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Phytoplankton, with emphasis on potentially toxic cyanobacteria, from Amor Island, Alter do Chão (Santarém, Pará, Brazil)

O fitoplâncton, com ênfase nas cianobactérias potencialmente tóxicas, da Ilha do Amor, Alter do Chão (Santarém, Pará, Brasil)

Fitoplancton, con énfasis en cianobacterias potencialmente tóxicas, de Ilha do Amor, Alter do Chão (Santarém, Pará)

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Abstract
Amor Island (Alter do Chão, Santarém, Pará, Brazil) is considered a region of great natural beauty and a national and international tourist attraction. The aim of this study was to determine the composition and density of the phytoplankton of the Amor Island, with emphasis on heterocytous cyanobacteria. Water samples for phytoplankton were collected and analyzed at seven points in October 2021. Filaments of heterocytous cyanobacteria were cultured and analyzed, measuring the cells of the first 30 trichomes. The abundance, diversity and evenness of phytoplankton species were calculated and balneability of beach in relation to algal blooms was evaluated. 106 generic and infrageneric taxa were identified, highlighting the Chlorophyta. Phytoplankton density was higher at Verde Lake (145.6 ± 22.0 org.L^{-1}), diversity and evenness were higher at point 03 (Lake Verde) with 3.0 bits.cell^{-1} and 0.47, respectively. The most abundant species were: *Aphanothece minutissima*, *Ankistrodesmus* sp., *Aphanothece* sp., *Aphanizomenon* sp., *Mougeotia* sp., *Merismopedia* sp. and *Quadrigula* sp. The average density of cyanobacteria was higher in the Tapajós River (517.0 cell.mL^{-1}). No algal and cyanobacterial blooms were identified, indicating water appropriate for bathing. Through morphological and morphometric description, the cultivated species were identified as *Aphanizomenon gracile* and *Cylindrospermopsis*/*Raphidiopsis raciborskii*, cited among the cyanobacteria with the highest occurrence of toxic blooms in the world. The region deserves phytoplankton monitoring studies, due to reports of cyanobacterial blooms, and greater knowledge of its planktonic biodiversity, since it is a threatened region.
Keywords: *Aphanizomenon*; *Cylindrospermopsis/Raphidiopsis;* Balneability; Nostocales; Environmental Teaching.

Resumo
A Ilha do Amor (Alter do Chão, Santarém, Pará, Brasil) é considerada uma região de grande beleza natural e atração turística nacional e internacional. O objetivo do estudo foi determinar a composição e a densidade do fitoplâncton da Ilha do Amor com ênfase nas cianobactérias heterocitadas. Amostras de água para análise do fitoplâncton foram coletadas e analisadas em sete pontos, durante outubro de 2021. Os filamentos de cianobactérias heterocitadas foram cultivados e analisados, medindo-se as células dos 30 primeiros tricomas. Foram calculadas a abundância, diversidade

e equitabilidade das espécies do fitoplâncton e avaliada a balneabilidade da praia em relação a florações de algas. Foram identificados 106 táxons genéricos e infragenéricos, destacando- se as Chlorophytas. A densidade do fitoplâncton foi maior no Lago Verde (145,6 ± 22,0 org.L^{-1}), a diversidade e a equitabilidade foram maiores no ponto 03 (Lago Verde) com 3,0 bits.$cell^{-1}$ e 0,47, respectivamente. As espécies mais abundantes foram: *Aphanothece minutissima, Ankistrodesmus* sp.*, Aphanothece* sp.*, Aphanizomenon* sp.*, Mougeotia* sp.*, Merismopedia* sp. e *Quadrigula* sp. A densidade média das cianobactérias foi mais elevada no Rio Tapajós (517,0 cel.mL^{-1}). Não foram identificadas florações de algas e cianobactérias, indicando águas próprias para banho. Pelo descritivo morfológico e morfométrico, as espécies cultivadas foram identificadas como *Aphanizomenon gracile* e *Cylindrospermopsis/Raphidiopsis raciborskii*, citadas entre as cianobactérias de maior ocorrência de florações tóxicas no mundo. A região merece estudos de monitoramento do fitoplâncton, devido à relatos de florações de cianobactérias, e maior conhecimento de sua biodiversidade planctônica, visto ser uma região ameaçada.
Palavras-chave: *Aphanizomenon*; *Cylindrospermopsis/Raphidiopsis*; Balneabilidade; Nostocales; Educação Ambiental.

Resumen
Isla do Amor (Alter do Chão, Santarém, Pará, Brasil) es considerada una región de gran belleza natural y atractivo turístico nacional e internacional. El objetivo del estudio fue determinar la composición y densidad del fitoplancton Isla do Amor, con énfasis en las cianobacterias heterocíticas. Se recolectaron y analizaron muestras de agua para análisis de fitoplancton en siete puntos durante octubre de 2021. Se cultivaron y analizaron filamentos de cianobacterias heterocíticas, midiendo las células de los primeros 30 tricomas. Se calculó la abundancia, diversidad y uniformidad de las especies de fitoplancton y se evaluó la calidad de las aguas para el baño de la playa en relación con la proliferación de algas. Se identificaron un total de 106 taxones genéricos e infragenéricos, destacándose los Chlorophyta. La densidad de fitoplancton fue mayor en Lago Verde (145,6 ± 22,0 org.L^{-1}), la diversidad y uniformidad fueron mayores en el punto 03 (Lago Verde) con 3.0 bits.cel^{-1} y 0.47, respectivamente. Las especies más abundantes fueron: *Aphanothece minutissima*, *Ankistrodesmus* sp., *Aphanothece* sp., *Aphanizomenon* sp., *Mougeotia* sp., *Merismopedia* sp. y *Quadrigula* sp. La densidad promedio de cianobacterias fue más alta en el Río Tapajós (517.0 cel.mL^{-1}). No se identificaron floraciones de algas y cianobacterias, lo que indica un agua apta para el baño. A partir de la descripción morfológica y morfométrica, las especies cultivadas fueron identificadas como *Aphanizomenon gracile* y *Cylindrospermopsis*/*Raphidiopsis raciborskii*, citadas entre las cianobacterias con mayor ocurrencia de floraciones tóxicas en el mundo. La región amerita estudios de monitoreo de fitoplancton, por reportes de florecimientos de cianobacterias, y un mayor conocimiento de su biodiversidad planctónica, ya que es una región amenazada.
Palabras clave: *Aphanizomenon*; *Cylindrospermopsis/Raphidiopsis;* Las aguas de baño; Nostocales; Enseñanza Ambiental.

