






First report of harmful *Microcystis* sp. and microcystin in two tributaries of Paraná River in Misiones, Argentina

Primeiro registro de *Microcystis* sp. nociva e microcistina em dois afluentes do Rio Paraná em Misiones, Argentina

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Abstract: Aim: Characterize cyanobacterial accumulations detected for the first time from November 2020 to February 2022 along the coastal zone of the Paraná River and in the Zaimán and Mártires streams in Posadas (Misiones, Argentina), by identifying the dominant species, determining their potential toxicity, and monitoring their progression using satellite images. **Methods:** This work analyzes the presence of cyanobacteria by optical microscopy, the production of microcystin (MCY) by molecular and analytical methods, and the detection of these cyanobacterial accumulations by satellite image analysis. **Results:** Optical microscopy analysis revealed the presence of *Microcystis* spp. colonies in all collected samples and *Dolichospermum* sp. in the Mártires stream. Using PCR amplification of a conserved region of the *mcyE* gene we confirmed the presence of MCY-encoding genes in all samples and MCY in the Zaimán stream using UPLC MS/MS analysis. Complementary satellite image analysis showed blooms in the Zaimán and Mártires streams were detectable during all the sampling periods. The presence of *Microcystis* spp., and MCY could affect the population, as well as the flora and fauna in the streams and the ecosystems along the Paraná River basin. **Conclusions:** In this study, we successfully characterized cyanobacterial accumulations along the Paraná River coast and in the Zaimán and Mártires streams, revealing the presence of *Microcystis* spp. colonies and MCY-encoding genes, with potential implications for local populations and ecosystems.

Keywords: cyanobacterial bloom; Paraná River basin; water quality.

Resumo: Objetivo: Caracterizar o acúmulo de cianobactérias detectadas pela primeira vez de novembro de 2020 a fevereiro de 2022 ao longo da zona costeira do rio Paraná e nos riachos Zaimán e Mártires em Posadas (Misiones, Argentina). Espécies dominantes e seu potencial de toxicidade foram identificadas e sua progressão foram monitoradas usando imagens de satélite. **Métodos:** Este trabalho analisa a presença de cianobactérias por microscopia óptica, a produção de microcistina (MCY) por métodos moleculares e analíticos e a detecção dessas acumulações de cianobactérias por análise de imagens de satélite. **Resultados:** A análise de microscopia óptica revelou a presença de colônias de *Microcystis* spp. e *Dolichospermum* sp. em amostras coletadas no riacho Mártires. Utilizando amplificação



por PCR de uma região conservada do gene *mcyE*, confirmamos a presença de genes codificadores de MCY e de MCY no Zaimán usando análise UPLC MS/MS. A análise complementar de imagens de satélite mostrou que as florações nos riachos Zaimán e Mártires foram detectáveis durante todos os períodos de amostragem. A presença de *Microcystis* spp. e MCY poderia afetar a população, bem como a flora e a fauna dos riachos e ecossistemas ao longo da bacia do rio Paraná. **Conclusões:** Neste estudo, foram caracterizadas com sucesso o acúmulo de cianobactérias ao longo da costa do Rio Paraná e nos riachos Zaimán e Mártires, revelando a presença de colônias de *Microcystis* spp. e genes que codificam MCY com potenciais implicações para as populações locais e os ecossistemas.

Palavras-chave: floração de cianobactérias; bacia do Rio Paraná; qualidade da água.

1. Introduction

Cyanobacteria, oxygenic photosynthetic microorganisms, are widely distributed in aquatic and terrestrial environments. Their ability to undergo rapid growth and form visible accumulations on the water surface, known as blooms, has become increasingly prevalent worldwide in recent decades (Massey et al., 2022). Eutrophication resulting from human-related activities has been identified as a significant factor contributing to the development of Cyanobacterial Harmful Algal Blooms (cyanoHABs). Also, the combination of rising global temperatures and prolonged drought periods can alter the water column structure, leading to stratification that further promotes bloom formation (Huisman et al., 2018; Paerl & Barnard, 2020). Therefore, massive cyanobacterial proliferation can increase turbidity and pH levels, reduce dissolved CO₂, and in severe cases, deplete dissolved oxygen, resulting in the mortality of aquatic fauna (Havens, 2008). In Argentina, the occurrence of cyanoHABs in freshwater bodies is of growing environmental concern (Aguilera et al., 2017).

