

# Occurrence of toxic cyanobacterial bloom in the left margin of the Tapajós river, in the Municipality of Santarém (Pará State, Brazil)

Ocorrência de uma floração de cianobactérias tóxicas na margem direita do rio Tapajós, no Município de Santarém (Pará, Brasil)

Floración de cianobacterias tóxicas en la orilla derecha del río Tapajós, en el Municipio de Santarém (Pará, Brasil)

Lena Lillian Canto de Sá

Laboratório de Microbiologia Ambiental, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

José Maria dos Santos Vieira

Faculdade de Farmácia, Instituto de Ciências da Saúde, Universidade Federal do Pará, Belém, Pará, Brasil

Rosivaldo de Alcântara Mendes

Laboratório de Toxicologia, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Samara Cristina Campelo Pinheiro

Laboratório de Microbiologia Ambiental, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Elivam Rodrigues Vale

Laboratório de Microbiologia Ambiental, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Francisco Arimatéia dos Santos Alves

Laboratório de Microbiologia Ambiental, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Iracina Maura de Jesus

Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Elisabeth Conceição de Oliveira Santos

Diretoria, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Vanessa Bandeira da Costa

Laboratório de Microbiologia Ambiental, Seção de Meio Ambiente, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

## ABSTRACT

The presence of cyanobacterial blooms and their subproducts interferes directly in water quality and may cause negative effects, both aesthetically and to public health, due to the production of potentially toxic and carcinogenic compounds. The most common type of intoxication involving cyanobacteria is caused by microcystin-LR (hepatotoxin), which can cause severe damage to the liver. The objective of this study was to identify the genera that caused cyanobacterial blooms in the Tapajós river (Santarém, Pará, Brazil) in March 2007, as well as to execute acute toxicity bioassays in Swiss-webster mice. Sample collection was performed at five sampling points throughout the left margin of the Tapajós river, by horizontal dragging with the aid of a 20  $\mu$ m plankton net. Samples of raw water (5,000 ml) were also collected in amber propylene bottles. Optical microscopy was applied to identify the organisms, and the determination of microcystin-LR was executed through ELISA and HPLC. The analyses showed that, at P01 and P02, there was an ecological imbalance in the phytoplanktonic community, characterized by an intense proliferation of the genera *Anabaena* and *Microcystis*. The concentrations of microcystin-LR reported in the raw water samples were below the maximum values permitted by Brazil's legislation for drinking water. However, it is important to note that the blooming observed *in loco* occupied around 10 cm of the water column surface and therefore presented cyanobacterial cells enough to cause rashes in people who swam or bathed in the rivers during this period.

**Keywords:** Cyanobacteria; Microcystins; Water Quality.

## Correspondence / Correspondência / Correspondencia:

Lena Lillian Canto de Sá

Instituto Evandro Chagas, Seção de Meio Ambiente

Rodovia BR 316, km 7, s/nº, Bairro: Levilândia

CEP: 67030-000 Ananindeua-Pará-Brasil

E-mail: lenasa@iec.pa.gov.br

## INTRODUCTION

Cyanobacteria are prokaryotic organisms capable of fixing carbon through photosynthesis. As part of the phytoplankton community, they are responsible for a large portion of the primary productivity and energy flow in aquatic ecosystems<sup>17</sup>. These microorganisms inhabit a wide range of environments (freshwater, brackish, marine and terrestrial)<sup>24</sup> and are present in all aquatic biotopes (water/air interface, water column and sediment)<sup>14</sup>.

The presence of cyanobacteria in a body of water is associated with a group of environmental factors (concentration of nitrogen and phosphorus, high temperatures and availability of light) that, when altered, can cause blooming. This phenomenon is characterized by the intense growth of these microorganisms in the water<sup>10</sup>.

The occurrence of blooms has usually been attributed to the accelerated eutrophication process in aquatic environments, which is produced mainly by human activity (domestic and agro-industrial sewage). The presence of cyanobacterial blooms and their byproducts in rivers, lakes and reservoirs along the water supply line directly interferes with water quality. They may potentially add negative aesthetic and organoleptic features, such as undesirable colors, odors and tastes. They may also have a negative impact on public health due to the production of potentially toxic and carcinogenic compounds<sup>7</sup>.

