Topics of limnological research in Mexico

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Universidad de Colima

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This book is dedicated to
Dr. Singaraju Sri Subrahmanya Sarma,
in gratitude for all his teachings in the world of limnology.

Index

Towards Molecular, Genetic, and Optical Monitoring of Potentially Harmful Cyanobacteria Blooms in Mexican Freshwater Bodies

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Abstract

Programs for safe cyanotoxins-free water use in drinking and irrigation water are fundamental to avoid potential health risks and should be implemented. Data on limnological waterbodies, either natural or artificial, related to cyanobacterial bloom occurrence are essential to estimate the frequency of possible dangerous events and to establish strategies for monitoring and prevention. In Mexico, predominant cyanobacteria in freshwater algal blooms are *Microcystis* sp., *Planktothrix* sp., and *Raphidiopsis* spp. Cyanotoxins bioaccumulate in soil and plants through irrigation water and transfer to edible vegetables affecting plant physiology and food crop productivity. The harmful cyanobacterial blooms and cyano-

toxins production affect microbial diversity in waterbodies and soil. This work emphasizes standardized protocols using chemical, genomic, and optical techniques for potential toxic blooms in water bodies in the early stages.

Keywords

Cyanotoxins, microcystin, cyanobacterial bloom, cyanotoxin bioaccumulation, molecular detection, abundance monitoring.

Introduction

Bloom formation is a natural phenomenon where phytoplankton and cyanobacteria grow excessively, favored mainly by increased nutrients and the change of water flow conditions, and ultimately, by global warming. However, when toxins are produced, generally by cyanobacteria, they are known as cyanobacterial harmful blooms. These blooms have adverse environmental effects, including damage to flora and fauna, economic losses, and jeopardy to human health (Huisman et al., 2018; Anderson et al., 2021; Karlson et al., 2021). The control of harmful blooms in Latin America has gained greater importance in recent decades due to: a) the increasing scarcity of water, b) the deterioration of its quality, c) the lack of common regulations, monitoring efforts, and public access to the data generated, and d) protocols to evaluate harmful cyanobacteria (Aguilera et al., 2023).

Through the detection, monitoring, and prediction of cyanobacteria blooms, the quality of the water bodies can be determined to guarantee their safe consumption (Cannizzaro et al., 2019). The purpose of this chapter is to review the current knowledge on harmful cyanobacterial blooms, primarily in Mexico. Additionally, it aims to highlight the advancements in detection methods of cyanotoxins, to design a practical monitoring scheme for these increasingly prevalent and impactful phenomena. Among the techniques addressed in this chapter are mass spectrometry-based metabolomics, 16S or 23S rRNA gene sequencing, cyanotoxins biosynthesis gene amplification by polymerase chain reaction (PCR), quantitative-PCR (qPCR)-based probes, reverse transcription PCR (RT-PCR) and DNA arrays, high-throughput sequencing metagenomics, third generation sequencing technologies, as well as remote sensing, radiative transfer, as well as bio-optical sensing strategies are explored.

Diversity of Cyanobacterial Blooms in Mexico and Latin America: Status

The microbial population dynamics on freshwater bodies are correlated to seasons leading to different nutrient loads, and temperatures. However, gradual increases in temperature, mainly due to global warming, and anthropogenic perturbations, such as discharges of wastewater, and agriculture runoffs, among other factors, trigger the eutrophication phenomena in which taxonomic and functional diversity is reduced, as well as other abiotic

variables. These changes ultimately harm the stability of the ecosystem (Tomasin-Ortiz, 2012; Zhu et al., 2019).

In a recent review (Aguilera et al., 2023) Latin American countries such as Argentina, Chile, Brazil, and Uruguay presented the highest number of harmful cyanobacterial taxa. The most frequently found genera were *Aphanizomenon, Dolichospermum, Microcystis, Planktothrix, Pseudanabaena,* and *Raphidiopsis.* Among them, *Microcystis* was continuously reported with values more than 25 % of the cases per country. In Mexico, blooms of harmful cyanobacteria in freshwater bodies have been studied, some recent examples are presented in Table 1.

Table 1. Microbial Diversity Reported Studies of Cyanobacterial Blooms in Natural and Artificial Water Bodies Located in Mexico.

Water and Location (State)	Dominant Species Detected	Year of Study	Predominant Toxin Detected	Reference
Natural lake Zumpango, Reservoir, Chapultepec and Cuemanco (CDMX)	*M. aeruginosa, Planktothrix agardhii, M. flos-aquae*, *M. panniformis*, and *M. novacekki, M. wesenbergii*, *M. aeruginosa*, *Woronichinia naegeliana* and *Lyngbya birgei*	2010	MC-LR	Vasconcelos et al., 2010
Santa María del Oro Crater Lake (Nayarit)	*Limnoraphis robusta; Microcystis aeruginosa*	2015	MC-WR, MC-LR, MC-LA, MC-HilR, MC-LF, MC-YR, and MC-LY.	Bustillos-Guzmán et al., 2020
Lagunas de Montebello, National Park (Chiapas)	*Planktothrix agardhii*	2017	Not Measured	Yanez-Montalvo et al., 2022
Lake Alberca de Tacámbaro. (Michoacán)	*M. aeruginosa*	2018 -2019	Not Measured	Montero et al., 2021
The Apatlaco River (Morelos)	*Acinetobacter, Arcobacter, and Myroides* phyla	2019	Not Measured	Breton-Deval et al., 2019
The Valle de Bravo Reservoir (CDMX)	*M. wesenbergii*, *M. aeruginosa*, *Woronichinia naegeliana* and *Lyngbya birgei*	2019	Not Measured	Nandini et al., 2019
Zumpango Lake, Chapultepec Lake, Alameda Oriente Lake, and Virgilio Uribe Olympic Rowing-Canoeing Track (CDMX)	*Microcystis, Planktohtrix*	2020	MC-LR	Pineda-Mendoza et al., 2020

