International Journal of Hygiene and Environmental Health 219 (2016) 288–293

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Improving microcystin monitoring relevance in recreative waters: A regional case-study (Brittany, Western France, Europe)

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ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form
16 December 2015
Accepted 17 December 2015

Keywords:
Cyanobacteria
Microcystin
Monitoring efficiency
Exposure
Public health survey

ABSTRACT

Cyanobacteria and their toxins are known as a health hazard in recreative and distributed waters. Monitoring data from 2004 to 2011 were collected at regional scale to characterize exposition parameters to microcystins in Brittany (Western France). The data show that cyanobacteria populations are experiencing a composition shift leading to a longer duration of cell densities higher than WHO alert levels 2 and 3. Microcystins however appear to be more frequently detected with subacute concentrations in low cell density samples than in high cell density samples or during bloom episodes. Positive relations are described between microcystin concentrations, detection frequencies and cyanobacteria biovolumes, allowing for a novel definition of alert levels and decision framework following WHO recommendations.

Introduction

Toxic cyanobacteria are known as a potential health hazard since the 1990s, and three major exposure routes are currently recognized: (i) through dermal contact and accidental inhalation/ingestion during recreational activities in raw waters (Osborne et al., 2007; Stewart et al., 2006; Backer et al., 2008, 2010); (ii) through ingestion of drinking water produced from a contaminated resource (Byth, 1980; Griffiths and Saker, 2003; Merel et al., 2013) and (iii) through the ingestion of cyanobacteria-based food ingredients, either directly as dietary compliments (Gilroy et al., 2000; Heussner et al., 2012; Rellán et al., 2009; Vichi et al., 2012) or indirectly via contaminated seafood, fishes or shellfishes (Ibelings and Chorus, 2007). Low-level chronic exposure through these routes is currently suspected of the highest human health impact (Spoof, 2005; Li et al., 2011; Weirich and Miller, 2014).

Water exposition routes are the most documented since the publication of WHO provisional guidelines for recreative and treated waters (Chorus and Bartram, 1999). Recreational water surveys involve a tiered management framework based on biomass proxies (chlorophyll a, cyanobacteria cell density), and the proposed decision scheme has been implemented by most countries where cyanobacteria are monitored (Ibelings et al., 2014). To summarize, this decision framework requires cyanobacteria to be quantified, and cell densities to be compared to Alert Level values: Alert Level 2 (20,000 cell/ml) implies water users information, whereas above Alert Level 3 (100,000 cell/ml) microcystin occurrence is likely and microcystin analysis is recommended. Microcystin concentrations are then compared with guideline values calculated according to a daily No Observed Adverse Effect Level (NOAEL) intake of 40 μg/kg bodyweight (Falconer et al., 1994; Codd et al., 2005). WHO guideline for recreational activity suspension is 20 μg/l microcystin LR, whereas French regulation, based on different intake hypotheses, adopted a chronic exposure guideline of 13 μg/l and an acute exposure guideline of 80 μg/l since 2013.

While proliferation episodes are widely documented and subjected to research work, chronic exposure, subacute toxin concentration and low-biomass effects are on the other hand less often considered. They can however be seen as a health hazard for particular populations such as children, pregnant women, asthmatic or atopic people (Li et al., 2011; Weirich and Miller, 2014). Cyanobacterial toxins are for example known to be associated with carcinogenesis (Zegura et al., 2011), cytotoxicity (Young et al., 2008) and have been shown to act as endocrine disruptors

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http://dx.doi.org/10.1016/j.ijheh.2015.12.004
1438-4639/

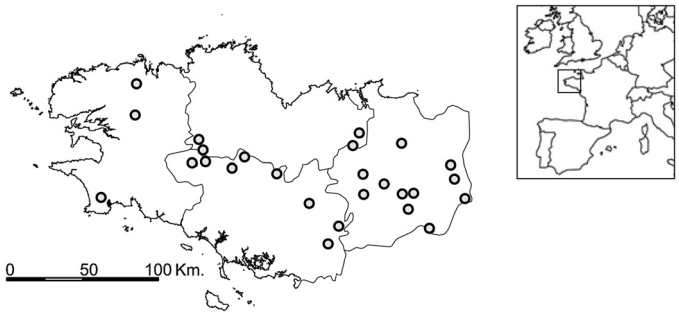

Fig. 1. Spatial distribution of the 26 investigated sites in Brittany (France).

(Sychrová et al., 2012) or allergenic/pyrogenic agents, either as aerosols (Kirkpatrick et al., 2011; Cheng et al., 2007) or through direct contact with raw waters (Pilotto et al., 2004; Kirkpatrick et al., 2010, 2011; Ohkouchi et al., 2015).

Public health surveys of recreational inland waters in Britany (north western France) are held since 2003, and monitoring data have shown subacute toxin concentrations to be the most frequently encountered. In this context the present exploitation of water quality data from 26 recreational lakes collected between 2004 and 2011 was aimed at answering two questions: first, does the current monitoring framework derived from the 1999 WHO recommendations accurately account for microcystin exposure in recreational waters? Secondly, if this is not the case how can it be improved in terms of exposure representation and cost efficiency?

