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Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey

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ABSTRACT

Dissolved (extracellular) microcystin (MC) concentrations were determined at 3 sampling stations on Lake Kovada, Turkey. The dominant species of cyanobacteria found in August and September of 2006 were *Microcystis aeruginosa*, *Synechococcus* sp., *Phormidium limosum, Phormidium formosa and Planktothrix limnetica.* MC concentrations in water were measured by ELISA and MC variants were examined by HPLC-PDA. Quantitative analysis by HPLC indicated that five MC variants (MC–LR, –RR, –LA, –LW, –LF) were identified in water samples from Kovada Lake. The maximum concentration of dissolved MC–LW was 98.9 µg l^{-1} in October. MC–LR was only detected in May at a concentration of 0.5 µg l^{-1}. The cross reactivity of the antibody (MC10E7) to variants such as MC–LA MC–LW & MC–LF was low. Hence the results determined by ELISA were lower than those determined by HPLC in September and October samples due to differences in the specificity of the antibody to MC variants. Total extraceullar MCs was quantified by ELISA and ranged from 0.73 to 48.5 µg MC–LR equivalents l^{-1}, which in some cases exceeded the WHO provisional Guideline Value for MC–LR in drinking water. This study confirms that the lakes of Turkey should be monitored for toxic cyanobacteria and for MCs to avoid or reduce the potential exposure of people to these health hazards.

1. Introduction

The frequent dominance of eutrophic freshwaters by cyanobacteria (blue-green algae) is of increasing concern because these organisms can produce several types of potent toxins (cyanotoxins), among which the most commonly detected are the microcystins (MC) (Sivonen, 1996; Sivonen and Jones 1999). MCs are produced and mainly retained within the intact cyanobacterial cells in which they are produced and are principally released into the surrounding water when cells die and lyse (Lawton and Robertson Peter, 1999). Intracellular MC can enter a waterbody after cell lysis which may occur due to bacterial or viral (cyanophage) attack. Cyanobacterial cell lysis by microbial pathogens (cyanophages, fungi and heterotrophic bacteria) has long been recognised (see Fogg et al., 1973) and these naturally-occurring agents can be major causes of cyanobacterial bloom collapse (Suttle, 1990; Gons et al., 2002; Zwart et al., 2005).

MCs can be produced by species of *Microcystis*, *Anabaena*, *Nostoc*, *Planktothrix* and several other cyanobacterial genera (Sivonen and Jones, 1999; Codd et al., 2005). There is a risk that blooms of these cyanobacteria contaminate water supplies that are used for recreation, fisheries, agriculture, livestock-watering and for abstraction for drinking water Currently over 80 structural variants of MC are known and these differ widely in their toxicities (Meriluoto and Codd, 2005; Falconer, 2005a,b). The most commonly found MCs include the leucine–arginine (MC–LR), arginine–arginine (MC–RR), tyrosine–arginine (MC–YR) and leucine–alanine (MC–LA) variants (Mhadhbi et al., 2006). MCs are potent hepatotoxins and inhibitors of several serine/threonine protein phosphatases, leading to the disruption of normal cell metabolism and function (Mackintosh et al., 1990; Eriksson et al., 1990; Hastie et al., 2005).

Toxic cyanobacterial blooms have been described in numerous countries (e.g. Frank, 2002; Ballott et al., 2005; Codd et al., 2005; Albay et al., 2005) and several cases of animal and human intoxication have been reported (Falconer, 1996; Chorus et al., 2000; Codd, 2000; Hitzfeld et al., 2000). The most severe human health episode known to be attributed to direct exposure to MCs was that of the deaths of haemodialysis patients in Brazil in 1996 (Jochimsen et al., 1998; Pouria et al., 1998). The increased incidence of primary liver cancer in areas of China has also been correlated with the presence of MCs in surface drinking water (Yu, 1995; Ueno et al., 1996). Many countries in Europe have experienced problems with cyanobacterial contamination in drinking water reservoirs (Wiedner et al., 2001; Westrick, 2003; Hoeger et al., 2005). Guidelines for cyanotoxins in water exist in several countries worldwide (WHO, 1998). The World Health Organization (WHO) has proposed a provisional Guideline Value of 1 µg l^{-1} MC–LR in drinking water. Additional guidelines are necessary to prevent health impairments from cyanobacteria in recreational waters and a single guideline value is not sufficient to apply to all water-uses and potential cyanotoxin exposure routes. Rather a series of guideline values associated with incremental severity and probability of health effects is defined at three levels (Falconer et al., 1999).

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These can be designed to reduce the risk of potential adverse health outcomes (Meriluoto and Codd 2005).

In the European Union (2000/60EC), which characterises high-priority water pollutants, toxin-producing cyanobacteria have been specifically identified as potential key hazardous pollutants (Funari et al., 2000; Hitzfeld et al., 2000).

To date, several screening and analytical methods have been developed for cyanotoxin detection and quantification. These methods should ideally be able to detect MC concentrations below the WHO Guideline Value for MC–LR. Enzyme-linked immunosorbent assays (ELISAs), developed for MC's, are highly specific, sensitive, quick to perform, inexpensive, and require minimum sample processing (Metcalf and Codd, 2003; Hawkins et al., 2006). For the trace analysis of MCs in water, various ELISA systems using either polyclonal (Metcalf et al., 2000; Young et al., 2006) or monoclonal (Nagata et al., 1995) antibodies have been developed. As detection by ELISA is based on analyte structure (not on toxicity), immunoassays may indicate false positives from a toxicological point of view as the toxicity of individual microcystin variants can differ by many orders of magnitude. Confirmatory techniques including high performance liquid chromatography (HPLC) can be used which allow the identification and quantification of individual MC variants. However these physico-chemical methods require expensive equipment, relatively long procedures and analysis times, analytical standards (not always commercially available) and trained personnel (Lawton et al., 1994; Tsuji et al., 1994). Nevertheless, ELISAs as screening methods are very useful for a first examination and quick cyanotoxin detection (Metcalf and Codd, 2003; Campás and Marty 2007). Ideally, screening plus confirmatory methods should be carried out.