Water and Location (State)	Dominant Species Detected	Year of Study	Predominant Toxin Detected	Reference
El Palote Dam (Guanajuato)	*Planktothrix* spp., *Raphidiopsis* spp., and *Limnothrix redekei*	2020	Not measured	Valdés-Santiago et al., 2021

A common feature of harmful cyanobacteria blooms is the changes in microbial community composition over time, such as in the monomictic Alberca de Tacámbaro Lake. Although this lake was oligotrophic in 2006, with high microbial diversity with *Chroococcus minutus* and *Gloeocapsiopsis crepidinum* as dominant species, in 2018 and 2019, when a eutrophication phenomenon was evident, only the cyanobacterium *M. aeruginosa* was detected as the dominant species with a greatly reduced microbial diversity mainly due to anthropogenic activities (Hernández-Morales et al., 2011; Montero et al., 2021). A similar phenomenon was recorded in the "Lagunas de Montebello" National Park, where in recent years some of its lakes have shown eutrophication with *Planktothrix agardhii* as the dominant species with almost 97 % relative abundance, in contrast to the oligotrophic lakes, where the cyanobacteria *Cyanobium* was relatively more abundant and a greater relative diversity was seen (Yanez-Montalvo et al., 2022).

Seasonal changes in microbial diversity are also well documented. In 2019, in the Valle de Bravo Reservoir, it was observed that *M. aeruginosa* was the dominant cyanobacterium in January, while *Woronichinia naegeliana* was dominant in September (Vasconcelos et al., 2010; Nandini et al., 2019). In the same way, the immediate effect of human activities on eutrophication is also clearly established, since in the Apatlaco River the phyla Proteobacteria and Bacteroidetes were abundant in almost all the sites sampled. In the contaminated ones, Acinetobacter, Arcobacter, and Myroides were the dominant phyla (Bretón-Deval et al., 2019).

The Effects of Cyanotoxins and the Tools for their Identification

The presence of cyanotoxins in irrigation water, agricultural soil, and contaminated vegetables is concerning because it represents an indirect route of human exposure. The genera most recurrently reported producing cyanotoxin are *Microcystis, Anabaena, Oscillatoria,* and *Planktothrix*. In freshwater ecosystems from North and Central America, the most frequently reported cyanotoxins were microcystins (MC's), followed by anatoxins, cylindrospermopsins, and saxitoxins, and their producing genera identified were *Microcystis* spp., *Anabaena* spp., *Aphanizomenon* spp., *Lyngbya* spp., and *Raphidiopsis* spp. (Aguilera et al., 2018; Svirčev et al., 2019).

Soil accumulates cyanotoxins through irrigation with contaminated water which affects microbial diversity, microfauna, and plant development (Bouaïcha & Corbel, 2016).

The presence of MCs also leads to changes in bacterial diversity in soil (El Khalloufi et al., 2016). Plants can take up and bioaccumulate microcystin-LR (MC-LR) and cylindrospermopsin, in tissues, roots, seedlings, leaves, stems, and edible parts, affecting the physiology and morphology of them, hence, causing the loss of production (Machado et al., 2017; Levizou et al., 2020).

Studies carried out between 2000 and 2015 show that the cyanotoxins most frequently detected in water bodies in central Mexico are: microcystins, nodularins, cylindrospermopsins, and saxitoxins (Pérez & Sánchez, 2017). Cyanobacteria and cyanotoxin analysis techniques are divided into qualitative and quantitative methods to identify and measure their presence. Qualitative methods such as fluorescence microscopy which allows the identification of cyanobacteria by using specific fluorochromes (Jin et al., 2018), or flow cytometry which allows rapid and accurate quantification of cyanobacterial cells (Peniuk et al., 2016). In contrast, quantitative methods such as liquid chromatography coupled to mass spectrometry (LC-MS) are used to identify and quantify cyanotoxins in water samples (Oehrle et al., 2010).

Molecular Aspects of Cyanotoxins and Analytical Tools for their Identification

Microcystins are cyclic heptapeptides. The great diversity in the structures of cyanotoxins determines their molecular targets leading to a range of toxic effects in exposed organisms (Baliu-Rodriguez et al., 2022). MC-LR is one of several structural variants. It is the most studied since it is widely distributed and because of its toxicity, and it is considered a human carcinogen under chronic exposure (Huisman et al., 2018; Hernández et al., 2023). In contrast, cylindrospermopsin and anatoxin are heterocyclic alkaloids that possess a multi-organ toxicity with severe lesions of the liver, kidneys, and gastrointestinal tract among others (Yang et al., 2021). Metabolomic analysis for cyanotoxins involves a comprehensive approach that seeks to identify and quantify the complete set of metabolites present in soil and water at a specific time, to understand their distribution pathway, their toxicity, and their effects on human health and the environment (Rinehart, 1998). The precise identification can be performed following a global analysis of metabolites under certain conditions and/or focusing on a specific analysis of a group of known and related metabolites in which their mass-to-charge (m/z) ratios and retention times are often identified using reference standards. As a powerful analytical tool, liquid chromatography (LC) is common in metabolomic analysis. High-performance LC (HPLC) separates compounds based on their interactions in stationary and mobile phases. Gas chromatography (GC) can also be used, especially for volatile compounds. In the case of cyanotoxins, once separated, these compounds are introduced into the mass spectrometer, where they are ionized and fragmented; ionization can be performed by different techniques such as electrospray (ESI) or

chemical ionization (CI) in LC-MS, and electron impact ionization (EI) in GC-MS. Detection is based on the mass-to-charge ratio (m/z) of the ions generated, which allows the identification of cyanotoxins and related metabolites. High-resolution mass spectrometry (HRMS) provides higher accuracy in ion mass determination, improving selectivity and reliability in identifying cyanotoxins. For compound identification, databases and spectral libraries are used to identify peaks obtained by mass spectrometry, and subsequently mass spectra are compared and correlated with known profiles of cyanotoxins and other metabolites (Lee et al., 2010). Finally, the data obtained is subjected to multivariate statistical analysis to identify patterns, correlations, and significant differences between samples. It is important to note that these techniques are currently the most widely used because certified standards offer some advantages in terms of sensitivity, selectivity, and accuracy in the quantification of cyanotoxins in complex matrixes, such as environmental samples.

