Article

Microcystin-LR equivalents and their correlation with *Anabaena* spp. in the main reservoir of a hydraulic system of Central Mexico

José L. Alillo-Sánchez, Martha L. Gaytán-Herrera, Víctor M. Martínez-Almeida, and Pedro Ramírez-García*
Universidad Nacional Autónoma de México, Research and Postgraduate Division (CyMA Project, Bacteriological Laboratory), Estado de México, México
** Corresponding author: micro@unam.mx; cianobacter08@gmail.com*

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Abstract

The occurrence of cyanobacterial blooms is a characteristic of eutrophic inland water bodies. Valle de Bravo reservoir (Mexico State, Mexico) is the main source of water for the Cutzamala Hydraulic System, which supplies drinking water to the west of Mexico City (~6 million consumers) and suburban areas of Mexico State. The goal of this study was to determine the presence of microcystins (MC-LR equivalents) and their relationship with toxic populations of cyanobacteria recorded some years ago in this important reservoir. We measured the concentration of MC-LR equivalents using a commercial kit (EnviroLogix) based on the ELISA test. The calculation of abundance and biovolume was carried out monthly from February to November 2010. The presence of MC-LR equivalents was related to the biovolume of *Anabaena planctonica*. The values of this toxin from February to June exceeded the World Health Organization (WHO) provisional guideline (1 µg L^{-1}) for finished drinking water sources, particularly in April when the highest value was recorded (5.56 µg L^{-1}). In addition, in April, May, June, and August, the abundance of cyanobacteria exceeded the WHO moderate risk level (10×10^4 cells mL^{-1}) for recreational activities. This study furthers investigations ranging from the characteristics of the water column to benthic cyanobacteria and molecular biology tests to establish which species are toxic in the reservoir.

Key words: *Anabaena*, eutrophic reservoir, microcystin-LR, toxic cyanobacteria, Valle de Bravo reservoir, WHO guideline

Introduction

Cyanobacteria are a major group of bacteria that occur worldwide. Cyanobacterial blooms occur in all types of surface waters as a result of eutrophication and are of particular concern in waterbodies used for recreational activities and drinking water supplies (Schindler 2006, Bláhová et al. 2007). The high growth of these microorganisms deteriorates water quality and increases treatment costs (WHO 1998, Ramírez et al. 2004). Blooms of cyanobacteria can produce potent toxins (secondary metabolites), and more than one toxin could be present (APHA 1992, Falconer 2005).

The chemical structure of cyanotoxins is divided into cyclic peptides, alkaloids, and lipopolysaccharides (Sivonen and Jones 1999, O'Neil et al. 2012). The mechanisms of toxicity are diverse and are manifested in eukaryotes with neurotoxic effects as well as dermatotoxic, cytotoxic, hepatotoxic, and gastrointestinal damage (Hoeger et al. 2005, Dittmann et al. 2013). The most well-known are the microcystins (MCs), which are cyclic heptapeptides that act as potent inhibitors of type 1 and 2A phosphatases (PP1 and PP2A) (Roset et al. 2001). Prolongued exposure at levels >1.5 µg L^{-1} (Canadian regulation) cause chronic damage. In waterbodies used for recreation, values from 10 µg L^{-1} (Australian and German regulation) to 25 µg L^{-1} (Italian and French regulation) are considered a health risk (Chorus 2012). When ingested in high concentrations, MCs produce liver damage because of blood accumulation, causing an increase in the liver's volume with subsequent bleeding, leading to death (Falconer 2005). The potential for adverse human health

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effects (Azevedo et al. 2002) has led to increased research (Oliver and Ganf 2000). The major genera that produce MCs are *Microcystis*, *Anabaena* (recently changed to *Dolichospermum*; Waclin et al. 2009), and *Planktothrix* (Sivonen and Jones 1999, Fujii et al. 2002, Falconer and Humpage 2005).

