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Cyanobacteria, microcystins and cylindrospermopsin in public drinking supply reservoirs of Brazil

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ABSTRACT

Brazil has a history of blooms and contamination of freshwater systems by cyanobacterial toxins. The monitoring relevance of toxins from cyanobacteria in reservoirs for public supply is notorious given its high toxicity to mammals, included humans beings. The most recurrent toxins in Brazilian water bodies are microcystins (MC). However, the recent record of cylindrospermopsin (CYN) in northeastern Brazil, Pernambuco state, alerts us to the possibility that this could be escalating. This study reports occurrence of MC and CYN, quantified with ELISA, in 10 reservoirs, devoted to public drinking supply in northeastern Brazil. The composition and quantification of the cyanobacteria community associated with these water bodies is also presented. From 23 samples investigated for the presence of MC, and CYN, 22 and 8 out were positive, respectively. Considering the similarity of the cyanobacteria communities found in reservoirs from Pernambuco, including toxin-producing species associated to MC and CYN, we suggest that geographic spreading can be favored by these factors. These issues emphasize the need for increased monitoring of MC and CYN in drinking supply reservoirs in Brazil.

Key words: bloom, harmful algae, monitoring, toxin, water quality.

INTRODUCTION

Toxins from cyanobacteria are known to be harmful agents to human and animal health (Carmichael 1994, Jochimsen et al. 1998, Soares et al. 2006). Besides the high poisoning potential of these toxins, chronic effects in human populations due to long-term exposure to hepatic and neurotoxins have also been reported (Suganuma et al. 1988, Falconer and Buckley 1989, Falconer 1991, 1996, Nishiwaki-Matsushima et al. 1992, Carmichael 1994, Ito et al. 1997, Ding et al. 1999, Cox et al. 2003, Clark et al. 2007).

Microcystins (MC) are hepatotoxins produced by some species of the *Microcystis*, *Planktothrix* and *Anabaena/Dolichospermum* genera, among others,

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which act by inflicting damage to cells from the liver and other organs (Soares et al. 2007), possibly leading the organisms to death by hemorrhagic shock (Mackintosh et al. 1990, Hooser et al. 1991). Moreover, at sub-lethal doses, these toxins might act as tumor promoters (Ueno et al. 1996, Ding et al. 1999, Zhou et al. 2002).

Cylindrospermopsin (CYN) is a tricyclic alkaloid produced by *Cylindrospermopsis* (Ohtani et al. 1992, Berry and Lind 2010), *Aphanizomenon* (Banker et al. 1997, Preußel et al. 2006), *Umezakia* (Harada et al. 1994), *Anabaena* (Schembri et al. 2001, Spoof et al. 2006) and *Lyngbya* (Seifert et al. 2007). These are cytotoxins that irreversibly block protein synthesis, where the primary clinical symptoms are both hepatic and renal failure. They also act on tissues of the intestinal tract, vascular system and muscles (Terao et al. 1994, Falconer et al. 1999, Seawright et al. 1999, Froscio et al. 2008). Additionally, there are indications that CYN produces genotoxic, carcinogenic and mutagenic effects (Falconer and Humpage 2001, Saker et al. 2003). Following the first case of human intoxication provoked by CYN in Australia, 1979 (Hawkins et al. 1985), this toxin has been systematically found in other regions of the world (Carmichael et al. 2001, Li et al. 2001, Stirling and Quilliam 2001, Burns et al. 2002, Chonudomkul et al. 2004, Berry and Lind 2010), including Brazil (Bittencourt-Oliveira et al. 2011a).

