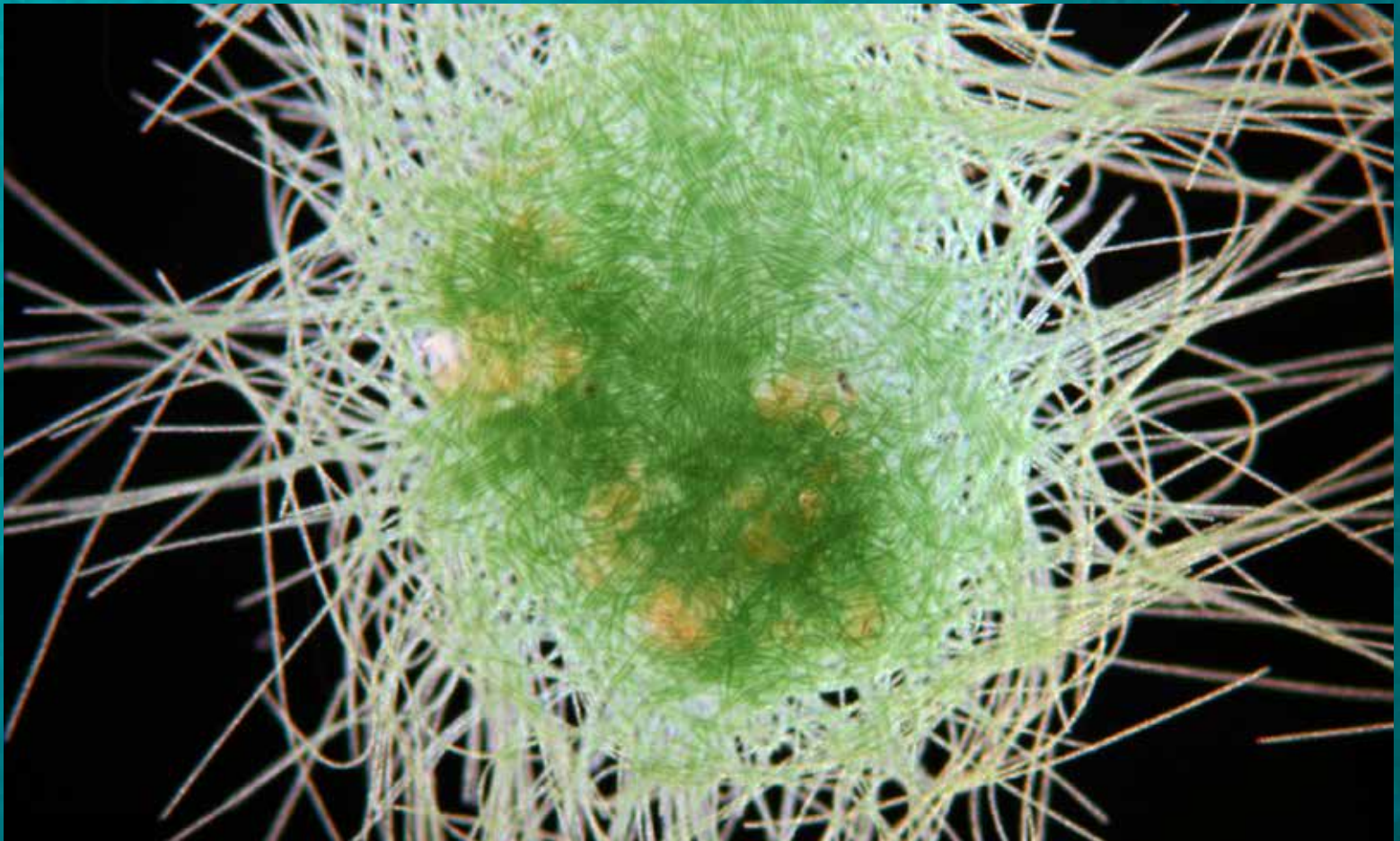


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Monitoring microcystin-LR-producing cyanobacterial bloom in La
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Monitoring microcystin-LR-producing cyanobacterial bloom in La Purísima Dam in Guanajuato, Mexico