Some countries have laws that regulate concentration limits of cyanotoxins, mainly MCs, most of which are based on the provisional guideline established by the World Health Organization (WHO 1998), which is 1 μg L^{-1} MC-LR for finished drinking water. For recreational activities, the parameters utilized for risk management and regulations are number of cyanobacterial cells mL^{-1}, biovolume $mm^3 L^{-1}$, or chlorophyll concentration μg L^{-1} (Chorus 2012).

Despite evidence of hepatic, renal, pulmonary, and intestinal damage, as well as muscular paralysis caused by cyanotoxins, the permissible limits in Mexico are not yet regulated. Currently no drinking water policies establish the maximum permissible limit of MCs or other kinds of cyanotoxins, possibly because few studies (Ramírez et al. 2004, Vasconcelos et al. 2010) show the presence of MCs in Mexican continental waters.

We studied the Valle de Bravo reservoir (VB) belonging to the large Cutzamala Hydraulic System (CHS), which comprises 6 other dams. The CHS is the largest in the center of Mexico and contributes ~15.6 m^3 s^{-1} to the southwest of Mexico City and suburban areas of Mexico State. Its contribution is 30% of drinking water consumed in these metropolitan areas, supplying ~6 million users (Ramírez et al. 2004). The reservoir's maximum water capacity is 391 hm^3, and it contributes 38% of water to the CHS (6 m^3 s^{-1}). Its natural surroundings have allowed major tourism development in the region as well as the growth of water sports such as yachting, water-skiing, and windsurfing. The water input from 4 rivers feeds the reservoir with 177.6 hm^3 yr^{-1} (Merino-Ibarra et al. 2008); the water quality of these rivers is affected by fish farming. The reservoir also receives diffuse pollution from farmland runoff that contributes to the deterioration of water quality (Olvera-Viascán et al. 1998).

Before 1999, attention was solely placed on water quality monitoring of VB without considering the presence of potential toxin-producing cyanobacteria. In June 1999, however, a high abundance of *Microcystis* spp. was reported in the reservoir (Ramírez et al. 2002). In addition, Valadez et al. (2005) reported the presence of potentially toxic species in VB such as *Anabaena* spp., *Planktothrix* spp., *Aphanizomenon flos-aquae*, and *Cilindrospermopsis raciborskii*. Subsequently, from July 2000 to July 2001, the presence of 12 species of cyanobacteria was recorded, but only *Microcystis botrys*, *M. flos-aquae*, *M. wesenbergii*, *Snowella septentrionalis*, *Anabaena* spp., and *Aphanizomenon yezoense* reached a high abundance (Gaytán et al. 2011). A large bloom of *Lyngbya birgei*, *Woronichinia naegeliana*, and *Microcystis wesenbergii* appeared in June 2012. After that, the populations remained codominant with *M. aeruginosa* until October 2012 (CNA 2012).

While Ramírez et al. (2004) reported levels of 4 μg L^{-1} MC-LR in July 2001, Vasconcelos et al. (2010) emphasized the absence of MC-LR equivalents in samples taken from VB in 2008, dominated by *M. wesenbergii*. We hypothesized that the discrepancy between the presence and absence of MC-LR could be due to a temporary change in populations of cyanobacteria in the waterbody. Our aim was therefore to establish the presence of MC-LR equivalents and their relationship with the potentially toxic cyanobacteria identified in the study.