Blooms of toxic cyanobacteria have been recorded worldwide and have become more numerous in both fresh and marine water (Hudnell et al. 2008, Paerl and Huisman 2009, Paerl et al. 2011, O'Neil et al. 2012). Parallel to an increase in documentation regarding these events, new species have been found to be responsible for these toxins (Bormans et al. 2005, Cox et al. 2005, Richardson et al. 2007, Berry and Lind 2010, Sant'Anna et al. 2011, Smith et al. 2011). As well, new occurrences of several toxins have been recorded in regions where they were historically thought not be present (O´Neil and Dennison 2005, Paul et al. 2005, Berry and Lind 2010, Bittencourt-Oliveira et al. 2011a, Smith et al. 2012, Mohamed and Al-Shehri 2013). With regard to Brazil, the observation of toxins from cyanobacteria in water intended for public drinking supply has been recorded in several regions, particularly in the south and the northeast (Molica et al. 2005, Anjos et al. 2006, Costa et al. 2006, Sotero-Santos et al. 2006, 2008, Moschini-Carlos et al. 2009, Bittencourt-Oliveira et al. 2010, 2012a).

The monitoring relevance of cyanobacteria toxins in reservoirs for public supply is notorious. Brazil was the first country to enforce a specific legislation for the control of cyanobacteria and their toxins in water used for drinking supply (Brasil 2011). Monitoring of reservoirs requires cell counting of potentially toxic cyanobacteria and toxin quantification (New Zealand, Ministry of Health 2008, Brasil 2011, NHMRC, NRMMC 2011).

The most recurrent toxins in Brazilian water bodies are the MCs. However, the recent record of CYN in three reservoirs for public supply in the northeast region alerted to the possibility that it could be spreading (Bittencourt-Oliveira et al. 2011a).

Reservoirs in Pernambuco state were designed to fulfill different roles and multiple uses which, to some extent, make management difficult and, at the same time, increase the instability of the aquatic biota. These issues demand frequent environmental studies, plus investigation on the cyanobacteria communities and, in particular, their toxins. The occurrence of cyanobacteria toxins in these reservoirs (northeastern Brazil) was already recorded by a few authors using different techniques (Bouvy et al. 1999, Molica et al. 2005, Bittencourt-Oliveira et al. 2010, 2011a, 2012a).

This study shows, for the first time, a wide and profound vision on the reservoirs in the northeastern region of Brazil with regard to cyanobacteria and their toxins. In addition to providing a glimpse of the current status for future research, this study can aid in comparative studies of diagnosis and monitoring, both on a regional and global scale, given the expansion of species of toxic cyanobacteria and their impact on the human population.

This study presents data gathered from 10 reservoirs of the state of Pernambuco, northeast of Brazil, destined for public drinking supply. The occurrence of MC and CYN, as well as the composition and quantification of the cyanobacteria community, is shown.

MATERIALS AND METHODS

Studied Reservoirs and Samplings

The investigated reservoirs supply cities with high demographic densities. These reservoirs are located in regions where climates are warm but rain regimes can range from regular (Zona da Mata, Agreste) to sparse (Sertão, 37°C yearly average). Importance was given to reservoirs for public supply identified as those frequently having cyanobacteria blooms (Table I). The sampling sites were, preferentially, at the center of each reservoir.

Twenty three samples from 10 reservoirs in the state of Pernambuco (Table II) were collected, preferentially, in two different seasons, dry and rainy. Samples for cyanobacteria identification were gathered by surface dragging, using 20-μm mesh plankton net or a wide neck bottle in the case of high population density. Samples for quantification analyses were gathered using a van Dorn bottle in the subsurface (0.5 m).

TABLE I
Reservoirs, coordinates, use, phytogeographic region, water capacity (m^3), maximum depth (m), target community and trophic state. S. Public Supply. W. Watering. F. Fishing. ND. No data.