Monitoreo de floración cianobacteriana productora de microcistina-LR en la Presa La Purísima en Guanajuato, México

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ABSTRACT

Toxic cyanobacterial blooms are a growing concern due to their increasing frequency. The presence of cyanotoxins, classified as emerging contaminants, in freshwater sources poses a serious threat to aquatic ecosystems and human health. Assessing cyanotoxin levels in water bodies is essential to safeguard public well-being and ensure the continued availability of this vital resource. In Guanajuato, the water from dams and reservoirs is primarily used for crop irrigation, as well as for recreational activities and fishing. La Purísima Dam is a Protected Natural Area with a background of eutrophication and home to migratory birds. This study reports the presence of

Planktothrix, *Microcystis* and *Raphidiopsis*. The genes *mcyC*, *mcyD*, *cyrA*, and *sxtA*, which are involved in the synthesis of microcystins, cylindrospermopsin and saxitoxin, were detected at DNA level, and MC-LR was quantified.

Key words: cyanotoxins, *Cylindrospermopsin*, reservoirs, *Planktothrix*, saxitoxin.

RESUMEN

Las floraciones tóxicas de cianobacterias son una preocupación creciente debido a su incremento. La presencia de cianotoxinas, clasificadas como contaminantes emergentes, en fuentes de agua dulce representa una seria amenaza tanto para los

ecosistemas acuáticos como para la salud humana. Conocer los niveles de cianotoxinas en los cuerpos de agua es esencial para garantizar el bienestar público y la disponibilidad de este recurso vital. En Guanajuato, el agua de presas y embalses se utiliza principalmente para el riego de cultivos vegetales, así como para actividades recreativas y pesca. La presa La Purísima es un Área Natural Protegida con antecedentes de eutrofización y es hábitat de aves migratorias. Este estudio reporta la presencia de los géneros de cianobacterias *Planktothrix*, *Microcystis* y *Raphidiopsis*. Adicionalmente, los genes *mcyC*, *mcyD*, *cyrA*, y *sxtA*, que están involucrados en la síntesis de microcistinas, cilindropermopsinas y saxitoxina se detectaron a nivel de ADN y se cuantificó la MC-LR.

Palabras clave: cianotoxinas, *Cylindropermopsina*, embalses, *Planktothrix*, saxitoxina.

INTRODUCTION

Cyanobacterial blooms are a growing global concern due to their ability to produce cyanotoxins, specialized metabolites harmful to both ecosystems and public health. Some cyanobacterial species can synthesize a wide variety of toxins, which are classified according to the primary organ they affect: hepatotoxins (e.g., microcystins), neurotoxins (e.g., anatoxins and saxitoxins), cytotoxins, and dermatotoxins (Chorus & Welker 2021). Microcystin-LR (MC-LR), the most studied and prevalent variant, is particularly hazardous due its stability in water, resistance to conventional water treatment, and ability to bioaccumulate in aquatic food webs (Akbar *et al.* 2022; Zhang *et al.* 2023).

Recreational activities in water bodies such as dams represent a significant route of human exposure to cyanotoxin through ingestion, skin contact, or inhalation of aerosolized particles. Such exposure to cyanotoxin has been linked to liver damage, gastrointestinal disorders, and potential long-term carcinogenic effects (Chorus & Welker 2021; Graham *et al.* 2020). At the same time, eutrophication driven by anthropogenic nutrient inputs, along with climate change-related factors like increased temperature and light availability, strongly favors the formation and persistence of cyanobacterial blooms (López-Hernández *et al.* 2022; Paerl & Otten 2013). Human activities in water reservoirs contribute to the proliferation of cyanobacterial blooms, adversely affecting water quality and public health. Therefore, it is imperative to establish monitoring protocols for the prevention and remediation of cyanotoxin contamination.

