

## ***Dominance of potentially toxic cyanobacteria on the waterfront of Santarém, Tapajós River, Brazilian Amazon***

The present study carried out an evaluation of physicochemical parameters of waters from the low Tapajós river, and the identification of dominant cyanobacteria in the blooms that occurred in a transect bordering the Santarém city waterfront, in the State of Pará, Brazilian Amazon. Five (5) sampling stations were selected along the waterfront (RT-E1, RT-E2, RT-E3, RT-E4, RTE5), where water, phytoplankton and general environmental data were collected during 10 field campaigns, at intervals of 48 hours, between January 13th and 31st 2015, at the very beginning of the rainy season. The sample collections were conducted at an average depth of 2.3 meters, always early in the morning and following the procedures of the Collection and Preservation Guide and Water Samples of the Brazilian National Water Agency. Quantitative analyses of the physicochemical parameters were performed, namely: hydrogenionic potential (pH); water temperature (°C); turbidity (NTU); electrical conductivity (EC); Dissolved Oxygen (DO); nitrate (NO-3); nitrite (NO-2); ammonium ion (NH4); ammonia (NH3); orthophosphate (PO43-); phosphate (P2O5); phosphorus (P); iron (Fe) and chlorophyll a (Chlo a). General descriptive statistics were calculated. For counting of individuals, the efficiency was superior to 80%, reaching a total of 3,910 individuals, from which 38% are species of the Cyanophyta division. The following species were dominant: *Synechocystis aquatilis*, *Microcystis aeruginosa*, *Dolichospermum spiroides*, *Nostoc* sp. Except for *S. aquatilis*, all the others are cyanotoxin producers that can be released into the water after cell disruption. The presence of cyanobacteria potentially producing cyanotoxins is of concern and should be considered in the diagnosis of waterborne diseases, such as hepatic infections that affect the riverine population of the Tapajós river.

**Keywords:** Physicochemical parameters; the Bloom; Cyanotoxins; South America.

## ***Domínio de cianobactérias potencialmente tóxicas na beira do Rio de Santarém, Rio Tapajós, Amazônia Brasileira***

O presente estudo realizou uma avaliação dos parâmetros físico-químicos das águas do baixo rio Tapajós e a identificação de cianobactérias dominantes nas flores que ocorreram em um transecto na orla da cidade de Santarém, no estado do Pará, Amazônia brasileira. Cinco (5) estações de amostragem foram selecionadas ao longo da orla (RT-E1, RT-E2, RT-E3, RT-E4, RTE5), onde foram coletados dados de água, fitoplâncton e dados ambientais gerais durante 10 campanhas de campo, em intervalos de 48 horas, entre 13 e 31 de janeiro de 2015, bem no início da estação chuvosa. As coletas de amostras foram realizadas a uma profundidade média de 2,3 metros, sempre de manhã e seguindo os procedimentos do Guia de Coleta e Preservação e Amostras de Água da Agência Nacional de Águas. Foram realizadas análises quantitativas dos parâmetros físico-químicos, a saber: potencial hidrogeniônico (pH); temperatura da água (°C); turbidez (NTU); condutividade elétrica (CE); Oxigênio Dissolvido (DO); nitrato (NO-3); nitrito (NO-2); íon amônio (NH4); amônia (NH3); ortofosfato (PO43-); fosfato (P2O5); fósforo (P); ferro (Fe) e clorofila a (Chlo a). Estatísticas descritivas gerais foram calculadas. Para a contagem de indivíduos, a eficiência foi superior a 80%, atingindo um total de 3.910 indivíduos, dos quais 38% são espécies da divisão Cyanophyta. As seguintes espécies foram dominantes: *Synechocystis aquatilis*, *Microcystis aeruginosa*, *Dolichospermum spiroides*, *Nostoc* sp. Com exceção de *S. aquatilis*, todos os outros são produtores de cianotoxinas que podem ser liberadas na água após a ruptura das células. A presença de cianobactérias potencialmente produtoras de cianotoxinas é preocupante e deve ser considerada no diagnóstico de doenças transmitidas pela água, como infecções hepáticas que afetam a população ribeirinha do rio Tapajós.

**Palavras-chave:** Parâmetros físico-químicos; Bloom; Cianotoxinas; América do Sul.

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## INTRODUCTION

The excess of nutrients in aquatic ecosystems, particularly nitrogen and phosphorus, resulting from their use as fertilisers in the agriculture, untreated sewage discharge, erosion, high rates of urbanisation, and lack of basic sanitation, among other factors, lead and/or intensify the bloom of algae in waterbodies, in a process known as cultural eutrophication (PAPPAS et al., 1996). Among the algae that form the bloom in fresh and brackish waters, we find some species of cyanobacteria which are vertical migrants and floaters, and thus may dominate the bloom whenever the water column stabilises (ESTEVEZ, 2011).

The Phylum Cyanobacteria Stanier ex Cavalier-Smith, according to Algaebase, there are over 4700 names (ANA, 2012), is a typical representative of the diverse species-rich group of microorganisms distributed environments worldwide. They are photosynthetic organisms and primary producers as eukaryotic algae, but do not hold cellular content and membrane-delimited structures, resembling to bacteria (SIOLI, 1991). Under normal conditions, the cyanobacteria are less efficient in terms of nutrient assimilation than microalgae (green or diatomaceous). However, a higher offer of nutrient facilitates the sudden assimilation and growth under the form of bloom (HUSZAR et al., 2000; AZEVEDO et al., 2003).

The presence of the bloom with dominance of cyanobacteria directly interferes on the water quality, with negative effects for public health due to the production and liberation of potentially toxic and carcinogenic compounds (cyanotoxins). In Brazil, the most notorious case of cyanotoxin contamination occurred in Caruaru, State of Pernambuco, where the presence of microcystins-LR (MCLR) was detected, which is an hepatotoxin that can cause severe damage to the liver tissue. The MCLR was present at the Tabocas Local Water Reservoir (*açude*), which provided water to the reservoir of the Institute of Renal Diseases of Caruarú City, and cause the death of 70 haemodialysis patients back in 1996 (MATTHIENSEN et al., 1999). This case became known as 'The Caruarú Case' and was preponderant for the bloom of toxic cyanobacteria in waters to considered as a serious public health issue, which in turn was further regulated by the Brazilian National Council for the Environment (CONAMA) through the Resolution #357/05 (MMA, 2005) as well as the Ministry of Health Instruction 2.914/2011, turning mandatory the monitoring of cyanobacteria at sites of surface water catchments (MMA, 2005), and recommends that the chlorophyll  $\alpha$  parameter be weekly monitored for re-evaluation of the cyanobacteria sampling frequency.