Study site

Valle de Bravo is a tropical high altitude (1780 m a.s.l.) reservoir located at 19°11′50″N; l00°09′13″W (Fig. 1). The reservoir and CHS are situated in the high basin of the Balsas River that belongs to the Mexican Hydrologic Region 18 (RH-18). The climate is subhumid, warm to temperate, with pronounced dry (Nov–May) and rainy (Jun–Oct) seasons. The reservoir is classified as warm monomictic, stratified for 9 months, with an anoxic hypolimnion (Mar–Oct), and with complete mixing from November to January (Olvera-Viascán et al. 1998, Merino-Ibarra et al. 2008). The surface area is 19 km^2 and represents 3.5% of the drainage basin; the mean depth (Z) is 21.1 m, and the maximum depth (Z_{max}) is 38.6 m. During the stratification period, Secchi depth (Z_{sd}) was <2 m, soluble reactive phosphorus (SRP) was 9 μg L^{-1} in the epilimnion and 39 μg L^{-1} in the hypolimnion, dissolved inorganic nitrogen (DIN) was 43 μg L^{-1} in the epilimnion and 508 μg L^{-1} in the hypolimnion, and ammonium was at its highest levels with 427 μg L^{-1}. During mixing, Z_{sd} was 3.4 m, SRP 10 μg L^{-1}, and DIN 336 μg L^{-1} (Merino-Ibarra et al. 2008).

Methods

From February to November 2010, we collected monthly samples from a subsurface depth (0.5 m) with a van Dorn bottle at 5 sample points (Tizates 1, Tizates 2, Tizates 3, Center, and Wall dam; Fig. 1). The following water quality variables were recorded at each location: pH, temperature (T, °C), Secchi depth transparency (Z_{sd}, m), dissolved oxygen concentration (DO, mg L^{-1}), and electric conductivity (K_{25}, μS cm^{-1}). From the 2 L samples, 50 mL was filtered through Whatman Grade No. 40 quantitative filter

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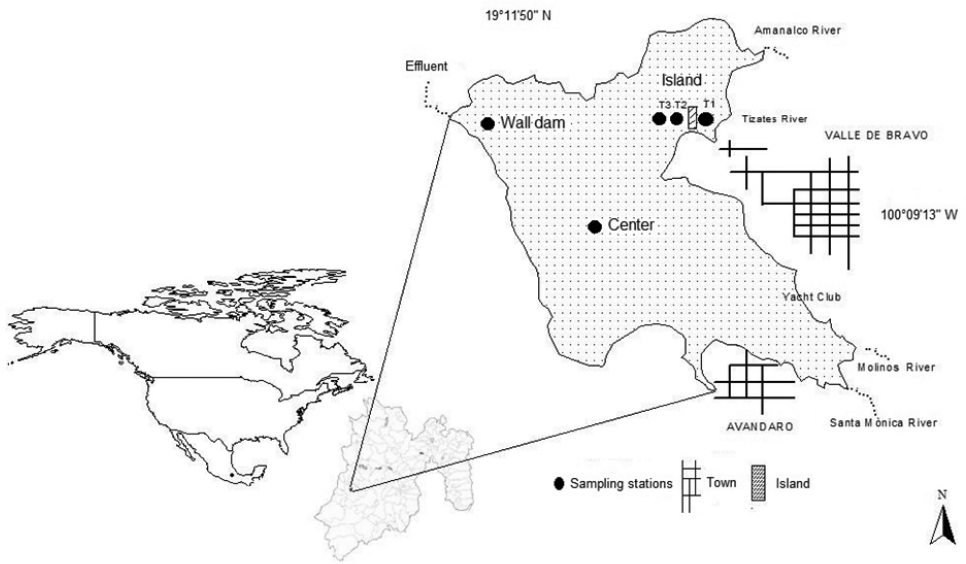

Fig. 1. Sampling sites in Valle de Bravo reservoir, State of Mexico, Mexico

paper (particle retention 8 µm) to determine nutrients: SRP by spectrophotometric method with stannous chloride (LOD 0.6 µg L^{-1} PO_4-P; APHA 1992); DIN calculated as the sum of nitrites (LOD 2 µg L^{-1} NO_2-N) and nitrates (LOD 0.5 µg L^{-1} NO_3-N), both by the diazotization method; and ammonium by the modified phenol hypochlorite method (LOD 1 µg L^{-1} NH_4-N; APHA 1992).

