International Scholars Journals

International Journal of Public Health and Epidemiology ISSN: 2326-7291 Vol. 5 (4), pp. 252-258, April, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A study of cyanobacteria and absence of cyanotoxins in a public water supply source

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Accepted 02 January, 2016

Current analysis, involving measurements of biotic and abiotic factors, determined which factor favored cyanobacteria with subsequent concentrations of microcystins in water collected from a public water supply source during the dry and rainy periods and which received residual water from agricultural production systems. Since no microcystins were detected, waters fitted within the maximum limits of 1μg.L^{-1} for MC-LR. Nevertheless, if aquiculture production systems are not properly administered, especially for the diet factor, great risks will exist in the contamination and pollution of fresh water. This fact may cause intoxication to the population that use the water and to the aquatic plants and animals which make it their habitat.

Key words: HPLC, nitrogen, organic matter, microcystins, phosphorus.

INTRODUCTION

Bloomings of phytoplanktonic algae are a direct consequence of the eutrophization of water courses. In the context of several microplanktons, the development of cyanobacteria bloomings is an extremely important issue owing to the ability of these organisms in pro-ducing secondary metabolites which are highly toxic for living beings, including humans (Haider et al., 2003; Figueiredo et al., 2004).

Cyanotoxins, extremely different in their chemical structures and in toxicity, are normally classified as dermotoxins, neurotoxins and hepatotoxins, according to their toxic effects on animals. Special attention should be given to microcystins which are hepatoto-xins. This is not merely due to their capacity in causing acute poisoning but also to their ability in triggering cancer in humans through chronic exposures to low concentrations of microcystins in the water
supply (Rippka et al., 1979; Haider et al., 2003).

Although there are more than 60 isoforms of microcystins, microcystin-LR (MC-LR) is the most investi-gated variant. Human exposure to microcystins may occur by a direct source as, for instance, drinking water (Ueno et al., 1996; WHO, 1998; Zhou et al., 2002), by water in which sports and games are enacted (WHO, 2003) and haemodialysis (Pouria et al., 1998), or indirectly as in the case of food (Amorim and Vasconcelos, 1999; Magalhães et al., 2003).

The danger of triggering tumors through chronic exposures to microcystins in supply water has been the main reason for defining a maximum rate of 1 μg.L^{-1} in the case of MC-LR, stipulated by the World Health Organization (WHO, 1998) and by the ministerial decree 518 of the Brazilian Ministry of Health (Brasil, 2004).

Brazilian Ministry of Health's decree 518 (Brasil, 2004) makes mandatory that water for human consumption should have a pH between 6.0 and 9.5 and maximum turbidity rate of 5.0 UNT. In the case of cyanobacteria bloomings the same decree enacts procedures related to control and caution in the quality of water for human consumption and drinking stan-dards. Procedures are classified as Caution, Alert 1, Alert 2 and Alert 3 levels, as follows:

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a) Caution level is characterized when a colony, or five filaments, of cyanobacteria per milliliter of crude water, up to 10,000 cells. mL^{-1} is detected (initial stage of development of cyanobacterial blooming). Monthly monitoring of cyanotoxins should be done; they should be fortnightly when there is a history of blooming in the environments.

b) Alert 1 level occurs when 10,000 to 20,000 cells. mL^{-1} are detected (initial establishment of cyanobac-terial blooming is confirmed). Weekly monitoring of cyanotoxins should be undertaken.

c) Alert 2 level occurs when 20,000 to 100,000 cells. mL^{-1} are detected (establishment of cyanobacterial blooming is confirmed with problems in the water qua-lity). Operational measures in monitoring and health risk prevention should be imposed. Weekly cyano-toxins analyses should be undertaken in water outputs and in water inputs of haemodialysis clinics and industries.

d) Alert 3 level is characterized by over 100,000 cyano-bacteria cells. mL^{-1} (well defined toxic blooming with imminent risk to population's health). Operational measures in monitoring and health risk prevention should be undertaken.