Molecular Techniques for Monitoring Potentially Harmful Cyanobacteria Blooms

In recent years, genetic and genomic strategies have been used to identify the presence of toxigenic species of cyanobacteria, as well as cyanotoxin biosynthesis operons. 16S rRNA V3-V4 region is commonly used to identify bacteria using amplicon sequencing. Identification of cyanobacteria relies on other genetic markers, including the *rpoC1* gene, intergenic regions, and conserved sequences of the 16S rRNA ribosomal gene (Rudi et al., 2000, Fergusson & Saint, 2003). Furthermore, other PCR-based methods, combined with indirect quantification using quantitative PCR (qPCR), provide high sensitivity (Asai et al., 2002; Rueckert et al., 2007). Specific cyanobacteria 16S rRNA gene amplification has been used before, as well as fluorescence probes targeting unique sequences in the 16S rRNA gene (Al-Tebrineh et al*.,* 2010). DNA arrays targeting the 16S rRNA of various cyanobacteria groups have shown good results, particularly in detecting genera like *Anabaena* and *Microcystis* (Castiglioni et al., 2004).

Moreover, a qPCR assay capable of detecting and quantifying toxin biosynthesis genes from the microcystins, nodularins, cylindrospermopsins, and saxitoxins biosynthesis pathways have been developed (Al-Tebrineh et al*.,* 2010). The *mcy* genes responsible for microcystins synthesis have been used to identify the toxigenic potential for the extensively studied microcystins. The *mcy* operon shares significant sequence homology with the nodularin synthesis operon (*nda* genes) and is commonly used to evaluate toxigenic potential using the same primer pairs (Pearson et al., 2016). In contrast, the cylindrospermopsin gene cluster (*cyrA-O* genes), spanning 43 Kb, presents gene arrangements in several cyanobacteria genera. Due to the limited conservation of *cyrN* and *cyrO* genes in many strains, *aoaA*, *aoaB*, and *aoaC* genes have been proposed as reliable markers (Mihali et al., 2008). Saxitoxin, synthesized by the *sxt* genes cluster, is well-characterized in speci-

fic genera. The characterization of this cluster has facilitated the investigation of molecular mechanisms, such as the regulatory effects of the genes *sxtY*, *sxtZ*, and *ompR* on the gene cluster (Kellmann et al., 2008). Anatoxin-a synthesis involves the *ana* gene cluster, displaying variations in the position and direction of certain genes. Understanding these gene clusters aids in unraveling molecular mechanisms and regulatory effects on toxin production (Mihali et al., 2011).

High-throughput Sequencing for Toxigenic Cyanobacteria Identification and Abundance Monitoring in Freshwater Ecosystems

The process to determine toxic and non-toxic cyanobacteria poses challenges due to the high similarity in the 16S rRNA region. Exploring operon clusters becomes essential for accurate differentiation. Studies on *Microcystis* reveal effective differentiation through combined amplification of 16S rRNA and 23S rRNA (Otsuka et al., 1999). For microcystin operon detection, *mcyA*, *mcyB*, and *mcyE* are commonly employed (Ouahid et al., 2009). Techniques combining hybridization and PCR of the genes *mcyE* and *ndaF* have been developed for the simultaneous detection of microcystin and nodularin clusters in various cyanobacteria genera (Castiglioni et al., 2004; Rantala et al., 2008; Al-Tebrineh et al., 2010). Analyses using qPCR establish relationships between toxigenic genotypes and cyanobacterial populations and provide insights into spatiotemporal dynamics (Briand et al., 2009). However, identifying active biosynthesis clusters remains challenging (Al-Tebrineh et al., 2010). To overcome challenges in identifying active biosynthesis clusters, the application of reverse transcription PCR (RT-PCR) methods is promising. These techniques establish a significant correlation between gene expression levels and cyanotoxin production, offering valuable insights into environmental influences. Their exceptional sensitivity and specificity make them suitable for monitoring potentially toxigenic cyanobacteria, aiding the application of predictive methods for estimating cyanotoxin concentrations under specific conditions (Duan et al., 2022).

The previous strategies are based on the application of the amplification and sequencing of 16S rRNA genes or genes involved in cyanotoxin biosynthesis. Previous studies have shown that although these strategies are useful for identifying potential cyanotoxin-producing genera and species, some strains may not necessarily possess the complete biosynthesis pathway or regulation to produce cyanotoxins, *e.g., Microcystis* mcyE gene (Linz et al., 2023). Hence, other strategies such as metagenomics using high-throughput sequencing are necessary to confirm the presence of cyanotoxin-producing species or to monitor the abundance of species with the critical genes for cyanotoxin production (Casero et al., 2019). Furthermore, deep metagenomics data can be useful to evaluate the abundance and dynamics of cyanotoxin-producing species.

Nanopore Sequencing Rapid On-site Cyanobacteria Identification.