Reservoir (Use)	Coordinates	Phytogeographic Region	Water Capacity	Max. Depth	Target community	Trophic state
Alagoinha (S, W)	8°27'31.9"S, 6°46'33.5"W	Agreste	2.0×10^4	5.0	11,886	ND
Arcoverde (S, W, F)	8°33'32.5"S, 6°59'07.5"W	Agreste	1.7×10^8	20.0	55,902	Eutrophic[a]
Carpina (S, F)	7°53'03.8"S, 5°20'37.8"W	Zona da Mata	2.7×10^8	15.0	124,520	Eutrophic[b]
Duas Unas (S, W, F)	8°05'02"S, 35°30.6"W	Zona da Mata	2.4×10^7	14.0	914,144	Eutrophic[c]
Ingazeira (S, W, F)	8°36'41.2"S, 6°54'23.7"W	Agreste	4.8×10^6	5.0	9,311	Hypertrophic[d*]
Ipojuca (S, F)	8°20'43,7"S, 36°22'31,5"W	Agreste	3.1×10^7	15.0	110,558	Eutrophice
Jucazinho (S, W, F)	7°59'03"S, 35°48'36.7"W	Agreste	3.2×10^8	40.0	55,766	Eutrophic[c]
Mundaú (S, W, F)	8°56'42.8"S, 36°29'27.4"W	Agreste	1.9×10^6	9.0	115,950	Eutrophic[f]
Tapacurá (S, W, F)	8°02'31.9"S, 35°11'46.5"W	Zona da Mata	9.4×10^7	9.7	940,224	Eutrophic[c]
Venturosa (S, W)	8°34'43.6"S, 36°52'47.3"W	Agreste	12.0×10^4	6.0	9,311	Eutrophic[d*]

a. Bittencourt-Oliveira et al. (2012b); b. Moura et al. (2011); c. Dantas et al. (2012); d*. Trophic State Index calculated from data of chlorophyll *a* of Bouvy et al. (2000) using trophic delineation according Forsberg and Ryding (1980); e. A.N. Moura (unpublished data); f. Dantas et al. (2010).

TABLE II

Diversity, partial density (x 10^6 cell.mL^{-1}), total density (10^6 cell.mL^{-1}), partial biomass (mg.L^{-1}) and total biomass of cyanobacteria in samples investigated. (%). Corresponding percentages of cell.mL^{-1} or mg.L^{-1} of cyanobacteria. (*n*). average number of cells per organism. Concentrations of microcystins (MC) and cylindrospermopsin (CYN) (ng.g^{-1} freeze-dried cells) in the environmental samples obtained by ELISA (three replicates). SD. Standard deviations. (*). Abundant species (°). Dominant species. (s). straight, (c). coiled. (-) absent.

Reservoir (Date sampling) Season	Cyanobacteria (*n*)	Partial Density	%	Total Density	Partial Biomass	%	Total Biomass	MC (SD)	CYN (SD)
Alagoinha (04.14.2009) Rainy	*Cylindrospermopsis raciborskii* s, c (34) *	2.16	42.06		27.51	51.84			
	Microcystis aeruginosa (420) *	0.88	17.18		8.36	15.75			
	Microcystis panniformis (419)*	1.21	23.49	5.14	6.55	12.34	53.07	836,280.0	-
	Gleitlerinema amphibium (61)	0.51	9.95		5.11	9.63		(±16,122.0)	
	Planktothrix agardhii (57)	0.28	5.52		4.43	8.35			
	Others	0.093	1.80		1.11	2.09			
Alagoinha (10.13.2009) Dry	*Planktothrix agardhii* (75) °	10.43	89.67		163.10	86.88			
	Sphaerospermopsis aphanizomenoides (45)	0.27	2.28	11.63	15.25	8.12	187.73	13,542.0	-
	Cylindrospermopsis raciborskii **s** (30)	0.41	3.54		5.25	2.80		(±118.8)	
	Others	0.52	4.51		4.13	2.20			
Arcoverde (05.12.2009) Rainy	*Cylindrospermopis raciborskii* **s,c** (26) °	95.70	77.93		12.44	82.98		306.1	33.3
	Geitlerinema amphibium (40)	24.20	19.71	122.80	2.42	16.14	14.99	(±16.8)	(±4.0)
	Others	2.90	2.36		0.13	0.88			
Carpina (04.06.2009) Rainy	*Planktothrix agardhii* (81) °	5.96	74.63		77.50	69.44			
	Cylindrospermopsis raciborskii **s**, **c** (24)	1.10	13.74	7.99	8.78	7.87	111.61	135.7	
	Sphaerospermopsis aphanizomenoides (44)	0.19	2.33		8.77	7.86		(±9.9)	-
	Geitlerinema amphibium (85)	0.32	4.05		4.21	3.77			
	Others	0.42	5.25		12.36	11.07			
Carpina (10.06.2009) Dry	*Planktothrix agardhii* (80) °	12.90	92.18		167.51	88.59			
	Planktothrix isothrix (88)	0.43	3.04	14.0	7.65	4.05	189.08	93.9	-
	Geitlerinema amphibium (174)	0.42	3.01		5.46	2.89		(±8.3)	
	Others	0.25	1.77		8.45	4.47			
Duas Unas (03.10.2009) Dry	*Sphaerospermopsis aphanizomenoides* (53) °	2.10	92.84		84.53	96.78			
	Cylindrospermopsis raciborskii **s** (24)	0.09	3.92	2.27	1.07	1.23	87.34	62.7	-
	Others	0.07	3.24		1.74	1.99		(±4.4)	
Duas Unas (05.04.2009) Rainy	*Microcystis panniformis* (540) °	3,356.7	99.15	3,385.50	31.79	98.92	32.13	170.3	143.0
	Cylindrospermopsis raciborskii **s** (30)	28.8	0.85		0.34	1.07		(±10.0)	(±14.0)