In Mexico, the National Water Commission has defined 757 hydrological basins, organized into 37 hydrological regions, grouped into 13 hydrological-administrative regions. The state of Guanajuato, located in hydrological regions 12 and 26, comprises nine major dams that are vital for various human activities, including agriculture, livestock, fishing, industry, domestic use, electricity generation, and the preservation of protected flora and fauna (Sistema Nacional de Información del Agua 2024). Knowing the cyanotoxin content in water bodies is essential to ensure public welfare and the availability of this vital liquid (Paerl & Otten 2013; Zahra *et al.* 2020).

Despite their importance, technical reports studies that evaluate the presence of cyanobacteria and cyanotoxins in Guanajuato's reservoirs are notably scarce, particularly those involving molecular detection techniques or toxin quantification. Given the high ecological and social value of these water bodies, systematic monitoring protocols are essential not only to mitigate risks to human and animal health, but also to inform water management policies and conservation strategies (Rodríguez *et al.* 2024). This study aims to fill this knowledge gap by monitoring cyanobacterial blooms and Microcystin-LR levels in Purísima Dam, an artificial reservoir of ecological significance in Guanajuato, Mexico, using morphological identification, molecular tools, and toxin quantification assays.

METHODOLOGY

For the sample collection, preservation, and transportation procedures, the protocol outlined by NOM-230-SSA1-2002 was followed. Sterile glass containers with ground-glass stopper were used to collect the samples from a surface body, the flask was submerged in the water with the neck pointing downward to a depth of 15 to 30 cm. The sampling was conducted on March 11, 2024. Collected point locations were MLP1: 20° 53' 41" N, 101° 16' 49" W (20.894722, -101.280278); MLP2: 20° 53' 45" N, -101° 16' 45" W (20.895833, -101.279167); MLP3: 20° 52' 20" N, -101° 17' 34" W (20.872222, -101.292778); MLP4: 20° 52' 09" N, -101° 17' 28" W (20.869167, -101.291111).

Morphological identification and quantification were performed using a compound microscope (Leica DMLS) following the key guidelines of Komárek & Anagnostidis (1999). Cell counts were conducted using Sedgwick-Rafter chamber, as outlined by APHA (2017).

For microcystin quantification, samples were analyzed using the Microcystin ADDA ELISA kit, following the manufacturer's instructions (Abraxis Eurofins® product #520011OH).

Before pelleted cells from environmental samples, 400 mL of the sample was homogenized and poured into an ultrasonic sonicator, treated for 90 seconds at 42 kHz. Aliquots of 50 mL were then centrifuged at 5,200 rpm for 10 minutes. The supernatant was decanted, and this procedure was repeated until a pellet of at least 50 mg wet weight was obtained. The cell pellets were stored at -70 °C before use. Genomic DNA was extracted using the DNA mini-prep kit (Zymo Research) according to the instructions of the manufacturer. Amplification of genes 16S, *mcyE*, *cyrA* and *sxtA* by PCR technique was performed with the Dream Taq super mix reaction mixture (Thermo Scientific) with oligos 16SF (AGCCCACTGGGACTGAGACA), 16SR (TCGCCATTGCGGAAA), *mcyF* (AATAAATCATAATTTAGAACS GGVGATTTAGG), *mcyR* (AATAAATCATAACGRBTVADTTGRTATTCAATTTCT), *cyrF* (GTCTGCCACGTGATGTTATGAT), *cyrR* (CGTGACCGCCGTGACA); *sxtF* (GGAGTGGATTCAACACCAGAA), *sxtR* (GTTTCCCAGACTCGTTTCAGG) (Al-Tebrineh *et al.* 2012), with the following program: an initial denaturation for 5 min at 95 °C, followed by 25 cycles at 94 °C for 1 min, 53.6 °C for 50 s, and 72 °C for 2 min, with a final extension step at 72 °C for 5 min. The PCR

products were observed using 1 % agarose gel electrophoresis, staining, and visualized.