The objectives of the present study were to detect MCs present in water by ELISA and identify MC variants by HPLC and characterize cyanobacterial biota from a heavily-used waterbody, with a history of cyanobacterial bloom production: Kovada Lake, in Southern Turkey.

2. Materials and methods

2.1. Study site

Lake Kovada is situated in a Turkish National Park, in Southern Anatolia (Fig. 1). It is a small lake, 9 km wide and 20.6 km long with a depth of 6–7 m. The mean summer total phosphorus concentration is 0.90 mg l^{-1} (data not shown). Surface water is generally utilized and abstracted simultaneously for several purposes including drinking, garden watering, fisheries, recreational purposes and for generating electricity. Recently, studies showed that water quality is adversely affected by the diffuse run-off of agricultural fertilizers and pest-control chemicals into the lake, triggering growth of cyanobacteria. Physicochemical characteristics of the lake are summarized in Gurbuz (2008).

2.2. Sampling and measurements

Samples were collected from three locations in Kovada Lake. The sites were chosen on the basis of wind direction, wastewater discharge areas and the point of entry of a canal to the Lake. K1 is in an inlet, K2 is at the entry of the canal which brings excess water from Lake Egirdir, and K3 is on the south shore (Fig. 1). Sampling

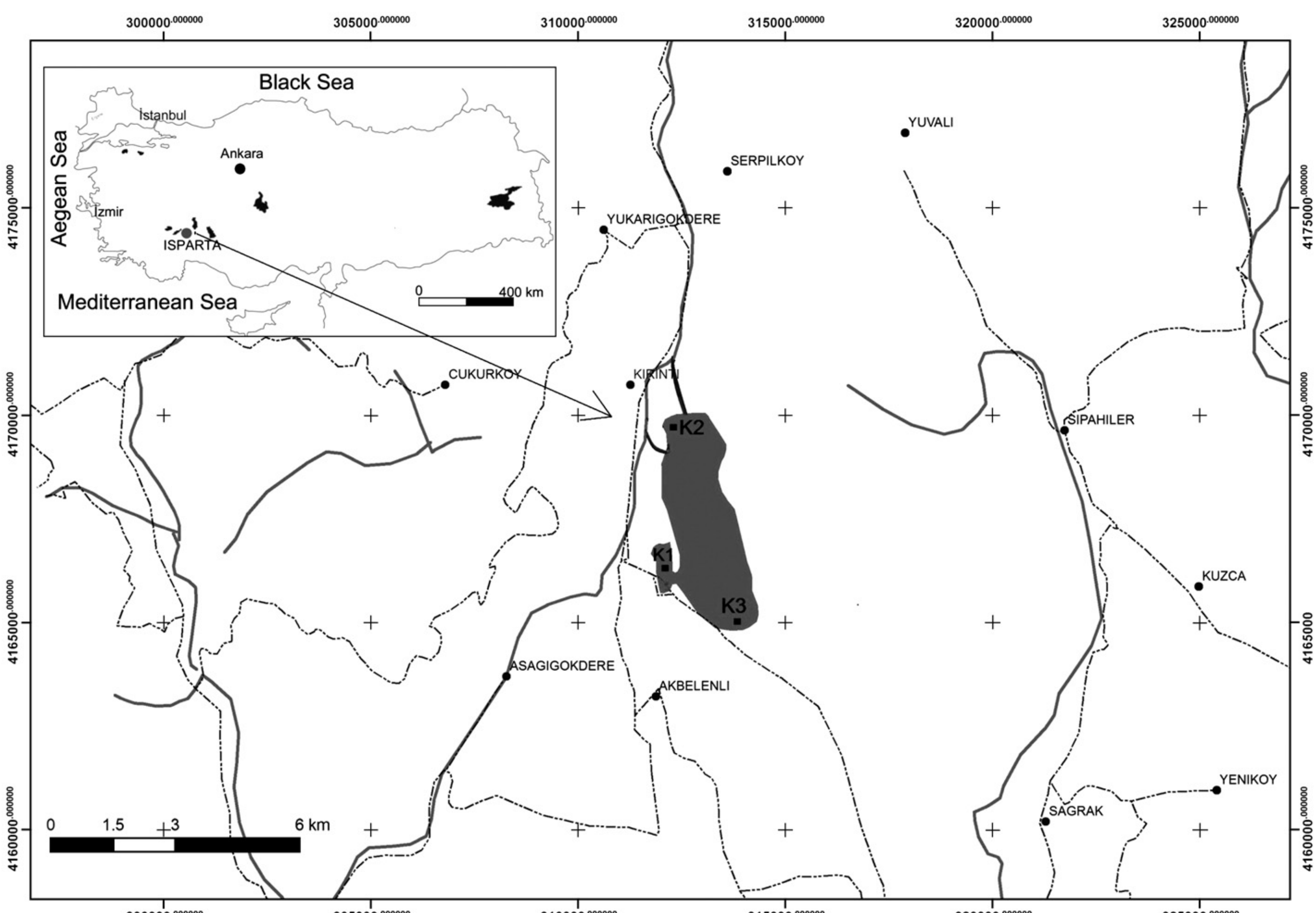

Fig. 1. Kovada Lake and sampling locations K1 to K3.