TABLE II (continuation)

Reservoir (Date sampling) Season	Cyanobacteria (*n*)	Partial Density	%	Total Density	Partial Biomass	%	Total Biomass	MC (SD)	CYN (SD)
Ingazeira (04.14.2009) Rainy	*Planktothrix agardhii* (77) °	10.30	69.70		160.58	76.77			
	Cylindrospermopsis raciborskii **s, c** (18) *	2.47	16.71	14.78	32.09	15.34	209.18	3,399.7	-
	Geitlerinema amphibium (117)	1.64	11.10		12.32	5.89		(±299.2)	
	Others	0.37	2.48		4.19	2.00			
Ingazeira (10.13.2009) Dry	*Geitlerinema amphibium* (117) °	4.26	52.76		52.43	39.59			
	Planktothrix agardhii (77) *	2.64	32.67	8.07	39.53	29.85	132.44	2,032.0	-
	Cylindrospermopsis raciborskii **s, c** (18)	0.87	10.79		27.93	21.09		(±78.4)	
	Others	0.30	3.78		12.54	9.47			
Ipojuca (05.26.2010) Dry	*Planktothrix isothrix* (115) °	1.33	92.37		25.41	95.81			
	Geitlerinema amphibium (54)	0.055	3.77	1.44	0.49	1.85	26.52	-	3.43
	Others	0.053	3.86		0.62	2.34			(±0.5)
Ipojuca (05.03.2010) Rainy	*Planktothrix isothrix* (115) °	1.40	91.69		26.58	93.86			
	Cylindrospermopsis raciborskii **s, c** (35)	0.12	7.63	1.53	1.16	4.10	28.32	10.3	201.5
	Others	0.01	0.68		0.58	2.05		(±1.1)	(±26.8)
Jucazinho (02.17.2009) Rainy	*Planktothrix agardhii* (118) °	22.20	41.57		31.08	30.73			
	Sphaerospermopsis aphanizomenoides (41)	19.5	36.52		58.93	58.27	101.13	64.0	76.3
	Cylindrospermopsis raciborskii **s, c** (16)	8.4	15.73		8.40	8.31		(±8.8)	(±10.7)
	Others	3.3	6.18	53.40	2.72	2.69			
Jucazinho (03.24.2009) Dry	*Planktothrix agardhii* (117) °	101.6	52.13		14.22	47.46			
	Geitlerinema amphibium (43)*	41.2	21.14	194.90	4.94	16.50			
	Sphaerospermopsis aphanizomenoides (41)	28.5	14.62		8.61	28.74	29.97	489.2	2,718.0
	Cylindrospermopsis raciborskii **s, c** (16)	15.2	7.80		1.52	5.07		(±44.1)	(±279.0)
	Others	8.4	4.31		0.67	2.24			
Jucazinho (10.27.2009) Dry	*Planktothrix agardhii* (118) °	61.90	79.77		86.66	80.13			
	Geitlerinema amphibium (43)	6.90	8.89	77.60	8.28	7.66	108.15	117.7	0.5
	Cylindrospermopsis raciborskii **s, c** (16)	6.40	8.25		6.40	5.92		(±8.7)	(±0.1)
	Others	2.40	3.09		6.81	6.30			
Jucazinho (04.28.2009) Rainy	*Planktothrix agardhii* (117) °	333.4	74.42		46.68	71.53			
	Geitlerinema amphibium (43)	63.7	14.22	448.00	7.64	11.71	65.25	83.6	201.4
	Sphaerospermopsis aphanizomenoides (41)	29.3	6.54		8.85	13.57		(±3.7)	(±90.0)
	Cylindrospermopsis raciborskii **s, c** (16)	17.4	3.88		1.74	2.67			
	Others	4.20	0.94		0.34	0.51			