One of the most found genus in cyanoHABs is *Microcystis* sp., which includes various species such as *M. aeruginosa* Kützing, *M. novacekii* (Komárek) Compere ex Komárek, *M. flos-aquae* (Wittrock) Kirchner, *M. wesenbergii* (Komárek) Komárek in Kondrateva, among others. However, the morphological features of these species may not always provide sufficient information for their identification through microscopic analysis. (Komárek & Komárková, 2002). Within a bloom, multiple species from the *Microcystis* genus can coexist, forming what is commonly referred to as the *Microcystis aeruginosa* complex (MAC) (Harke et al., 2016). One morphological characteristic of *Microcystis* is the ability to form colonies of different sizes and with gas vesicles inside the cells, which allow them to control buoyancy and utilize light more efficiently

(Komárek & Komárková, 2002). It is well-known that many species can produce a wide variety of secondary metabolites, including microcystin (MCY), hepatotoxins that affect both human and animal health (Massey et al., 2022).

The city of Posadas is located on the coast of the Paraná River and is surrounded by two urban streams, Zaimán and Mártires, which flows directly into the river. These streams have undergone modifications to their course as a result of the construction of the Yacyretá Dam, 70 km downstream (Corrientes, Argentina). Particularly, the Zaimán stream experienced an increase in its elevation in tandem with the river during the dam construction process generating a wetland of about 200 ha, inhabited by numerous species (Lecertua et al., 2009). Reports of cyanoHABs in Misiones are scarce and there is no record of the presence of cyanotoxins (Absi & Meichtry de Zaburlin, 1987; Meichtry de Zaburlín, 1994; Motta Bedoya, 2018; O'Farrell et al., 2019).

This study aims to report the occurrence of cyanobacteria and their toxins during blooms observed between 2020 and 2022, focusing primarily on the Mártires and Zaimán streams. This research represents the first documentation of *Microcystis* sp. and the presence of MCY in these environments, which could have negative implications for the local ecosystem and human health within the area and downstream the Paraná River. Furthermore, considering the dispersal capabilities of these microorganisms, their presence in these streams raises concerns about the possibility of blooms occurring in other environments in the basin as well.

2. Materials and Methods

2.1. Study area

Posadas is in the province of Misiones, Argentina and is surrounded by the Paraná River as well as two streams, Zaimán and Mártires (Figure 1). The population of Posadas is approximately