Resolution 357 (Conama, 2005) defines and classifies fresh, saline and brine water for the whole Brazilian territory. Class II drinking water is proper to aquiculture and fishing activities featuring the following biotic and abiotic characteristics: (a) Maximum cyanobacteria count up to 5,000 cells mL^{-1}; (b) Turbi- dity rates up to 100 UNT; (c) Maximum concentrations of biochemical oxygen demands (BOD) up to 5 mgL^{-1}; (d) Concentrations of dissolved oxygen (DO) higher than 5 mgL^{-1}; (e) Maximum concentrations of total phosphorus in lentic environments up to 0.03 $mg.L^{-1}$; (f) Maximum concentrations of total phosphorus in intermediate environments up to 0.05 mgL^{-1}; (g) Maxi -mum concentrations of total nitrogen up to 3.7 $mg.L^{-1}$, when pH is less than or equal to 7.5; up to 2.0 $mg.L^{-1}$, when pH lies between 7.5 and 8.8; 1 $mg.L^{-1}$ when pH is higher than or equal to 8.5.

Several researches undertaken since the last century reported the occurrence worldwide of hepatotoxic development with the production of several microcystins associated with a predominance of *Microcystis aerugi-nosa* (Figueiredo et al., 2004). Reports on the issue were published in the Brazilian states of Paraná (Hirooka et al., 1999), Rio Grande do Sul (Matthiensen et al., 2000), Per-nambuco (Pouria et al., 1998) and Rio Grande do Norte (Chellappa et al., 2000).

Whereas, algae development in many tropic and subtropical water systems has become a further problem due to management deficit, current research investigates the physical and chemical factors which promote the development of cyanobacteria blooms with subsequent microcystin concentrations in water from a public water supply source during the dry and rainy seasons while receiving residual waters from aquaculture production systems.

MATERIALS AND METHODS

Current research was undertaken between the dry and rainy sea-sons, during 12 months. Sampling sites consisted of four places of the stream Rico, the water supply source of Jaboticabal SP Brazil. The four sites were previously chosen by the Water and Sewerage Service of Jaboticabal (SAAEJ).

The hydrographic basin of the stream Rico (from the source till the water receiving site in Jaboticabal SP Brazil) is linked to the set of River Mogi Guaçu Basins in the Central-Northern region of the state of São Paulo, the administrative region of Ribeirão Preto which comprises the municipalities of Monte Alto, Jaboticabal, Santa Ernestina, Taquaritinga and Guariba.

The four collection sites of the public supply source, stream Rico, were the stream source; the area behind the urban area of Monte Alto; the water receiving system of the Water and Sewerage Service of Jaboticabal (SAAEJ); the area behind the SAAEJ.
Each site of the stream Rico was divided into two sampling zones. However, due to the low water volume, collection in the water column was unfeasible, especially during the dry season. Thus, the number of total samples was actually sixteen.

Register of abiotic conditions was the first thing done in all collection sites by U-10 multiparameter probe for water analysis (Horiba, 1991). Temperature, pH, dissolved oxygen, conductivity, turbidity and salinity were the parameters obtained by the equipment. 25-µm-plankton nets were employed to collect surface (between 0.2 and 0.3 m) phytoplankton. 100 ml samples were fixed (1:100 ml) in Lugol solution for the identification and quantification of the microplankton community. Water volumes of 4000 ml were collected from the surface (between 0.2 and 0.3 m) with four polyethylene flasks, 1000 ml each, for the analysis of organic matter and nutrients.

Samples were then carried in ice- filled isothermal boxes and taken to the Laboratories of Limnology and Plankton Culture of the UNESP Aquaculture Center (CAUNESP).

Oxygen biochemical requirements (OBR) readings were obtained by Apparatus Model AL 320 (Aqualytic, 1997). Results, expressed in mgL^{-1}, were equal to those obtained by methodology described by Apha (1998).

Oxygen chemical requirements (OCR) rates were obtained by the calorimetric method with Hach's Spectrophotometer DR-2000 and by Hach's digester block for DQO. Methodology described in the apparatus instructions book (Hach, 1998, 2000) produced results, expressed in $mg.L^{-1}$, similar to those by Apha (1998).

So that macronutrients could be determined, digestion was first undertaken by digester Digesdahl Hach (Hach, 1999), based on a single total digestion of organic matter with sulfuric acid and hydrogen peroxide.

Rates of Kjeldahl Total Nitrogen (KTN) were obtained by modi-fied Nessler method (method 8075) from the digestion-produced extract, using Hach's spectrophotometer DR-2000 (Hach, 1998, 2000). Results were given in mgL^{-1}. Methodology is equivalent to that described by Apha (1998).

Phosphorus concentration rates were determined by colorimetry with metavanadate and ammonium molybdate, as described by Apha (1998).