For quantitative and qualitative analyses of cyanobacteria, we took 600 mL samples with a van Dorn sampler, immediately fixed with 1 mL of Lugol solution. Following the Utermöhl method (APHA 1992), we settled a 10 mL sample, enumerating the total cells present in approximately 40 fields located in strips across the center of the bottom of the sedimentation camera. Counts of microphytoplankton were made at 640× and nanophytoplankton at 1008× (approximately 30 fields) using a Zeiss inverted microscope; abundance was reported as cells mL^{-1}. Identification was based on taxonomic keys of Komárek and Anagnostidis (1999, 2005). Taxa belonging to Nostocales were determined according to Cronberg and Annadotter (2006) and Komárek (2010). The biovolume calculation was based on geometric models and volume formulas (Sun and Liu 2003).

The determination of MC-LR concentration (hereafter MC-LR refers to equivalents) was performed on concentrate samples (600 mL) from a 90 L filtration of water from the reservoir in a phytoplankton net (20 µm) to determine the level of extracellular MCs in water samples. The concentrate samples were stored in a freezer (−20 °C) until analysis. The samples were thawed and then filtered to remove phytoplankton; from the supernatant; 200 µL were subjected to an ELISA test using the Quantiplate Kit for Microcystin (EnviroLogix, USA) with an optimal detection range from 0.16 to 2.5 µg L^{-1}. The values reported were determined from a curve calculated from the [MC-LR] standard versus absorbance.

A Pearson product-moment correlation coefficient between biovolume and cyanotoxin was calculated using MS-Excel software. Relationships between the MC-LR equivalents and variable physicochemical and cyanobacteria biovolume data (except rare species, frequency <10%) were calculated using the partial canonical correspondence analysis (pCCA; Canoco for Windows 4.5) considering period of time (months) as a covariable. Variables and biovolume values, except for pH, were transformed by adding one unit and then calculating the $\log_{10}$.

Results

Water quality variables

The pH ranged from 7.8 to 9.0 during the first 9 months but fell to 7.1 in November. The water temperature ranged between 18.5 and 25 °C, with the highest temperatures from May to August. The average Z_{sd} was 1.46 m, except in November when it rose to 4.9 m. The DO in the reservoir was at a mean level of 8.2 mg L^{-1} from February to October but in November fell sharply to 3.8 mg L^{-1}. The K_{25} increased during the first 4 months to 185 µS cm^{-1}, gradually decreased to 160 µS cm^{-1} in October, and rose again in November. SRP concentrations ranged from 0.6 to 94 µg L^{-1} PO_4^{-3}-P, with pulses in March, June, and November. DIN had 2 pulses, one in April (200 µg L^{-1}) and another in November (290 µg L^{-1}; Fig. 2 A–G).