TABLE II (continuation)

Reservoir (Date sampling) Season	Cyanobacteria (*n*)	Partial Density	%	Total Density	Partial Biomass	%	Total Biomass	MC (SD)	CYN (SD)
Mundaú (09.02.2008) Rainy	*Microcystis panniformis* (827) °	1.43	99.66		7.69	95.17		630.9	
	Others	0.05	3.34	1.48	0.39	4.83	8.08	(±41.1)	-
Mundaú (03.17.2009) Dry	*Microcystis panniformis* (827) °	16.11	87.92		86.68	77.13			
	Others	0.63	3.42	18.3	13.02	11.59	112.38	16,858.4	-
	Cylindrospermopsis raciborskii **s, c** (17)	1.59	8.66		12.69	11.29		(±1,314.1)	
Mundaú (05.05.2009) Rainy	*Microcystis panniformis* (1143) °	7.21	75.99		38.79	68.73		3,316.5	
	Cylindrospermopsis raciborskii **s, c** (23)	2.08	21.97	9.49	16.67	29.54	56.42	(±271.5)	-
	Others	0.21	2.05		0.96	1.70			
Mundaú (11.09.2009) Dry	*Microcystis panniformis* (1143) °	8.27	85.88		44.48	81.38			
	Cylindrospermopsis raciborskii **s, c** (17)	1.16	12.05	9.63	9.28	16.98	54.66	2,366.1	-
	Others	0.20	2.07		0.90	1.65		(±114.5)	
Tapacurá (03.10.2009) Dry	*Microcystis panniformis* (78) °	1.49	71.95		39.33	85.93		52.7	
	Planktothrix agardhii (73)	0.26	12.59	2.07	3.65	7.97	45.77	(±5.4)	-
	Others	0.32	15.48		2.79	6.1			
Tapacurá (05.04.2009) Rainy	*Microcystis panniformis* (8653) °	2.27	88.91		59.80	93.54		30.4	
	Planktothrix agardhii (73)	0.13	5.11	2.55	1.82	2.85	63.93	(±1.5)	-
	Others	0.15	5.98		2.31	3.61			
Tapacurá (10.05.2009) Dry	*Sphaerospermopsis aphanizomenoides* (56) °	1.73	43.10		70.85	65.64			
	Microcystis panniformis (3593)*	0.63	15.60	4.02	16.54	15.33	107.93	62.3	-
	Cylindrospermopsis raciborskii **s, c** (16)	0.63	15.64		9.43	8.74		(±1.1)	
	Others	1.03	25.66		11.11	10.3			
Venturosa (10.13.2009) Dry	*Merismopedia tenuissima* (16) °	11.76	99.95	11.77	19.05	99.57	19.13	17.3	-
	Others	0.006	0.05		0.08	0.43		(±1.4)	

