kestemont, 2006). Microcystins affect a large number of fish organs, such as liver, intestine, kidney (Fischer & Dietrich, 2000a), heart (Best et al., 2001) and gills (Carbis et al., 1997; Bury et al., 1998a, b). Haematological disorder (Koop & Hetesa, 2000), increased activity of some serum enzymes (Carbis et al., 1997),
inhibition of protein phosphatases activity (Sahin et al., 1995) and death (Tencalla et al., 1994) have also been reported.

Aquatic animals not only can be damaged by cyanobacterial toxins but can bioaccumulate them as well (Tencalla et al., 1994; Mohamed et al., 2003; Soares et al., 2004). Since the uptake of cyanobacterial toxins by fishes results primarily following oral ingestion of toxic cyanobacterial cells or contaminated tissues and many fishes, such as those living in small eutrophicated lakes and aquaculture ponds, are not able to avoid the ingestion of these toxic materials through food (Tencalla & Dietrich, 1997; Magalhães et al., 2001), the accumulation of MC in fishes via the food chain could be a threat to human food safety. In a recent study, MC were found to be transferred mainly from contaminated aquatic animals to a chronically exposed human population (fishermen at a Lake Chaohu, China) together with indication of hepatocellular damage (Chen et al., 2009).

In aquatic environments, surface aggregations of some cyanobacteria may accumulate to form massive scum with high cell density and toxin concentrations. This phenomenon often occurs in shallow littoral areas, which are the primary environments for newly hatched fish larvae. As these larvae have limited ability for escaping exposure and their diet allow for the possible ingestion of toxin-containing cells, significant exposures could occur, leading to chronic effects or even death of the organisms (Sivonen & Jones, 1999; Chorus, 2001). Studies on the development of newly hatched fishes are therefore critical, contributing to the overall evaluation of the ecological effects of MC toxicity in aquatic systems. Zhang et al. (2008) showed that high MC concentrations retarded egg development (2–10 h delays) and larval growth, reduced hatching rate (up to 45%) and caused high malformation rate (up to 15%) and hepatocytes damage.

The purpose of the present study was to compare the effect of dietary intake of two M. aeruginosa bloom samples on growth performance and MC accumulation in larvae of the common carp Cyprinus carpio L. Cyprinids are especially important fish species in freshwater ecosystems because of their role as direct consumers of phytoplankton and zooplankton, their potential for biological management of cyanobacterial blooms and their value as food (Opuszynski & Shireman, 1995; Xie & Liu, 2001). These characteristics of cyprinids make them suitable to study the effect of natural variations of MC composition (type and number of MC variants) in the same toxic cyanobacterial strain. Indeed, the type of MC variants produced by a given strain is controlled by multienzymatic complexes (NRPS/PKS-I) involved in MC biosynthesis (Dittmann & Wiegand, 2006). These multienzymatic complexes are assembled into a modular structure, with each module responsible for the activation, thiolation, modification and condensation of one amino acid substrate (Arment & Carmichael, 1996; Kleinkauf & von Döhren, 1996; Marahiel et al., 1997). Peptides produced by this mechanism are small (two to 48 residues) with diverse structures and a broad spectrum of biological activities.

MATERIALS AND METHODS

BLOOM SAMPLING

The cyanobacterial bloom material was collected on 14 (A) and 28 (B) September 2005, from Lalla Takerkoust reservoir situated at 35 km south-west of Marrakesh (31° 36' N;
8° 2' W), with a 27 μm phytoplankton net. Samples were collected close to shore from the surface layer. The bloom-forming species was mainly *M. aeruginosa* identified by both microscopy and polymerase chain reaction (PCR) detection. The collected samples were freeze-dried and stored at −26°C until microcystins quantification by high performance liquid chromatography with photodiode-array detection (HPLC-PDA) analysis.

