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Occurrence of microcystins in water, bloom, sediment and fish from a public water supply

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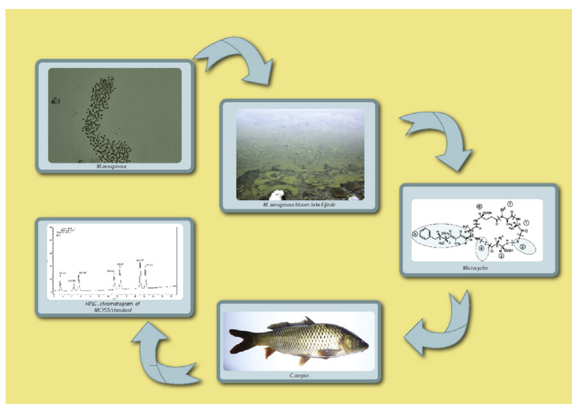
HIGHLIGHTS

- Microcystin accumulation was determined in liver and muscle tissues of fish species.
- MC–YR, were detected at high concentrations in both liver and muscle tissues.
- MC–YR were also most identified variants in the water, sediment and bloom samples.
- In some cases, the HPLC results of edible tissues showed MC exceeded the TDI
- The tested samples from the study area, containing MCs may pose a human health risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Microcystin (MC) accumulation was determined in the liver and muscle of two omnivorous fish species which are consumed and are economically important, and in a planktivorous–carnivorous fish from Lake Eğirdir, Turkey. Free extractable MCs in fish tissue samples were detected by enzyme-linked immunosorbent assay (ELISA) with confirmation by high performance liquid chromatography with photodiode array detection (HPLC-PDA). MC-LA and -YR, were detected in both liver and muscle, followed by MCs -LY, -LF, -RR and -LR respectively. The MC concentrations varied between 0.043 and 1.72 μg/g dry weight in liver and muscle tissues. MCs were also determined in samples of water, sediment and a bloom sample of *Microcystis aeruginosa* from the lake by HPLC-PDA. MC-LY and -YR were most commonly identified in water samples, with total MC concentrations ranging from 2.9 ± 0.05 to 13.5 ± 2.3 μg/L. Sediment analyses, showed that MC-YR was present in samples between 7.0 and 17.6 μg/g dw, especially in October, November and December when no MC-YR was recorded in water, followed by MC-LW. The findings indicate that water and sediment contained MCs, and more importantly that fish were contaminated with MCs that may pose an MC-associated human health risk.

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1. Introduction

The appearance of toxic cyanobacterial blooms in eutrophic lakes, reservoirs and recreational waters is a common worldwide problem (Codd et al., 2005). Among the cyanobacterial toxins (cyanotoxins) which are commonly produced, microcystins (MCs), with their hepatotoxic and tumour-promoting activities, are considered to be one of the most hazardous groups (Chorus and Bartram, 1999). MCs are a large group of cyclic heptapeptides, with more than 100 structural variants, produced by numerous species of freshwater cyanobacteria. Toxic populations of e.g. *Microcystis*, *Planktothrix*, *Anabaena* and *Aphanizomenon* have been reported worldwide (Sivonen and Jones, 1999) and blooms have been associated with human and animal intoxications (Codd, 2000; Metcalf and Codd, 2012). It has been shown that cyanobacteria, including MC-producing genera, are ingested by aquatic consumers (Mohamed et al., 2003; Ou et al., 2005). MCs may enter aquatic consumers by direct feeding of cyanotoxin-containing cyanobacterial cells, or indirectly via food web-mediated intake of cyanotoxin-containing food (Rohrlack et al., 2003, 2005). A variety of aquatic organisms including molluscs, shrimp and fish have been shown to accumulate MCs in their tissues (Panosso et al., 2003; Ou et al., 2005; Smith et al., 2008).

Provided that MC concentrations in diets are sub-lethal, the extent of their accumulation in consuming organisms will depend on a variety of factors, including the MC profile, concentration in the diet, assimilation rates, and consumer detoxification and elimination processes. Fast removal of MCs from tissues has been tracked in many fish species, including common carp, *Cyprinus carpio* and silver carp, *Hypophthalmichthys molitrix* (Adamovsky et al., 2007). Physiological status, including temperature, availability of alternative food and length of exposure (Soares et al., 2004; Adamovsky et al., 2007; Deblois et al., 2008) may also modulate the concentrations and effects of MCs in aquatic organisms.

Extensive laboratory experiments and field investigations have been conducted on the bioaccumulation and distribution of MCs in a variety of aquatic organisms (Xie et al., 2004; Chen and Xie, 2005a,b; Zhang et al., 2007). However, studies on the transfer and accumulation of MCs in foodwebs are scarce (Ibelings et al., 2005; Xie et al., 2005). Such reports indicate that MCs can be transferred through food chains (Ibelings et al., 2005; Smith and Haney, 2006), and suggest a potential risk to higher trophic level species, including humans, through the consumption of contaminated aquatic products. The tolerable daily intake (TDI) for humans, as determined by the World Health Organization, assuming life-time exposure, is 0.04 μg of MC-LR/kg body weight/day (Sivonen and Jones, 1999).