1. Introduction

Phytoplankton are a group of organisms, mostly microscopic, that float in the water and are composed of several phyla and inhabit different aquatic environments. Phytoplankton are the most important primary producers on the planet and are estimated to account for half of global primary production (Field et al., 1998; Ellegaard & Ribeiro, 2017).

Cyanobacteria are prokaryotic organisms that are included in the artificial algae group of phytoplankton because they perform oxygenic photosynthesis. They can be found in different environments, but planktonic aquatic cyanobacteria are the most studied and, consequently, the best known, due to their ability to generate blooms in eutrophic aquatic environments. Many blooms cause water quality to deteriorate, harming people, animals, aquatic ecosystems, the economy, drinking water supplies, and recreational activities, including swimming and commercial and recreational fishing (Sinha et al., 2017; Paerl, 2018).

Cyanobacterial blooms usually accompany the production and release of toxins (cyanotoxins), which cause adverse effects on human and animal health (Wood, 2016), with 183 cyanotoxin poisonings in humans and/or animals reported worldwide (Svirčev et al., 2019).

Climate change and eutrophication are believed to be synergistic enhancers of cyanobacterial blooms (Bláhová et al., 2008; Huisman et al., 2018; Paerl, 2018), mainly due to increased temperature, changes in rainfall patterns, and low precipitation to evaporation ratio (Wang et al., 2021).

In the Amazon, the blooms are known as "river slime" and is, at first glance, a natural phenomenon that occurs due to the seasonality marked by heavy rainfall with the input of organic compounds from the continent. In the Tapajós River region there are reports of extensive blooms associated with the arrival of rainfall, with species of the genera *Microcystis* and *Dolichospermum* (formerly *Anabaena*) being the main culprits. These blooms are accompanied by the production of microcystin (Sá et al., 2010; Silva et al., 2019).

It is noted through long-term monitoring studies, a gradual increase of cyanobacteria in the rivers of the Eastern Amazon, especially in deep rivers, of great hydrodynamics as the estuary of the Pará River that recorded cyanobacteria blooms 22 times above the allowed for safe waters according to Brazilian legislation (Carneiro, 2021).

In general, phytoplankton is little studied in Amazonian waters, but efforts have been made to expand the knowledge about the composition and dynamics of these organisms in different types of water in the Amazon region, such as the studies of Torres et al. (2020), in the clear waters of the Tapajós River; Paiva et al. (2006) and Sena et al. (2015), in the white waters of the Guajará Bay and Pará River, respectively; and Sousa et al. (2015) in the black and white waters of the rivers that cut through the Charapucú State Park, mouth of the Amazon River.

This paper investigates the composition, phytoplankton density, with emphasis on cyanobacteria, and morphological characterization of heterocytous cyanobacterial species in the clear waters surrounding Amor Island, Alter do Chão, a major tourist site in Brazil (Santarém, Pará) during the Amazon summer.

2. Methodology

2.1 Study area

The Alter do Chão Village is one of the administrative districts of Santarém (Pará, Brazil), located about 40 km upstream from the center of Santarém. The Amor Island, which belongs to the Alter do Chão Village, is considered a region of great natural beauty and a national and international tourist attraction. On the island is an extensive strip of sand bathed by the clear waters of the Tapajós River and the waters of the Verde Lake (Figure 1).

The Tapajós basin presents a monomodal flood pulse, with a flood period from February to May and an ebb period fromAugust to November. The rising water level (~6 m) floods a large area in this basin, completely covering the sand belt of Amor Island (Junk & Krambeck, 2000).

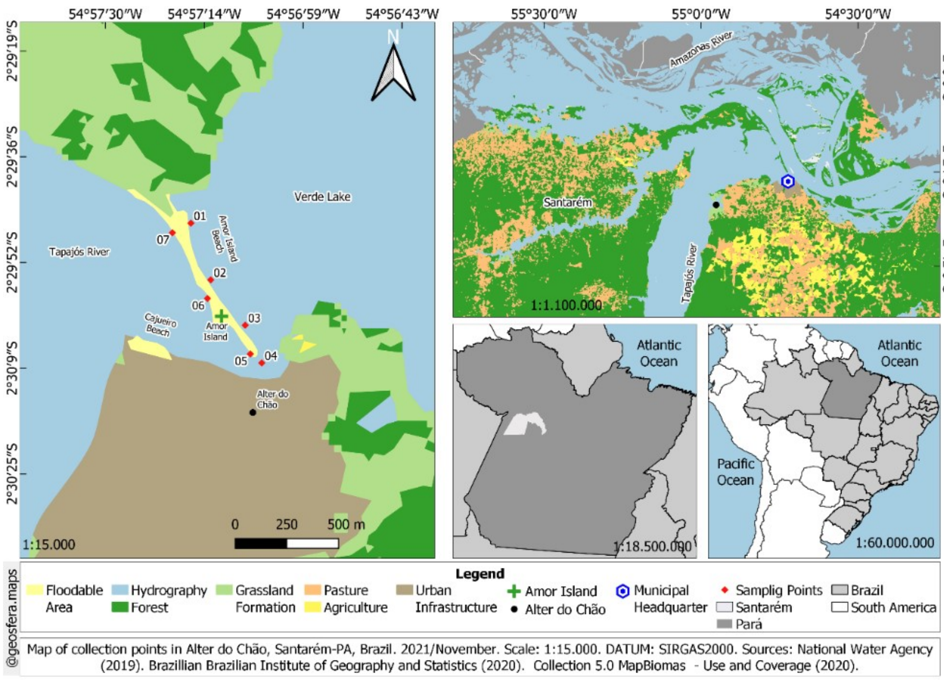
The region's climate is of the Am climatic type, that is to say, tropical, hot and humid characterized by a high annual rainfall total and moderate dry season (Alvares et al., 2013). The annual average reaches 1,886 mm and more than 75% of this total is concentrated between the months of January and June. The dry period goes from August to November and the months of July and December are considered transition months for the dry and rainy seasons, respectively.

