RISK LEVELS OF TOXIC CYANOBACTERIA IN PORTUGUESE RECREATIONAL FRESHWATERS

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_INTRODUCTION

Portuguese surface freshwaters are widely used as a source of drinking water as well as bathing water. Cyanobacterial blooming in these water resources are common and are often associated with cyanotoxin production. The Portuguese legislation for drinking water (Decreto-Lei nº306/2007) establishes the regulatory level of 1 µg/L for total microcystins in treated water. This parameter is determined when eutrophication of water is suspected and when the number of potentially toxic cyanobacteria exceeds 2000 cells/mL. Conversely, the Portuguese legislation concerning the quality of bathing water (Decreto-Lei nº 135/2009), that was transposed form the European Directive nº2006/7/CE, do not include any guideline for cyanobacterial cells nor microcystins concentrations. It only recommends that when the bathing water profile indicates a potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks. When cyanobacterial proliferation is detected visually, it is the responsibility of the local health delegate to evaluate the associated risk. If any risk has been identified or presumed, health and environmental authorities should implement the adequate management measures to prevent exposure, including information to the public.

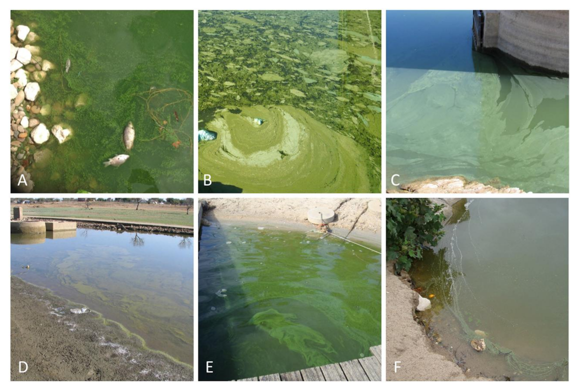

Fig. 1 | Cyanobacteria blooms in Portuguese lakes. A – Fish mortality in Patudos recreational lake undergoing a cyanobacterial bloom (source: Publico newspaper by Marisa Soares, Sept. 9th, 2014); B – *Aphanizomenon* bloom in a water reservoir in central Portugal, May 2015; C – *Planktothrix* bloom in a drinking water reservoir in south of Portugal, Sept. 2005; D – *Aphanizomenon* and *Microcystis* bloom in a water reservoir in central Portugal, Sep. 2005; E – *Microcystis* bloom in a recreational Lake in central Portugal, Oct. 2012; F – *Planktothrix* bloom in a recreational Lake in central Portugal, Oct. 2012.

According to national specificities, some European countries complemented the European Directive nº2006/7/CE and implemented their own guidance or regulations, based on cyanobacterial cell numbers, biovolumes, pigments and/or cyanotoxin concentrations (Ibelings et al., 2015; Chorus, 2012). Prior to establishing regulatory or guideline values, it will be fundamental to characterize Portuguese inland bathing waters concerning the frequency, density, specie composition and toxicity of cyanobacterial blooms. These data are available but not systematized at a national scale. In this work we present the results of the monitoring of cyanobacteria and microcystins in 8 freshwater reservoirs located in the centre of Portugal largely used for bathing and recreational activities. These results will contribute to identify the cyanobacterial blooms profile and to assess the risk level of toxic cyanobacteria in Portuguese recreational freshwaters.

_MATERIALS AND METHODS

Cyanobacteria and microcystins were monitored in surface water samples collected at 8 freshwater reservoirs. The monitoring period in those reservoirs varied between 2 and 14 years, as shown in Table 1.

Cyanobacteria were identified and quantified by optical microscopy according the Utermöhl technique described in the European Standard EN15204. Total microcystins (MC) were analyzed by Enzyme-linked immunosorbent-assay (ELISA) kit (Abraxis, ADDA ELISA), according to the manufacturer instructions.

We calculated the risk levels for each reservoir based on two criteria: the cyanobacterial cell density and the microcystin concentration (Table 2), according to the WHO Guidelines in bathing waters (WHO, 2003).

Table 1 | Sampled freshwater reservoirs, years of monitoring and number of collected samples.

Freshwater reservoirs	Years of monitoring	Number of samples
A1	14	109
A2	10	40
A3	9	56
A4	14	112
A5	3	21
A6	2	13
A7	8	32
A8	11	53
		Total = 436

Table 2 | Criteria used to determine the risk levels in the reservoirs.