Third-generation sequencing technologies, such as Oxford Nanopore Technologies (ONT) platforms, have several advantages that can be exploited to monitor toxic cyanobacteria presence in freshwaters. This technology can obtain sequences of a wide range of fragment lengths from short (20 bases) to ultra-long (>4 million bases), for each of the individual DNA or RNA fragments in a sample. Furthermore, the library preparation protocols and computational requirements are minimal with an accessible cost (Potvin et al., 2023). The MinION flow cell can generate an average of 20-25 Gigabases (Gb) of sequencing data after 72 h, this is enough data to obtain complete cyanobacterial genomes. PCR-free library preparation protocols reduce the cost of equipment and laboratory processing time and can generate highly accurate data. This can be used to evaluate cyanobacterial abundance in freshwater through direct sequencing. ONT sequencing data can be processed in real-time. The number of sequenced reads written in each file can be controlled, *e.g.*, 1,000 reads to generate a file; thus, each generated file can be analyzed in a bioinformatic workflow while sequencing is running. Data is available to take actions according to results, for example, if potential toxigenic cyanobacteria are identified, amplification steps can be used to evaluate the presence of cyanotoxin biosynthesis clusters by specific PCR amplification according to identified species (Fig. 1). A recent study shows a strategy for rapid on-site detection of harmful algal blooms (RosHAB). RosHAB's study consists of freshwater sampling, genomic DNA preparation, metagenome sequencing, and data analyses using a MinION device. When positive or inconclusive results are obtained, PCR or amplicon sequencing can be useful to identify the toxigenic potential of the cyanobacteria present in samples by comparison with genes previously used to confirm toxigenic potential (Duan et al., 2022; Potvin et al., 2023). Examples of biosynthesis operons of the main cyanotoxins: nodularin, cylindrospermopsin, microcystin, saxitoxin, and anatoxin are shown in Figure 1 (Kellmann et al., 2008; Pearson et al., 2010; Mihali et al., 2011). Even so, if sequencing leads to concerning or inconclusive results, the sample can be reanalyzed using high-throughput metagenomics. Then, data is deposited in the International Cyanobacterial Toxin Database (ICYATOX; www.icyatox.ca).

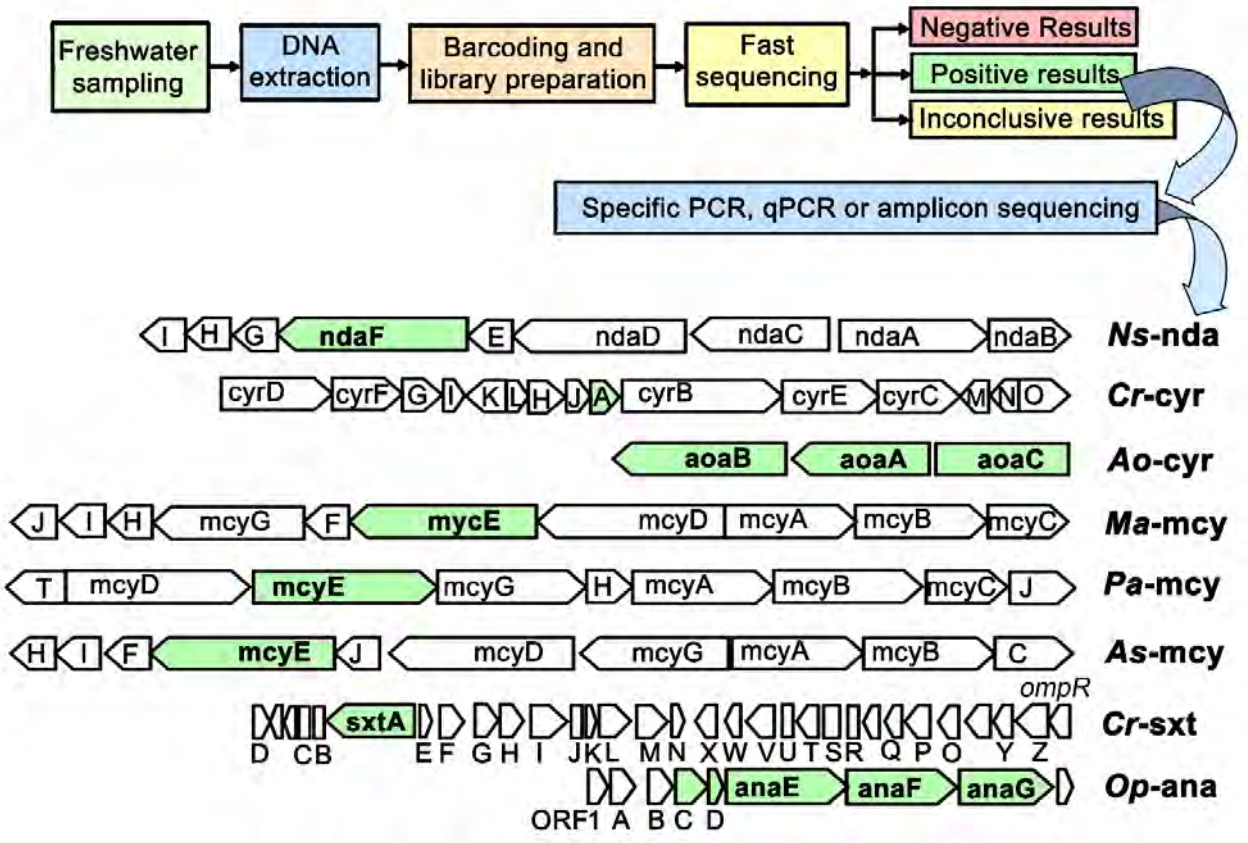

Figure 1. Rapid On-site Detection of Harmful Algal Blooms Strategy and Cyanotoxins Biosynthesis Clusters. The Biosynthesis Operons of *Nodularia spumigena* nodularin (*Ns*-nda); *Cylindrospermopsis raciborskii* cylindrospermopsin (*Cr*-cyr); *A. ovalisporum* cylindrospermopsin (*Ao*-cyr); *Microcystis aeruginosa* microcystin (*Ma*-mcy); *Planktothrix agardhii* microcystin (*Pa*-mcy); *Anabaena* sp. microcystin (*As*-mcy); *Cylindrospermopsis raciborskii* saxitoxin (*Cr*-sxt); *Oscillatoria* pcc. anatoxin (*O*p-ana) are shown.

Each new cyanobacterial genome and its metadata contributes to developing a more accurate reference database which will increase in length over time. Potvin et al. (2023) results show that a 30-minute Flongle cell sequencing run was sufficient to obtain reliable cyanobacterial relative abundance profiles, obtaining real-time results at the species level. Using this method, they identified *Anabaena, Calothrix, Chamaesiphon* spp, *Dolichospermum, Planktothrix, Pseudanabaena, Oscillatoria,* and *Nostoc*. Therefore, similar strategies can be used in Ibero-America to monitor locations where cyanobacterial harmful algal blooms have been reported monitoring their population abundance. If this strategy is made through seasons, and obtained data is correlated with other features, it can be useful to predict dangerous blooms in multiple locations.