The average temperature in the municipality varies little during the year, with the highest averages registered for the dryperiod with an average temperature of 25 ºC. The relative humidity in the region is above 80% throughout the year. The winds are predominantly from the east (trade winds), but there is a considerable influence of the breeze coming from the Tapajós River, occurring on 50% of the days, which can change the temperature and humidity conditions in the region (Silva, 2017).

2.2 Phytoplankton collection and analysis

Phytoplankton sampling was conducted along the beach strip region, at seven points, in the month of October 2021, where three points were located on the part of the beach facing Verde Lake: point 01 (S 02° 29' 45.9" and W 054° 57' 16.5"), point 02 (S 02° 29' 54.8" and W 054° 57' 13.4") and point 03 (S 02° 30' 1.9" and W 054° 57' 8.0"); one intermediate point between the lake and the Tapajós River: point 04 (S 02° 30' 7.8" and W 054° 57' 5.4"); and three points facing the region of greater influenceof the Tapajós River: point 05 (S 02° 30' 6.4" and W 054° 57' 7.2"), point 06 (S 02° 29' 57.7" and W 054° 57' 13.9"), and point 07 (S 02° 29' 47.4" and W 054° 57' 19.4") (Figure 1).

Figure 1. Map of the study area and the distribution of the collection points, at Amor Island, Alter do Chão Village Santarém,Pará, Brazil).



Sources: Authors

Samples were collected directly from the subsurface of the water with 500 mL polyethylene vials, fixed with 4% neutral formaldehyde and analyzed by sedimentation method according to method 10200F (APHA, 2017) using inverted optical microscopy (Axio Vert. A1, Carl Zeiss, Germany).

The water quality parameter was based on the CONAMA Resolutions 357/2005 (Brasil, 2005) (classification/framing of water bodies) and 274/2000 (Brasil, 2000) (balneability).

In the laboratory analyses, all cells, cenobia, fructules, colonies, filaments were considered as one organism ($org.L^{-1}$). Cyanobacteria were counted in cells per milliliter ($cell.mL^{-1}$). Identification, nomenclature, and taxonomic framework were based on specialized literatures (Round et al., 1990; Van Den Hoek et al., 1996; Bicudo & Menezes, 2017; Komárek & Anagnostidis, 2005; 2008; Komárek, 2013).

Aphanizomenon and *Cylindrospermopsis*/*Raphidiopsis* filaments were developed in a non-axenic sample in BG-11 medium (RIPPKA, 1979) plus cycloheximide, for 60 days, being analyzed under triocular light microscopy (Axio Lab. A1, Carl Zeiss, Germany). with measuring eyepieces coupled to a camera (AxiocamMRc, Carl Zeiss), measuring the cells of the first 30 trichomes. The morphological and morphometric characteristics of the filaments (length and diameter of vegetative cells, akinets and heterocytes, when present, color of the cells) and of the culture (color, form of incrustation of the culture, odor and formation of gases and mucilages). The coloring was established according to the Faber Castell Supersoft Color Ecolápis color palette ®.

2.3 Data Analysis

The abundance (Lobo & Leighton, 1986), diversity (Shannon, 1948) & equitability (Pielou, 1977) of phytoplankton species were calculated. A non-parametric Kruskal-Wallis (H) and Dunn's post hoc analysis of variance was performed to identify if there were differences between the points in the Verde Lake portion (points 01, 02 and 03) and the points in the Tapajós River portion (points 05, 06 and 07). The statistical tests were performed in the Past 4.06b program (Hammer et al., 2001).

3. Results

3.1 Phytoplankton

A total of 106 generic and infrageneric taxa were identified, containing 53 species and 04 varieties, distributed in 11 classes, with Chlorophyceae (26%), Cyanophyceae (22%), Bacillariophyceae (15%) and Zygnemaphyceae (13%) standing out. The species *Aphanothece* sp., *Ankistrodesmus* sp., *Closterium acutum* Brébisson, *Cosmarium* sp., *Planktolyngbya* sp., *Peridinium umbonatum* Stein, *Staurastrum* sp. and *Cylindrospermopsis*/*Raphidiopsis* occurred at all points on the beach (Table 1).

Table 1. List of generic and infrageneric phytoplankton taxa recorded at Amor Island, Alter do Chão Village (Santarém,Pará, Brazil), in October 2021.

	Verde Lake			**Lake- River**	**Tapajós River**		
Species list/points	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Cyanophyceae							
**Aphanizomenon* sp.		X	X		X	X	X
Aphanocapsa delicatissima West & G.S.West				X		X	X
Aphanocapsa holsatica (Lemmerm.) Cronberg & Komárek	X	-	X	-	X	X	X
Aphanocapsa incerta (Lemmerm.) Cronberg & Komárek	-	-	-	X	X	-	X
Aphanocapsa sp.	X	X	-	-	-	-	-
Aphanothece minutissima (W. West) Komárk.-Legn. & Cronberg	-	-	-	X	-	-	-
Aphanothece sp.	X	X	X	X	X	X	X
Chroococcus sp.	-	-	-	-	X	X	X
***Cylindrospermopsis/Raphidiopsis*	X	X	X	X	X	X	X
Dolichospermum sp.	-	X	X	-	-	X	X
Eucapsis densa M.T.P.Azevedo et al	-	X	X	-	X	-	-
Epigloesphaeria sp.	-	-	-	-	-	-	X
Merismopedia glauca (Ehrenberg) Kützing	X	-	-	-	X	-	-
Merismopedia minima Beck	-	-	X	-	-	-	-
Merismopedia tenuissima Lemmermann	X	X	X	-	-	-	X
Merismopedia sp.	-	X	X	X	X	X	X
Microcrocis pulchella (Buell) Geitler	-	-	-	-	-	-	X
Microcystis sp.	-	X	-	-	-	-	-
Oscillatoria limosa C.Agardh ex Gomont	-	-	X	-	-	-	-
Phormidium sp.	-	X	-	-	-	X	-
Planktolyngbya sp.	X	X	X	X	X	X	X
Pseudanabaena sp.	-	-	-	X	-	-	-

Table 1. List of generic and infrageneric phytoplankton taxa recorded at Amor Island, Alter do Chão Village (Santarém,Pará, Brazil), in October 2021.