There are several records of poisoning deaths in cattle, horses, pigs, sheep, dogs, fish and invertebrates caused by the ingestion of/contact with these toxic blooms<sup>16</sup>. Another recorded example of the action of these toxins was the death of 60 hemodialysis patients in Caruaru, Pernambuco because of the presence of hepatotoxins in the water<sup>34</sup>.

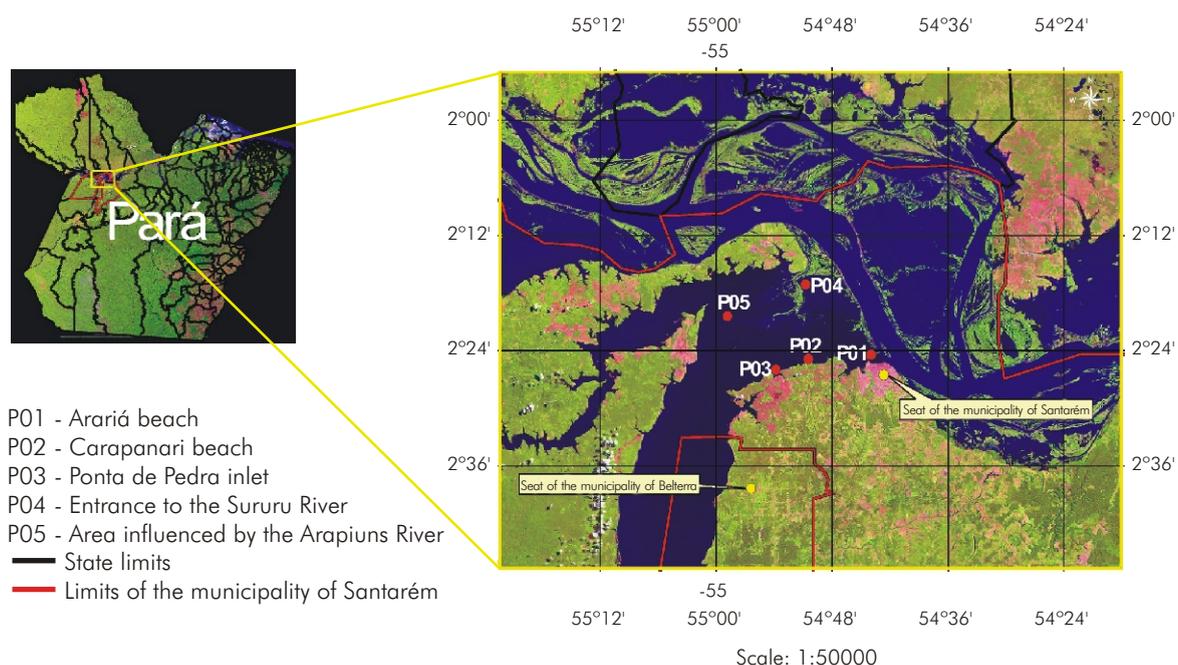
According to Falconer<sup>16</sup>, the toxins produced by cyanobacteria can be divided into neurotoxins, dermatotoxins and hepatotoxins, according to their toxic effects on mammals. The species that have been identified as producing hepatotoxins are included in the genera *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, *Nostoc* and *Cylindrospermopsis*<sup>10</sup>. The species *Microcystis aeruginosa* is thought to have the widest distribution in Brazil, and *Anabaena* is the genus with the highest number of potentially toxic species, according to Carmichael<sup>25</sup>. The most common type of intoxication involving cyanobacteria is caused by hepatotoxins, mainly microcystins (-LR, -LL and -YA), which can cause severe liver damage<sup>23</sup>.

To better understand the problems related to cyanobacterial blooms and water quality in general, our aims in this study were to identify the genera that caused a cyanobacterial bloom along the right shore of the Tapajós River (Santarém, Pará, Brazil), to verify that microcystins were produced by this bloom and to determine the toxicity of these cyanobacteria using toxicity tests in mice.