The dominance of cyanobacteria in aquatic environments depends on the coincidence of four main factors: phosphorus concentrations higher than 10 mg. L<sup>-1</sup>, temperatures above 20 °C, stability of the water column, and pre-existing populations that contain vesicles (known as aerotopic), which allow the fluctuation and permanence of cyanobacteria on the water surface (AZEVEDO et al., 2003).

The Tapajós River, one of the most important tributaries of the Amazon River, presents these factors, and even though in their headwaters the values of the measured Trophic State Index (TSI) follow the limits allowed by the CONAMA Resolution # 357/05 (being the maximum of 50.000 cells of cyanobacteria per 1mL.L<sup>-1</sup> for waters class 2 as the ones of the Tapajós), a huge the bloom of cyanobacteria has been observed over the last years on the shores of this large river, in front of the City of Santarém, western region of the State of

Pará. In 2010, a the bloom of around 5 kilometres long occurred, which was documented by and by the Water Quality Project . The analysis of this the bloom confirmed the presence of two genera of cyanobacteria that are potentially toxin producers (*Dolichospermum* and *Microcystis*), which in turn obliged the State Sewage Company to avoid catching waters of the river, instead using underground water for consumption of the population.

Indeed, natural and anthropogenic variables contribute to patterns of variation that reflect on the algae population's cycles (REBOUÇAS et al., 2006). Human activities have been causing an increasing artificial enrichment of aquatic ecosystems by such nutrients as nitrogen and phosphorus that come from different sources: the use of agricultural fertilisers, the discharge of industrial and domestic sewage without adequate treatment, erosion caused by destruction of riparian forests, high rates of urbanisation and lack of basic sanitation (CARVALHO et al., 2008).

Since the availability of these nutrients in association with environmental factors like radiation, temperature and water pH, and with anthropic factors, such as, occupation of the physical environment and socioeconomic activities, all influence water quality and phytoplankton productivity, it is important and necessary to distinguish the natural characteristics of the waterbodies from those caused and changed by human actions (ESTEVEZ, 2011). Thus, the present study assessed physicochemical parameters of waters from the low Tapajós river, and identified dominant cyanobacteria in a the bloom that occurred in a transect that covers the waterfront of the City of Santarém, western Pará, in the Brazilian Amazon.

## **METHODOLOGY**

### **Study area**

The municipality of Santarém has 294,580 inhabitants, an area of 22,887 square kilometres and a demographic density of 12.8 inhabitants per square kilometres (IBGE, 2010). Its annual mean temperature is 27.7 °C, and its rainfall is of 2,200 millimetres per annum. Assuming the Köppen-Geiger climate classification, Santarém is classified as Am, with more rainy periods comprised between the months of December to June, and less rainy from July through November (MIRANDA et al., 2009).

This city is located on the right bank of the Tapajós river (2°25'30" southern latitude and 54°42'50" western longitude). The waterfront is one of its postcards, where one can see the meeting of the waters from the Amazon and Tapajós. In this area, there are daily activities of tourism, leisure, commerce, port and aquatic activities that were all crucial at the time of selecting the sampling sites on the low Tapajós river over a transect that covers the city's waterfront (Fig. 1).

The year of 2015 was subject to the phenomenon of *El Niño*, and there was a delay of the rainy season, which commenced only at the second half of January. Two months earlier (November and December 2014), it was possible to carry out the environmental recognition of the study area, with previous visits to the waterfront of the Tapajós river and photographic documentation and preparation of a waterfront sketch, at the same time identifying points of sewage discharge from commercial and residential sources. At some

points these open-air sewers run with more intense fluxes, dragging waste that is dumped untreated, directly into the river. Thus, the choice of water sampling stations was made due to the port activity and magnitude of the nearby sewage points, considering the magnitude of the sewage size on a scale of 1 to 5, with 1 being smaller and 5 maximum magnitude (Table 1).

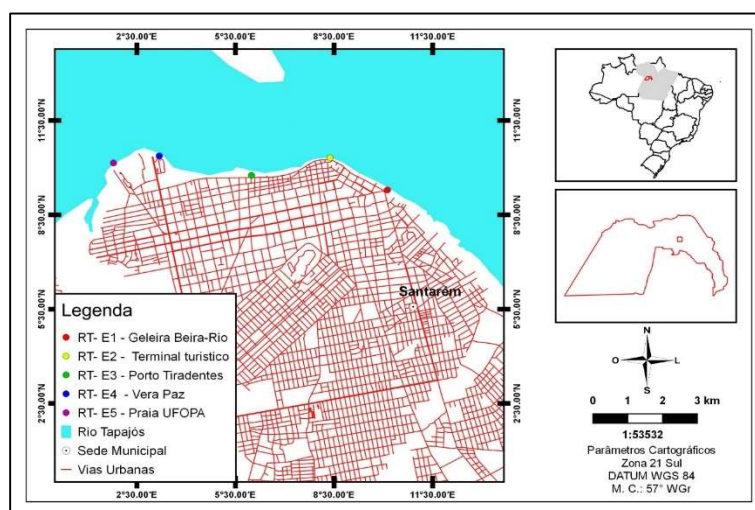


Fig. 1: Map of the transect location on the border of the Municipality of Santarém (Produced using the. Quantum GIS Software, Version 2.14).

Table 1: Characterisation and location of sampling sites.