**MICROCYSTINS DETECTION AND QUANTIFICATION**

The toxin extraction and pre-purification were done according to Lawton *et al.* (1994). Briefly, 250 mg of lyophilized cyanobacterial cells were extracted three times with 70% methanol (50–75 mg dry cells ml\(^{-1}\) of methanol). For each extraction, the suspension was centrifuged at 4000 g (10 min, 4°C). Afterwards, the supernatant was retained and the pellet was further extracted. The three methanol extracts were diluted with Milli-Q ultra-pure water to a final methanol concentration of 20% (v:v). For the microcystin pre-purification, the final extract was passed through octadecyl silicagel ODS-C18 environmental (1 g) Sep-Pak cartridges (Waters, Chromatography Division, Millipore Corp.; www.waters.com). In this procedure, the ODS columns were previously activated with 20 ml of methanol (100%) and washed with 20 ml 20% methanol. Then the diluted methanolic extract was applied to the cartridges and washed with 10 ml of 20% methanol. The MC were finally eluted with 10 ml of 70% methanol. The last collected fraction containing the toxins was completely evaporated at 40°C and resuspended in 1 ml of methanol–Milli-Q ultra-pure water (50:50, v:v) and filtered through a GF/C glass filter before being subjected to HPLC.

Chromatographic analysis was performed by HPLC Waters equipment (model 2695; www.waters.com) with a photodiode-array detector (model 996). The column used was Chromolith C18 (250 mm × 4.6 mm, 5 μm; www.merck-chemicals.com). The mobile phase was: (A) water (H\(_2\)O) + 0.05% (v:v) trifluoroacetic acid (TFA) and (B) acetonitrile (MeCN) + 0.05% (v:v) TFA. During the HPLC running time of 55 min, the separation was achieved using the solvent gradient from 70 to 0% (A). The sample volume injected was 50 μl, and the mobile phase run at 1 ml min\(^{-1}\). The UV spectrum for each separated fraction was checked and the microcystins variants were preliminarily identified by their characteristic UV spectrum (maximum absorbency at 238 nm). Standard MC-LR, MC-YR and MC-RR were purchased from Calbiochem (Merck Chemicals). MC-FR and MC-WR were purified in the Laboratory of Plant Physiology of the Autonomous University of Madrid. Other MC different from the previous ones were quantified using MC-LR as a standard. The results are presented as MC-LR equivalent by adding the mass of all the variants found. MC-(H4)YR was identified by liquid chromatography–mass spectrometry. The LC-MS experiments were carried out on an Agilent 1100 series HPLC system (Agilent Technologies; www.chem.agilent.com), consisting of a vacuum degasser, a binary pump, an autosampler and diode array detector (DAD), coupled to a hybrid quadrupole time of flight (QTOF) instrument (QStar/Pulsar i; Applied Biosystems; www.appliedbiosystems.com) equipped with a turbospray ion source interface. The column used was Teknokroma, MED SEA18, and the mobile phase was a gradient of two eluents: (A) H\(_2\)O + 0.1% TFA and (B) MeCN + 0.1% TFA. The mobile phase ran at 1 ml min\(^{-1}\). The chromatogram obtained in HPLC-MS was identical to those obtained using HPLC-DAD.

Full scan mass spectrometry (MS) spectra were acquired in the positive ion mode, using a source potential of 5000 V, over the mass range of 50–1500 at 1 s. Electrospray ionization (ESI)–MS and collision induced dissociation (CID)–MS were measured using N\(_2\) as a collision gas (collision energy, 3 kV) in the pressure range of 65 bar. The N\(_2\) drying temperature was set at 300°C, and the cone voltage was fixed at 70 V. Mass signals of unknown compounds with sufficient intensities (>1000 counts in accumulated spectra) were analysed, and fragment patterns were compared with those from known or partly characterized MC. All chemicals were of chromatographic grade (Scharlau Chimie; www.scharlau.com).