## MATERIALS AND METHODS

### DESCRIPTION OF THE STUDY AREA AND SAMPLING

Samples were collected on March 21, 2007, at five sites along the right shore of the Tapajós River in Santarém, Pará, Brazil. The sampling sites were: P01 – Arariá beach (54°44'10.28" S, 2°24'33.20" W); P02 – Carapanari beach (54°44'29.66" S, 2°24'55.01" W); P03 – Ponta de Pedra inlet (54°53'43.71" S, 2°26'04.78" W); P04 – in front of the entrance to the Sururu River (54°51'02.36" S, 2°17'06.24" W), which is influenced by the Amazon River at this time of the year; and P05 – the river bed (54°58'44.59" S, 2°20'20.29" W), an area influenced by the Arapiuns River (Figure 1).



Source: LabGeo/IEC/SVS/MS

**Figure 1** – Study area with sampling stations (●) located along the Tapajós River (Santarém, Pará, Brazil)

## METHODS

### Sample collection

The samples used for qualitative study of the cyanobacteria were collected by horizontal hauls on the surface of the water using a plankton net with a 20- $\mu$ m mesh size. Approximately 2,000 L of water were filtered. A 100-mL aliquot was fixed in formalin, and a 250-mL aliquot was refrigerated. We also collected untreated surface water using a 5,000-mL amber-type polypropylene bottle. These samples were stored in a polystyrene box with recyclable ice packs until analysis.

### Species identification

In the laboratory, the samples were analyzed by observing temporary slides under a binocular microscope. The species identification and nomenclature were carried out according to specialized literature<sup>2,3,18,15</sup>.

### Determination of microcystins in the water using an ELISA technique

In the laboratory, a 100-mL aliquot of untreated water was sonicated to promote cell lysis and the release of toxins into the water and then filtered using a Millipore AP20 filter system. A 20- $\mu$ L aliquot of the filtrate was analyzed using the methods described by An and Carmichael<sup>1</sup> with an EnviroLogix Inc. EP-022 kit, according to the manufacturer's instructions. The kit detects microcystin-LR using polyclonal antibodies. All the analyses were performed in duplicate, and the average of the results was considered the sample concentration.

### Determination of microcystins in the water using HPLC

The determination of microcystins in the water samples was performed by extracting 2L of a water sample using solid-phase extraction (SPE) followed by High Performance Liquid Chromatography (HPLC) analysis. The SPE extractions were performed using the methodology proposed by Tsuji et al<sup>28</sup> that consisted of: activating a C18-ODS cartridge with 20 mL of methanol and 20 mL of deionized water; adding, under a vacuum, 2 L of a water sample to the activated C18-ODS cartridge; adding 20 mL of deionized water to "clean-up" the activated C18-ODS cartridge; adding 20 mL of methanol to the activated C18-ODS cartridge to elute the toxin; completely drying the methanol fraction, which contains the toxin, in a rotating evaporator; resuspending the sample in 1 mL of methanol; and filtering the solution through a 0.45- $\mu$ m nylon filter. For the chromatographic analysis of the samples, we used the following conditions for HPLC (Varian): C-18 reverse phase column, ODS, 5- $\mu$ m, 250 mm x 4 mm (Varian); acetonitrile mobile phase: 20 mM ammonium acetate, pH 5.0 (28:72 v/v), flow - 1.0 mL/min; photodiode detection array (PDA) at 238 nm; injection volume 20  $\mu$ L; time of analysis of 30 minutes; and Microcystin-LR pattern from *M. aeruginosa* (Sigma M-2912).

### Determination of microcystins in lyophilized cells using HPLC

After lyophilizing 250 mL of the sample collected by horizontal hauls using a plankton net (20/ $\mu$ m), the lyophilized sample was extracted with butanol:methanol:double deionized water (1:4:15, v/v) in a ratio of 20 mL for each 100 mg of sample, according to the methodology described by Krishnamurthy et al<sup>19</sup> and modified by Domingos et al<sup>13</sup>. The material obtained was agitated for one hour, and the fragmented cells were removed by centrifugation at 3,000 g for ten minutes. The procedure was repeated twice to ensure the total extraction of microcystins. The extraction supernatants were combined, evaporated at 400°C to 30% of their initial volume and passed through an octadecylsilane cartridge (C18) (Bond-Elut Varian). Afterwards, the supernatants were sequentially washed in 20 mL of deionized water and 20 mL of 100% methanol to elute the toxins. The fraction eluted with methanol was collected, evaporated until dry and diluted in 1 mL of 50% methanol. The sample was then filtered using a 0.45- $\mu$ m nylon filter, and the toxins were identified by HPLC using the same chromatographic conditions as for the water samples.