RESULTS

The La Purísima Dam area is part of the Silao-Romita aquifer. This dam receives effluents from Guanajuato rivers. Chapín and Trinidad rivers supplies water for irrigation. Samples were collected from four points around the dam, specifically in areas with greater green coloration, the presence of scum, and the locations of water pumps. Locations MLP1 and MLP2 are near to the Molinero community, where a strong fishy odor was detected in the area, that could be associated with the presence of geosmin, a compound linked to cyanobacterial blooms (Fig. 1A-1C). The MLP2 sampling point was notable because residents were pumping water from this location for irrigation for cultivating coriander and zucchini (Fig. 1C). At the MLP3 sampling point, scum was observed and MLP4 sample was taken near the water pump that supplies water to cultivation land (Fig. 1D, 1E). At the collection sample time, La Purísima dam was medium, with a fill percentage of 54 % relative to the ordinary maximum water level.

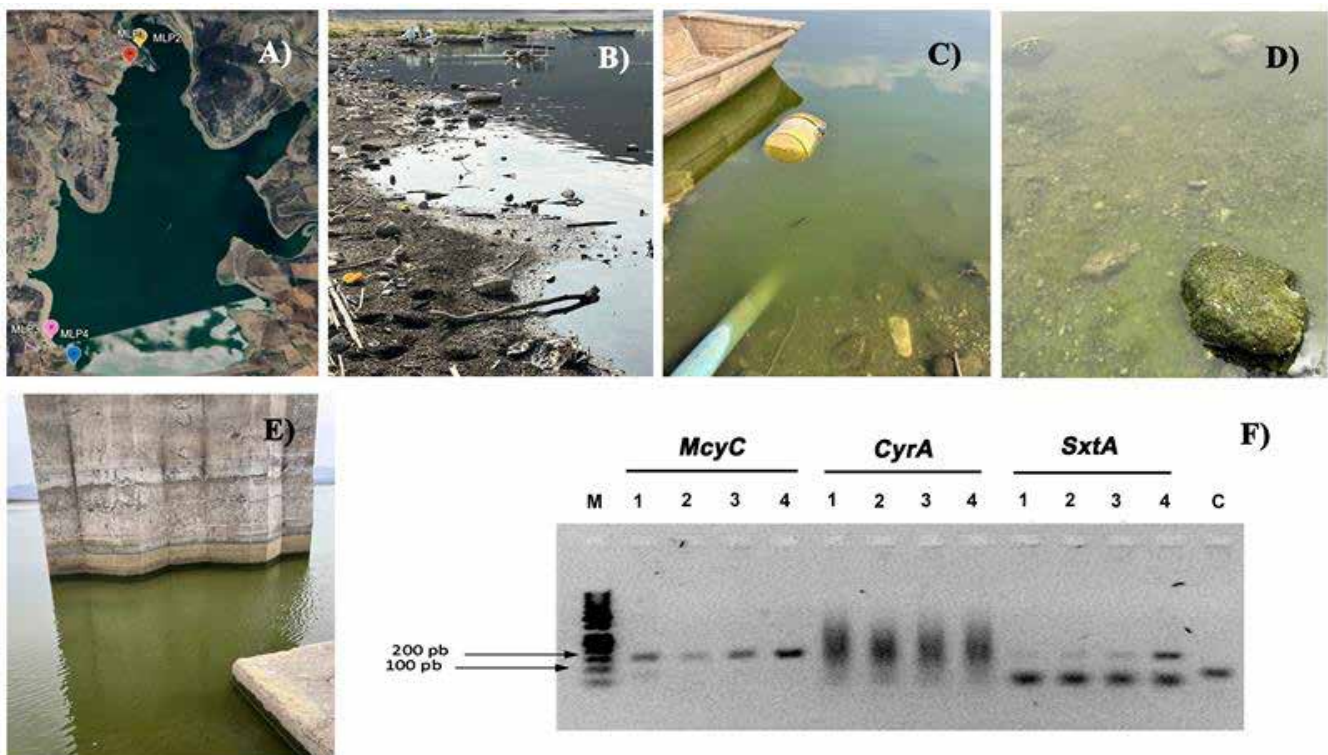


FIGURE 1. Collecting points at the La Purísima dam on March 11th, 2024. A. A) Satellite map of La Purísima dam, with sampling points MLP1, MLP2, MLP3, and MLP4 highlighted. B) MLP1. C) MLP2. D) MLP3. E) MLP4. F. Amplification of DNA regions encoding genes involved in cyanotoxin synthesis: *mcyC*, *cyrA* and *sxtA* at the four sampling points (1: MLP1; 2: MLP2; 3: MLP3; 4: MLP4). M: Molecular weight marker.