STATION	REFERENCE	DESCRIPTION
Tapajós River Station 1 RT- E1	S 02º 25' 14.4", W 054º 42' 12.1"	Fishing port and ice supply. It has 3 points of medium size sewers discharge (magnitude 3)
Tapajós River Station 2 RT- E2	S 02º 24' 56.5", W 054º 42' 42.3"	River tourist terminal. It has 2 discharge points of large sewage (4)
Tapajós River Station 3 RT- E3	S 02º 24' 56.5", W 054º 42' 42.3"	Right side of the Tiradentes Port. Greater port activity. 1 sewer of greater magnitude (5)
Tapajós River Station 4 RT- E4	S 02º 24' 55.3", W 054º 44' 11.7"	Vera Paz Port, near the Cargill Port. It has 2 points of large sewer discharge (4)
Tapajós River Station 5 RT- E5	02º 24' 59.3", W 054º 44' 35.8"	UFOPA beach, reform of boats and rafts. It has 2 discharge points of small to medium sewage (2)

### Sample collection and storage procedures

Ten water sampling campaigns were conducted at the beginning of the rainy season, with a 48-hour interval between campaigns, and performed at an average depth of 2.3 meters, always in the morning and following the procedures described in the Collection Guide and Preservation of Water Samples from the Environmental Technology and Sanitation Company of the National Water Agency (ANA, 2012; APHA, 1915).

After a thorough review of the literature, 15 physicochemical parameters of the water were selected for analysis within the present study (ESTEVEZ, 2011; AZEVEDO et al., 2003; GENTIL et al., 2008; MIRANDA et

al., 2009; SÁ et al., 2010) namely: dissolved oxygen concentration (DO mg/L); Dissolved oxygen saturation (DO%); Hydrogenation potential (pH); Water temperature (°C); Turbidity (NTU); Electrical conductivity (EC), nitrate (NO<sub>3</sub>); Nitrite (NO<sub>2</sub>); Ammonium ion (NH<sub>4</sub>); Ammonia (NH<sub>3</sub>); Orthophosphate (PO<sub>4</sub><sup>3-</sup>); Phosphate (P<sub>2</sub>O<sub>5</sub>); Phosphorus (P); Iron (Fe) and chlorophyll a (Chlo α). These parameters were analysed quantitatively, except for phytoplankton that was also analysed qualitatively.

For the quantitative study, the samples were collected using a vertical Van Dorn acrylic bottle (3 liters), 50 cm from the bottom and at the base of the euphotic zone, composing integrated samples. They were then stored in polyethylene bottles of 300 mL, one sample from each station fixed in 5% Lugol and the rest refrigerated at 4° C for nutrient analysis.

In the RT-E2 and RT-E4 sample stations (Table 1), 600 mL were stored in dark polyethylene bottles, which after arrival in the laboratory were filtered through a glass fiber filter (47 mm of diameter with porosity between 0.45 and 1.0 μm) for phytoplankton compaction. The filters of the samples and replicates were duly waterproofed and frozen in the freezer of the Laboratory of Environmental Chemistry of UFOPA – Tapajós Campus.

Samples for the qualitative analysis of phytoplankton were collected with plankton netting with 20 μm mesh, which was drawn horizontally and fixed in 4% formalin solution. The physical parameters were measured *in loco*, attesting values for: hydrogenation potential (pH - MS pH TECNOPON®); Water temperature (T° - YSI® probe); Electrical conductivity (EC - Metalyser® conductivity meter); Concentration of dissolved oxygen (OD-LOVIBOND Oximeter) and turbidity (NTU -Turbidimeter POLICONTROL®).

### Sample analyses

In the laboratory of Applied Chemistry (Institute of Water Science and Technology, Federal University of Western Pará - ICTA/UFOPA), the nutrient analysis was carried out, according to *Standard Methods*, using multi-parametric and photometric probe (HANNA Instruments model HI 830999), set at 25 °C, following the sample preparation method and using reagents described in the manufacturer's guide, for the analysis of each nutrient, values were obtained: NO<sub>3</sub>; NO<sub>2</sub>; NH<sub>4</sub>; NH<sub>3</sub>; PO<sub>4</sub><sup>3-</sup>; P<sub>2</sub>O<sub>5</sub>; P and Fe.

For the counting of the cyanobacteria, the samples fixed in Lugol 5% and the method of (UTERMÖHL, 1958) for sedimentation were used, following to an inverted Zeiss Axiovert 125 microscope, where each cell, colony, colonnade or filament was considered as an individual (GENTIL, 2008). For the counting of the number of fields, 80% efficiency was accepted, by the method of (PAPPAS et al., 1996), according to the equation 1:

$$Efficiency (\%) = 1 - \frac{Number\ of\ Species}{Number\ of\ Individuals} \quad (1)$$

For the determination of Chlorophyll a, filters and frozen replicates were used, where the compacted phytoplankton was analysed by the methodology described by modified by (STRICKLAND et al., 1965) and (TEIXEIRA, 1973), and available in. In the spectrophotometer, the optical densities were determined in the wavelengths, aiming to obtain the result of the total photosynthetic pigment or chlorophyll a calculated by

the following equation 2:

$$chlo \alpha = \frac{11,6 \times D_{665} - (1,31 \times D_{645} + 0,14 \times D_{630} + D_{750}) \times V_1}{V_2 \times L} \quad (2)$$

where:

11.6; 1.31 and 0.14 are constants;

D - Absorbance readings at wavelengths 665 nm, 645 nm, 630 nm and 750 nm;

V1 - 90% acetone volume (0.01 L or 10 mL);

V2- volume of the filtered sample in litres (0.3 L or 300 mL);

L- Bucket optical path (1 cm).

For the taxonomic analyses, slides and coverslips were prepared. By using a binocular microscope Zeiss Axio with photographic camera coupled to the optical system, we took photomicrographs to assist in the identification of cyanobacteria species. The results obtained in the analysis of the physicochemical parameters and of chlorophyll 'a' were analysed by descriptive statistics and using the Excel Software Version 2013 of the Microsoft Office Package.

## RESULTS AND DISCUSSION

### Environmental characteristics of study area

Table 2 presents descriptive statistics for the physicochemical parameters assessed during the 10 field campaigns conducted throughout the study (n=50 samples), in addition to values found for Chlorophyll a, the latter only for the samples from the stations RT-E2 e RT-E4 (Table 2).

**Table 2:** Descriptive statistics of water physicochemical variables on the studied transect, Waterfront of Santarém (Pará).