**DIET PREPARATION**

A control diet (Table I) was prepared by mixing the solid powdered ingredients (previously ground to <100 μm) followed by the addition of the lipids emulsified in water, in order to
Table I. Composition and chemical analysis of cyanobacterial diets fed to *Cyprinus carpio* larvae

<table>
<thead>
<tr>
<th>Ingredients (g kg$^{-1}$)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilized beef liver</td>
<td>370·0</td>
</tr>
<tr>
<td>Brewers yeast</td>
<td>550·0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>10·0</td>
</tr>
<tr>
<td>Soybean lecithin (50%)</td>
<td>40·0</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>10·0</td>
</tr>
<tr>
<td>Choline chloride (50%)</td>
<td>10·0</td>
</tr>
<tr>
<td>Mineral mix†</td>
<td>10·0</td>
</tr>
<tr>
<td>Lyophilized cyanobacteria‡</td>
<td>0·1</td>
</tr>
</tbody>
</table>

Chemical analysis

- Dry matter (% $M_D$) 94·0
- Crude protein (% $M_D$) 51·1
- Crude lipid (% $M_D$) 6·1
- Ash (% $M_D$) 7·2
- Gross energy (kJ g$^{-1}$ $M_D$) 19·3
- Microcystins (ng g$^{-1}$ $M_D$)‡ 60·0

*Per kg of vitamin mix: retinol, 1 800 000 IU; calciferol, 200 000 IU; α-tocopherol, 3·5 g; ascorbic acid, 5 g; thiamin-HCl, 1·5 g; riboflavin, 2·5 g; Ca pantothenate, 5 g; nicotinic acid, 20 g; pyridoxine-HCl, 0·5 g; folic acid, 1 g; cyanocobalamin, 2 g; menadion sodium bis., 1 g; biotin, 0·15 g; inositol, 40 g.
†Per kg of mineral mix: cobalt sulphate–7H$_2$O, 0·04 g; copper sulphate–7H$_2$O, 0·5 g; iron carbonate, 4 g; potassium iodide, 0·06 g; magnesium oxide, 50 g; manganese oxide, 2 g; sodium selenite, 0·03 g.
‡Only for diets A and B.
$M_D$, dry mass.

form a moist blend. Diets A and B were obtained from this basal diet through the addition of 0·1 g kg$^{-1}$ of freeze-dried material of *M. aeruginosa* bloom A and B, respectively, including the same MC content (60 ng g$^{-1}$ dry matter). The blend was pelleted using a grinder and the resulting pellets were dried at 40°C. Dry pellets were crushed and sieved to obtain particles with graded diameter (100–200, 200–400 and 400–600 μm).

Chemical analyses of diets (Table I) were performed according to the following procedures: dry matter after drying in an oven at 105°C until constant mass; ash by incineration in a muffle furnace at 450°C for 16 h; protein (N × 6·25) by the Kjeldahl method after acid digestion; lipid by petroleum ether extraction in a Soxtec system (Tecator; www.tecator.se) apparatus; energy by direct combustion in an adiabatic bomb calorimeter.

**FISH AND EXPERIMENTAL SET UP**

First-feeding 7 days-old larvae [mean ± s.e. 2·36 ± 0·15 mg wet mass ($M_W$) and 7·64 ± 0·19 mm standard length ($L_S$)] of *C. carpio* were used in the present experiment. Larvae were from eggs obtained through induced spawning of a broodstock with pituitary extract and incubated at 25°C. At the beginning of the experiment, larvae were randomly assigned to nine rearing units filled with dechlorinated tap water. Each rearing unit consisted of a set of two plastic tanks, an internal 5 l tank with lateral screened windows (mesh size of 500 μm), inside of which larvae (100 per tank) were placed, and an external 8 l tank, as described by Charlon & Bergot (1984). The water temperature was kept at mean ± s.e. 25·3 ± 1·2°C and the photoperiod at 16 L (PAR of $1·9 \times 10^{-6}$ μmol m$^{-2}$ s$^{-1}$);8D throughout the experimental period. Every 24 h, larvae from each rearing unit were transferred (as in Charlon & Bergot, 1984) to a clean unit with renewed water, to avoid the accumulation of excreted ammonia and the formation of a bacterial film at the bottom, as well as to ensure acceptable levels of oxygen (≥5·6 mg l$^{-1}$) and pH (7–8). The experiment lasted 12 days.
Diets (control diet, diet A and diet B) were tested in triplicate. Larvae were fed by hand twice daily (0900 and 2000 hours) at a ratio of 1 mg diet larva$^{-1}$ day$^{-1}$, corresponding to 0.06 ng MC day$^{-1}$ or a total of 0.72 ng MC in the 12 days period per larva. All the diets given to fish were consumed in c. 2 h.