	Risk level	Cyanobacterial density (nº of cells/mL)		Risk level	Microcystins (µg MC / L)
	No Risk	Not detected		No Risk	Not detected or not analysed*
	Low Risk	< 20.000		Low Risk	< 20
	Moderate Risk	20.000 – 100.000		High Risk	≥ 20
	High Risk	> 100.000			

*When the density of potential toxic cyanobacteria was bellow 2000 cells/mL.

_RESULTS

MONITORING OF CYANOBACTERIA AND MICROCYSTINS IN FRESHWATER RESERVOIRS

The 8 freshwater reservoirs exhibited distinct profiles concerning the variation of cyanobacteria and microcystins (Figure 2):

Reservoirs A1, A2, A3_ Bloom frequency and cyanobacterial density were low; non-toxic species were predominant but toxic *Microcystis* spp. occurred, although microcystins were never detected.

Reservoir A4_ Bloom frequency and cyanobacterial density were moderate; toxic cyanobacteria were dominant (*Microcystis* spp.) and associated with low microcystin levels.

Reservoirs A5, A7_ Blooms were frequent and cyanobacterial density was high; several toxic cyanobacterial genera were present (*Microcystis*, *Aphanizomenon*, *Anabaena*, *Oscillatoria*); microcystins occurred punctually.

Reservoirs A6_ Blooms were persistent with high cell densities; toxic filamentous cyanobacteria were dominant (*Anabaena* sp., *Aphanizomenon* sp. and *Oscillatoria* sp.); microcystins were persistently detected, but bellow 20 µg/L.

Reservoirs A8_ Blooms were persistent with high cell densities; several potentially toxic species were present; microcystins were persistent and peaks above 20 µg/L coincided with the dominance of *Microcystis* spp. and *Aphanizomenon* spp.