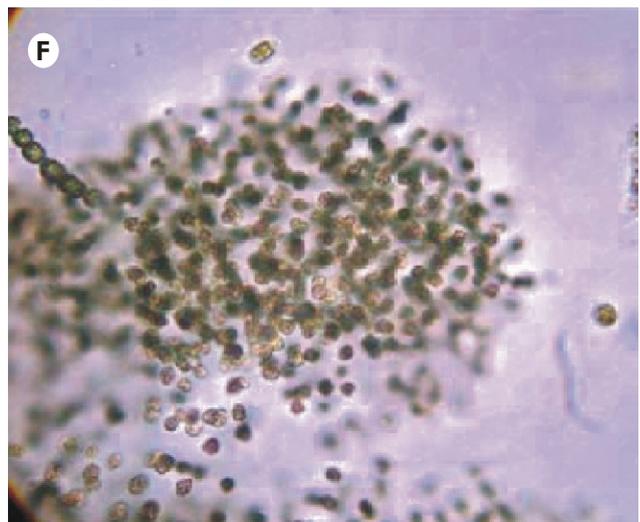
### Bioassays of acute toxicity in mice

The lyophilized material was dissolved in a sterile saline solution and sonicated for five cycles of 30 s at 100 W to release the toxin<sup>22</sup>. Toxicity was determined by intraperitoneal injection of lyophilized cell extract diluted in saline solution, at concentrations of 1,200, 1,000 and 100 mg/Kg, into Swiss-Webster male mice weighing between 20-25 g. The median lethal dose (LD<sub>50</sub>) was determined using five mice for each dose. To obtain the expected concentration we injected 0.1 mL/10 g of mouse body weight of solution into each mouse. The signs of intoxication, survival time and post-mortem examination of the liver confirmed acute toxicity and were used to determine the LD<sub>50</sub><sup>5,6,26,20</sup>. For each concentration tested, two control animals were inoculated with the saline solution used to dilute the extract.

## RESULTS

While collecting samples at the P01 and P02 sites, we observed a clear ecological imbalance in the phytoplankton community, characterized by the appearance of a bloom (Figures 2A and B). The analysis of the samples collected at these sites confirmed the presence of the genera *Anabaena* (Figures 2C and D) and *Microcystis* (Figures 2E e F). The genus *Anabaena* comprises species with coiled trichomes and a worldwide distribution that are commonly found in different bodies of water, especially in eutrophic environments. *Microcystis* is composed of microorganisms with dense mucilage that are recognized for frequently forming surface blooms.

Table 1 presents the results of the analyses performed using direct microscopy and the microcystin quantification by ELISA and HPLC of concentrated (lyophilized) and untreated water samples.



**Figure 2** – Photographic records of the bloom on the shores of the Tapajós River (A and B) and the genera *Anabaena* (C and D) and *Microcystis* (E and F) present in the water. C and E were taken with the 10x objective and D and F with the 40x objective

**Table 1** – Results of the analyses using direct microscopy to identify the cyanobacteria genera, and ELISA and HPLC methods for quantification of microcystins in surface water samples

POINTS	Prevalent genera of cyanobacteria	Type of cyanotoxin produced	ELISA P/ MICROCYSTIN-LR concentrated sample	ELISA P/ MICROCYSTIN-LR untreated water sample	HPLC P/ MICROCYSTIN-LR concentrated sample	HPLC P/ MICROCYSTIN-LR untreated water sample
P01	<i>Anabaena</i> sp.	Microcystin-LR	Positive (higher than 3.0 ppb)	Negative	3.25 µg.g <sup>-1</sup>	0.23 mg/L
P02	<i>Anabaena</i> sp.	Microcystin-LR	Positive (higher than 3.0 ppb)	Negative	12.39 µg.g <sup>-1</sup>	0.55 mg/L
P03	Autochthonous phytoplankton	N/A	Negative	Negative	Not analyzed	Not analyzed
P04	Autochthonous phytoplankton	N/A	Negative	Negative	Not analyzed	Not analyzed
P05	Autochthonous phytoplankton	N/A	Negative	Negative	Not analyzed	Not analyzed

The ELISA for detecting microcystins in the untreated water samples was negative for all sites. The only concentrated (lyophilized) samples that were positive at concentrations above 3 ppb were those from the P01 and P02 sites. The microcystin-LR analysis using HPLC recorded cyanotoxin concentrations of 3.25 µg.L<sup>-1</sup> for P01 and 12.39 µg.L<sup>-1</sup> for P02 in untreated water samples, and 0.23 µg.L<sup>-1</sup> for P01 and 0.55 µg.L<sup>-1</sup> for P02 in concentrated and lyophilized water samples (Table 1).