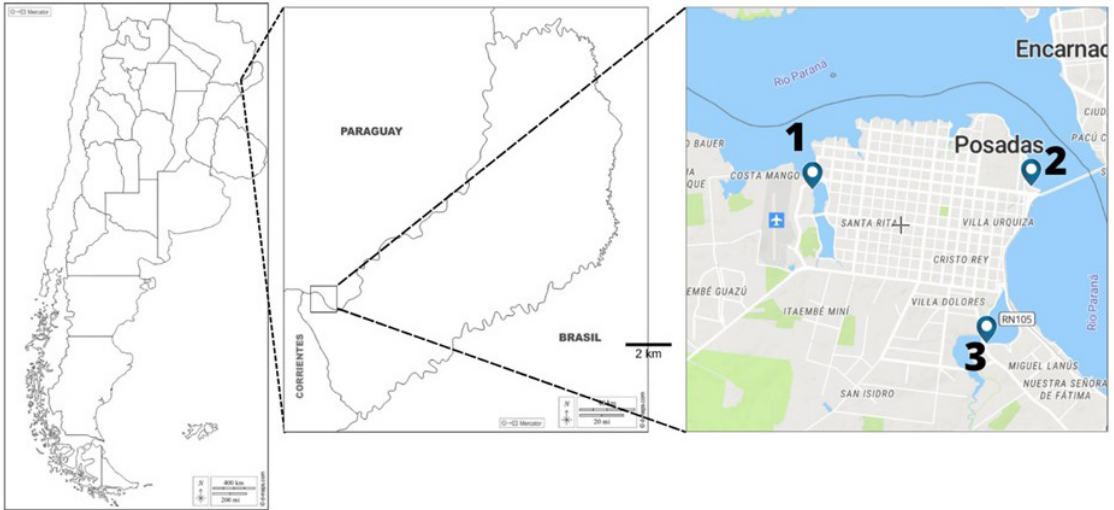


Figure 1. Cyanobacteria in the urban area of Posadas, Misiones, Argentina. During the November 2020–March 2022, cyanobacterial accumulations were detected, and samples were taken in: (1) Arroyo Mártires; (2) Río Paraná and (3) Arroyo Zaimán.

Table 1. Sampling sites and dates.

Date	Location (Latitude -Longitude)
13 Nov 2020	Paraná River 27°22'16.4"S- 55°53'05.5"W
11 Mar 2021	Zaimán Stream 27°25'06.8"S- 55°53'58.8"W
12 Dec 2021	Paraná River 27°22'16.4"S- 55°53'05.5"W
17 Feb 2022	Mártires Stream 27°22'50.3"S- 55°57'14.1"W
18 Feb 2022	Zaimán Stream 27°25'06.8"S- 55°53'58.8"W
28 Feb 2022	Zaimán Stream 27°25'06.8"S- 55°53'58.8"W
18 Mar 2022	Mártires Stream 27°22'50.3"S- 55°57'14.1"W

400,000 inhabitants and both streams receive discharges from human activities.

2.2. Sampling and microscopic analysis

Sampling in the Paraná River, Zaimán, and Mártires was carried out between 2020 and 2022 (Figure 1, Table 1). Surface water samples were taken from the shore where cyanobacterial accumulation was visible. In the laboratory, samples were filtered using glass fiber paper (Whatman GF/F) and stored at -20 °C. Fresh samples were immediately analyzed under an optical microscope and negative staining with nigrosin was performed to highlight colony morphology.

2.3. Molecular analysis

Environmental DNA was extracted from 100 mg of filter using the enzymatic lysis protocol according to Burns et al. (2004). To standardize the amount of template in the PCR reactions, amplification of the *cpcBA* gene (PCbF 5'GGCTGCTTGTTTACGCGACA3' and PCaR 5'CCAGTACCACCAGCAACTAA3')

was performed as a marker for the presence of cyanobacteria, as it amplifies the intergenic region of the genes encoding the B and A subunits of phycocyanin. To evaluate the toxigenic potential, primers that amplify the *mcyE* gene (*mcyE*-F 5'TTTGGGGTAACTTTTTGGGCATAGTC3' and *mcyE*-R 5'AATTCITGAGGCTGTAAATCGGGTTT3') and *anaC* (*anaC*-F 5'TCTGGTATTCAGTCCCCTCTAT3' and *anaC*-R 5'CCCAATAGCCTGTCATCAA3') were used, targeting MCY and anatoxin encoding genes.

PCR reactions were performed in 20 µL containing 1x PCR buffer (Inbio Highway), 0.4 mM of each dNTP (Genbiotech), 0.25 mM of each primer, 2 mM MgCl₂ (Inbio Highway), and 1 U T-Plus DNA polymerase (Inbio Highway). The amplification conditions were initial denaturation for 10 min at 95 °C, followed by 35 cycles at 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and a final step of 5 min at 72 °C to allow complete extension of the PCR products. The PCR products were visualized on 1% agarose gels stained with ethidium bromide.