Variable	RT-E1			RT-E2			RT-E3			RT-E4			RT-E5		
	Min - Max	Mean	SD	Min - Max	Mean	SD	Min - Max	Mean	SD	Min - Max	Mean	SD	Min - Max	Mean	SD
NO <sub>3</sub> (mg.L <sup>-1</sup> )	1,9-10,5	6,83	2,9	1-15,9	7,15	5,11	4,6-15	9,67	4,11	4,8-15,1	8,37	3,49	0,2-17,8	7,51	5,78
NO <sub>2</sub> (mg.L <sup>-1</sup> )	0,02-0,08	0,03	0,0	0,02-0,11	0,04	0,03	0,02-0,06	0,04	0,01	0,02-0,05	0,04	0,01	0,03-0,06	0,04	0,01
NH <sub>4</sub> (mg.L <sup>-1</sup> )	0-0,73	0,42	0,2	0,38-0,59	0,49	0,09	0-0,66	0,40	0,24	0,39-0,93	0,51	0,16	0-1,91	0,53	0,51
NH <sub>3</sub> (mg.L <sup>-1</sup> )	0-0,69	0,39	0,1	0,35-0,56	0,47	0,08	0-0,62	0,37	0,22	0,37-0,88	0,48	0,15	0-1,8	0,51	0,48
P <sub>034</sub> (mg.L <sup>-1</sup> )	0,4-6,5	1,71	1,8	0,3-2,3	0,93	0,77	0,5-3,6	1,46	1,15	0,4-1,8	0,94	0,43	0-8	1,87	2,44
P <sub>205</sub> (mg.L <sup>-1</sup> )	0,3-4,9	1,29	1,3	0,2-1,7	0,68	0,56	0,4-2,7	1,11	0,86	0,3-1,4	0,69	0,33	0-6	1,39	1,83
P (mg.L <sup>-1</sup> )	0,1-2,1	0,55	0,6	0,1-0,8	0,3	0,27	0,2-1,2	0,49	0,38	0,1-0,6	0,3	0,15	0-2,6	0,6	0,80
Fe (µg.L <sup>-1</sup> )	106-400	186,5	97,7	48-380	188,3	100,9	132-381	227,3	88,7	70-388	191,5	92,5	85-392	195,1	93,5
T (°C)	28-29	28,6	0,3	26,8-29,2	28,5	0,73	28,4-29,3	28,8	0,28	28,5-29,7	29,1	0,35	28,2-29,9	29,2	0,49
CE (µS.cm <sup>-1</sup> )	12,8-31,6	19,3	6,1	6,7-17,3	14,2	2,96	7,1-22,4	14,4	4,88	6,7-16,1	13,4	3,60	6,8-16,1	13,0	3,41
Turbidity (NTU)	9-23	14,1	4,8	8-16	10	2,31	10-18	12,9	2,56	9-26	12,3	5,21	9-17	11,1	2,33
pH	5,44-7,35	6,13	0,7	5,52-7,53	6,17	0,69	5,66-6,97	6,11	0,41	5,68-6,75	6,09	0,38	5,46-6,53	6,00	0,35

<b>OD</b> (mg.L <sup>-1</sup> )	0,1- 13,1	3,92	5,7	0,1- 11,2	1,38	3,45	0,1- 1,1	0,49	0,36	0,3- 14,2	2,06	4,27	0,1- 1,1	0,61	0,28
<b>DO (%)</b>	17- 32,2	22,7	5,5	17,4- 41,1	24,7	7,38	16,9	24,9	6,24	15,5- 93,6	30,5	22,6	14,8	21,6	5,01
<b>CHLO-α</b> (μg.L <sup>-1</sup> )				0,424	0,78	0,36				0,579	1,60	2,24			
				-						-					
				1,621						7,910					

When analysing the concentrations found for nitrogen and phosphate nutrients, we observe they are below the reference values determined by the Brazilian National Council for the Environment (CONAMA), according to Resolution # 357/2005, however phosphorus is a growing concern, as it is related to the eutrophication of waters. Table 3 presents some comparative values between the averages found and the reference values.

**Table 3:** Comparison of the analysed nutrients with regulatory limits.

NUTRIENT	REFERENCE VALUE <sup>1</sup>	MEAN VALUE FOUND
Total phosphorus (P)	0,1 mg/L	0,448 mg/L
Orthophosphate (PO <sub>4</sub> <sup>3-</sup> )	1 a 5mg/L	1,382 mg/L
Nitrate (NO <sub>3</sub> <sup>-</sup> )	10,0 mg/L	7,99 mg/L
Nitrite (NO <sub>2</sub> <sup>-</sup> )	1,0 mg/L	0,014 mg/L

<sup>1</sup>According to CONAMA Resolution #357/2005.

According to the Water Sample Collection and Preservation Guide, it is not recommended to take samples close to the banks of rivers, channels and at the point of dumping, except when these regions are of specific interest. In this case, the identification of the sampling site must take into account the existence of industrial and/or domestic liquid effluents, as well as the presence of tributaries in the area of influence of the sampling point, as these may alter the water quality of the sampling point. body.

In RT-E3, note that the nitrate values are always higher, precisely because of the contamination of the supply of organic matter from domestic sewage in the urban area and from vessels.

The Tapajós River has availability of nutrients and favorable climate, influenced by periods of flood and ebb and by not having significant differences in water depth and temperature between the collection stations. The average value of the water temperature was 28.9 ° C, ideal for cyanobacteria, which in general have a higher tolerance to high temperatures, when compared with other algae.

In the newly formed domestic sewage, the predominant form of orthophosphate is HPO<sub>4</sub><sup>-</sup>, which mostly originates from the phosphates, but as the sewage ages the orthophosphate takes the form of PO<sub>4</sub><sup>3-</sup>, which is more available to aquatic organisms. On the other hand, particulate organic matter contains about 70% or more of the total organic phosphorus, whereas the rest is in dissolved or colloidal form, and its distribution is influenced by biotic factors (e.g., activity of autotrophic and heterotrophic organisms) as well as abiotic factors such as pH and temperature (ESTEVEZ, 2011).

Due to the lack of significant differences in depth, and assuming there were no large variations in the water temperature between the collection stations, the average temperature was 28.9 °C, ideal for cyanobacteria, which are generally more tolerant to high temperatures when compared to other algae.

The pH values throughout the seasons varied from 5.4 to 7.5, which are justified in a scenario of the

blooms, high intensity of sunlight and the influence of the Amazon River that sometimes invades the studied area. According to (SIOLI, 1991), the water chemical analyses give indications about the soil types and the cationic layer of the clay fraction of the suspended material.