For MC analysis in larvae, 10 larvae were randomly sampled from each tank on days 3 and 12, starved for 1 day and frozen at $-26^\circ$C until MC extraction. Observations on fish development were made under a Zeiss Stemi DV4 stereomicroscope (www.zeiss.com). Photographs were taken using a Canon PowerShot A620 digital camera (www.canon.com) and analysed using Adobe Photoshop CS3 (www.adobe.com). Larval standard length ($L_S$) was measured from these images, using the UTHSCSA Image-Tool v3.00 programme (developed at the University of Texas Health Science Center at San Antonio; www.utsi.utexas.edu). Each day, 10 larvae were used after anaesthesia for measurement of $L_S$ and $M_W$. Fish were handled in accordance with E.U. regulations concerning the protection of experimental animals.

**DETERMINATION OF TOTAL MC CONTENT IN FISH TISSUE BY ELISA**

For MC analysis in fish tissue, larvae were anaesthetized, euthanized, weighed and measured before freezing. MC extraction procedure was performed as described by Smith & Haney (2006) with minor modification. Fish tissue was homogenized with an Ultraturrax homogenizer (www.ika.net) for 5 min. The suspension was then sonicated with an ultrasonic processor for 2 min at c. 80 amplitude (Sonics Materials, Vibra Cell 50; www.sonic.com). Samples were extracted for 24 h in 80% methanol at 4$^\circ$C. Extracted samples were then clarified by centrifugation at 3000 g for 10 min and filtered through a 0.2 µm filter (Acrodisc, Polyethersulphone, VWR International; www.vwr.com). Fish extract was evaporated to dryness and resuspended in ultra-pure water (Milli-Qs, Millipore; www.millipore.com). Enzyme-linked immunosorbent serologic assay (ELISA) was performed as described by the Envirogard Microcystins Plate Kit (Strategic Diagnostic; www.sdix.com). The absorbency was determined in a DENARIO We-Scan ELISA reader at a wavelength of 450 nm. Results are reported as MC-LR equivalents.

**CALCULATIONS AND STATISTICS**

Means, s.d. and s.e. for all experimental variables were calculated using the Microsoft Excel 2007. Statistical analysis of data was performed by one-way ANOVA at a probability level of 0.05 and means were compared by the Tukey test using the SPSS 11.5 software (www.spss.com).

**RESULTS**

Despite comparable content of MC in both *M. aeruginosa* blooms [968 µg g$^{-1}$ dry mass ($M_D$) in bloom A and 976 µg g$^{-1}$ $M_D$ in bloom B], differences were found regarding the type, number and percentage of MC variants, as revealed by HPLC-PDA analysis (Fig. 2 and Table II). MC-LR clearly dominated in bloom A (74-05%), while no dominance of a specific MC was detected in bloom B. MC-(H4)YR was identified by liquid chromatography–mass spectrometry (LC-MS) analysis (Fig. 3).