In the mouse toxicity tests, there were no deaths during or after the seven days of observation at any of the concentrations tested, although the animals showed limited mobility and signals of abdominal contractions within minutes of the application. Thus, these data suggest that the observed blooms did not have sufficient concentrations of toxins to induce acute toxicity that would cause immediate harm to human health.

## DISCUSSION

In Brazil, until the mid-1990s, the relationship between the degradation of water supplies and public health was restricted to water contaminated with the causative agents of waterborne diseases, especially several species of bacteria, protozoa, worms and some viruses. Only after the tragic 1996 deaths of about 60 patients with chronic renal failure who underwent hemodialysis at a clinic in the city of Caruaru, Pernambuco, did authorities realize that another important but often disregarded factor could be responsible for human death via water ingestion: biologically produced toxins, which may be present in the water supply<sup>25</sup>. After this event, blooms of toxic cyanobacteria were recognized as a public health problem and maximum allowable limits of these toxins in the water supply<sup>21</sup> and in multi-use water<sup>12</sup> were established.

The toxic effects of cyanotoxins have received increasing attention from researchers around the world. Microcystins with hepatocarcinogenic effects in mice are the cyanotoxins most frequently found in bodies of water worldwide<sup>4</sup>. In Brazil, the occurrence of toxic cyanobacteria in reservoirs of water intended for human consumption has been observed in several states<sup>5</sup>. In Pará, we have been

following significant events of toxic cyanobacterial blooms since 1999, indicating the alarming nature of this issue. In 1999, during a cyanobacteria monitoring study near the Utinga dam, which supplies the city of Belém, Pará, toxic strains of *Radiocystis fernandoi* and *Microcystis viridis* were found along with the presence of microcystins in untreated water from the dam<sup>31,29,32,30</sup>. During a bloom of *Cylindrospermopsis raciborskii* in the Iriri and Xingu Rivers (Altamira, Pará), there was a large fish die-off, and saxitoxins were found in the water<sup>33</sup>.

In the present study, the concentrations of microcystin-LR found in the untreated water samples from the Tapajós River are below the maximum allowable levels under the Brazilian legislation for water consumption<sup>21</sup>, which discards, at the moment, the possibility of acute poisoning to humans. However, it is important to emphasize that a cumulative effect of these heptapeptides on the body may lead to future health problems.

All genera of cyanobacteria have dermatotoxins (LPS) in their cell walls. These toxins may cause irritation upon contact with the skin<sup>27</sup> as well as eye irritation, conjunctivitis, hives, nasal obstruction and asthma<sup>8</sup>.

Cases of contact dermatitis in humans associated with recreational water use have been reported<sup>9</sup>. Thus, we cannot discard the possibility of eventual skin irritation in persons, especially children, the immunosuppressed and the elderly, who frequent the Arariá and Carapanari resorts, which are among the most visited in the municipality of Santarém, Pará.

The confirmation that the bloom observed on the right shore of the Tapajós River comprised two genera of cyanobacteria (*Anabaena* and *Microcystis*) that potentially produce cyanotoxins calls attention to the need for environmental monitoring in this region to determine the causes and/or origins of these occurrences.

## CONCLUSION

Considering its importance for public health and to improve our understanding of the local biodiversity and the natural and anthropogenic processes that may be related

to these events, which have been occurring year after year in the Tapajós River, it is necessary to monitor the cyanobacteria in these river waters. This necessity arises because, during the study period, we found blooms of two genera of cyanobacteria (*Anabaena* and *Microcystis*) that produce toxins and also detected microcystin-LR in the untreated water. Over the years, these blooms may become increasingly abundant and cause health risks to the local

population who use this water for consumption, fishing and recreation.