Turbidity in the studied transect showed a variation between 8 NTUs (Nephelometric Turbidity Unit) in RT-E2 and 26 NTUs in RT-E4, averaging 12.1 NTUs, and the RT-E4 station sheltering the Cargill bulk carrier port. These grains are dumped from a high conveyor belt, and during their launch into the holds of the ships they are aerated by the constant wind of the Santarém waterfront. At that moment, films and particles of these grains are dissipated, composing a dust that is deposited on the adjacent surface waters. Also, near this RT-E4 sampling station is located the State of Pará Port Authority as well as sewage and vegetation from the former Vera Paz beach area. All these elements result in the collection and accumulation of suspended particles that may explain the variation of the turbidity found in this and previous studies, such as the one by (MIRANDA et al., 2009) in the same area and period, where 76.4 NTUs were obtained, very different from the value found by (ABDO et al., 1997), which was 6.5 NTUs. All these values were below the 100 NTUs allowed by CONAMA Resolution 357/2005.

The maximum value of electrical conductivity recorded on RT-E1 was  $31.6 \mu\text{S}\cdot\text{cm}^{-1}$ , within the allowed range for natural freshwater, which should be less than  $500 \mu\text{S}\cdot\text{cm}^{-1}$ . The electrical conductivity is directly related to the concentration of dissolved ions, mainly inorganic. RT-E1 is the station with the highest dissolution power because it is in the most open part of the river mouth, and this station also has the lowest flow of vessels, receiving fishing boats that harbor for ice supply.

For dissolved oxygen, low values were found, such as a minimum of  $0.11 \text{ mg}\cdot\text{L}^{-1}$ , registered on RT-E3 that receives direct influence from the Tiradentes harbour, where there is the largest point of sewage, in addition to the continuous disposal of waste *in natura* from the toilets of riverboats arriving and departing daily with passengers to neighbour municipalities, to the State capital of Belém and to other states. The CONAMA Resolution 357/2005 establishes the maximum limit for dissolved oxygen of  $5 \text{ mg}\cdot\text{L}^{-1}$ , but in the samples collected on the Tapajós river transect bordering the Santarém waterfront, the mean dissolved oxygen concentration was  $1.69 \text{ mg}\cdot\text{L}^{-1}$ , indicating water pollution in the area, at least up to the studied depth.

It is common to observe that different factors may act together to contaminate areas similar to our study site, such as the sewers of the urban collecting network; erosion and deforestation, as well as the remains of public roads that are carried by the rainwater to the river (SANTOS et al., 2014); waste, sanitary waste from riverboats and garbage as well as wastes from the sinks of vessels that can act as organic and inorganic pollutants (MIRANDA et al., 2009); in addition, during the supply of ships with grain in the bulk carrier port of the multinational, a powder is loaded by the wind and deposited on the waters. This 'soybean dust' is a mixture of grain films, straws and other residues that could contribute to the nutrient supply at the RT-E4 station. The nutrient load, resulting from all these factors, is discharged into the low Tapajós river, in front of the Santarém waterfront, without any treatment, and we strongly believe that it may play an important role in influencing the occurrence of the blooms in this area. For, there is evidence that the flowering in lotic environments in Brazil is due to the association of extensive summer periods, in which the absence of rainfall



causes a low water flow rate, together with an ideal balance of nutrients that revolve in the first rains and, to the elevation of the temperature, providing conditions favorable to the occurrence of cyanobacteria the blooms.

During the low water period, the water level and flow decreases, reducing the dissolution capacity of this organic matter. Therefore, after the maximum point of the drought, in November (Fig. 2), there is a residue of this load that settled and was accumulated in the bottom, being revolved as soon as the rainy season begins, over the months of December or January, as observed in 2015. With the gradual increase of the water level of the low Tapajós river, the sediments and organic matter are revolved and the nutrients made available, and this contributes to the increase of the phytoplankton productivity and the appearance of the blooms that fill the spaces between the riverboats and port areas, modifying the landscape of the border of Santarém. Fig. 2 at RT-E4 station, where Cargill's bulk carrier port and the former Vera Paz beach are currently located.

The values marked on the ruler of the Brazilian Navy in Santarém for the years between 2005 and 2014 demonstrate how the level of the low Tapajós river at the mouth follows the pluviometric pattern, coinciding the peak of the flood (high waters) with the period of higher rainfall intensity, and the peak of drought (low water) with the lowest volume of rainfall in the region (Fig. 3). However, due to climatic differences in the distribution of rainfall in the Tapajós river basin, the mouth presents the highest percentage of headwater, and this differentiation of rainfall and drainage network behaviour in the basin (SANTOS et al., 2014) associated with a pattern of land use and occupation tends to aggravate the consequences of the less rainy season, especially in the waterfront of Santarém.



Fig. 2: The RT-E4 Station area during the dry season.

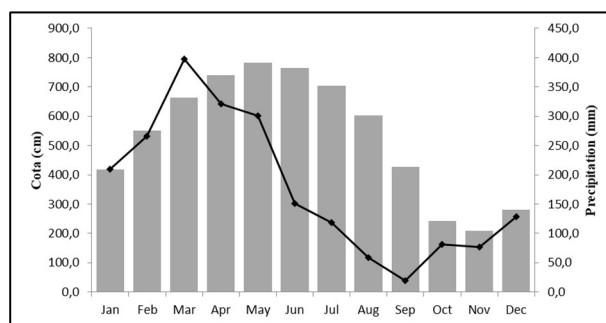


Fig. 3: Monthly means of Cota and precipitation on the Tapajós River (2005-2014). **Source:** Brazilian Navy Office in Santarém for the Cota; Station 82246 of the Brazilian National Institute for Meteorology (Belterra Municipality) for (precipitation).

According to (MATTHIENSEN et al., 1999), the bloom result from the interaction of physical, chemical and biological factors, and they are characterised by an explosive growth of the microorganisms of one or a few species, which although self-limiting and of short duration, often produce visible colorations on the natural waterbodies, generating problems that may range from landscape aesthetics to serious public and environmental health problems. As concerns phytoplankton algae, it is important to emphasize that their presence in freshwater is an important element for the evaluation of environmental conditions (GENTIL, 2008), since the low values of dissolved oxygen (DO) in water show a tendency for eutrophication and greater

potential for the bloom of algae, especially cyanobacteria. Dissolved oxygen also influences the increase of iron (Fe) values in water, and according to (MIRANDA et al., 2009) the decrease of oxygen dissolved in water increases the reducing tendency of the medium, according to the equilibrium shown in equation 3:



The values found for iron varied between 48 and 400  $\mu\text{g. L}^{-1}$ , different from the ones found by (MIRANDA et al., 2009) that varied from 4.77 to 15.91  $\mu\text{g. L}^{-1}$ , showing an increase in the studied perimeter in recent years states that in surface waters the level of iron increases during the rainy season due to the land cover and the occurrence of erosion processes of the banks. The presence of industrial effluents and/or industrial products used in activities to remove the oxidised layer (rust) also contributes to this increase.