2.4. Detection of *Microcystis* by UPLC MS/MS

Sampling was performed in March 2020 in Zaimán stream. Cells were collected by filtration (Whatman GF/F) from 1 liter of sample to detect intracellular MCY and the filtrate was used to determine the dissolved MCY. For identification and quantification, MCY-LR (Sigma-Aldrich®) was used as the standard. The chromatographic system used was a WATERS TQD UPLC MS-

MS with positive electrospray ionization (ESI+). A C18 column with dimensions of $150 \times 2.1 \text{ mm} \times 3 \mu\text{m}$ was used, with the mobile phase consisting of component A: 0.1% formic acid in water and component B: 0.1% formic acid in acetonitrile. The column temperature was set at $50 \text{ }^\circ\text{C}$, and the sample injection volume was $50 \mu\text{L}$ at a flow rate of 0.40 mL/min . The control corresponds to $500 \mu\text{g L}^{-1}$ of MCY-LR for ion 995.4-996.64.

2.5. Satellite images analysis

Satellite images were obtained from Sentinel-2 using the EO Browser (<https://apps.sentinel-hub.com/eo-browser>). We selected the area of interest to obtain satellite images and used the Custom Script tool from the EO Browser. The R script from the Cyanolakes (GitHub, 2023) was used to analyze the images (Kravitz & Matthews, 2020). This script provides an estimation of chlorophyll-a based on the simulated dataset for cyanobacteria *Microcystis aeruginosa* using a normalized difference chlorophyll-a index for the use of L1C Sentinel-2 image data (Kravitz & Matthews, 2020). Satellite images were selected based on cloud coverage was less than 90% around the time of sample collection.

3. Results

In all samples collected, colonies belonging to *Microcystis aeruginosa* complex (MAC) of varying sizes surrounded by mucilage were observed (Figures 2a-c). In the sample from March 2022, collected from the Mártires stream, we were also able to identify *Dolichospermum* sp. (Figure 2b).

Molecular analyses revealed that *cpcBA* gene can be successfully amplified from samples collected

during the summer of 2020-2021 from the Paraná River and Zaimán stream, confirming the presence of cyanobacterial DNA. Additionally, amplification of the *mcyE* gene indicates that these samples have the potential to produce MCY (Figure 3).

The presence of this toxin was confirmed by UPLC MS/MS in samples from March 2021 obtained from the Zaimán stream. The result showed an intracellular concentration of $\sim 250 \mu\text{g/L}$ MCY-LR equivalents (Figure 4b). Although this represents a high value, the sample was taken on the shore of the stream where there was cyanobacteria scum. During the summer of 2021-2022 the potential for MCY production was also verified by PCR (Figure 3). Following the identification of *Dolichospermum* sp. in the Mártires stream sample, we conducted amplification of the *anaC* gene to specifically target the presence of anatoxin-encoding genes. However, our analysis did not reveal the presence of these genes in the sample.

The satellite image record showed that the presence of cyanobacteria in the Paraná River during 2020 was not intense enough to be detected (Figure 5), although cyanobacteria scum was evident on the shore. To analyze the chlorophyll-a concentration in the Zaimán stream, we specifically chose the images captured on days when the cloud coverage was less than 90% around the time of sample collection. These images consistently displayed a high level of chlorophyll-a, aligning with our field observations. Similarly, the same criteria were applied to select images from the Mártires stream, which clearly depicts the extent of the cyanobacterial accumulation in the water body and its elevated chlorophyll index.

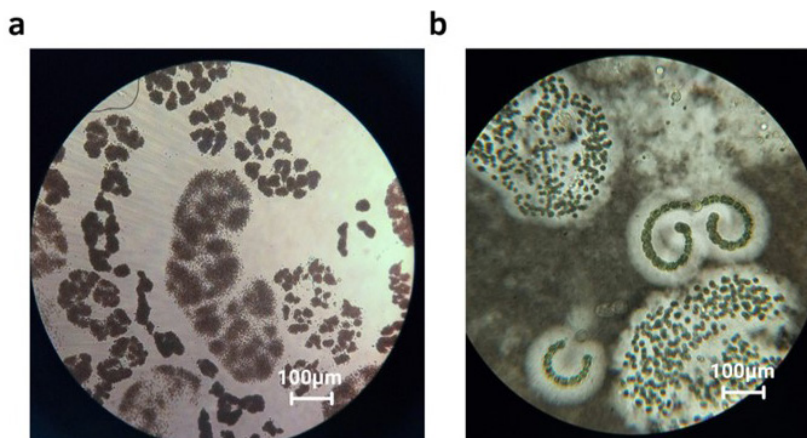


Figure 2. Colonies of *Microcystis* spp. were observed under an optical microscope (400X) in samples obtained from Paraná (a) and Mártires (b) and *Dolichospermum* sp. in the sample from the Mártires stream (b) on March 18, 2022.