No fish died during the 12 days of the experiment and no differences in fish behaviour could be distinguished among the treatments and the control. Fish continued to consume diets A and B at the same rate throughout the experimental period. Significant differences in larval growth were observed among all groups. From day 9, $M_W$ and $L_S$ in fish fed toxins-containing diets were significantly lower than in fish fed the control diet (Figs 4 and 5), which was reflected in the specific growth
Fig. 2. High performance liquid chromatography with photodiode-array detection chromatogram showing the microcystins variants (see Fig. 1) and their percentages in the freeze-dried material of *Microcystis aeruginosa* natural bloom (a) A and (b) B. Microcystins variants were determined according to the available standard samples.
Table II. Amount of total microcystin (MC) and MC profile (see Fig. 1) in the two bloom samples (A and B) collected in Lalla Takerskoust reservoir used in the assay.

<table>
<thead>
<tr>
<th>Bloom</th>
<th>Total MC μg g⁻¹ dry mass</th>
<th>MC-LR (%)</th>
<th>DMC-LR (%)</th>
<th>MC-FR (%)</th>
<th>MC-LY (%)</th>
<th>MC-RR (%)</th>
<th>MC-YR (%)</th>
<th>MC-WR (%)</th>
<th>MC-(H4)YR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>976</td>
<td>74·05</td>
<td>4·22</td>
<td>10·02</td>
<td>8·59</td>
<td></td>
<td></td>
<td></td>
<td>4·45</td>
</tr>
<tr>
<td>B</td>
<td>968</td>
<td>33·26</td>
<td></td>
<td></td>
<td></td>
<td>25·62</td>
<td>19·83</td>
<td>3·74</td>
<td>14·55</td>
</tr>
</tbody>
</table>

rate ($G$) (Table III). Moreover, between toxin fed groups, group fed diet B was significantly more affected than that fed diet A (Figs 4 and 5 and Table III).

The MC accumulation by fish fed diets A and B appeared to increase with experimental time, and was always significantly higher in fish fed diet B (Fig. 6). After 3 days of feeding, the MC content was 10·87 ng g⁻¹ and 16·21 ng g⁻¹ in groups A and B, respectively. In both groups, MC were rapidly accumulated in fish tissues after 12 days of continuous supply of toxins-containing diet (Fig. 6), but at the end the accumulation became much more important in fish nourished with diet B (26·96 ng g⁻¹) than with diet A (17·32 ng g⁻¹).

DISCUSSION

The intake of low and repeated doses of MC from cyanobacterial cells can inhibit growth, which may be due to toxic effects on *C. carpio* larvae. There are several studies describing the oral toxicity of MC in fishes after feeding them toxic algae (Fischer & Dietrich, 2000a; Li et al., 2004; Zhao et al., 2005), generally the toxic effect being attributed to the main MC congener in cyanobacterial cells. These cells are thought to be lysed in the fish gut after ingestion, and digestion releases intercellular MC into the intestinal lumen of fishes (Falconer, 1993). Following intestinal absorption, the toxin is taken up into hepatocytes via a carrier-mediated transport system, and then inhibits the activity of serine and threonine protein phosphatases 1 and 2A (Runnegar et al., 1993). This inhibition could disturb the cellular phosphorylation balance and cause hyperphosphorylation of a variety of functional proteins, which leads to apoptosis and necrosis of hepatocytes (Tencalla et al., 1994; Carbis et al., 1996; Dawson, 1998; Fischer et al., 2000).

The analysis performed in this work revealed an important difference in MC congeners present in two different *M. aeruginosa* blooms, with almost the same total MC content. The variation in MC composition between the two blooms seems to affect significantly the uptake by *C. carpio*, and hence MC toxicity. This might be due to the molecular affinity of the organic ion transporters responsible for carrying MC across cell membranes, which would differ for each type of MC. Dietrich & Hoeger (2005) agree, on the basis of experiments by Meriluoto et al. (1990) using different epimers of ³H-dihydro-MC-LR, that minimal structural changes in the MC molecule can have major implications for the uptake, organ distribution and excretion kinetics of these toxins. In this study, evidence for different uptake of total MC based on their different accumulation in tissues of *C. carpio* fed bloom materials with diverse MC composition was found. It could be that more hydrophilic variants are better taken...
Fig. 3. Full scan mass spectra of MC-(H4)YR (see Fig. 1).