Thus, the mean values found were higher at the RT-E3 station (227.3  $\mu\text{g. L}^{-1}$ ), where is located the Port of Tiradentes with greater flow of port activity and the largest sewage. In addition, iron in the presence of organic matter dissolved in the surface waters may have its complexes altered and its solubility increased, presenting higher values. The second highest mean value was recorded at the sampling station RT-E5 (195.1  $\mu\text{g. L}^{-1}$ ), where boat and ferry reform activities are carried out with the use of chemical products, such as solvents (Fig. 4). These products come into contact with the water and their packaging is thrown on the beach, contaminating the sand and consequently the river when loaded with rainwater.



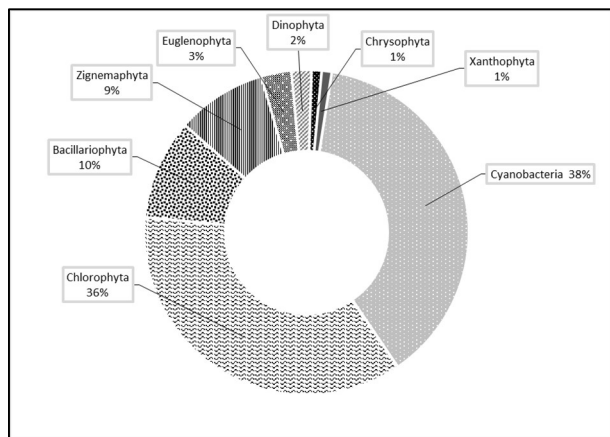
**Fig. 4:** The RT-E5 station area with open-air yards.

### Phytoplankton Community Analyses

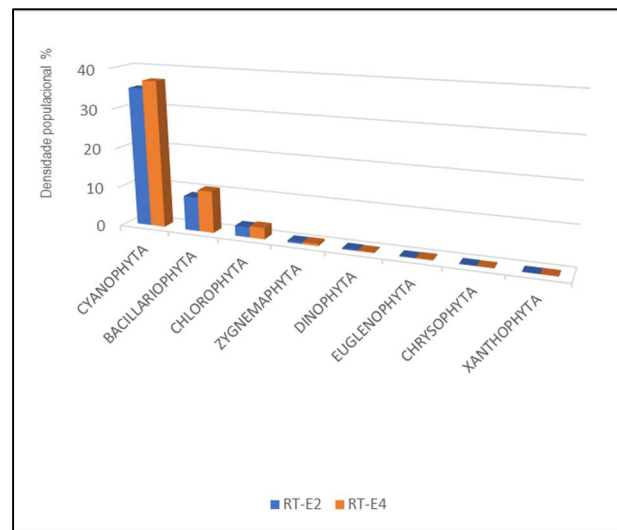
We found 103 taxa, distributed into 8 taxonomic classes or divisions, as follows: Cyanophyta (39); Chlorophyta/Chlorophyceae (36); Bacillariophyta/Bacillariophyceae (10); Zignemaphyta/Zygnemaphyceae (08); Euglenophyta/Euglenophyceae (04); Chrysophyta/Chrysophyceae (03); Dinophyta/Dinophyceae (02); Xanthophyta/Xanthophyceae (01). In the distribution of the taxonomic classes, the class Cyanophyceae (38%) and Chlorophyceae (35%) were the ones that contributed the most to the richness of taxa in the phytoplankton community (Fig. 5).

After separation of the samples collected at the RT-E2 and RT-E4 stations, where the Chlorophyll 'a' analysis were performed, each sample was checked until a counting efficiency of more than 80% was reached

(PAPPAS et al., 1996), comprising a total of 3,910 individuals from which 2,855 belong to the Cyanophyta division. Fig. 6 shows the distribution of population density by percentage in both sampling stations.



**Fig. 5:** Relative contribution of phytoplankton taxonomic classes.



**Fig. 6:** Percentage contribution of the phytoplankton population density in the stations RT-E2 and RT-E4.

Because they are extremely adaptable and intelligent, cyanobacteria use mechanisms that allow them to have dominance over the other taxonomic classes, in a system that more closely resembles cooperation with a percentage of advantage, than to competition over the other taxonomic classes. As for the presence of cyanobacteria in the blooms occurring in the study area, the following species were dominant: *Synechocystis aquatilis* (%), *Microcystis aeruginosa* (%), *Dolichospermum spiroides* (%) e *Nostoc* sp. (%).

## Toxins

Except for *S. aquatilis*, which was abundantly found, all other dominant cyanobacteria species in the analysed samples are potentially producers of cyanotoxin, which can indeed be released into the water after cell disruption.

There is also the bloom that form in the channel of the river and spread and cause the accumulation of the scum in the margins. Over two or three days, fragments of a the bloom can descend toward the mouth and enter the spaces between the riverboats in the port area, and accumulate on the banks in front of the edge of Santarém. Fragments of senescence the blooms present the greatest potential for cyanobacterial cell disruption with release of toxic substances in the water, especially when they get in friction with boats entering and exiting the ports. However, the toxicity of cyanobacteria varies from species to species, and within the same genus there may be producing and non-toxin producing strains, also called cyanotoxins. Cyanotoxins are classified according to the pharmacological action, such as hepatotoxins, neurotoxins and dermatotoxins, and human exposure may occur through dermal contact, by inhalation, or by oral ingestion (e.g., water supply or accidentally in recreational activities), via intravenous (in the case of haemodialysis), and by bioaccumulation in the food chain (ALVES, 2006), Table 4.

**Table 4:** Toxic groups cyanobacteria present in the lower Tapajós River Basin considering class, mechanism of action, tolerable daily intake, lethal dose (rats), primary target (affected organs) and effects on human health.