Microplankton composition was determined by Utermöhl techni-que (1958). Identification of microplankton was undertaken at class level (Desikachary, 1959; Dodge, 1985; Maeda and Carey, 1985;

Table 1. Arithmetic mean of chlorophyceae and cyanobacteria (in cell mL^{-1}) obtained from water at four sites in the stream Rico, water supply source of Jaboticabal SP Brazil, between August and September 2006 (dry period) and between November and December 2006 (rainy period).

Phytoplankton community	Collection sites							
	Source		Behind Monte Alto's town area		Saaej receiving water		Behind Saaej area	
	dry	rainy	dry	rainy	dry	rainy	dry	rainy
				Chlorofíceas				
Scenedesmus quadricauda	0Aa	0Aa	45Aa	5Ba	40Aa	8Ba	0Aa	0Aa
Scenedesmus arcuatus	0Aa	0Aa	34Ab	2Bb	6Ab	6Aa	0Aa	0Aa
Staurodesmus convergens	0Aa	0Aa	32Ab	2Bb	18Ac	7Ba	5Ab	4Ab
				Cyanobacteria				
Microcystis aeruginosa	0Aa	0Aa	1.161Aa	556Ba	58Aa	9Ba	10Aa	0Ba
Cylindrospermopsis raciborskii	0Aa	0Aa	10Ab	0Bb	57Aa	8Ba	0Ab	0Aa
Merismopedia tenuissima	0Aa	0Aa	22Ac	12Bc	0Ab	0Ab	0Ab	0Aa

In each line rates followed by different capital letters differ among themselves by Tukey's test at 5 and 1%. In each column, rates followed by different small letters differ among themselves by Tukey's test at 5 and 1%.

Priddle and Fryxell, 1985; Komárek and Anagnostidis, 1986; Sournia, 1986; Ricard, 1987; Anagnostidis and Komárek, 1988; Balech, 1988; Komárek and Anagnostidis, 1989).

Quality and quantitative analyses of microcystins, within and outside the cells, were determined by producing the extracts, following Krishnsmurthy et al. (1986), Tsuji et al. (1994), Matthiensen et al. (1999) and Magalhães et al. (2003) and, at a later stage, by readings undertaken with high performance liquid chromatography (HPLC-DAD).

RESULTS

Highest cyanobacteria counts, highest turbidity rates and highest concentrations of KTN and P (Table 2) were obtained during the dry period at the public supply water source (Table 1) behind the Monte Alto's town region.

Chlorophyceae were positively correlated with temperature and OCR rates in the public water supply source, stream Rico (Table 3), during the dry period, and with turbidity, DO and rates of nitrogen and phosphorus (N:P) during the dry and rainy periods. *M. aeruginosa* was positively correlated with DO rates during the dry period, with N:P rates during the rainy period and with turbidity and OCR in both the dry and rainy periods.

Table 3 shows Pearson's correlation rates between the number of chlorophyceae and *M*. *aeruginosa* cells and other parameters. Temperature, pH, DO and N:P in the water of aquaculture systems and turbidity and OCR in the river water were the parameters with significant correlation index with number of cells.

Turbidity had a positive correlation with *M. aeruginosa* and chlorophyceae development obtained from the water of the public water supply source (Table 3) in the dry and in the rainy periods. Positive correlations between DO concentrations and cyanobacteria development were visible in the entire public water supply source (Table 3).

Correlations between OBR/OCR concentrations, Chlorophyceae and *M. aeruginosa* development were only positive in river water (Table 3). Such a narrow relationship was more visible in *M. aeruginosa* development.

Although N:P positively correlated with highest chlorophyceae and *M*. *aeruginosa* counts in the river water, correlation only occurred during the rainy season (Table 3) and failed to coincide with highest *M*. *aeruginosa* counts during the dry period (Table 1).

It should be highlighted that microcystin MC-LR was not found in any of the researched sites of the stream Rico.

DISCUSSION

Temperature, pH, dissolved oxygen (OD) and turbidity rates (Table 2) were similar to those in other research works (Sipaúba-Tavares, 1995; Briand et al., 2002; Istvánovics et al., 2002; Chellappa and Costa, 2003; Xie et al., 2003; Douterelo et al., 2004; Matsuzaki et al., 2004; Albay et al., 2005; Mercante et al., 2005; Sotero-Santos et al., 2006). However, conductivity rates differed from those obtained by Douterelo et al. (2004) and Sotero-Santos et al. (2006) and similar to those reported by Chellappa and Costa (2003).