	Verde Lake			Lake- River	Tapajós River		
Species list/points	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Romeria sp.	-	-	X	X	X	-	-
Snowella lacustris (Chodat) Komárek & Hindák	-	X	-	-	X	X	-
Chlorophyceae							
Ankistrodesmus bernardii Komárek	-	-	X	-	-	-	-
Ankistrodesmus spiralis (W.B.Turner) Lemmermann	-	-	X	X	X	X	-
Ankistrodesmus sp.	X	X	X	X	X	X	X
Coelastrum microporum Nägeli	X	-	-	-	-	-	X
Coelastrum pulchrum Schmidle	-	-	-	X	-	-	-
Coelastrum sp.	-	-	X	-	-	-	-
Coenocystis subcylindrica Korshikov	-	-	X	-	-	-	-
Crucigenia tetrapedia (Kirchner) Kuntze	X	X	-	X	-	X	X
Crucigenia sp.	-	-	X	-	-	-	-
Desmodesmus sp.	X	X	-	-	-	-	-
Dimorphococcus lunatus A. Braun	-	-	-	-	-	-	X
Golenkinia sp.	-	X	-	-	-	X	-
Kirchneriella sp.	X	-	-	-	-	-	-
Monoraphidium arcuatum (Korshikov) Hindák	-	-	X	-	X	-	-
Monoraphidium contortum (Thur.) Komárk.-Legn	-	-	X	X	-	X	-
Monoraphidium obtusum (Korsikov) Komárk.-Legn	-	-	X	X	X	-	-
Monoraphidium tortile (W.West & G.S.West) Komárk.-Legn	-	-	-	-	-	-	X
Monoraphidium sp.	X	X	X	-	-	-	-
Quadrigula closterioides (Bohlin) Printz	X	-	X	X	X	-	-
Quadrigula lacustris (Chodat) G.M.Smith	-	-	-	-	-	X	X
Quadrigula sp.	X	X	X	X	-	-	-
Scenedesmus obliquus (Turpin) Kutzing	-	-	-	-	-	-	X
Scenedesmus obtusus Meyen	-	-	-	-	-	-	X
Scenedesmus sp.	-	X	X	X	X	X	X
Tetradesmus acuminatus (Lagerheim) M.J.Wynne	-	-	X	-	-	-	-
Treubaria setigera (W.Archer) G.M.Sm	-	X	-	-	-	-	-
Treubaria sp.	-	X	-	-	-	-	X
Klebsormidiophyceae	-	-	-	-	-	-	-
Elakatothrix genevensis (Reverdin) Hindák	X	-	-	X	X	X	X
Treuboxiophyceae							
Botryococcus braunii Kützing	-	-	-	-	-	-	X
Botryococcus terribilis Komárek et Marvan	-	-	-	-	-	-	X
Dictyosphaerium ehrenbergianum Nägeli	-	-	-	-	-	-	X
Dictyosphaerium sp.	-	X	-	-	X	X	X
Oocystis lacustris Chodat	-	-	-	-	-	-	X
Oocystis marssonii Lemmerm	X	X	X	X	X	-	X

Table 1. List of generic and infrageneric phytoplankton taxa recorded at Amor Island, Alter do Chão Village (Santarém,Pará, Brazil), in October 2021.

	Verde Lake			Lake- River	Tapajós River		
Species list/points	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Oocystis sp.	X	X	X	X	-	-	X
Zygnemaphyceae							
Closterium acutum Brébisson	X	X	X	X	X	X	X
Closterium sp.	-	-	-	X	-	-	-
Cosmarium monomazum P.Lundell	-	X	-	-	-	-	-
Cosmarium quadratum Ralfs ex Ralfs	X	-	-	-	-	-	-
Cosmarium regnellii Wille	-	X	-	-	-	-	-
Cosmarium sphagnicolum West & G.S.West.	-	-	X	-	-	-	-
Cosmarium sp.	X	X	X	X	X	X	X
Desmidium sp.	-	X	-	-	-	-	-
Hyalotheca sp.	-	-	-	-	X	X	-
Micrasterias sp.	-	-	-	-	-	-	X
Mougeotia sp.	-	-	-	-	X	X	X
Staurastrum tetracerum (Kütz.) Ralfs	-	-	-	X	-	-	-
Staurastrum sp.	X	X	X	X	X	X	X
Xanthidium sp.	-	-	-	-	-	-	X
Euglenophyceae							
Euglena sp.	X	-	-	-	-	-	-
Trachelomonas volvocina (Ehrenberg) Ehrenberg	-	-	X	-	X	X	-
Trachelomonas sp.	X	X	-	X	X	-	X
Dinophyceae							
Glochidinium sp.	-	-	-	-	-	-	X
Peridinium umbonatum Stein	X	X	X	X	X	X	X
Xanthophyceae	-	-	-	-	-	-	-
Centritractus africanus F.E.Fritsch & M.F.Rich	-	-	-	-	-	-	X
Centritractus belenophorus Lemmerm	-	-	-	-	-	-	X
Centritractus sp.	-	X	-	-	-	-	-
Chrysophyceae							
Dinobryon sertularia Ehrenberg	-	-	-	-	-	-	X
Dinobryon sp.	-	-	-	-	-	-	X
Coscinodiscophyceae							
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	-	-	-	-	X	-	-
Aulacoseira granulata var. *granulata* (Ehrenberg) Simonsen	-	X	-	-	-	X	-
Aulacoseira spp.	X	-	X	X	X	X	X
Paralia sulcata (Ehrenberg) Cleve	-	X	-	-	-	-	X
Urosolenia eriensis var. *eriensis* (H.L.Smith) Round & Crawford	-	-	-	-	-	X	-
Urosolenia eriensis var. *morsa* (West & G.S.West) L.N.Bukhtiyarova	X	X	X	X	X	-	X
Urosolenia longiseta (O.Zacharias) Edlund & Stoermer	X	-	X	X	X	X	X
Bacillariophyceae							

Table 1. List of generic and infrageneric phytoplankton taxa recorded at Amor Island, Alter do Chão Village (Santarém,Pará, Brazil), in October 2021.