IDENTIFICATION AND EVALUATION OF DENSITY AND BIOMASS

Cyanobacteria identification was carried out under a light microscope (Nikon YS100, Melville, NY, USA), and by comparison with specialized literature. Samples for density evaluation (cells.mL^{-1}) were maintained in acetic Lugol's solution and settled in counting chamber according to Utermöhl (1958). Densities were converted into biovolume following the procedure in Hillebrand et al. (1999) and transformed into biomass, assuming a mass density of 1 mg.L^{-1} (Wetzel and Likens 2000). Average values were used for biovolume measurement (n = 50, for abundant species and n = 10, for less frequent species). Abundance and dominance were evaluated according to Lobo and Leighton (1986). Abundant species were those with densities above the community average density, while dominant species were those with density surpassing 50% of the community total density.

Microcystin and Cylindrospermopsin Analysis by Immunoassay Method

Samples with approximately 20 L were collected, centrifuged, frozen in liquid nitrogen, and lyophilized. The lyophilized cells from environmental samples (~10 mg) with 3 mL of Milli-Q water were disrupted by ultrasonication (Microson Ultrasonic Cell Disruptor, Misonix, USA) and used directly for analysis of MC and CYN. Toxin quantification was carried out using a commercial ELISA kit (Beacon Analytical Systems Inc., Portland, ME, USA), following the manufacturer's protocols. The high and low detection limits for MC and CYN by ELISA were 2.0 and 0.1 $\mu g.L^{-1}$, respectively. Negative and positive controls for the ELISA analysis are included in the commercial kit. Triplicate analyses were performed and the values were averaged.

RESULTS

Occurrence of MC was found in all reservoirs (Table II). Only in one sample, out of 23 samples MC was absent (Ipojuca reservoir, 5/26/2010). It was found that in all samples cyanobacteria reported in literature as potentially toxin-producing (MC, CYN and SX), except for *M. tenuissima*. With respect to CYN, eight out of the 23 analyzed samples were positive in four reservoirs (Arcoverde, Duas Unas, Ipojuca, and Jucazinho). In these reservoirs MC and CYN were found simultaneously.

Diversity of cyanobacteria communities, their dominant and abundant taxa, densities and biomasses (total and partial), as well as the presence of MC and CYN in 23 samples were in the Table II. Cyanobacteria communities in the reservoirs were constituted, basically, by *Cylindrospermopsis raciborskii* (Woloszynka) Seenayya & Subba Raju, *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, *Sphaerospermopsis aphanizomenoides* (Forti) Zapomělová, Jezberová, Hrouzek, Hizem, Reháková & Komárková and *Geitlerinema amphibium* (Gomont) Anagnostidis. *C. raciborskii* and *P. agardhii* were dominant and abundant in five reservoirs, which had MC or CYN. *Planktothrix* species (*P. agardhii* and *P. isothrix* (Skuja) Komárek & Komárková) were dominant in five reservoirs and were responsible for more than 50% of cyanobacteria total biomass. In addition, they were the highest occurring genus in blooms, followed by *Microcystis panniformis* Komárek et al. in four reservoirs. The species *Planktothrix agardhii* and *Microcystis panniformis* reached the highest densities, surpassing 10^7 $cells.mL^{-1}$. *Planktothrix agardhii*, *P. isothrix*, *Sphaerospermopsis aphanizomenoides*, *M. panniformis* and *Merismopedia tenuissima* Lemmermann constituted monospecific blooms, accounted for up to 85% of cyanobacteria total biomass in the sample (Table II).

Generally speaking, there was no correlation between dry and rainy seasons, increase or decrease of superficial cyanobacteria biomasses or densities, in the reservoirs.

DISCUSSION

Cyanotoxins were found in 100% of the set of reservoirs (ten), where MCs were detected in 95.65% of the samples investigated for MC and CYN.