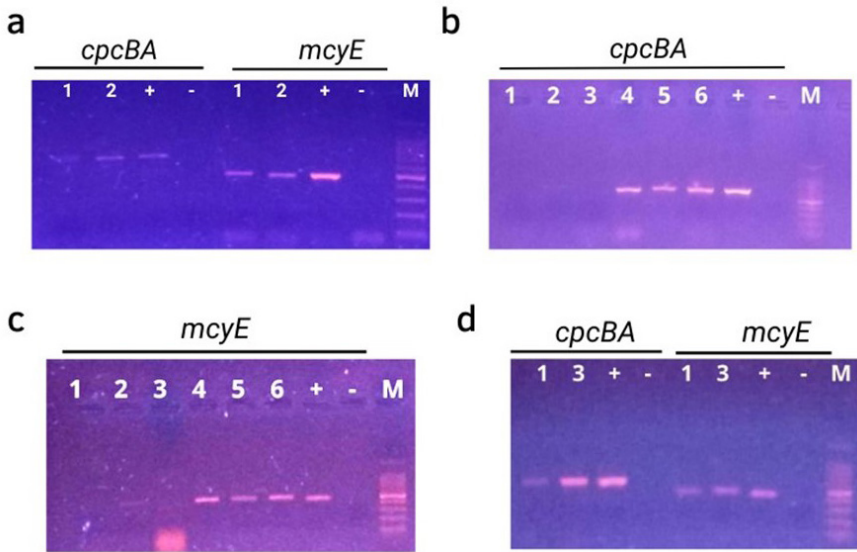


Figure 3. Molecular detection of toxigenic genotypes and microcystins. (a) DNA was extracted from samples from the Paraná River in November 2020 (lane 1) and those obtained from the Zaimán stream in March 2021 (lane 2). The *cpcBA* gene (intergenic spacer of the phycocyanin B and A gene) and the *mcyE* gene were amplified by PCR. Control (+) *M. aeruginosa* PCC7806. The DNA obtained from samples from December 2021 to March 2022 was used to amplify the *cpcBA* gene (b) and the *mcyE* gene (c) by PCR: Zaimán 03/11/2021 (lane 1), Paraná 12/12/21 (lane 2), Mártires 02/22 (lane 3), Zaimán 02/18/22 (lane 4), Zaimán 02/28/22 (lane 5), Mártires 03/18/22 (lane 6). A new DNA extraction was performed from samples 1 and 3, obtaining positive amplification of both genes (d). Control (+) *M. aeruginosa* PCC7806.