Optical Techniques for Monitoring Potentially Harmful Cyanobacteria Blooms

The need to monitor cyanobacteria in water bodies to prevent the release of toxins, odors, and water discoloration has led researchers to develop different sensing techniques (Moreira et al., 2014). Unique radiative and bio-optical properties must be exploited in optical methods for detecting cyanobacteria. For detection and quantification *in situ*, *in vivo*, in real-time, and in an automated manner, spectrofluorometric or spectroradiometric techniques must be used. In this way, it is important to determine the absorbance and/or reflectance spectra by non-destructive optical technique.

Through detection, monitoring, and prediction of cyanobacteria blooms, water quality in aquatic environments can be determined to guarantee its safe consumption and use (Cannizzaro et al., 2019). Cyanobacteria can be detected primarily using optical biosensors, spectrophotometry, and remote sensing (radiometer). Remote sensing is preferred for studying large water bodies where unmanned vehicles such as drones or satellites are used to perform indirect contact monitoring using optical devices. Using an appropriately structured algorithm will increase the relevant quality and resolution of data. The reflectance and spectral absorption characteristics of the optical sensors used should allow the determination of the presence, growth, and effects of cyanobacteria without having direct contact with them. Recent advances are driving the use of biosensors due to their excellent reproducibility, robust platform, high sensitivity, low cost, ease of use, and real-time identification. The main disadvantage is the lack of approval for it to be used as a verified method for determining quantitatively toxins. Biosensors use platforms based on immunoassays, optical principles, or based on highly sensitive nanomaterials to perform qualitative and/or quantitative determination of toxins. Biosensors can be exploited using four different strategies to quantify toxins: limit of detection (LOD) and/or dynamic range (DR), variation between measurements, assay time, and feasibility of the point-of-care (POC). For example, biosensors can use optical methods such as chemiluminescence, fluorescence, and optical immunoassay. Ultraviolet-visible (UV-Vis) spectrophotometry can be used to reach the detection limit of toxins produced by cyanobacteria (Malhotra et al., 2021). Among the water quality parameters that can be reviewed are total suspended solids (TSS), ultraviolet transmittance (UVT), dissolved organic matter (DOM), biological oxygen demand (BOD), and chemical oxygen demand (COD). The presence of cyanobacteria is verified in the range of 540 to 685 nm.

Recently, machine learning (ML) models from neural networks in optical radiometers have been introduced to quantify the presence of cyanobacteria in reservoirs, lakes, as well as inland and coastal waters using the wide spectral resolution of the phycocya-

nin (PC) photosynthetic pigments contained in them (Zolfaghari et al., 2022). Through the radiometer, it is possible to obtain hyperspectral images using remote optical reflectance sensing to detect cyanobacterial toxins in water bodies. The difficulty in detecting these toxins is that they tend to accumulate on the surface of water consumed by humans and animals since these are present in different species of cyanobacteria with very different toxicity, morphology, and cell sizes. Finally, pigments in water allow remote sensing of cyanobacteria through changing reflectance to obtain hyperspectral images using drones over wide areas (Kwon et al., 2020).

Conclusions

The global increase in cyanobacterial blooms is an undeniable reality that continues to become more complicated by pollution. Chemical and molecular cyanotoxin analysis should be standardized, validated, and must be made accessible to communities using cyanotoxin-contaminated water. In addition, practical guidelines should be provided to prevent risks to human health and the ecosystem. It is necessary to investigate the effect of cyanotoxins on freshwater ecosystems and food crops, as well as alternatives for their degradation and control. Similarly, the constant monitoring of microbial populations is crucial to generate concrete actions and new strategies to avoid the causes that lead to this type of bloom and establish the original natural state of water bodies. This is an open field of research in Mexico that requires immediate attention.

Authors' Contributions

JNGC, CPRC, RAR, ASRS and JGCM Writing. LVS, JLCG and RVB writing, review and editing.

References

Aguilera, A., Gómez, E. B., Kaštovský, J., Echenique, R. O., & Salerno, G. L. (2018). The polyphasic analysis of two native *Raphidiopsis* isolates supports the unification of the genera *Raphidiopsis* and *Cylindrospermopsis* (Nostocales, Cyanobacteria). *Phycologia*, *57*(2), 130-146. https://doi.org/10.2216/17-2.1

Aguilera, A., Almanza, V., Haakonsson, S., Palacio, H., Benitez Rodas, G. A., Barros, M. U. G., Capelo-Neto, J., Urrutia, R., Aubriot, L., & Bonilla, S. (2023). Cyanobacterial bloom monitoring and assessment in Latin America. *Harmful Algae*, *125*, 102429. https://doi.org/10.1016/j.hal.2023.102429

Al-Tebrineh, J., Mihali, T. K., Pomati, F., & Neilan, B. A. (2010). Detection of saxitoxin-producing cyanobacteria and *Anabaena circinalis* in environmental water blooms by quantitative PCR. *Applied and Environmental Microbiology*, *76*(23), 7836-7842. https://doi.org/10.1128/AEM.00174-10

Anderson, D. M., Fachon, E., Pickart, R. S., Lin, P., Fischer, A. D., Richlen, M. L., Uva, V., Brosnahan, M. L., McRaven, L., Bahr, F., Lefebvre, K., Grebmeier, J. M., Danielson, S. L., Lyu, Y., & Fukai, Y. (2021). Evidence for massive and recurrent toxic blooms of *Alexandrium catenella* in the Alaskan Arctic. *Proceedings of the National Academy of Sciences (PNAS)*, *118*(41), e2107387118. https://doi.org/10.1073/pnas.2107387118

Asai, R., Nakamura, C., & Ikebukuro, K. (2002). Detection technique of asymmetric RT-PCR-based amplified single-stranded DNA and its application to biosensor for detection of mRNA for cyanobacteria, *Anabaena variabilis*. *Biotechnology Letters*, *24*, 1677-1682. https://doi.org/10.1023/A:1020609702434