Early reports showed that toxic cyanobacteria have dominated in warmer waters of the tropics and subtropics. Within the regions with tropical and sub-tropical climates, Australia shows some similarity to the northeast of Brazil, due to its area, its many reservoirs, shallows, rivers and, mainly, its historical records of frequent blooms of toxic cyanobacteria (Burford et al. 2007, Burford and Davis 2011). Toxin production records are common in Brazil and Australia, and is less in other areas of the world (Fastner et al. 2003, Saker et al. 2003, Burford and Davis 2011).

Brazil has a history of contamination of freshwater systems by cyanotoxins (Teixeira et al. 1993, Jochimsen et al. 1998). Over the last ten years, there have been frequent records of cyanobacteria blooms in reservoirs throughout the country (Anjos et al. 2006, Costa et al. 2006, Chellappa et al. 2008, Dantas et al. 2011, Bittencourt-Oliveira et al. 2011a, b, 2012a, Piccin-Santos and Bittencourt-Oliveira 2012).

In spite of monospecific bloom occurrences in the reservoirs of Ingazeira, Jucazinho, Mundaú and Tapacurá, those blooms constituted by more than one species were the most frequent. Costa et al. (2006), Bittencourt-Oliveira et al. (2011a, 2012b), Moura et al. (2011), and Lira et al. (2011) reported multi-species blooms of cyanobacteria constituted mostly by the genera *Planktothrix* and *Cylindrospermopsis*, with possible alternation of predominance according to the climate conditions.

Apart from high temperatures, blooms in the northeast of Brazil are also enhanced by the hydric regime characterized by long and accentuated dry periods and by the length of time that the water remains in the reservoirs. Conditions associated with dry and rainy seasons, such as stratification and de-stratification of water bodies, as well as water turbidity, are appointed as favoring factors, allowing the establishment of other species with high population densities and forming multi-species blooms of cyanobacteria (Dantas et al. 2011, Bittencourt-Oliveira et al. 2012b). Bittencourt-Oliveira et al. (2012b) observed in the Arcoverde reservoir an overwhelming increase of *C. raciborskii* populations during the dry season (de-stratification), characterized by thermally stratified water where, in the rainy season, the cyanobacteria biomass dropped substantially due to the presence of other cyanobacteria in the community. These authors pointed out that species being favored by stratification cannot be considered a rule because in other reservoirs in the same region the population density of *C. raciborskii* increased all through the thermal de-stratification. These differences in the behavior of populations of cyanobacteria, particularly of *C. raciborskii*, also vary between Brazilian and Australian water bodies (Hawkins and Grifftiths 1993, Bittencourt-Oliveira et al. 2011b).

In all samples with MCs, potentially toxic cyanobacteria were found, as described in the literature. The single exception of nontoxic cyano-bacterium was *M. tenuissima* in the Venturosa reservoir (10.13.2009), even in high densities. Despite the high cellular density, the biomass was low (19.05 $mg.L^{-1}$). Picoplanktonic cyanobacteria, such as *Aphanocapsa cumulus* Komárek & Cronberg (Domingos et al. 1999) and *Epigloeosphaera brasilica* Azevedo et al. (Bittencourt-Oliveira et al. 2012a), were already reported in Brazil as toxin-producing. It should be emphasized that no cyanobacteria have to be discarded with respect to toxin production.

Diversity of the cyanobacteria community, mainly constituted by toxin-producing species (*Cylindrospermopsis raciborskii, Planktohrix agardhii*, *Sphaerospermopsis aphanizomenoides* and *Geitlerinema amphibium*), was similar in all reservoirs, except for *Merismopedia tenuissima* (Venturosa reservoir). Such homogeneity of cyanobacteria communities in reservoirs of the state of Pernambuco corroborate previous studies (Bouvy et al. 2000, 2003, Dantas et al. 2011, Lira et al. 2011, Moura et al. 2011, Bittencourt-Oliveira et al. 2011a, b, 2012a).