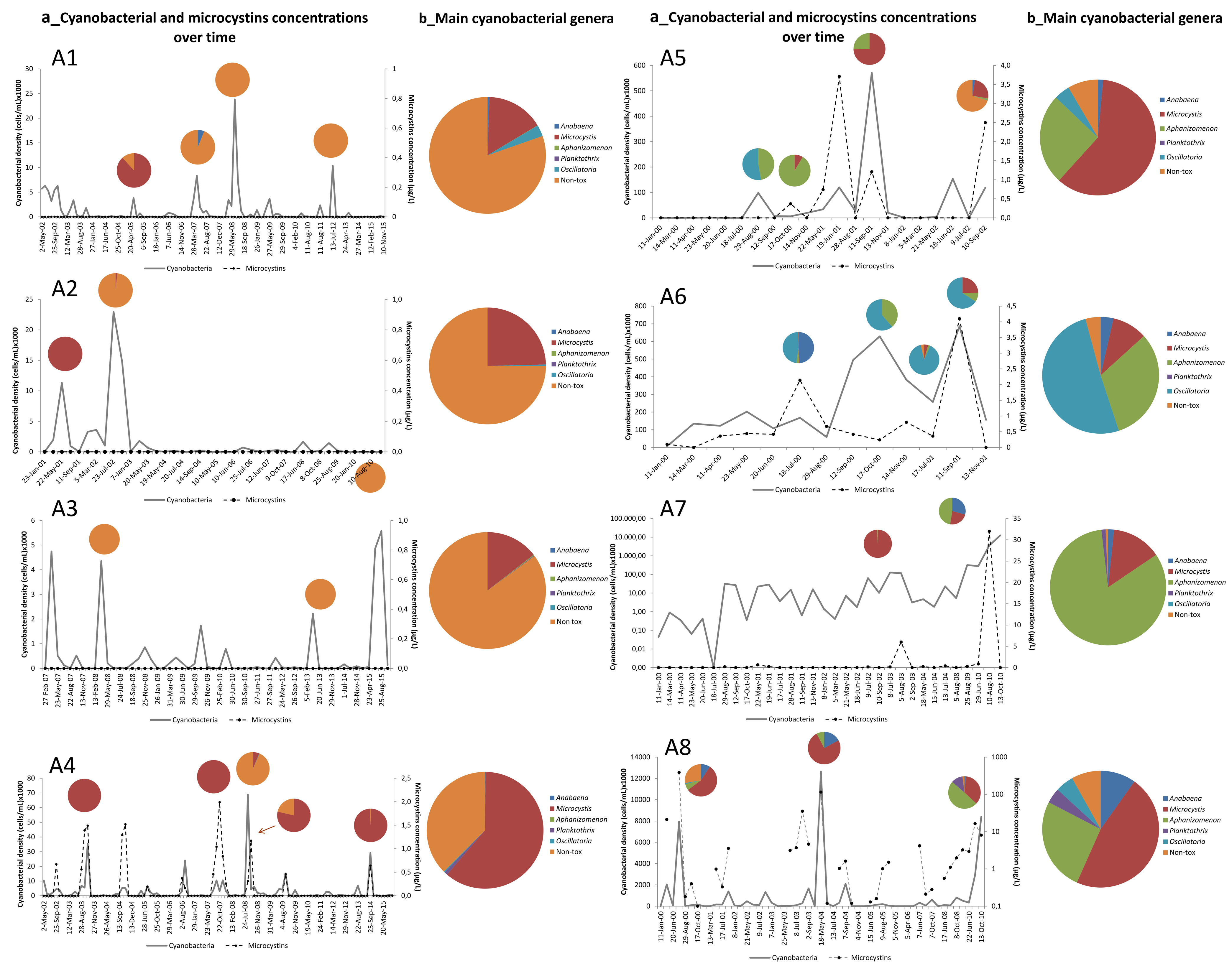

Figure 2 | a) Cyanobacterial and microcystins concentrations over the monitoring periods; b) Circular graphics indicate the main cyanobacterial genera calculated as the average of all the samples for each reservoir (b) and in the indicated time points in (a).

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The risk levels calculated for each reservoir were based on the criteria referred in Table 2. According to the cell-based criteria, 4 of the reservoirs exhibited a high risk level in 20-80% of the samples (Figure 3). However, according to the microcystin-based criteria the severity of the risk was reduced. In this case, only 2 of the reservoirs showed a high risk level in approximately 7% of the samples.

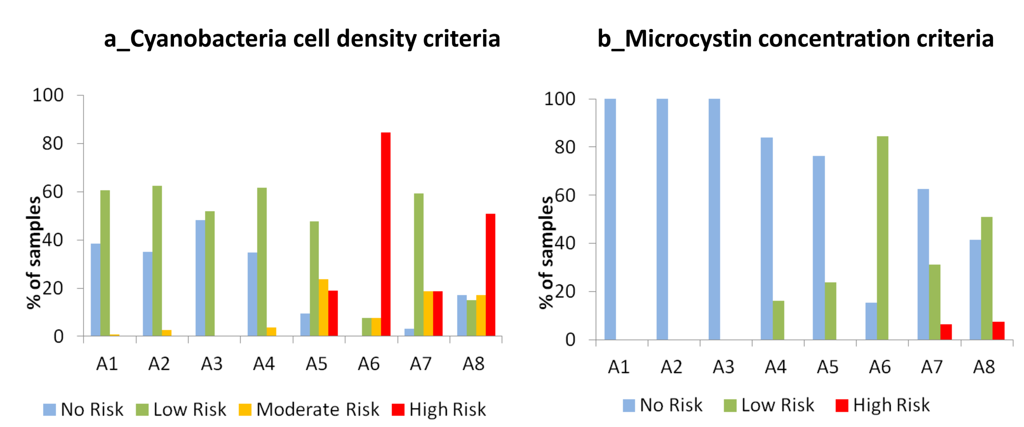

Figure 3 | Risk levels of the 8 freshwater reservoirs based on cyanobacterial cell density (a) and microcystins concentration (b).

_CONCLUSIONS

- Portuguese recreational freshwaters reservoirs have distinct cyanobacteria proliferation scenarios: in some reservoirs blooms were absent; in other reservoirs cyanobacterial blooms were frequent and often persistent;
- Potentially toxic cyanobacteria occurred and in some cases, associated with microcystins production (other cyanotoxins were not included in the monitoring programs);
- The risk levels are more severe when the evaluation criteria is based on cyanobacterial density, comparing to the risk levels based on microcystins criteria;
- The risk evaluation criteria should include other cyanotoxins in order to overcome the misevaluation of the real risk posed by toxic cyanobacteria in recreational freshwater reservoirs.

_References

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