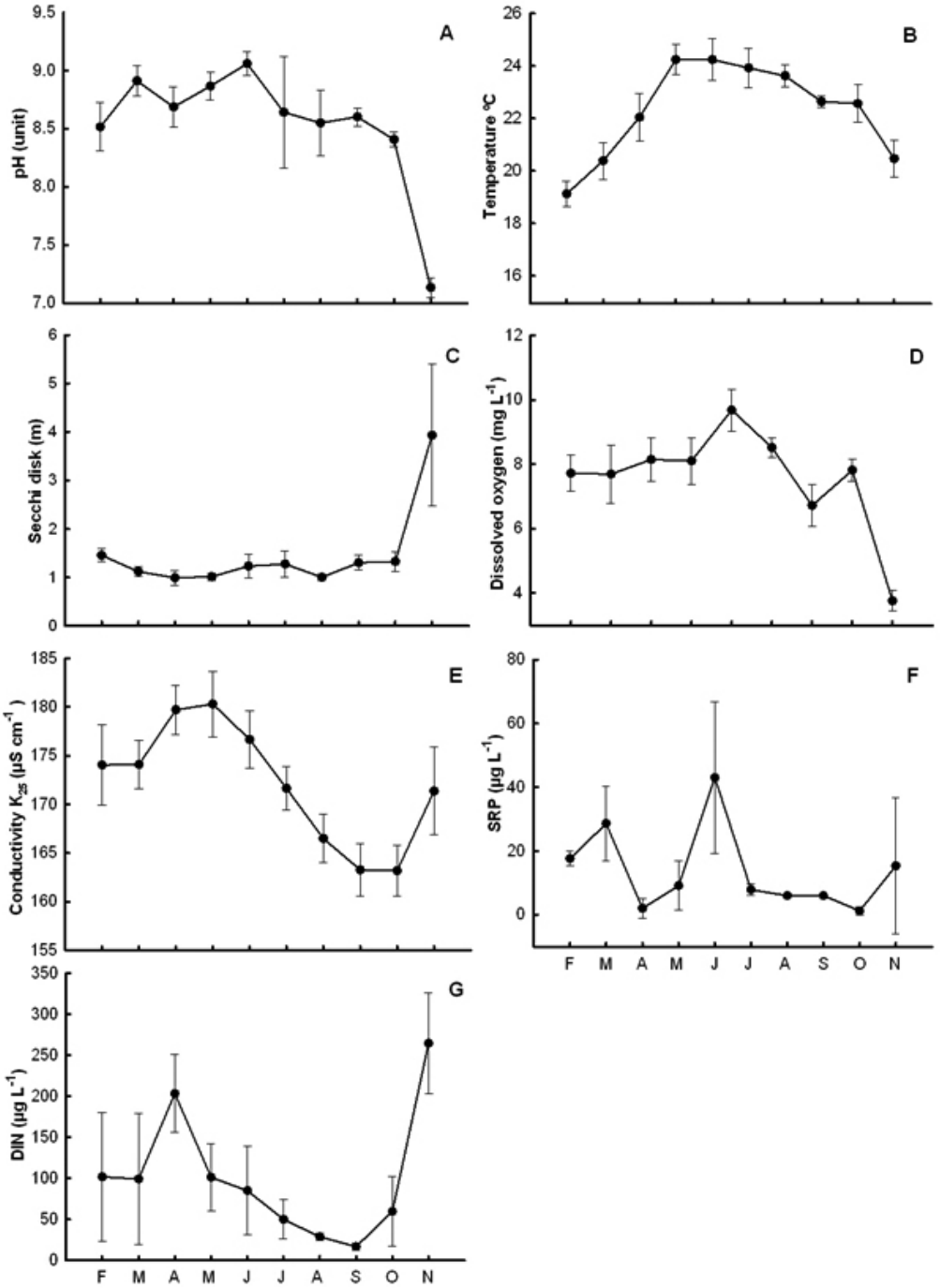

Fig. 2. Variation of physicochemical variables from surface water samples in Valle de Bravo reservoir from February to November 2010. Mean ± SD.

Species richness, abundance, and biovolume of cyanobacteria

Species richness in the VB reservoir consisted of 18 taxa (Table 1), with 6 belonging to order Chroococcales, 4 to Oscillatoriales, and 8 to Nostocales. The highest number of taxa (10–15) was observed in the warm season and early rainy season (Apr, May, and Jul; Fig. 3); during this period, Nostocales were well represented by 8 taxa, and Oscillatoriales were represented by 2–4 taxa. In the months that followed, Chroococcales were dominant, with only one taxon belonging to Oscillatoriales.

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Table 1. List of cyanobacteria encountered during February to November of 2010 in Valle de Bravo reservoir.