Highest cyanobacteria counts and highest turbidity, KTN and P in the stream Rico were probably obtained due to non- located nitrogen sources such as discharge of rain water, water from agriculture areas and autochthonous recycling of nutrients (Chellappa and Costa, 2003).

An increase in the prevalence of chlorophyceae and cyanobacteria occurred as a response to different types of river pollution, such as eutrophization, increase in organic pollutant levels, increase in fecal coliform concentrations and the presence of rural residues. However, a general response cannot be pinpointed for all as a group

Table 2. Arithmetic mean of physical and chemical variables in the water collected at the four sites of stream Rico, water supply source of Jaboticabal SP Brazil, between August and September 2006 (dry period) and between November and December 2006 (rainy period).

Variables	Public water supply source							
	Source		Behind town area		Saaej water collection		Behind Saaej	
	Sc	Cv	Sc	Cv	Sc	Cv	Sc	Cv
pH	6.4Aa	6.0Aa	7.5Ab	6.4Ba	6.2Aa	7.0Bb	7.2Ab	7.2Ab
Temperature (ëC)	22.5Aa	22.0Aa	18.5Ab	23.0Ba	18.9Aa	22.9Bb	19.8Aa	23.5Ab
Turbidity (UNT)	0.2Aa	0.3Aa	55.7Ab	35.0Bb	46.3Ab	32.5Bb	4.0Ac	3.0Ac
Conductivity ($mS.cm^{-1}$)	1.5Aa	0.14Ba	1.51Aa	0.12Ba	1.09Ab	0.13Ba	1.5Aa	0.10Ba
OD (mgL^{-1})	6.7Aa	5.3Ba	4.3Ab	4.8Ba	5.1Ab	7.6Bb	6.8Aa	4.7Ba
DBO (mgL^{-1})	20.0Aa	3.0Aa	6.0Ab	9.0Ab	7.0Ab	5.0Ab	1.0Aa	1.0Aa
DQO (mgL^{-1})	4.0Aa	10.0Ba	30.0Ab	63.0Bb	12.0Aa	32.0Bb	5.0Aa	40.0Ba
NTK (mgL^{-1})	15.0Aa	3.75Ab	291.1Ab	109.0Bb	233.0Ab	86.3Bb	53.0Ac	1.0Bc
P (mgL^{-1})	0.1Aa	0.3Aa	2.6Ab	1.9Ab	2.5Ab	0.0Ba	0.4Aa	0.2Aa

Sc = dry, Cv = rainy, DO = Dissolved Oxygen, OBR = oxygen biochemical requirements, OCR = oxygen chemical requirements, KTN = Kjeldahl Total Nitrogen, and P = Phosphorus. In each line rates followed by different capital letters differ among themselves by Tukey's Test at 5 and 1%. In each column, rates followed by different small letters differ among themselves by Tukey's Test at 5 and 1%.

Table 3. Correlation coefficients (r) between physical and chemical parameters and chlorophyceae and *Microcystis aeruginosa* counts in water collected from the ten production aquaculture systems and from the four sites of the public water supply source. Statistically significant: ($p < 0.01$ and $p < 0.05$).

Correlated variables	Stream Rico	
	Dry period	Rainy period
Chlorophyceae vs. pH	0.059**	0.257**
Chlorophyceae vs. temperature	0.562	0.386**
Chlorophyceae vs. turbidity	0.966	0.634
Chlorophyceae vs. conductivity	0.088**	0.001**
Chlorophyceae vs. OD	0.875	0.836
Chlorophyceae vs. DBO	0.105**	0.157**
Chlorophyceae vs. DQO	0.886	0.077**
Chlorophyceae vs. N:P	0.780	0.514
Microcystis aeruginosa vs. pH	0.301**	0.315**
M. aeruginosa vs. temperature	0.241**	0.123**
M. aeruginosa vs. turbidity	0.536	0.433
M. aeruginosa vs. conductivity	0.067**	0.001**
M. aeruginosa vs. OD	0.659	0.088**
M. aeruginosa vs. DBO	0.051**	0.798
M. aeruginosa vs. DQO	0.951	0.672
M. aeruginosa vs. N:P	0.248**	0.537

DO = Dissolved Oxygen; OBR = oxygen biochemical requirements; OCR = oxygen chemical requirements; ** = no correlation.

group, since responses of single species may be completely different (Douterelo et al., 2004; Eler and Espíndola, 2006). Flow and hydro-geometric conditions coupled to environment factors affect cyanobacteria dynamics in River systems. Phosphorus is a limiting nutrient since certain cyanobacteria species may fix nitrogen in the atmosphere. In rivers, however, phosphorus release model from specific sources may make nitrogen a limiting nutrient (Guven and Howard, 2006).