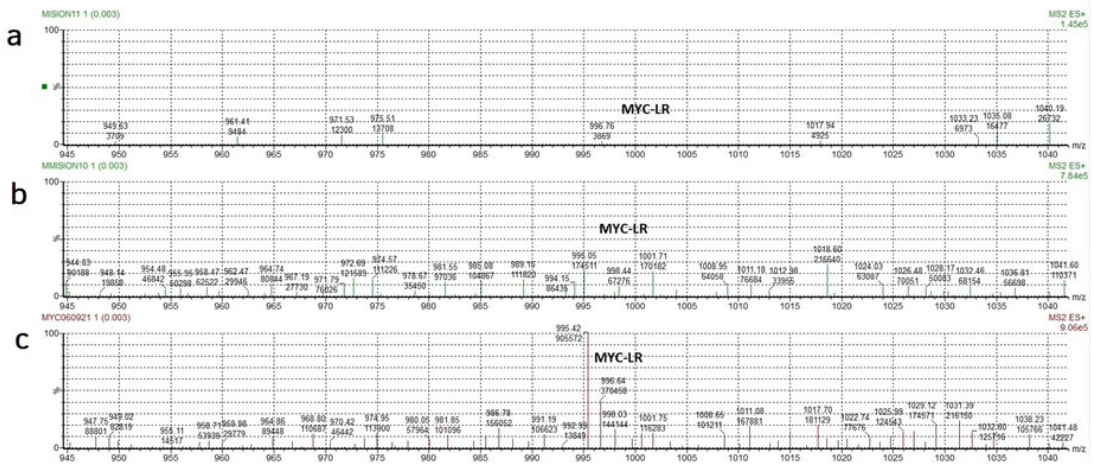


Figure 4. Detection of dissolved (a) and intracellular (b) MCY in samples from Zaimán stream from March 2021 by UPLC-MS/MS using as standard MCY-LR (c).

4. Discussion

Both the presence of *Microcystis* sp. and MCY were detected by microscopic, molecular, and analytical methods in two urban water bodies and along the coast of the Paraná River in the city of Posadas, Misiones, Argentina. This is the first report of toxic cyanobacteria in the area, which represents a risk not only for human and animal health, but

also can cause ecosystem disturbances and reduction in biodiversity.

CyanoHABs are an emerging problem due to their negative impact on water quality. In Argentina, records of these phenomena date back to 1990 and have been increasing in recent years (Aguilera et al., 2017). During this study, we identified *Microcystis* sp. as the predominant genus in bloom samples,