	Verde Lake			Lake- River	Tapajós River		
Species list/points	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Cymbella sp.	X	X	X	-	-	X	X
Eunotia sp.	X	-	X	-	X	X	X
Fragilaria sp.	X	X	X	-	-	-	X
Gomphonema sp.	X	X	X	-	-	-	X
Gyrosigma sp.	-	-	-	-	X	-	-
Nitzschia palea (Kützing) W.Smith	-	-	-	-	-	-	X
Nizschia sp.	-	X	X	-	-	-	-
Nupela sp.	-	-	-	-	-	-	X
Pinnularia brauniana (Grunow) Studnicka	-	-	-	-	-	X	-
Pinnularia sp.	-	-	-	-	-	-	X
Placoneis sp.	-	-	-	-	-	X	-
Surirella sp.	X	X	X	-	-	-	X
Synedra cf. *acus* Kützing	X	-	-	-	-	-	-
Synedra fusiformis Grunow	-	-	-	-	X	-	-
Synedra sp.	-	-	-	-	X	-	-
Tabellaria fenestrata (Lyngbye) Kützing	X	-	-	-	-	-	-

*After cultivation the species was identified as *Aphanizomenon gracile* Lemmermann; **after cultivation the species was identified as *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* (Woloszynska). Sources: Authors.

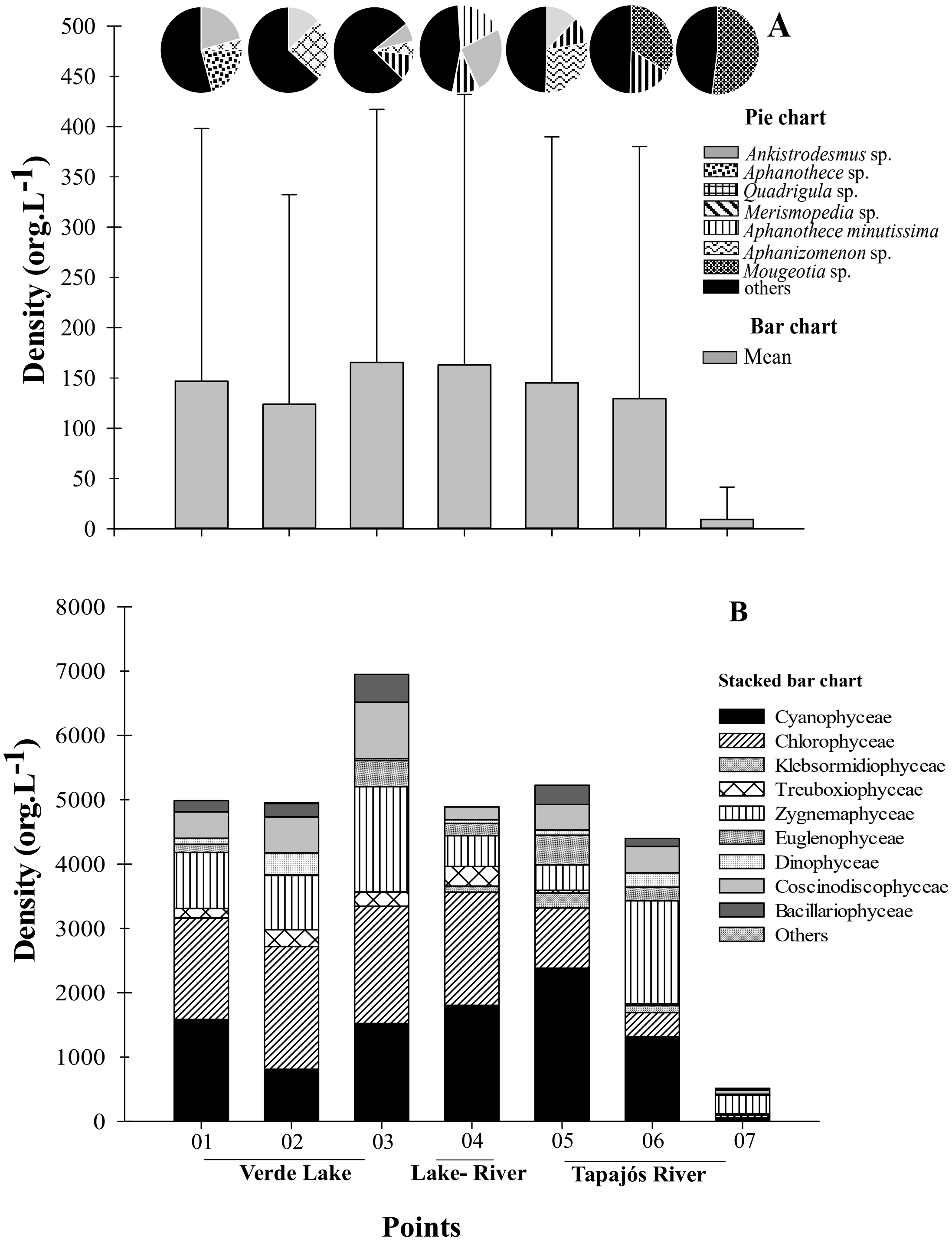
The density varied significantly between the two portions of the beach ($H= 24.2$; $p= 0.0008$), being the phytoplankton density higher in the waters facing Verde Lake (145.6 ± 22.0 org.L^{-1}) than the waters under the direct influence of Tapajós River (81.1 ± 17.5 org.L^{-1}). The point 07 presented, significantly, the lowest average density (Figure 2A).

The most abundant species were *Aphanothece minutissima* (point 04, with 19.0%), *Ankistrodesmus* sp. (points 01 and 04 with 21.0% and 25.0%, respectively), *Aphanothece* sp. (point 01, with 20.0%), *Aphanizomenon* sp. (point 05, with 26.0%), *Mougeotia* sp. (points 06 and 07, with 31.0% and 47.0%, respectively), *Merismopedia* sp. (point 06, with 15.3%), *Quadrigula* sp. (point 02, with 22.3%) (Figure 2A). The remaining species had a density percentage of less than 10%.

The total density ranged from 506.8 org.L^{-1} (point 07) to 6,950.0 org.L^{-1} (point 03), with cyanobacteria (Cyanophyceae)contributing more to the density in the Tapajós River points and chlorophytes (Chlorophyceae) for the waters facing Verde Lake (Figure 2B).

Diversity was higher at point 03 (Verde Lake), with 3.0 bits.cell^{-1}, where equitability was higher (0.47). Equitability was lower at point 07 (0.22) due to the high contribution of *Mougeotia* sp. to the density at this point.

Figure 2. Spatial variation of the phytoplankton densities, mean and total, along the beach of Ilha do Amor, Alter do Chão (Santarém, Pará, Brazil): A - mean density (bar chart) and percent abundance of phytoplankton species (pie chart: other category, species with percent abundance lower than 10%) and; B - total density by phytoplankton class.