Baliu-Rodriguez, D., Peraino, N. J., Premathilaka, S. H., Birbeck, J. A., Baliu-Rodriguez, T., Westrick, J. A., & Isailovic, D. (2022). Identification of Novel Microcystins Using High-Resolution MS and MSn with Python Code. *Environmental science and technology*, *56*(3), 1652-1663. https://doi.org/10.1021/acs.est.1c04296

Bouaïcha, N., & Corbel, S. (2016). Cyanobacterial toxins emerging contaminants in soils: a review of sources, fate and impacts on ecosystems, plants and animal and human health. In Larramendy, L. L., Soloneski, S. (Eds.), *Soil Contamination - Current Consequences and Further Solutions.* (1st ed.) InTechOpen. https://doi.org/10.5772/64940

Breton-Deval, L., Sanchez-Flores, A., Juárez, K., & Vera-Estrella, R. (2019). Integrative study of microbial community dynamics and water quality along The Apatlaco River. *Environmental Pollution*, *255*, 113158. https://doi.org/10.1016/j.envpol.2019.113158

Briand, E., Escoffier, N., Straub, C., Sabart, M., Quiblier, C., & Humbert, J. F. (2009). Spatiotemporal changes in the genetic diversity of a blood forming *Microcystis aeruginosa* (cyanobacteria) population. *The ISME Journal, 3*, 419-429. https://doi.org/10.1038/ismej.2008.121

Bustillos-Guzmán, J. J., Turner, A., Hernández-Almeida, O. U., Band-Schmidt, C. J., Romero-Bañuelos, C. A., Hernández-Sandoval, F. E., Núñez-Vázquez, E. J., & Palomino-Hermosillo, Y. A. (2020). Presence of Cyanotoxins in a mexican subtropical monomictic crater lake. *Applied Sciences*, *10*(19), 6719. https://doi.org/10.3390/app10196719

Cannizzaro, J. P., Barnes, B. B., Hu, C., Corcoran, A. A., Hubbard, K. A., Muhlbach, E., Sharp, W. C., Brand, L. E., & Kelble, C. R. (2019). Remote detection of cyanobacteria blooms in an optically shallow subtropical lagoonal estuary using MODIS data. *Remote Sensing of Environment*, *231*, 111227. https://doi.org/10.1016/j.rse.2019.111227

Casero, M. C., Velázquez, D., Medina-Cobo, M., Quesada, A., & Cirés, S. (2019). Unmasking the identity of toxigenic cyanobacteria driving a multi-toxin bloom by high-throughput sequencing of cyanotoxins genes and 16S rRNA metabarcoding. *The Science of the total environment*, 665, 367-378. https://doi.org/10.1016/j.scitotenv.2019.02.083

Castiglioni, B., Rizzi, E., Frosini, A., Sivonen, K., Rajaniemi, P., Rantala, A., Mugnai, M. A., Ventura, S., Wilmotte, A., Boutte, C., Grubisic, S., Balthasart, P., Consolandi, C., Bordoni, R., Mezzelani, A., Battaglia, C., & De Bellis, G. (2004). Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. *Applied and Environmental Microbiology*, *70*(12), 7161-7172. https://doi.org/10.1128/AEM.70.12.7161-7172.2004

Duan, X., Zhang, C., Struewing, I., Li, X., Allen, H., & Lu, J. (2022). Cyanotoxin-encoding genes as powerful predictors of cyanotoxin production during harmful cyanobacterial blooms in an inland freshwater lake: Evaluating a novel early-warning system. *Science of The Total Environment, 830*, 154568. https://doi.org/10.1016/j.scitotenv.2022.154568

El Khalloufi, F., Oufdou, K., Bertrand, M., Lahrouni, M., Oudra, B., Ortet, P., Barakat, M., Heulin, T., & Achouak, W. (2016). Microbiote shift in the *Medicago sativa* rhizosphere in response to cyanotoxins extract exposure. *Science of The Total Environment*, 539, 135-142. https://doi.org/10.1016/j.scitotenv.2015.08.127

Fergusson, K. M., & Saint, C. P. (2003). Multiplex PCR assay for *Cylindrospermopsis raciborskii* and cylindrospermopsin-producing cyanobacteria. *Environmental Toxicology, 18*(2), 120-125. https://doi.org/10.1002/tox.10108

Hernández-Morales, R., Ortega, M. R., Sánchez, J. D., Alvarado, R., & Aguilera, M. S. (2011). Distribución estacional del fitoplancton en un lago cálido monomíctico en Michoacán, México. *Biológicas*, *13*, 21-28. https://www.biologicas.umich.mx/index.php?journal=biologicas&page=article&op=view&path%5B%5D=116

Hernández, B. Y., Zhu, X., Nagata, M., Loo, L., Chan, O., & Wong, L. L. (2023). Cyanotoxin exposure and hepatocellular carcinoma. *Toxicology*, *487*, 153470. https://doi.org/10.1016/j.tox.2023.153470

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbio*logy, *16*, 471-483. https://doi.org/10.1038/s41579-018-0040-1

Jin, C., Mesquita, M. M. F., Deglint, J. L., Emelko, M. B., & Wong, A. (2018). Quantification of cyanobacterial cells via a novel imaging-driven technique with an integrated fluorescence signature. *Scientific Reports,* 8, 9055. https://doi.org/10.1038/s41598-018-27406-0

Karlson, B., Andersen, P., Arneborg, L., Cembella, A., Eikrem, W., John, U., West, J. J., Klemm, K., Kobos, J., Lehtinen, S., Lundholm, N., Mazur-Marzec, H., Naustvoll, L., Poelman, M., Provoost, P., De Rijcke, M., & Suikkanen, S. (2021). Harmful algal blooms and their effects in coastal seas of Northern Europe. *Harmful Algae*, *102*, 101989. https://doi.org/10.1016/j.hal.2021.101989.