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## Ocorrência de uma floração de cianobactérias tóxicas na margem direita do rio Tapajós, no Município de Santarém (Pará, Brasil)

### RESUMO

A presença de florações de cianobactérias e seus subprodutos interfere diretamente na qualidade da água, podendo introduzir efeitos negativos, tanto de ordem estética, como de saúde pública, devido à produção de compostos potencialmente tóxicos e carcinogênicos. O tipo mais comum de intoxicação envolvendo cianobactérias é ocasionado por microcistina-LR (hepatotóxica), a qual pode causar severos danos ao fígado. Assim, o objetivo deste estudo foi identificar os gêneros causadores de uma floração de cianobactérias no rio Tapajós (Santarém, Pará, Brasil) no mês de março de 2007, bem como realizar bioensaios de toxicidade aguda, utilizando camundongos *Swiss-webster*. As amostragens foram realizadas em cinco pontos de coleta distribuídos na margem direita do rio Tapajós, onde foram realizados arrastos horizontais, com o auxílio de uma rede para plâncton de 20  $\mu\text{m}$ ; e foram também coletadas amostras de água bruta (5.000 mL) em garrafas de polipropileno do tipo âmbar. Para a identificação dos organismos utilizou-se microscopia ótica. A determinação de microcistinas-LR foi realizada por meio das técnicas de Ensaio Imunoadsorvente Enzima-Associado e Cromatografia Líquida de Alta Pressão. As análises demonstraram que na altura dos pontos P01 e P02 ocorreu um desequilíbrio ecológico na comunidade fitoplanctônica, caracterizado pela proliferação intensa dos gêneros *Anabaena* e *Microcystis*. Nas amostras de água bruta, as concentrações de microcistina-LR registradas estão abaixo dos valores máximos permitidos na legislação brasileira para água de consumo; entretanto, é importante ressaltar que a floração, visualizada *in locu*, ocupava cerca de 10 cm da superfície da coluna d'água, e que, portanto, continha células de cianobactérias suficientes para provocar irritações cutâneas em pessoas que usassem o rio como balneário nesse período.

**Palavras-chave:** Cianobactérias; Microcistinas; Qualidade da Água.

## Floración de cianobacterias tóxicas en la orilla derecha del río Tapajós, en el Municipio de Santarém (Pará, Brasil)

### RESUMEN

La presencia de floraciones de cianobacterias y de sus subproductos afecta directamente a la calidad del agua, pudiendo producir impactos negativos tanto estéticos como de salud pública, debido a la producción de compuestos potencialmente tóxicos y cancerígenos. El tipo más común de intoxicación involucrando cianobacterias se debe a la microcistina LR (hepatotóxicas), que puede causar graves daños al hígado. El objetivo de este estudio fue identificar el género causante de la floración de cianobacterias en el río Tapajós (Santarém, Estado de Pará, Brasil) en marzo de 2007, así como realizar bioensayos de toxicidad aguda utilizando ratones *Swiss-Webster*. Las muestras se tomaron en cinco puntos de muestreo, distribuidos en la margen derecha del río Tapajós, donde fueron realizados arrastres horizontales, con la ayuda de una red de plancton de 20  $\mu\text{m}$  y fueron recogidas también muestras de agua cruda (5.000 ml) en botellas de polipropileno color ámbar. Para la identificación de los organismos se utilizó la microscopía óptica, y la determinación de microcistina LR se realizó por medio de las técnicas de ELISA y HPLC. El análisis puso de manifiesto que a la altura de los puntos P01 y P02 ocurrió un desequilibrio ecológico en la comunidad de fitoplancton caracterizado por la proliferación de los géneros *Anabaena* y *Microcystis*. En las muestras de agua cruda, las concentraciones de microcistina LR registradas, están por debajo del valor máximo permitido en la legislación brasileña para el agua potable, aunque es importante destacar que la floración vista *in situ* ocupaba cerca de 10 cm de la superficie de la columna de agua y que, por lo tanto, contenía suficientes células de cianobacterias para ocasionar erupciones cutáneas en las personas que pudieran utilizar los bañarse en los ríos en ese período.

**Palabras clave:** Cianobacterias; Microcistinas; Calidad del Agua.



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