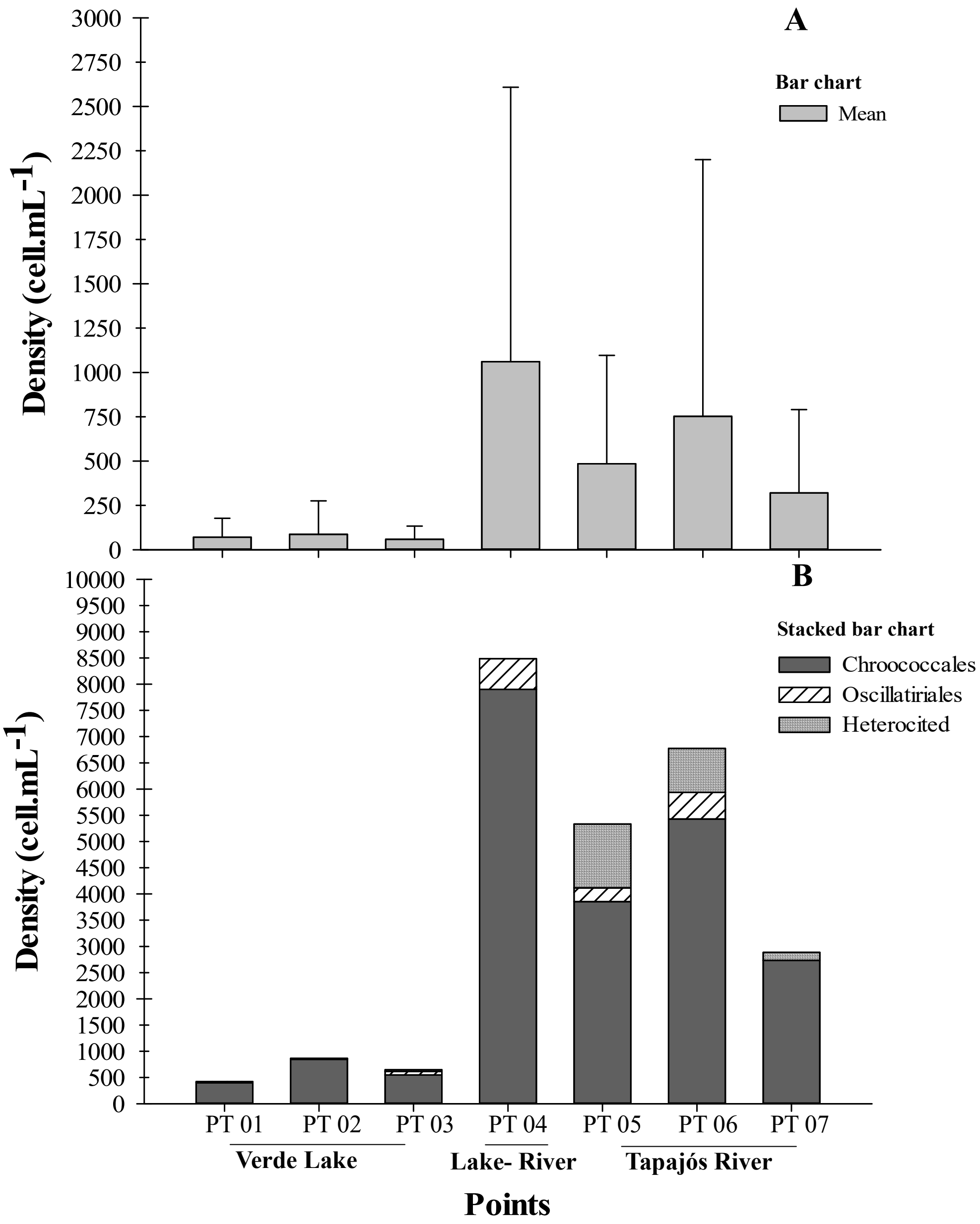
Sources: Authors

3.2 Cyanobacteria

The mean density varied significantly between the two portions of the beach, being higher in Tapajós River (517.0 cell.mL^{-1}) than the portion facing Verde Lake (72.0 cell.mL^{-1}) and there was a significant difference (H= 22.14; p= 0.001) between these portions, since points 01, 02 and 03 (Verde Lake) showed significantly lower densities than the other beach points (Figure 3A).

Total density ranged from 423.0 cell.mL^{-1} (PT 01, Verde Lake) to 8,487.0 cell.mL^{-1} (PT 04, Lago- Rio). The order Chroococcales was more dense at all points, but Oscillatoriales and heterocysted cyanobacteria contributed to the density from the transition point between lake- river (PT 04) to the points facing the Tapajós River (Figure 3B), but did not present blooms, a factor that guarantees a water suitable for bathing.

Figura 3. Spatial variation of the mean and total densities of cyanobacteria along the beach at Amor Island, Alter do Chão (Santarém, Pará): A- mean density (bar chart) and; B- total density, by order, of cyanobacteria (stacked bar chart).



Source: Authors.