Kellmann, R., Mihali, T. K., Jeon, Y. J., Pickford, R., Pomati, F., & Neilan, B. A. (2008). Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. *Applied and Environmental Microbiology*, *74*(13), 4044-4053. https://doi.org/10.1128/AEM.00353-08

Kwon, Y. S., Pyo, J. C., Kwon, Y. -H., Duan, H., Cho, K. H., & Park, Y. (2020). Drone-based hyperspectral remote sensing of cyanobacteria using vertical cumulative pigment concentration in a deep reservoir. *Remote Sensing of Environment*, *236*, 111517. https://doi.org/10.1016/j.rse.2019.111517

Lee, D. Y., Bowen, B. P., & Northen, T. (2010). Mass spectrometry-based metabolomics, analysis of metabolite-protein interactions, and imaging. *Biotechniques*, *49*(2), 557-565. https://doi.org/10.2144/000113451

Levizou, E., Papadimitriou, T., Papavasileiou, E., Papadimitriou, N. & Kormas, K. A. (2020). Root vegetables bioaccumulate microcystins-LR in a developmental stage-dependent manner under realistic exposure scenario: The case of carrot and radish. *Agricultural Water Management*, *240*, 106274. https://doi.org/10.1016/j.agwat.2020.106274

Linz, D. M., Sienkiewicz, N., Struewing, I., Stelzer, E. A., Graham, J. L., & Lu, J. (2023). Metagenomic mapping of cyanobacteria and potential cyanotoxin producing taxa in large rivers of the United States. *Scientific reports, 13*(1), 2806. https://doi.org/10.1038/s41598-023-29037-6

Machado, J., Campos, A., Vasconcelos, V., & Freitas, M. (2017). Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: A review of their *relevance* for agricultural plant quality and public health, *Environmental Research, 153*, 191-204, https://doi.org/10.1016/j.envres.2016.09.015

Malhotra, A., & Örmeci, B. (2021). Detection and identification of a mixed cyanobacteria and microalgae culture using derivative spectrophotometry. *Journal of Photochemistry and Photobiology B: Biology*, *238*, 112616. https://doi.org/10.1016/j.jphotobiol.2022.112616

Mihali, T. K., Kellmann, R., Muenchhoff, J., Barrow, K. D., & Neilan, B. A. (2008). Characterization of the gene cluster responsible for cylindrospermopsin biosynthesis. *Applied and Environmental Microbiology*. *74*(3), 716-22. https://doi.org/10.1128/AEM.01988-07

Mihali, T. K., Carmichael, W. W., & Neilan, B. A. (2011). A putative gene cluster from a *Lyngbya wollei* bloom that encodes paralytic shellfish toxin biosynthesis. *PLoS One*. *6*(2), e14657. https://doi.org/10.1371/journal.pone.0014657

Montero, E., Vázquez, G., Caballero, M., Favila, M. E., & Martínez-Jerónimo, F. (2021). Seasonal variation of *Microcystis aeruginosa* and factors related to blooms in a deep warm monomictic lake in Mexico. *Journal of Limnology, 80*(2). https://doi.org/10.4081/jlimnol.2021.2013

Moreira C., Ramos, V., Azevedo, J., & Vasconcelos, V. (2014). Methods to detect cyanobacteria and their toxins in the environment. *Applied Microbiology and Biotechnology*, *98*, 8073-8082. https://doi.org/10.1007/s00253-014-5951-9

Nandini, S., Sánchez-Zamora, C., & Sarma, S. S. S. (2019). Toxicity of cyanobacterial blooms from the reservoir Valle de Bravo (Mexico): A case study on the rotifer *Brachionus calyciflorus*. *Science of The Total Environment, 688*, 1348-1358. https://doi.org/10.1016/j.scitotenv.2019.06.297

Oehrle, S. A., Southwell, B., & Westrick, J. (2010). Detection of various freshwater cyanobacterial toxins using ultra-performance liquid chromatography tandem mass spectrometry. *Toxicon*, *55*(5), 965-972. https://doi.org/10.1016/J.TOXICON.2009.10.001

Ouahid, Y., & Del Campo, F. F. (2009). Typing of toxinogenic *Microcystis* from environmental samples by multiplex PCR. *Applied Microbiology and Biotechnology, 5*(2), 405-412. https://doi.org/10.1007/s00253-009-2249-4

Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S., & Watanabe, M. M. (1999). Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiology Letters*, *172*(1), 15-21, https://doi.org/10.1111/j.1574-6968.1999.tb13443.x

Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., & Neilan, B. (2010). On the chemistry, toxicology, and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. *Marine Drugs, 8*(5), 1650-1680. https://doi.org/10.3390/md8051650

Pearson, L. A., Dittmann, E., Mazmouz, R., Ongley, S. E., D'Agostino, P. M., & Neilan, B. A. (2016). The genetics, biosynthesis, and regulation of toxic specialized metabolites of cyanobacteria. *Harmful Algae*, *54*, 98-111. https://doi.org/10.1016/j.hal.2015.11.002

Peniuk, G. T., Schnurr, P. J. & Allen, D. G. (2016). Identification and quantification of suspended algae and bacteria populations using flow cytometry: applications for algae biofuel and biochemical growth systems. *Journal of Applied Phycology, 28*, 95-104. https://doi.org/10.1007/s10811-015-0569-6

Pérez, M. C., & Sánchez, J. A. (2017). Cyanotoxins in water bodies in central Mexico. *International Journal of Environmental Pollution*, *33*(4), 651-665.