Cyanobacteria	
Order Chroococcales	Family Phormidiaceae
Family Merismopediaceae	9. *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek 1988
1. *Merismopedia* sp.	Family Oscillatoriceae
2. *Snowella septentrionalis* Komárek & Hindák 1988	10. *Lyngbya birgei* G. M. Smith 1916
3. *Woronichinia naegeliana* (Unger) Elenkin 1933	**Order Nostocales**
Family Microcystaceae	Family Nostocaceae
4. *Microcystis* sp.	11. *Anabaena* sp.
5. *Microcystis aeruginosa* (Kützing) Kützing 1846	12. *Anabaena planctonica* (Brunnthaler 1903)
6. *Microcystis wesenbergii* (Komárek) Kommarék in Kondrateva 1968	13. *Anabaena* aff. *viguieri*
	14. *Anabaena* aff. *spiroides*
Order Oscillatoriales	15. *Anabaena crassa* (Lemmermann) Komárek-Leng & Cronberg 1992)
Family Pseudanabaenaceae	16. *Cuspidothrix issatschenkoi* (Usac.) Rajaniemi et al. 2005
7. *Pseudanabaena mucicola* (Naumann & Huber-Pestalozzi) Schwabe 1964	17. *Aphanizomenon yezoense* (Watanabe 1991)
8. *Limnothrix redekei* (Van Goor) Meffert 1988	18. *Cylindrospermopsis raciborskii* Seenaya & Subba Raju 1972

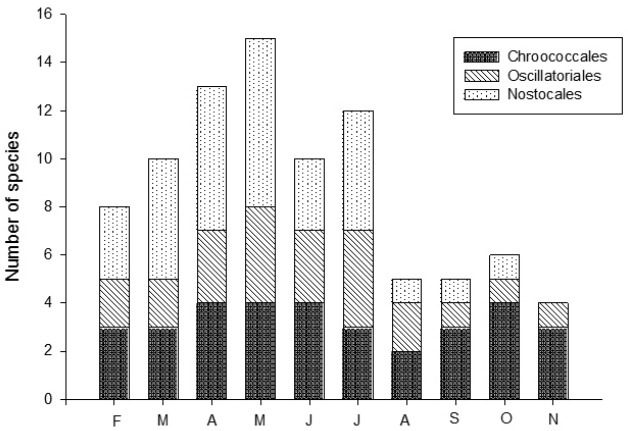

Fig. 3. Species richness by orders of cyanobacteria in VB reservoir.

The average abundance of cyanobacteria at 5 sample points had 2 peaks, one in June (14.3×10^4 cells mL^{-1}) and another in August (11.3×10^4 cells mL^{-1}; Fig. 4A). *Pseudanabaena mucicola* (4.1×10^4 cells mL^{-1}) was most abundant during the sampling months, followed by *Microcystis wesenbergii* (2.7×10^4 cells mL^{-1}). *Woronichinia naegeliana* was the third most abundant taxa with 0.2×10^4 cells mL^{-1}.

After transforming the abundance to biovolume, *Microcystis wesenbergii* contributed overwhelmingly to the highest value (Fig. 4B), followed by *Lyngya birgei* with larger cellular dimensions (3.2 µm length × 7.16 µm width), which maintained the biovolume curve at the abundance in July and August. *Pseudanabaena mucicola* was the third species that contributed to the biovolume in August, September, and November; however, because of their small size (3.2 × 1.2 µm), biovolume input was low and therefore did not match patterns of abundance. Unlike abundance patterns, biovolume pulses were apparent in April and June. The distribution of average biovolume in the sampling sites decreased in the following order: Tizates 1 > Tizates 3 > Tizates 2 > Wall dam > Center (Fig. 5).

Distribution of MC-LR

The highest concentration of MC-LR (5.56 µg L^{-1}) was in April and the lowest in September (0.25 µg L^{-1}). Of the 5 stations, the Wall dam site had the highest equivalents of the toxin and the Center site had the lowest (Fig. 6). Correlating biovolume with MC-LR equivalents, a higher

value was obtained for the *Anabaena* genera (Table 2). A high correlation was also obtained for *Anabaena planctonica* (r = 0.65, n = 50) and *A.* aff. v*iguieri* (r = 0.50, n = 50).

The pCCA showed certain groupings among variables and species. During the dry season, the higher conductivity, high temperatures, and DIN peak were associated mainly with Nostocales (Fig. 7B). *Microcystis wesenbergii* and its epiphyte *Pseudanabaena mucicola* were associated with increasing SRP (Fig. 7A).