3.3 Cultured cyanobacteria

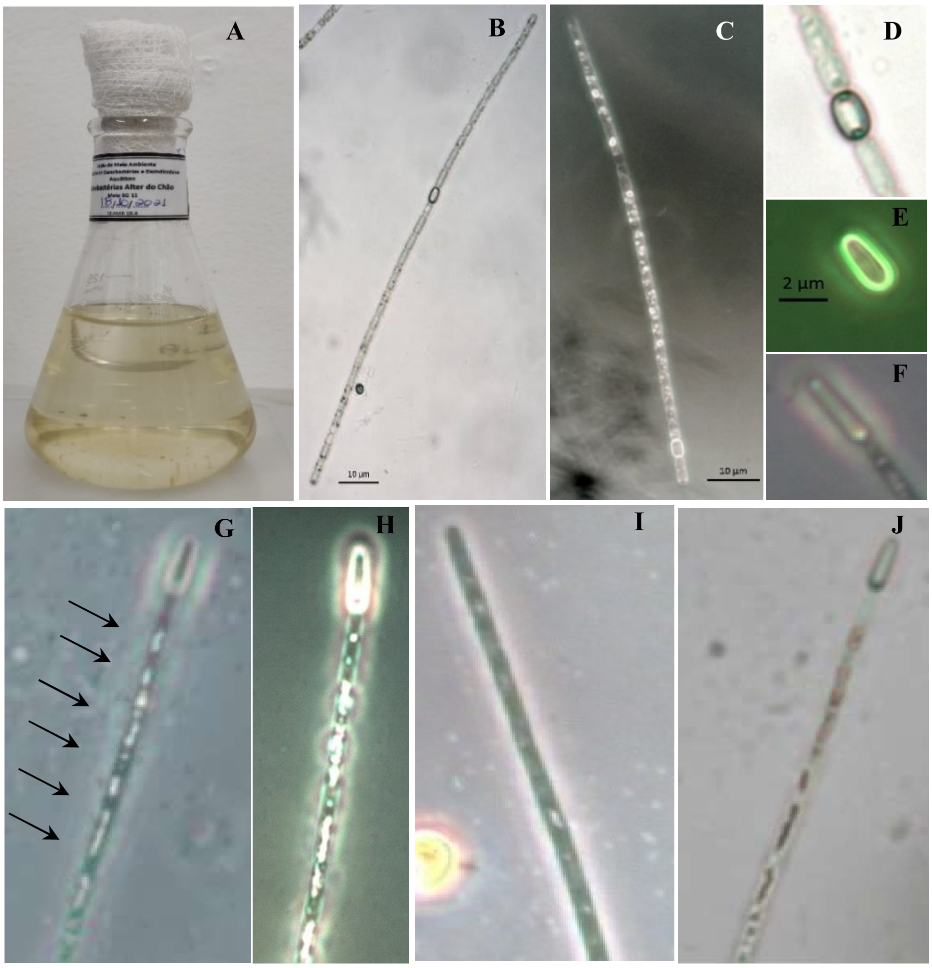
The cultivation of the genera *Aphanizomenon* and *Cylindrospermopsis*/*Raphidiopsis* showed a brownish green coloration (360, spring green) and little embedding of the populations on the wall of the surface substrate (culture glass). The filaments are planktonic and free-floating. The culture had no odor and consequently no gas was released (Figure 4A).

The trichomes of *Aphanizomenon* were straight to slightly arched, constricted, and terminal cells rounded and never pointed, but a slight narrowing toward the apical cells was found. The length ranged from 70.2 µm to 134.4 µm. The vegetative cells were short, quadratic to cylindrical, with length ranging from 2.2- 6.7 µm and diameter ranging from 2.5- 6.2 µm. Aerotopes, acinetos and heterocytes with two polar nodules were identified.

Aphanizomenon heterocytes were classified as intercalary and showed spherical or barrel shapes with diameter and length ranging from 3.8- 6.8 µm and 4.7- 10.0 µm, respectively. The intercalary acini measured between 4.9-7.0 µm in diameter and 7.7-10.0 µm in length. The color of the filaments was closest to light green (371 of the color Amor Island is suggested to be *Aphanizomenon gracile* (Figure 4B, C and D).

The filaments of *Cylindrospermopsis/Raphidiopsis* were straight with an average length of 145.5 µm, bright green (371 from the color palette) to pine green (374 from the color palette). Heterocytes had ellipse, lanceolate and ovoid shapes, which were found only at the end of the filament (Figure 4E and F). The vegetative cells had numerous aerotopes (Figure 4G and H) and an average length and width of 8.7 µm and 2.4 µm, respectively. From the descriptive the species was identified as *Cylindrospermopsis/Raphidiopsis raciborskii*.

Figure 4. Characteristics of cyanobacterial species cultivated in BG-11 medium: A- appearance of the culture; B- filament of *Aphanizomenon*; C- *Aphanizomenon* in phase contrast; D- detail of *Aphanizomenon* heterocyte with two polar nodules; E and F- heterocyte of *Cylindrospermopsis/Raphidiopsis* in phase contrast; G and H- filament of *Cylindrospermopsis/Raphidiopsis raciborskii* in phase contrast showing the aerotopes (arrows); I- filament of *Cylindrospermopsis/Raphidiopsis raciborskii* with vegetative cells; and J- filament of *Cylindrospermopsis/Raphidiopsis raciborskii* showing the terminal heterocyte.



Sources: Authors.

4. Discussion

The phytoplankton of Amor Island was characterized by a high abundance and species richness of chlorophytes (classes Chlorophyceae and Zygnemaphyceae), which is usual in clear water rivers as verified by Cunha et al., (2013), in Araguari River (Amapá, Brazil), Santos et al. (2020) in the Cxuruá- Una reservoir (Pará, Brazil), and Brito (2015) in Verde Lake, the same area of the present study.

The Tapajós River, near the edge of the city of Santarém, presents acidic to slightly alkaline waters, temperatures around 28.9 °C, low turbidity (< 100 UTN) and low electrical conductivity. In these conditions there is a predominance of chlorophytes followed by cyanobacteria (Torres et al., 2020), similar to the present study. The slight acidity and low conductivities in the water create a favorable condition for chlorophyceae (Lopez et al., 2009; Ramos et al., 2018) and this was possibly the condition of the waters of Amor Island.

In addition, the velocity of the waters of Amor Island is apparently low, creating a semi-lacustrine environment, capable of supporting phytoplankton growth, as suggested by Lobo et al. (2017) for the Tapajós River mouth region. About this, Novo et al. (2006) evaluated seasonal changes of phytoplankton in the stretch between Parintins (Amazonas, Brazil) and Almerim (Pará, Brazil) and verified in the Tapajós River a high abundance of phytoplankton downstream of the city of Santarém suggesting that the river may play an important role in the production of floodplain phytoplankton during the low water period in the Amazon River.

Roland et al. (2002) indicated that clear water environments are nutrient poor, but are as productive as those with white water, since phytoplankton primary production is regulated by solar radiation and not by nutrients. Therefore, the species diversity of Amor Island was considered medium to high in the study area.

This study expanded the knowledge about the diversity of planktonic species in Amazonian waters, in particular cyanobacteria, which 24 species were identified. In the study environment, located at the mouth of the Tapajós River, toxic cyanobacterial blooms are recorded, especially during the rainy months (Sá et al., 2010). In these blooms, high densities of *Microcystis* and *Dolichospermum* (formerly *Anabaena*) are reported, which had little representation in this study suggesting that there is a trigger of the blooms that act in the rainy season benefiting these species.