Pineda-Mendoza, R. M., Olvera-Ramírez, R., Martínez-Jerónimo, F. (2012). Mycrocystins produced by filamentous cianobacteria in urban lakes. A case of study in Mexico City. *Hidrobiológica, 22*(3), 290-298. https://www.scielo.org.mx/pdf/hbio/v22n3/v22n3a10.pdf

Pineda-Mendoza, R. M., Briones-Roblero, C. I., Gonzalez-Escobedo, R., Rivera-Orduña, F. N., Martínez-Jerónimo, F., & Zúñiga, G. (2020). Seasonal changes in the bacterial community structure of three eutrophicated urban lakes in Mexico City, with emphasis on *Microcystis* spp. *Toxicon*, *179*, 8-20. https://doi.org/10.1016/j.toxicon.2020.02.019

Potvin, M., Gauthier, J., Langevin, C., Mohit, V., da Costa, N. B., Deschênes, T., Pomerleau, M., Kukavica-Ibrulj, I., Verreault, D., Comte, J., & Levesque, R. C. (2023). Rapid on-site detection of harmful algal blooms: real-time cyanobacteria identification using Oxford Nanopore sequencing. *Frontiers in microbiology*, *14*, 1267652. https://doi.org/10.3389/fmicb.2023.1267652

Rantala, A., Rizzi, E., Castiglioni, B., de Bellis, G., & Sivonen, K. (2008). Identification of hepatotoxin-producing cyanobacteria by DNA-chip. *Environmental Microbiology*, *10*(3), 653-664. https://doi.org/10.1111/j.1462-2920.2007.01488.x

Rinehart, K. L., Harada, K., Namikoshi, M., Chen, C., Harvis, C. A., & Gopichand, Y. (1998). Chemistry and bioactivity of the marine cyanobacterial metabolite aplysiatoxin. *Environmental Health Perspectives*, *78*, 73-76.

Rudi, K., Skulberg, O. M., Skulberg, R., & Jakobsen, K. S. (2000). Application of sequence-specific labeled 16S rRNA gene oligonucleotide probes for genetic profiling of cyanobacterial abundance and diversity by array hybridization. *Applied and Environmental Microbiology*, *66*(9), 4004-4011. https://doi.org/10.1128/AEM.66.9.4004-4011.2000

Rueckert, A., Wood. S. A. & Cary S. C., (2007). Development and field assessment of a quantitative PCR for the detection and enumeration of the noxious bloom-former *Anabaena planktonica*. *Limnology and Oceanography Methods*, *5*, 474-483. https://doi.org/10.4319/lom.2007.5.474

Sviršev, Z., Lalić, D., Bojadžija Savić, G., Tokodi, N., Drobac Backović, D., Chen, L., Meriluoto, J., & Codd, G. A. (2019). Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings. *Archives of Toxicology, 93*, 2429-2481. https://doi.org/10.1007/s00204-019-02524-4

Tomasini-Ortiz, A.C., Moeller-Chávez, G., Sánchez Chávez, J.J. & Bravo Inclán, L.A. (2012). Cyanobacteria and Cyanotoxins in Lake Patzcuaro, Michoacan, Mexico. *Revista AIDIS de Ingeniería y Ciencias Ambientales: Investigación, Desarrollo y Práctica*, *5*(2), 93-101. https://revistas.unam.mx/index.php/aidis/article/view/32650

United States Environmental Protection Agency (U.S.E.P.A). (2015). *Drinking water health advisory for the cyanobacterial toxin microcystin. EPA 820R15100*. http://water.epa.gov/drink/standards/hascience.cfm

Valdés-Santiago, L., García-Chávez, J. N., & Castro-Guillén, J. L. (2021). Caracterización de las comunidades microbianas asociadas a un florecimiento cianoalgal en una presa de León, Gto. México mediante secuenciación de regiones variables de los genes que codifican la rARN 16S y 18S. *Hidrobiológica*, *31*(1), 93-105. https://doi.org/10.24275/uam/izt/dcbs/hidro/2021v31n1/Valdes

Vasconcelos, V., Martins. A., Vale, M., Antunes, A., Azevedo, J., Welker, M., Lopez, O., & Montejano, G. (2010). First report on the occurrence of microcystins in planktonic cyanobacteria from Central Mexico. *Toxicon, 56*(3), 425-431. https://doi.org/10.1016/j.toxicon.2010.04.011

Yanez-Montalvo, A., Aguila, B., Gómez-Acata, E. S., Guerrero-Jacinto, M., Oseguera, L. A., Falcón, L. I., & Alcocer, J. (2022). Shifts in water column microbial composition associated to lakes with different trophic conditions: “Lagunas de Montebello” National Park, Chiapas, México. *PeerJ, 10*, e13999. https://doi.org/10.7717/peerj.13999

Yang, Y., Yu, G., Chen, Y., Jia, N., & Li, R. (2021). Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin. *Journal of Hazardous Materials, 406*, 124653. https://doi.org/10.1016/j.jhazmat.2020.124653

Zhu, C., Zhang, J., Nawaz, M. Z., Mahboob, S., Al-Ghanim, K. A., Khan, I. A., Lu, Z., & Chen, T. (2019). Seasonal succession and spatial distribution of bacterial community structure in a eutrophic freshwater Lake, Lake Taihu. *Science of the Total Environment, 669*, 29-40. https://doi.org/10.1016/j.scitotenv.2019.03.087

Zolfaghari, K., Pahlevan, N., Binding, C., Gurlin, D., Simis, S. G. H., Verdú, A. R., Li, L., Crawford, C. J., VanderWoude, A., Errera, R., Zastepa, A., & Duguay, C. R. (2022). Impact of spectral resolution on quantifying cyanobacteria in lakes and reservoirs: A machine-learning assessment. *IEEE Transactions in Geoscience and Remote Sensing*, *60*, 5515520. https://doi.org/10.1109/TGRS.2021.3114635

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This book takes a significant step in showcasing the relevance of limnology to our survival. Freshwater habitats, though they cover less than 1 % of the Earth's surface, are home to a substantial portion of the world's biodiversity—at least 10 % of all known species. Freshwater habitats and the biodiversity they support are under threat. Moreover, our survival depends on access to high-quality freshwater. This book not only highlights the beauty of limnology and the scientific methods used to study it, but it also draws attention to the major causes of biodiversity loss in freshwater ecosystems. It shows all readers what it means to deal with inland waters as a scientist interested in understanding ecosystems and protecting them.

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