Materials and methods

Our study is based on public health weekly survey data from 26 lakes used as recreational areas or water resources monitored every year from 2004 to 2011 in Brittany (north-western France, Fig. 1). These sites were selected according to cyanobacteria survey continuity criteria. Only 26 sites out of a total of 40 could be considered as continuously monitored from June to September since 2004.

Cyanobacteria data, i.e. cell densities and microcystin concentrations, were collected from the regional public health authorities (French Agence Régionale de Santé of Brittany). All samples were analyzed by four different local laboratories for cyanobacteria composition and microcystin concentration.

Cyanobacteria were identified and counted with light microscope according to Utermöhl (1958). All monitoring data were first examined as cell densities, to comply with WHO alert level thresholds (Chorus and Bartram, 1999), and then converted to cell biovolumes using mean cell dimensions obtained from cyanobacteria reference works (Geitler, 1932; Komarek and Anagnostidis, 1999; Komarek and Anagnostidis, 2005; Komarek, 2013) and relevant geometric formulas (US Environmental Protection Agency, 2010). This conversion was meant to account for differences in biomass contribution induced by larger taxa associated with lower cell densities (e.g. Microcystis, Woronichinia, etc.) vs. smaller taxa associated with larger cell densities (e.g. Aphanocapsa, Aphanothece, etc.).

All toxin analyses were conducted on the same samples used for species composition analysis. Toxin analyses were systematically performed on all samples reaching WHO alert level 3, but could be performed on lower cell density (e.g. WHO alert level 2). Sample preparation involved a preliminary cyanobacteria cell lysis step with methanol (Lawrence et al., 2001) to account for total (dissolved and intra-cell) toxin concentrations. Depending on the laboratories, Microcystins (MCs) were analyzed either with LC/MS, HPLC or ELISA immunoassays. These methods quantification limits ranged from 0.05 to 0.2 μg/l. All laboratory results were harmonized to a common quantification limit, i.e. 0.2 μg/l.

From 2004 to 2011 in Brittany, cyanobacteria were analyzed in 3 278 water samples; MCs analyses were conducted on a total of 719 samples (i.e. 23% of all samples) and MCs were detected in 159 samples (i.e. 22% of all samples analyzed for MCs).

Frequency distribution analyses were carried out on grouped data with classes of 1, 2 and 5.10^i with $i = -1; 0; 1; 2$ for MCs concentrations, $i = 4; 5; 6$ for cell densities, and $i = 5; 6; 7$ for cell biovolumes.

The Monitoring Cost Index used from Section 3.3 onwards was calculated in two steps. First the mean analytical cost at regional scale was calculated for the current situation, i.e. 23% samples analyzed for MCs. Then a new mean regional cost was estimated for any proportion of samples analyzed for MCs according to monitoring hypothesis.

Minimum biovolume thresholds were graphically derived from MCs concentration classes. These thresholds were defined as the minimal biovolume for a positive detection of MCs class $(n-1)$. For MCs = 1 μg/l for example, the threshold biovolume was set as the minimum cell volume allowing an observation of $0.5 < MCs < 1$ μg/l; for a target MCs of 20 μg/l, the threshold was the minimal biovolume giving $5 < MCs < 10$ μg/l, etc. . .

Results and discussion

Cyanobacteria occurrences and biomass

All data together, maximal cell densities ranged every year from 1.9 to 6.5 10^6 cell/ml, with weekly average values from 10,600 to 452,000 cell/ml. Quantile distributions of cell densities and WHO alert thresholds have already been described in a previous work (Pitois et al., 2014a). To summarize, the mean weekly values tended to increase regularly from 2004 to 2011, in particular because of an increase of the lower, spring densities (Fig. 2). Cell densities above

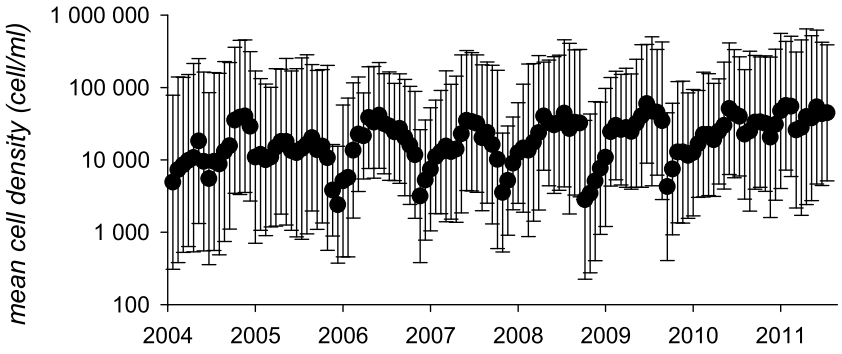

Fig. 2. Mean weekly cell densities from June 2004 to September 2011.