Further, variations in flow conditions are the main rule in initial cyanobacteria development whereas strong vertical mixture, caused by high river discharges, may eliminate the favorable conditions for cyanobacteria. When river discharge is at the surface, turbulence-caused vertical speed is negligible since the movement of colonies in the water column is considered to be produced only by density changes. This is based on the fact that the colony involved in the water mass moves from downstream to

upstream. The colony's horizontal speed is thus directly proportional to the river's mean speed (Borett et al., 2006; Guven and Howard, 2006).

Concentrations of dissolved oxygen, temperature rates and relationships between nitrogen and phosphorus may directly affect chlorophyceae and *M. aeruginosa* counts (Albay et al., 2005; Sipáúba-Tavares, 1995; Sipaúba-Tavares, 2005), which is clearly evident in current research (Table 3). It is well known that high temperatures cause a rise in the number of species, an increase in the speed of organic processes and an increase in metabolic activity (Matsuzaki et al., 2004).

Albay et al. (2005) also stated that temperature is an important environmental factor in the toxic development of M. aeruginosa. According to these authors, highest microcystin concentration was reported between 24.0 and 28.5ëC, with a direct correlation between temperature increase and microcystin concentration rise. However, in previous researches they had already reported highest microcystin concentrations at low temperatures, such as 8ëC.

Matsuzaki et al. (2004) reported that temperature rates and stratification and residence time remained high in water environments with *M. aeruginosa* and *Cylindrospermopsis raciborskii* development. Absence of herbivores has been reported through zooplanktons, migration of the phytoplanktonic community along the water column and a higher rate of nitrogen fixing.

Positive correlations between cyanobacteria development and temperature rates (Table 3), shows that temperature may be the key factor that triggers cyanobacteria development. According to Fleming et al. (2002), temperature rise causes the stabilizing of water column and an increase in light radiance which is available to the cyanobacteria community.

The growth phase of developing cyanobacteria is mainly restricted to the hot months due to the relationship between growth levels and temperature, with 25ëC as the best (Hense and Beckmann, 2006). Cyanobacteria development may be related to high air and water temperatures. According to Briand et al. (2002) and Istvánovics et al. (2002), temperatures between 22 and 23.5ëC and between 24 and 30^{O}C, respectively are ideal for the germination of akinetes. The best temperature is 25^{O}C (Hense and Beckmann, 2006).

Positive correlations between DO concentrations and cyanobacteria development may point out the importance of this chemical variable for cyanobacteria development. Concentration of dissolved oxygen in water is determined by algae and bacteria activities since algae produce dissolved oxygen during daytime and respiration is always predominant owing to the bacteria. Aerobic and anaerobic microbiological processes also affect other factors related to water quality such as pH and ammonia concentrations (Moriarty, 1997; Vidotti and Rollem, 2004).

Production of dissolved oxygen in the light zone during photosynthesis is equal or higher than respiration-caused oxygen consumption. The penetration of light in the tank is determined by turbidity and algae biomass. When both these factors are high, the light zone is shallow and only a small portion of algae produces dissolved oxygen during daytime (Moriarty, 1997; Vidotti and Rollem, 2004).

Oxygen goes into the tank by diffusion which is amplified by wind-caused mechanical movements or by photosynthetic activities. Since algae and other organisms beneath the light zone breathe dissolved oxygen, respiration rate is lower than supplied dissolved oxygen. The light zone should ideally extend itself completely throughout most of the water column (Moriarty, 1997; Vidotti and Rollem, 2004).

It should be highlighted that DO rates reported during the rainy period were very low, beneath the concentration rates needed for the maintenance of life in the water system. This fact is a source of concern since deficits in DO, condition fish to stress, and their development is consequently jeopardized (Sipaúba-Tavares, 2005).