Microscopic inspection revealed 11 different species, including *Chlamydomonas* sp., *Trichodesmium* sp., *Trachelomonas* sp., *Lemmernannia tetrapedia*, *Nitzschia* sp., *Mesodinium rubrum*, *Scenedesmus* sp., *Zygnema* sp., *Monoraphidium capricornutum*, *Oscillatoria* sp. and *Eudorina elegans* were identified (Table 1) according to morphological criteria. Cyanobacteria with toxic potential, such as *Planktothrix* as the dominant microcystin-producing genus co-occurring with *Microcystis* and, to lesser extent, *Raphidiopsis*, were also observed, as confirmed by metagenomics 16S (data not shown).

Regions of cyanotoxin synthesis gene clusters were amplified by PCR to detect toxigenic genotypes. *mcyC* for microcystin synthesis, *cyrA* for cylindrospermopsin synthesis and *sxtA* for saxitoxin synthesis gene (Al-Tebrineh *et al.* 2012). From all point samples, a PCR product was amplified corresponding to the molecular weight of each cyanotoxin biosynthesis gene. Thus, indicate the presence of the genes and genotype capacity for toxin production (Fig. 1F). The results for the concentration of MC-LR were below detection limit (0.1 ppb), indicating minimal microcystin levels in the water samples. Specifically, the concentrations were as follows: MLP1 and MLP2: 0.06 ppb; MLP3: 0.21 ppb; MLP4: 0.05 ppb. The lower detection limit was 0.56 ppb therefore, no significant MC-LR production was occurring.

DISCUSSION

On November 25, 2005, declared the area known as La Purísima Dam and its surrounding zone as a Protected Natural Area (ANP) in the Sustainable Used category by Mexican Government Decree No. 249. This designation, requested by the Civil Association *Colonos y Usuarios de la Presa La Purísima, A. C.*, and the State Ecology Institute, recognized the ecological relevance of the site due to its high aquatic biodiversity and its role in sustaining migratory bird population and aquatic life (Periódico Oficial 188). In addition to its ecological value, La Purísima serves as a recreational area and receives water inputs from the Guanajuato, Chapín and Trinidad rivers.

This dam supplies water to Irrigation District No. 11 and supports a wide range of aquatic species that are harvested for human consumption. However, irrigation with water contaminated by cyanobacteria poses a significant public health risk. Cyanotoxins produced during toxic blooms can adversely affect soil microbial communities, bioaccumulate in plant tissues, and ultimately enter the food chain, reaching fruits and vegetables consumed by the population (Bouaïcha & Corbel 2016; Levizou *et al.* 2020; Machado *et al.* 2017).

One of the persistent issues in La Purísima dam is its eutrophic condition, exacerbated by untreated domestic and agricultural waste, elevated temperatures, and poor water circulation (Tirado *et al.* 2023). These factors collectively promote phytoplankton growth and increase the frequency of cyanobacteria blooms, as confirmed in the present study (Table 1).

Our monitoring from 2021 to 2024 has consistently documented eutrophic conditions and the presence of potentially toxigenic cyanobacteria. The genera *Planktothrix*, *Raphidiopsis*, *Oscillatoria*, *Microcystis*, and *Anabaena/Dolichospermum* have been recurrently identified, all of which are known microcystin producers and are among the most common genera responsible for cyanotoxin blooms worldwide (De Alavision *et al.* 2023).

Quantification of microcystin-LR (MC-LR) across different years showed the following concentrations: 0.42 ppb ($\mu\text{g/L}$) in 2021; 0.30 ppb in 2022 (to be published); and 0.21 ppb in 2024 (this study). These levels, while below the World Health Organization's guideline value for drinking water (1 $\mu\text{g/L}$), indicate a persistent presence of cyanotoxins. Notably, these concentrations are up to ten times lower than those reported for other artificial reservoirs (Rodríguez *et al.* 2024), yet their chronic presence may still pose risks for long-term human exposure and ecosystem health.