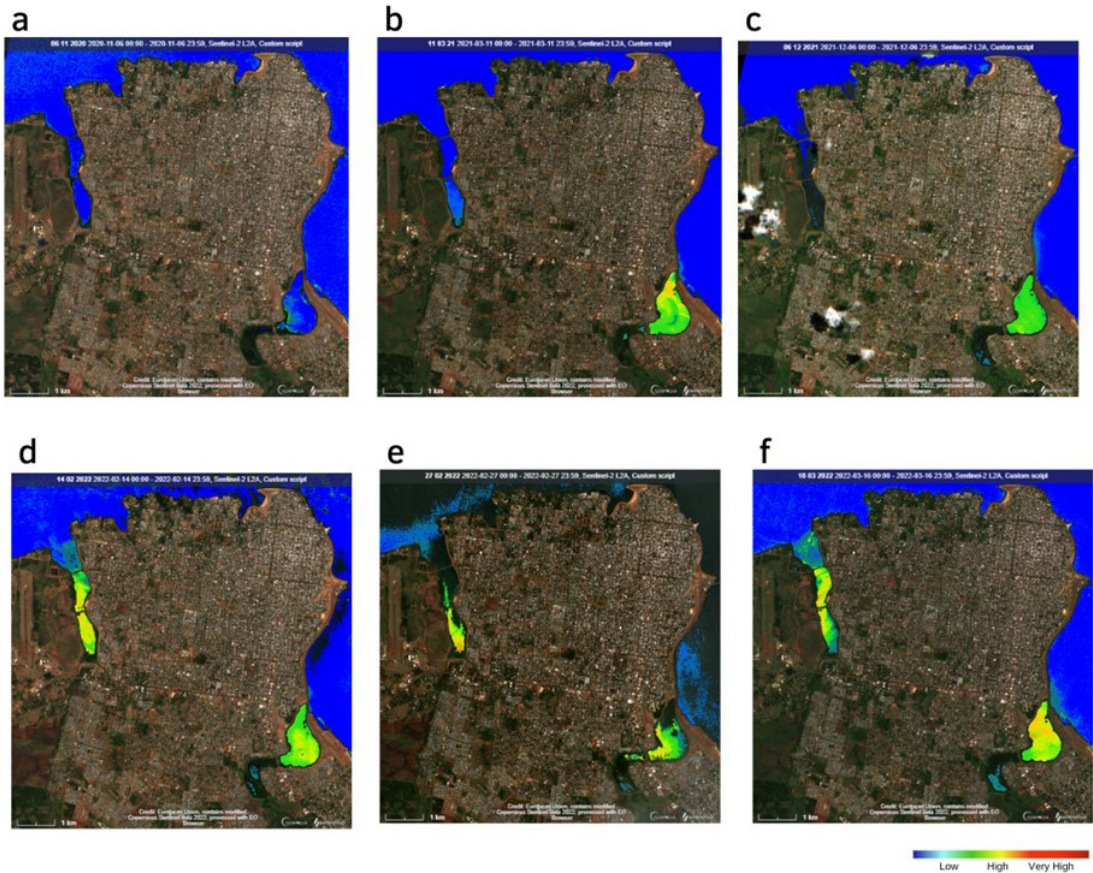


Figure 5. Satellite images of the coastal region of the Paraná River and the Zaimán and Martires streams. Images were obtained from the Sentinel 2 L1C analyzed with the Chlorophyll-a NDCI L1C image processor to estimate the chlorophyll-a of superficial cyanobacterial blooms. The images correspond to 11/06/2020 (a), 03/11/2021 (b), 12/06/2021 (c), 02/14/2022 (d), 02/27/2022 (e) and 03/16/2022 (f). The color scale that corresponds to different levels of chlorophyll is shown at the bottom of the figure.

as has been reported in most cyanoHABs in Argentina (Aguilera et al., 2017), and the presence of MCY was verified by analytical methods. Additionally, *Dolichospermum* sp. was found together with *Microcystis* in the Mártires stream, which is common in subtropical reservoirs and rivers (Aguilera et al., 2017). Both streams flow into the Paraná River, which spans 4965 km and is the second longest in South America, covering an area of 891000 km² across Brazil, Argentina, and Paraguay. Due to its high flow rate (16000 m³/s), 389 dams of various sizes have been built, mainly in its upper part in Brazil (Makrakis et al., 2019), which has a great impact on biodiversity and water quality along the basin and modifies the hydrological regimes of ecosystems in the streams and lagoons within the Paraná basin (Thomaz et al., 2007), as has occurred mainly in the Zaimán (Lecertua et al., 2009). In this regard, studies have been conducted on changes in phytoplankton

diversity and fluctuations during periods of low and high-water levels in some reservoirs and lagoons in Upper Paraná, Brazil, showing the presence of cyanobacteria, especially *Microcystis* sp. (Fonseca & Rodrigues, 2007; Vieira da Silva et al., 2022). In our work, we observe the appearance of cyanobacteria during a period of maximum river lowering, with a value of 975 cm at Posadas when the alert minimum 968 cm (INA, 2022). This was accompanied by mild to severe drought and very high temperatures during sampling dates as reported by the Servicio Meteorológico Nacional Argentino (SMN, 2022a, b), conditions that favors the appearance of cyanobacteria (Huisman et al., 2018).

The investigation of cyanoHABs in aquatic environments associated with rivers is relevant, as indicated by Kruk et al. (2021) findings in the Uruguay River where they concluded that environments, such as ponds, dams, and low-flow streams, may act as reservoirs of cyanobacteria.

When water levels increased due to rainfall events, cyanobacteria are transported to the main channel leading to blooms in various remote locations, negatively impacting ecosystems and water quality.

A complementary tool for studying blooms is the use of satellite images for estimating chlorophyll-*a*. In this study, a tool designed and validated using field samples and freely available was used (Kravitz & Matthews, 2020; Kravitz et al., 2021). Our results indicate that, during the sampling period, except for November 2020 was consistent with *in situ* observations during sampling (Figure 5). This tool proved to be useful as it allowed us to evaluate the extension of the bloom in the water body.

5. Conclusion

This study provides the first report of cyanobacteria and their toxins in the region, confirming the presence of *Microcystis* sp. and microcystin (MCY) toxin production. The identification of *Dolichospermum* sp. in conjunction with *Microcystis* highlights the complex nature of cyanobacterial assemblages in subtropical reservoirs and rivers.

The extensive length of the Paraná River, coupled with hydrological modifications resulting from dam constructions, further exacerbates the changes associated with water quality and biodiversity conservation in the streams that integrates the basin. Therefore, it is crucial to conduct comprehensive surveys to monitor nutrient variations, cyanobacterial taxa, and toxin production to establish alert systems.

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