At the level of biodiversity, the waters of the Amor Island reflect the effects of the waters of the Amazon River, acting on the Tapajos River, and the shallow environment (beach) of the island. The Amazon River through secondary channels makes connection with the Tapajos River, mainly in the flood months of the Amazon, increasing diatom species, since these microalgae constitute an important group of white water rivers, such as the Pará River (Sena et al., 2015), Guajará Bay (Paiva et al., 2006), Charapucu River (Sousa et al., 2015) and mouth of the Guamá River (Monteiro et al., 2009), highlighting the species *Aulacoseira granulata* that has a wide occurrence in these waters, as well as in the present study.

However, the representativeness of periphytic diatoms (class Bacillariophyceae) in the composition is considered important, which is possibly due to the characteristics of the shallow environment with a lot of water-sediment interaction and a water column under acidic and oligotrophic conditions with high light penetration (Bertassoli jr et al., 2017).

The phytoplankton presented higher density in the portion facing Verde Lake due to the more lacustrine condition. On the other hand, the cyanobacteria were denser in the portion facing the Tapajós River, because the species present in this portion had the highest number of cells per individual/colony. For example, the nanoplanktonic coccoid species *Aphanothece minutissima*, *Aphanocapsa delicatissima*, *Aphanothece* sp., which have cells smaller than 2.0 µm and colonies with more than 200 cells (Komárek & Anagnostidis, 2008).

It is noteworthy that in this portion of the region there is a small water circulation and a lot of bed-surface water interaction due to the movements of the *catraias*, the main means of transportation from the Alter do Chão Village and the Amor Island. Thus, we suggest that cyanobacterial biofilms are shed onto the water surface, which are observed with the naked eye during collection, and when analyzed microscopically, constitute a dense mucilage containing *Aphanothece*, *Aphanocapsa*, and filamentous species such as the heretocite *Aphanizomenon* sp. which together have a significant number of cells and increase the density of the phytoplankton. The species *Aphanocapsa* sp. has been cited as one of the most abundant in the Tapajós River during the dry period (Lobo et al., 2017).

The total density of cyanobacteria was 15,000 cell.mL^{-1}, in the river portion, 1,936 cell.mL^{-1}, in the lake portion, and 8,487.0 cell.mL^{-1} at the intermediate point lake-river. Taking into consideration the parameter of algae blooms, contained in the Brazilian legislation about balneability (Brasil, 2000), the waters are considered, during this month of investigation, suitable for bathing.

Aphanizomenon and *Cylindrospermopsis* (or *Raphidiopsis*) are planktonic cyanobacterial genera of the order Nostocales and, therefore, filamentous heterocystic diazotrophic, both have already been reported for the study region (Torres et al., 2020). Identification at a specific level was only possible from cultured samples that enabled us to visualize several specimens, which were characterized as *Aphanizomenon gracile* and *Cylindrospermopsis raciborskii*.

Cylindrospermopsis raciborskii is a widely occurring species worldwide and a producer of Cylindrospermopsin, a toxin with varied action, but mainly neurotoxic (Vehovszky et al., 2015). The species is believed to exhibit a wide metabolic plasticity (Bonilla et al., 2012) and different ecotypes that predominate under specific environmental conditions (Pagni et al., 2020). Polyphasic analyses have highlighted the genetic similarity between the genera *Cylindrospermopsis* and *Raphidiopsis*, and there is currently a trend to consider *Cylindrospermopsis raciborskii* as *Raphidiopsis raciborskii* (Aguilera et al., 2018). The analysis in this study was typically morphological, so it was chosen for the moment to consider it as *Cylindrospermopsis raciborskii*.

Thus, this species has already been reported during a bloom event with saxitoxin release in the Iriri and Xingu rivers (Altamira, Pará, Brazil), causing fish kills in the rivers (Brazil, 2003). Its morphological characteristics were compatible with observations by Komárek (2013) for *Cylindrospermopsis* with emphasis on the terminal heterocyte. In morphological descriptions of *Cylindrospermopsis*, the presence of terminal heterocytes at one or both ends in a drop-shaped shape is emphasized, which are formed from the terminal cells (Wu et al., 2011; Xie et al., 2017), similar to what was observed in the present study. A remark is made about the coloration of the strain (brownish green), quite different from the studies of blooms of the species, greenish.

Aphanizomenon gracile has been associated with freshwater blooms with neurotoxin (Saxitoxin) production (Cirés et al., 2017), considered Paralytic Shellfish Poisoning- PSP alkaloids (Cirés & Ballot, 2016; Yilmaz et al., 2018). The characteristics were compatible the morphometries presented by Komarek (2013) and the observations of Yilmaz et al., (2018). Although the *Cylindrospermopsis*/*Raphidiopsis raciborskii* and *Aphanizomenon gracile* species are not very dense in the samples, it is important to conduct studies with these organisms to evaluate the potential risks for the quality of the waters of Amor Island and for the population and tourists who practice primary contact activities there.

It is noteworthy that this region has currently reached a very high level of turbidity, darkening the waters of the Amor Island, a phenomenon recurring to rainy periods, but in a smaller proportion. It is believed that the high turbidity is due to tributaries that suffer from illegal mining activity such as the Crepori, Rato, and Jamaxim rivers, where the material dumped by miners is rich in heavy metals, especially mercury, and harmful to health (Diniz et al., 2022). Recurrent cyanobacterial blooms, mercury and other metal inputs, and increased turbidity make it even more important to conduct studies on phytoplankton in the region for the purpose of assessing environmental impacts.

5. Conclusion

Information about biodiversity is necessary and valid to support decisions about interventions in the Amazon region, which suffers constant threats and speculation from the energy sector, agribusiness and mining. Thus, knowledge about the phytoplankton of the Tapajós River is important to understand and/or evaluate the ecological integrity of the environment, especially in Amor Island, the main tourist attraction in Brazil.

This study contributes to increase the knowledge about the local biodiversity, becoming a document recording the phytoplankton algae present under preserved conditions in the waters of the mouth of the Tapajós River in a period of prolonged use by bathers. The flora is typical of the clear waters of the Amazon region with a predominance of chlorophytes, cyanobacteria and diatoms of the phytobenthos.

Studies should be carried out in the area addressing, above all, the seasonality of phytoplankton and its relationship with physicochemical characteristics, nutrients, and metals in the water, with emphasis on the behavior of cyanobacterial blooms.

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