Table 2. Correlation coefficient biovolume of genera and species vs. MC-LR equivalents in Valle de Bravo reservoir (n = 50).

Anabaena spp.	0.65
Microcystis spp.	0.24
Anabaena planctonica	0.65
Anabaena aff. *viguieri*	0.50
Planktothrix agardhii	0.42
Microcystis wesenbergii	0.24
Woronichinia naegeliana	0.21
Pseudanabaena mucicola	−0.014
Lyngya birgei	−0.0325

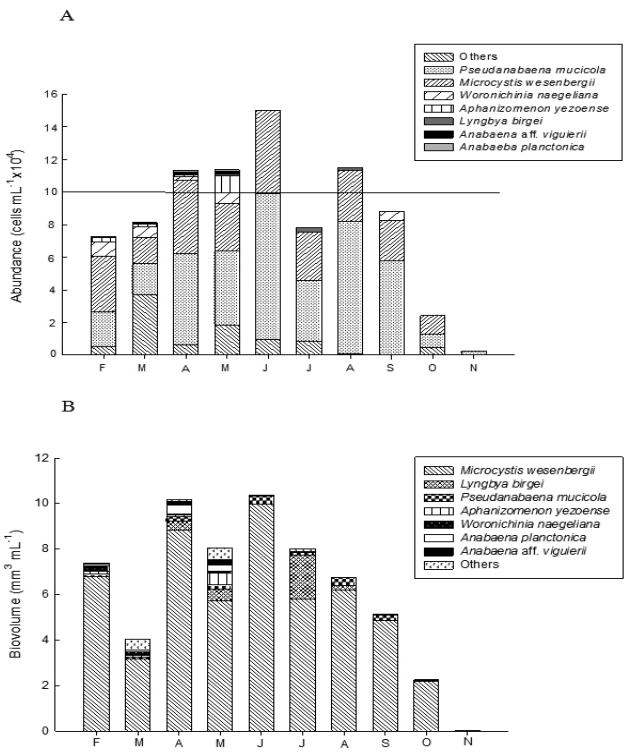

Fig. 4. (A) Abundance of cyanobacteria in Valle de Bravo reservoir. The line refers to 10 × 10^4 cells mL^{-1}. (B) Monthly percentage and total biovolume of cyanobacteria in Valle de Bravo reservoir (mm^3 mL^{-1}).

Discussion

Temperature values from March to November are typical for the prevailing subtropical climate in the country and provide favorable conditions for cyanobacterial growth (20–25 °C optimal) for most of the year (Oliver and Ganf 2000, Msagati et al. 2006, O'Neil et al. 2012). The active photosynthesis of these organisms produces the high DO concentrations and high pH values (O'Neil et al. 2012, Paerl and Paul 2012), in accordance with the reported optimal for these bacteria (6–9 pH units; Oliver and Ganf 2000, Msagati et al. 2006).

The SRP value exceeded 10 µg L^{-1} (optimum concentration for development of cyanobacteria; Oliver and Ganf 2000, Msagati et al. 2006) in March, June, and November. The average SRP value was 12.6 µg L^{-1}, indicating a

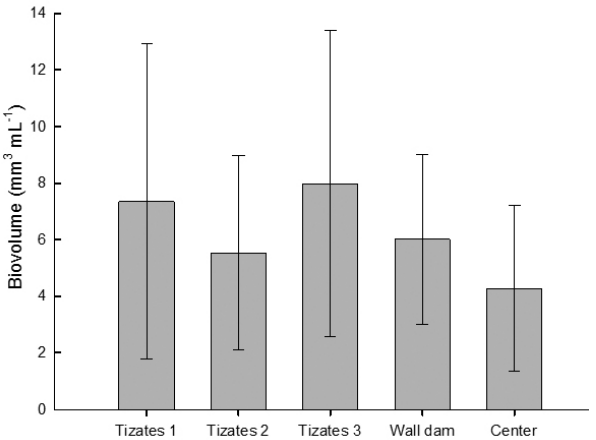

Fig. 5. Biovolume average and standard deviation of 5 sampling points.