Toxin Group	Class	Action mechanisms	Tolerable daily intake (TDI)	Lethal dose (µg/kg)	Primary target	Effects human health	Cyanobacterial species	Drinking water quality
<i>Cyclic peptide</i>								
Microcystine	Cyanotoxin	Protein phosphatase blockers	0,4 µg/kg	45-1000	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion	<i>Mirocystis</i> , <i>Mictrocystis aeruginosa</i> ; <i>Microsystis botrys</i> ; <i>Microsystis panniformis</i> ; <i>Microsystis protocystis</i> ; <i>Microcystis wasenbergii</i> <i>Nostoc</i>	0,3 <sup>5</sup> -1,0 <sup>6</sup> µg/kg (WHO, 1998)
<i>Alkaloids</i>								
Cylindropermo psin	Cytotoxin	Block protein synthesis; substantial cumulative toxicity	- <sup>2</sup>	200-2.100	multiple organs (liver, kidney, spleen, gastrointestinal tract, heart, thymus and skin)	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion	<i>Cylindropermo psis sp.</i>	0,7 <sup>5</sup>
Saxitoxin	Neurotoxin	Block acetylcholinesterase; block sodium channels	- <sup>2</sup>	10-30	Axon nerve	eye irritation, abdominal pain <sup>3</sup> ; paralysis, death <sup>4</sup>	<i>Dolichospermum sp.</i> ; <i>Dolichospermum spiroides</i> ; <i>Aphanizomenon sp.</i> ; <i>Cylidropermopsis sp.</i>	-
Anatoxin-a	Neurotoxin	Block post-synaptic depolarization	- <sup>2</sup>	250	sympathetic nerve	Tingling, burning, numbness, drowsiness, incoherent	<i>Dolichospermum sp.</i> <i>Dolichospermum spiroides</i> ; <i>Aphanizomenon</i>	-

						speech, salivation, respiratory paralysis leading to death	<i>n sp.</i> ; <i>Microcystis sp.</i> ; <i>Mictrocystis aeruginosa</i> ; <i>Microsystis botrys</i> ; <i>Microsystis panniformis</i> ; <i>Microsystis protocystis</i> ; <i>Microcystis wasenbergii</i> ; <i>Cylindropermo psis sp.</i>	
Homoanatoxina	Neurotoxin	-	- <sup>2</sup>	-	sympathetic nerve	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death	<i>Dolichospermum sp.</i> ; <i>Dolichospermum spiroides</i> ; <i>Aphanizomenon sp.</i> ; <i>Microcystis sp.</i> ; <i>Mictrocystis aeruginosa</i> ; <i>Microsystis botrys</i> ; <i>Microsystis panniformis</i> ; <i>Microsystis protocystis</i> ; <i>Microcystis wasenbergii</i> ; <i>Cylindropermo psis sp.</i>	-
						Irritating potential, affects any exposed tissue	All	-

*Lipossaccharides*<sup>1</sup>

<sup>1</sup> Liposaccharides (LPS) are components of the cell membrane of cyanobacteria, and are pyrogenic endotoxins, capable of producing skin irritations and allergies. These compounds are especially important in places at risk of exposure to cyanobacterial cells, such as those intended for bathing (CETESB, 2013).

<sup>2</sup> According *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management* (1999), the database is insufficient for the derivation of the TDI.

<sup>3</sup> (RAPALA et al., 2005)

<sup>4</sup> (TESTAI et al., 2016)

<sup>5</sup> EPA (Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems. 2019)

<sup>6</sup> World Health Organization (1998)

The dominant genus mentioned in this work (*Microcystis*, *Dolichospermum* and *Nostoc*), belong to the main cyanobacterial genera of toxins, of the hepatotoxins group, which comprises the nodularin, the cylindrospermopsin and the microcystin, that affect human liver cells and can generate persistent inflammatory processes. These species are more frequent and therefore pose a greater risk to health, since hepatotoxins have a bio-accumulative action and may at the molecular level act as an inhibitor of the proteins phosphatase 1 (PP1) and 2A (PP2A), and in the long term contribute to the development of hepatic cancerous tumours.

## Occurrence and Identification

In this study, the identification of cyanobacteria was made by observing the morphological and morphometric characteristics of the organisms, based on specific literature (BICUDO et al., 2006; ALVES, 2006; LAMPARELLI et al., 2014; SANT'ANNA, 2006), Table 5. The frequency of occurrence of each genus and/or species follows the classification described in the study entitled *Potential of Cyanobacteria The bloom in a Domestic Supply Reservoir in the State of Espírito Santo: Duas Bocas Reservoir - Cariacica - ES*, where the genera and species found are listed in their respective taxonomic divisions, followed by the letter (s) of occurrence frequency, as for example: *Dolichospermum spiroides* (Klebahn) Walcklin, Hoffmann & Komárek = D/CT (dominant species in numbers and constant in most fields counted per sample), Table 5.

**Table 5:** Distribution of frequency of algae in this study. **D** = dominant; **A** = abundant; **CT** = constant; **CM** = common; **R** = rare.