up and produce more drastic effects than more lipophylic ones. This is not expected since MC-LR is more toxic to mammals than the more hydrophilic MC-RR. Fishes do not respond the same way as mammals. Xie et al. (2004) showed that when silver carp Hypophthalmichthys molitrix (Valenciennes) fingerlings were exposed to a bloom containing MC-LR and MC-RR, no MC-LR was detectable in the muscle and blood samples of the fish in spite of the abundant presence of this toxin in the
intestines. On the other hand, the maximum MC-RR in the blood, liver and muscle of the fish was 49.7, 17.8 and 1.77 mg g⁻¹ M₉, respectively. They concluded that *H. molitrix* has a mechanism to degrade MC-LR actively and to inhibit MC-LR transportation across the intestines and that the depuration of MC-RR concentrations occurred slowly than uptakes in blood, liver and muscle, and the depuration rate was in the order of blood liver muscle (Xie *et al.*, 2004).

It is important to highlight that MC detected in fish tissues only refer to free toxins, since it was not possible to detect MC bound to protein phosphatases or glutathione using the standard MeOH extraction applied in this work (Amorim & Vasconcelos, 1999; Thostrup & Christoffersen, 1999; Zimba *et al.*, 2001; Magalhães *et al.*, 2003). A large number of MC congeners, those that contain methyldehydroalanine, form covalent bonds to protein phosphatases 1 and 2A in animal cells, and these covalently bound MC are not extracted using standard MeOH extraction (Williams *et al.*, 1997a, b; Pires *et al.*, 2004). Consequently, total MC concentration detected in fishes was underestimated. Williams *et al.* (1997a, b) reported that only c. 13% of the total MC load in fish liver was extractable via MeOH method, 24 h after exposure. The remaining toxin was thought to be irreversibly, covalently bound to protein phosphatases 1 and 2A, inhibiting protein phosphatases activity. In this study, fish from group A accumulated 36.81% of the administered toxin after 12 days of feeding, this rate increasing to 50.15% for fish from group B, indicating that MC structural changes may affect toxin transfer and uptake. Yet, this remains an important point to be confirmed in further experimental studies.
**Table III.** Mean ± s.e. initial ($M_{WI}$) and final wet mass ($M_{WF}$) and specific growth rate ($G^*$) of *Cyprinus carpio* larvae fed the experimental diets control A and B

<table>
<thead>
<tr>
<th>Diet</th>
<th>$M_{WI}$ (mg)</th>
<th>$M_{WF}$ (mg)</th>
<th>$G^*$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>2.44 ± 0.07^a</td>
<td>18.42 ± 0.39^a</td>
<td>17.77 ± 0.25^a</td>
</tr>
<tr>
<td>Diet A</td>
<td>2.21 ± 0.16^a</td>
<td>15.33 ± 0.40^b</td>
<td>15.94 ± 0.48^b</td>
</tr>
<tr>
<td>Diet B</td>
<td>2.42 ± 0.07^a</td>
<td>13.39 ± 0.26^c</td>
<td>14.25 ± 0.26^c</td>
</tr>
</tbody>
</table>

$G = 100(ln M_{WF} - ln M_{WI})t^{-1}$, where $t =$ time (days).

Means in the same column sharing different superscript lowercase letters are statistically different ($P < 0.05$).
toxicants and less important for hydrophilic compounds (De Maagd et al., 1999). Vesterkvist & Meriluoto (2003) also demonstrated that certain MC congeners may be more lipophilic than the hydrophilic MC-LR. These MC variants are believed to be more cell-permeable than the more hydrophilic MC (Kuiper-Goodman et al., 1999) and may be less dependent on the bile acid transporter system to pass a cell membrane (Sivonen & Jones, 1999). Additional research should be focused on these congener specificities, for a better understanding of MC uptake kinetics. This understanding may be crucial for proving risk assessment from MC exposure in natural conditions.

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References