In the studied Brazilian reservoirs, *C. raciborskii* shared dominance with species of *Planktothrix*, whereas *P. agardhii* and *P. isothri*x formed monospecific blooms which accounted for up to 85% of the total cyanobacteria biomass in the sample (Table II). *Cylindrospermopsis raciborskii, Sphaerospermopsis aphanizomenoides* previously enominated *Aphanizomenon aphanizomenoides* (Zapomělová et al. 2009, 2010), *G. amphibium*, *Microcystis* and *Planktothrix* species are potentially microcystin, cylindrospermopsin and saxitoxin-producing. On the other hand, of the mentioned species, only *C. raciborskii* (Ohtani et al. 1992, Li et al. 2001) and species of the genus *Sphaerospermopsis* (*Aphanizomenon* spp.) (Preußel et al. 2006, Yilmaz et al. 2008) have been described as producers of CYN.

Although populations of *C. raciborskii* and *Aphanizomenon*/*Sphaerospermopsis* spp. are common in the country, the toxin CYN was found only recently in Brazil (Bittencourt-Oliveira et al. 2011a). However, even though CYN occurs in reservoirs with high densities of C. *raciborskii* and *Sphaerospermopsis* no CYN-producing strain has been isolated in order to verify which species was responsible for producing this toxin. In Brazil no strain was reported as CYN-producing.

The Jucazinho reservoir had the greatest concentrations of CYN, associated with a high biomass of *Planktothrix agardhii, Sphaerospermopsis aphanizomenoides* and *Geitlerinema amphibium*. *P. agardhii* was the predominant species in three out of four samples in this reservoir, with total cyanobacteria biomass ranging from 30.73 to 80.13%, but there were no reports of an association with CYN production.

The sample from the Arcoverde reservoir taken on May 12, 2009 was the only one with an expressive predominance of *C. raciborskii* and also had the lowest concentrations of CYN (33.3 ng. g^{-1} freeze-dried cells). Therefore, biomass and density values of MC and CYN-producing cyanobacteria were not proportional to the concentrations of the measured toxins. This fact was already reported in previous studies undertaken in the reservoirs of the region (Piccin-Santos and Bittencourt-Oliveira 2012).

CONCLUSION

The similarity of cyanobacteria communities in the investigated reservoirs, associated with both the occurrence of MC and CYN and adequate environmental conditions, favors geographic spreading of toxic blooms in the region. This circumstance emphasizes the need for increased monitoring of MC and CYN in drinking supply reservoirs in Brazil.

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RESUMO

O Brasil tem um histórico de florações e contaminações por toxinas de cianobactérias nos seus ecossistemas de água doce. A relevância do monitoramento de toxinas de cianobactérias em reservatórios de abastecimento público é notória, devido à sua alta toxicidade para mamíferos, inclusive seres humanos. As cianotoxinas mais recorrentes em corpos de água brasileiros são microcistinas (MC). No entanto, o registro recente de cilindrospermopsina (CYN) no nordeste, estado de Pernambuco, nos alerta para a possibilidade do aumento de novas ocorrências. Este estudo relata a ocorrência de MC e CYN, quantificadas por ELISA, em 10 reservatórios destinados ao abastecimento público do nordeste do Brasil. Também são apresentadas a composição e a quantificação da comunidade de cianobactérias associadas a estes corpos de água. Das 23 amostras investigadas, 22 e 8 foram positivas em relação, respectivamente, a MC e CYN. Considerando a similaridade das comunidades de cianobactérias encontradas nos reservatórios de Pernambuco e a presença de espécies potencialmente produtoras de MC e CYN, nós sugerimos que uma expansão geográfica possa ser favorecida por esses fatores. Estas questões enfatizam a necessidade de aumentar o monitoramento de MC e CYN em reservatórios de abastecimento público no Brasil.

Palavras-chave: floração, algas nocivas, monitoramento, toxinas, qualidade de água.

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