Divisão	Species	Frequency
Cyanobacteria	<i>Aphanizomenon</i> sp. A.Morren ex É.Bornet & C.Flahault	R
	<i>Anathece</i> sp. (Komárek & Anagnostidis) Komárek, Kastovsky & Jezberová	CM
	<i>Aphanocapsa elachista</i> West & G.S.West	CT
	<i>Aphanocapsa</i> sp. C.Nägeli	R
	<i>Chroococcus</i> sp. Nägeli	CM
	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	CM/CT
	<i>Cylindrospermopsis</i> sp. G.Seenayya & N.Subba Raju	R
	<i>Coelomoron tropicale</i> P.A.C.Senna, A.C.Peres & Komárek	R
	<i>Coelosphaerium</i> sp. Nägeli	R
	<i>Cuspidothrix</i> sp. P.Rajaniemi, J.Komárek, R.Willame, P.Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen	R
	<i>Dolichospermum planctonicum</i> (Brunnthaler) Wacklin, L.Hoffmann & Komárek	CM
	<i>Dolichospermum</i> sp. (Ralfs ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek	CM
	<i>Dolichospermum spiroides</i> (Klebahn) Walcklin, Hoffmann & Komárek	D/CT
	<i>Geitlerinema</i> sp. (Anagnostidis & Komárek) Anagnostidis	CM
	<i>Merismopedia</i> sp. Meyen	CT
	<i>Merismopedia tenuissima</i> Lemmermann	R
	<i>Merismopedia trolleri</i> Bachmann	R
	<i>Microcystis aeruginosa</i> (Kützing) Kützing	CM / D
	<i>Microcystis botrys</i> Teiling	R
	<i>Microcystis panniformis</i> Komárek, Komárková-Legnerová, Sant'Anna, M.T.P. Azevedo, & P.A.C. Senna	R
	<i>Microcystis protocystis</i> Crow	R
	<i>Microcystis wesenbergii</i> (Komárek) Komárek ex Komárek	R
	<i>Microcystis</i> sp. Lemmermann	CT
	<i>Nostoc</i> sp. Vaucher ex Bornet & Flahault	CM / D
<i>Pseudanabaena galeata</i> Böcher	A/ CM	
<i>Pseudanabaena</i> sp. Lauterborn	A/CT	
<i>Rhabdoderma lineare</i> Schmidle & Lauterborn	A/CT	
<i>Sphaerocavum</i> sp. M.T. de P.Azevedo & C.L.Sant'Anna	CM	
<i>Synechocystis aquatilis</i> Sauvageau	A/ D	
<i>Woronichinia</i> sp. A.A.Elenkin	CM	
<i>Woronichinia naegeliana</i> (Unger) Elenkin	R	
Chlorophyta	<i>Ankistrodesmus</i> sp. Corda	R
	<i>Asterocapsa</i> sp. H.-J.Chu	R
	<i>Botryococcus</i> sp. Kützing	R/R
	<i>Chlorococcum</i> sp. Meneghini	CM
	<i>Chlorotetraedron incus</i> (Teiling) Komárek & Kováčik	R
	<i>Chodatellopsis elliptica</i> Korshikov	R
	<i>Closterium</i> sp. Nitzsch ex Ralfs	R
	<i>Cosmarium</i> sp. Corda ex Ralfs	CM
	<i>Crucigenia</i> sp. Morren	A/R
	<i>Desmodesmus armatus</i> (Chodat) E.H.Hegewald	CT
	<i>Desmodesmus intermedius</i> (Chodat) E. Hegewald	A/CM
	<i>Desmodesmus</i> sp. (Chodat) S.S.An, T.Friedl & E.Hegewald	CT
	<i>Desmodesmus spinosus</i> (Chodat) E. Hegewald	CM
	<i>Didymocystis</i> sp. Korshikov	R

	<i>Dimorphococcus</i> sp. Braun	CM
	<i>Elakatotrix gelatinosa</i> Wille	R
	<i>Eutetranoorus</i> sp. Walton	R
	<i>Geminela</i> sp. Turpin	R
	<i>Glaucocystis nostochinearum</i> Itzigsohn	R
	<i>Glaucocystis</i> sp. Itzigsohn	R
	<i>Keratococcus bicaudatus</i> (A. Braren) J. B. Petersen	CT
	<i>Koliella</i> sp. Hindák	R
	<i>Microspora</i> sp. Thuret	CM
	<i>Micrasterias</i> sp. C. Agardh ex Ralfs	R
	<i>Monoraphidium contortum</i> (Thuret) Komárkova – Legnerová	CT/CM
	<i>Nephrocytium</i> sp. Nägeli	CM
	<i>Onychonema</i> sp. Wallich	R
	<i>Pectodictyon</i> sp. Taft	R
	<i>Pediastrum duplex</i> Meyen	CT/CM
	<i>Pediastrum simplex</i> Meyen	A/D
	<i>Phytelios viridis</i> Frenzel	CM
	<i>Rhizoclonium</i> sp. Kützing	R
	<i>Scenedesmus acuminatus</i> (Lagerheim) Chodat	CM/CT
	<i>Scenedesmus linearis</i> Komárek	CM/CT
	<i>Schroederia</i> sp. Lemmermann	CM
	<i>Staurastrum</i> sp. Meyen ex Ralfs	A/CT
	<i>Treubaria</i> sp. C. Bernard	R
	Zinemaphyceae sp.1	CT/CM
	Zinemaphyceae sp.2	A/CM
Bacillariophyta	<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	A/CM
	<i>Aulacoseira</i> sp. Thwaites	A/CT
	<i>Diploneis</i> sp. Ehrenberg ex Cleve	R
	<i>Navicula</i> sp. Bory	CT/A
	<i>Urosolenia</i> sp. Round & Crawford	CM
	Bacillariophyceae sp. 1	A/CM
	Bacillariophyceae sp. 2	CT/A
Coccinodiscophyceae	R	
Euglenophyta	<i>Euglena</i> sp. Ehrenberg	A/CT
	<i>Heteronema</i> sp. Dujardin	R
	Euglenophyceae sp.1	A/CT
	Euglenophyceae sp.2	CM
Chrysophyta	<i>Chrysococcus ellipsoideus</i> Ettl	R
	<i>Dinobryon</i> sp. Ehrenberg	R
	<i>Chlamydomonas</i> sp. Ehrenberg	R
Dinophyta	<i>Peridiniopsis</i> sp. Lemmermann	CT/A
	Dinophyceae sp. 1	CM
Xanthophyta	<i>Raphidiella fascicularis</i> Pascher	R

As seen above, among the five-dominant species found in this study, four of them are potentially producing cyanotoxins, namely: *Microcystis aeruginosa*, *Dolichospermum spiroides* and *Nostoc*. They are species belonging to the main genera of toxin-producing cyanobacteria that may affect human liver cells and can generate persistent inflammatory processes, which in turn can sometimes confuse the diagnosis of diseases, as being derived from viral pathogenesis.

## CONCLUSIONS

The studied transect of the Tapajós river, in front of the border of Santarém, presented most of the physicochemical parameters within the allowed standard for the rivers classified as Class 2, according to CONAMA Resolution # 357/2005 (MMA, 2005). However, dissolved oxygen has been shown to be below the permitted standard, indicating water pollution in the margins up to the depth studied, i.e., the average of 2.3 meters of depth, depending on the access site and the sampling station.

The presence of cyanobacteria potentially producing cyanotoxins is of concern and may play a relevant role if considered while diagnosing local diseases, such as sudden outbreaks of hepatic infections observed in riverine communities in the region. However, there is a need for more in-depth studies on the toxicity potential to the human population and other aquatic organisms living in the Tapajós River, which in turn might be used to guide the population through public health and environmental education programs.

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