Oxygen biochemical requirement, defined as the concentration of DO required by bacteria when stabilizing degradable carbonaceous regions of organic matter under aerobic conditions and nitrification inhibition, indicates organic matter in the water (Samocha et al., 2004).

High OBR concentrations reported in current research may be explained by an increase in ratio rates, high fish population density and an increase in the concentration of dissolved organic compounds. High OBR and OCR concentrations generally reflect high concentrations of matter which may be biologically degradable. Since oxygen is consumed, there is a consequent decrease in DO concentrations in the water bodies which makes unfeasible life conditions for fish. Its elimination in water systems occurs by bacteria decomposition and sedimentation (Samocha et al., 2004). Most organic matter is decomposed by heterotrophic bacteria in the water column and on the sediment surface. The remaining organic matter is broken within the sediment's anoxic zone mainly by yeast-producing bacteria (Moriarty, 1997; Vidotti and Rollem Berg, 2004).

Positive correlation between N:P and highest phytoplankton counts reported in river water highlights the importance of these nutrients for development. In natural systems, however, as in the case of the public water supply source, this correlation may not have such high relevance.

Physical factors determine which genera and species establish and dominate specific ecosystems. Through changes in abiotic characteristics of ecosystems, seasonality influences the development of phytoplankton (Matsuzaki et al., 2004; Abrantes et al., 2006).

Phosphorus is an important metabolic nutrient and its availability determines productivity in natural water, even though it is dependent on pH (Samocha et al., 2004) and

nitrogen is generally essential in the manuring of fish tanks. However, phosphorus is a limiting factor for plankton development since it may be immediately incorporated to the food chain through phytoplankton and zooplankton as soon as it is placed in the environment (Matsuzaki et al., 2004; Jayatissa et al., 2006).

Local variations in phosphorus concentration may be one of the factors that cause disparities in cyanobacteria development. Since phosphate is linked to ferric iron in the sediment bottom and due to the stratification of the water column and the deposit of organic matter, the bottom of sediments may decrease and release phosphate. Phosphate may be transported to the light zone within the front regions through an intense mixture of the water column (Douterelo et al., 2004).

The absence of microcystin in the supply source may be due to the low population density of cyanobacteria (Table 1). During the dry period, there was also a negative correlation between ratios N:P and M. aeruginosa counting (Table 3). Contrastingly, Gkelis et al. (2005) found MC-LR, MC-RR and MC-Yr in seven rivers in Greece and reported geographic trends for the dominance and distribution of microcystins, featuring a non-dominance of producing microcystin strains between latitudes 33ë and 42ë N.

A low relationship between *M. aeruginosa* counts and MC-LR concentrations has been reported because of the latter's absence in these waters. Natural degradation kinetics of the toxin may be a possible explanation for the low correlation between cell density and counted microcystins (Hoeger et al., 2004; Kaya et al., 2005).

According to environment legislation, N and P concentrations in the stream Rico were outside maximum rates allowed by Resolution 357 (Conama, 2005). OBR and DO concentrations do not fit within Resolution 357 (Conama, 2005) in the river waters collected behind the Monte Alto town area. Finally, according to Decree 518 of the Brazilian Health Ministry, the waters of the stream Rico fit pH rates but do not with regard to turbidity rates (Brasil, 2004).

According to Decree 518 of the Brazilian Health Ministry (Brasil, 2004), collected waters were within monitoring levels, or rather, monthly analyses for cyanotoxins are recommended. However, when Resolution 357 (Conama, 2005), maximum permitted rates of 1 $\mu g.L^{-1}$ for MC- LR, mandatory by WHO (WHO, 1998) and Decree 518 of the Brazilian Health Ministry (Brasil, 2004) are taken into account, the analyzed water may be said to be in accordance.

Conclusion

No *microcystins* were detected in stream Rico, perhaps due to its lotic system and consequently to its low cyanobacteria population density. Waters collected fitted within the maximum limits of $1\mu g.L^{-1}$ for MC-LR.

If aquiculture production systems are not well planned and administered, especially with regard to food management, they may be a source of high contamination and pollution risk to fresh water and aquatic life. They may trigger intoxication events in the population making use of them.

ACKNOWLEDGEMENT

We would like to thank FAPESP (Research Foundation of the State of São Paulo) for funding the post-doctoral scholarship (Proc.: 05/58563-4) and Research help (Proc.: 05/59253-9).

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