Molecular analysis by PCR confirmed the presence of gene clusters responsible for the synthesis of microcystins (*mcyC*), cylindrospermopsins (*cyrA*), and saxitoxins (*stxA*) (Fig. 1F), supporting the toxigenic potential of the cyanobacterial community. These findings along with previous metagenomics data (unpublished), which detected *Planktothrix*, *Microcystis* and *Raphidiopsis* as dominant genera in the reservoir.

Given these results, it is essential to implement routine molecular and chemical monitoring of La Purísima Dam to detect early signs of toxigenic blooms and prevent potential health impacts. The integration of molecular tools like PCR and quantitative toxin assays (e.g. ELISA) into local water management protocols can serve as an early warning system.

Finally, this study represents a contribution to the understanding of cyanobacterial contamination in freshwater bodies of Guanajuato. To our knowledge, this is the first report to provide a comprehensive assessment combining field observations, morphotaxonomic identification, molecular detection of toxin genes, and quantification of MC-LR in La Purísima Dam an ecosystem of ecological and social importance for the region.

Table 1. Microbial diversity and cell density measured using Sedgwick-Rafter counting chamber.

Sample	Identification	Cell density (cells/L)	Taxonomic Group
MLP1	<i>Chlamydomonas</i> sp.	142,857	Chlorophyceae (Green algae)
	<i>Oscillatoria</i> sp.	85,714	Cyanophyceae (Cyanobacteria)
	<i>Trachelomonas</i> sp.	192,857	Euglenophyceae (Euglenoids)
	<i>Zygnema</i> sp.	64,285	Zygnematophyceae (Green algae)
	<i>Scenedesmus</i> spp	92,857	Chlorophyceae (Green algae)
	<i>Nitzschia</i> sp.	64,285	Bacillariophyceae (Diatoms)
	No identified	128,571	Unidentified
	<i>Crucigenia tetrapedia</i>	200,000	Chlorophyceae (Green algae)
	<i>Myrionecta rubra</i>	128,571	Cryptophyceae (Mixotrophic protists)
	<i>Selenastrum capricornctum</i>	85,714	Chlorophyceae (Green algae)
	$\Sigma=$	1,185,711	
MLP2	<i>Trichodesmium</i> sp.	16,564,285	Cyanophyceae (Cyanobacteria)
	<i>Chlamydomonas</i> sp.	1,971,428	Chlorophyceae (Green algae)
	<i>Nitzschia</i> sp.	42,857	Bacillariophyceae (Diatoms)
	<i>Zygnema</i> sp.	35,714	Zygnematophyceae (Green algae)
	<i>Scenedesmus quadricauda</i>	21,428	Chlorophyceae (Green algae)
	<i>Eudorina elegans</i>	42,857	Chlorophyceae (Green algae)
	$\Sigma=$	18,678,569	
MLP3	<i>Chlamydomonas</i> sp.	190,571,428	Chlorophyceae (Green algae)
	<i>Trichodesmium</i>	31,500,000	Cyanophyceae (Cyanobacteria)
	<i>Trachelomonas</i> sp.	71,428	Euglenophyceae (Euglenoids)
	$\Sigma=$	222,142,856	
MLP4	<i>Trichodesmium</i> sp.	17,414,285	Cyanophyceae (Cyanobacteria)
	<i>Chlamydomonas</i> sp.	6,785,714	Chlorophyceae (Green algae)
	<i>Nitzschia</i> sp.	71,428	Bacillariophyceae (Diatoms)
	$\Sigma=$	24,271,427	

CONCLUSIONS

Nontoxic cyanobacterial bloom containing the general *Planktothrix*, *Microcystis* and *Raphidiopsis* was found in La Purísima Dam. Genes involved in the production of microcystin, cylindrospermopsin and saxitoxin were detected by PCR. However, MC-LR level suggests that gene expression was not active at enough level to be detected, or it is post-transcriptionally or post-translationally regulated.

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