Many studies have indicated that cyanotoxins bioaccumulate in aquatic biota, (e.g. Sipia et al., 2001; Li et al., 2005) and that this may enhance the risk of exposure to biota higher up the food web, including humans, even though only small amounts of the cyanotoxins may be produced in the phytoplankton. Multiple possible exposure routes for MCs exist (Codd et al., 2005), including uptake of dissolved MCs via the epithelium and via the food web (Shen et al., 2005; Magalhaes et al., 2003; Cazenave et al., 2005, Zhang et al., 2009). Miller et al. (2010) also showed that exposure to MC may occur directly from the dissolved pool and hence bioconcentration is also possible and may be significant at times.

Due to potential health risks, studies have concentrated on investigating MC concentrations in fish tissues and organs and have shown that the toxins accumulate mainly in fish liver (Xie et al., 2005, 2007; Malbrouck et al., 2003; Mohamed et al., 2003), but also in other organs such as kidney (Williams et al., 1995; Mohamed et al., 2003), intestine (Mohamed et al., 2003; Xie et al., 2004, 2005) and, most importantly, edible muscle (Adamovsky et al., 2007; Schmidt et al., 2013).

Lake Eğirdir, is one of the most important fresh water lakes in Turkey. Furthermore, it has great importance for the region because it supplies potable water to the towns of Eğirdir and Isparta. The lake also has many other uses such as irrigation, fishing, and tourism (Gurbuz et al., 2009).

Blooms of *M. aeruginosa* have been observed in Lake Eğirdir over the past 7 years (Gurbuz et al., 2010). During the present investigations, the cyanobacterial bloom drifted downwind and accumulated along the shores, including the beach section and the shoreline of the centre of the town of Eğirdir. The major objectives of this study were to: (a) determine the extractable MC concentrations in the liver/hepatopancreas and edible muscles of two omnivorous fish (*C. carpio*, *Carasius gibelio*) which are consumed and are economically important in the local communities, and in the muscle tissue of a planktivorous-carnivorous fish (*Atherina boyeri*); (b) evaluate the potential threat of the contaminated fish to human health, in comparison with the distribution of MCs in lake compartments (water, sediment) and in bloom samples of *M. aeruginosa*.

2. Materials and methods

2.1. Study area

Lake Eğirdir is located within the Lake District in south west Turkey and is the second largest freshwater lake in Turkey with an area of 482 km^2. The length of the lake from North to South is 50 km; the length of the coastline is 150 km. The lake has a maximum depth of 13 to 14 m with an average depth between 8 and 9 m. During the past 10 years, with rapid economic development and agricultural pollution, the lake has undergone a steady increase in eutrophication, with regular occurrences of cyanobacterial surface blooms in the summer each year. Sample collections were made at locations (Fig. 1) where blooms of *M. aeruginosa* occurred.

2.2. Sample collection and preparation

2.2.1. Collection of fish, sediment, water and bloom samples

C. carpio is a widespread species and is economically important as a popular food source with specimens over 3 kg being especially favoured. *C. gibelio*, on the other hand is only consumed by the local population around Lake Eğirdir. However, both fish species are important due to their being a product of inland fisheries and aquaculture. Hence a health hazard may occur for humans eating these fish as a major part of their diet. *A. boyeri*, makes up 15% of the 44,082 tons of annual freshwater fish production in Turkey (TFS, 2007) and this species represents an important potential source for domestic fish meal production and is a rich source of animal protein (>70% of dry matter). Almost all the caught *A. boyeri* is used in fish farms as fresh feed (Gümüş et al., 2010). Furthermore, farmed fish fed with *A. boyeri* may also be exposed to microcystin.

Two omnivorous fish (*C. carpio*, *C. gibelio*) and the planktivorous–carnivorous fish *A. boyeri* were collected monthly. From five individuals of each of the three fish species, the liver (hepatopancreas for *C. carpio*) and muscle tissue were removed, lyophilised for 72 h and then individually homogenized with a mortar and pestle. Fish tissues, 200 mg to 1 g dry weight (dw) of liver or 1 to 3 g (dw) of muscle, were extracted with 80% (v:v) methanol in water (MeOH:H_2O) (Smith and Boyer, 2009).

Sediment samples were collected by sediment trap along with surface sediments for analysis. Samples were individually homogenized after lyophilisation. Equal known amounts (3 g dw of each sample) were extracted with 80% (v:v) MeOH:H_2O (Smith and Boyer, 2009).

Water samples were collected for MC analyses at a depth of 50 cm below the water surface by directly immersing sample containers in water. Five hundred ml water samples were filtered through a GF/C filter (Whatman) and the eluate concentrated by C18 SPE cartridge (Bond Elute Varian) according to Lawton et al. (1994).

Cyanobacterial bloom samples were collected in bottles from the water surface and examined by light microscopy for identification. Morphological identification was done using the criteria proposed by Komárek and Anagnostidis (1999). The samples were filtered through