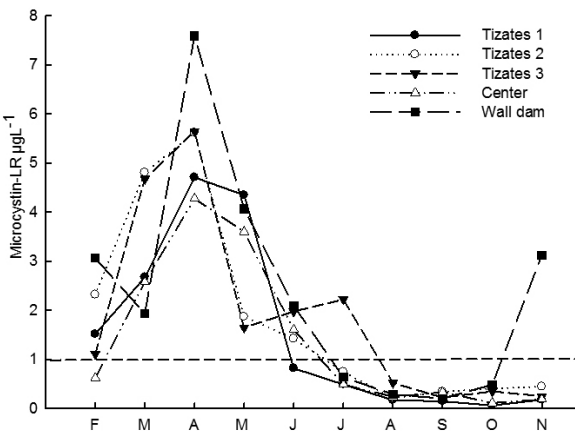

Fig. 6. Cyanotoxin equivalents in the different sampling sites of VB reservoir. Dotted line shows the provisional guidance value (1 µgL^{-1}) for finished drinking water (WHO 1998).

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mesotrophic condition, but the low transparency Z_{sd} (average 1.2 m) indicates that this waterbody is eutrophic (OECD 1982), and this trophic state coincides with previous reports (Olvera-Viascán et al. 1998, Gaytán et al. 2011).

Ammonium was the main contributor to DIN pulses in April and November (average for the 2 months 234 µg L^{-1}), but also during the sampling period (112 µg L^{-1}). Especially during February to June, the concentration of this resource (130 µg L^{-1}) was coupled to the optimum level of 100 µg L^{-1} reported for the growth of cyanobacteria (Oliver and Ganf 2000, Msagati et al. 2006, O'Neil et al. 2012). The average N:P in February and March was 12, indicating a limitation of N for phytoplankton growth. The concentration of DIN from February to June and N limitation during this period met the conditions for the development of Nostocales (Oliver and Ganf 2000, Jöhnk et al. 2011), as observed in high abundance and species richness in VB (Figs. 3 and 4B) and in the associated cluster of Nostocales–DIN in the pCCA (Fig. 7B).

From July to October, the SRP and DIN levels were less than optimal for the growth of cyanobacteria, and the decrease in species richness could be attributed to the drastic reduction of nutrients. SRP contribution from rainfall seems to favor *M. wesenbergii* and the epiphyte *Pseudanabaena mucicola*, as shown in the pCCA (Fig 7A). However, the dominance and persistence of *M. wesenbergii* throughout the study period (February to November), even when resources are limited, could be explained by their ability to move toward the thermocline during night and incorporate SRP and ammonium from this stratum (Shapiro, 1997, Chen et al. 2003, Merino-Ibarra et al. 2008, Vidal et al. 2009).

The K_{25} was inversely proportional to the level of the reservoir, with higher values in months of drought and lower values in rainy months due to dilution. Changes occurred in November, when stratification breaks (Fig. 2; Olvera-Viascán et al. 1998, Merino-Ibarra et al. 2008).

Cyanobacterial abundance peaks in April, May, June, and August exceeded the WHO (1998) guideline value of 10×10^4 cells mL^{-1} considered a moderate level of risk for recreational activities. Cellular counts exceeding this guideline in waterbodies should alert both authorities and users to avoid recreational contact; therefore, water not appropriately treated is also unsafe to drink because it increases negative health effects (Chorus and Bartram 1999). The high biovolume observed during June and July (Fig. 4B) is due to the contribution of nutrients from the inflow of the River Tizates in the rainy season, especially SRP in June (Fig. 2F), which resulted in the proliferation of cyanobacteria in this region of the reservoir (Fig. 5).

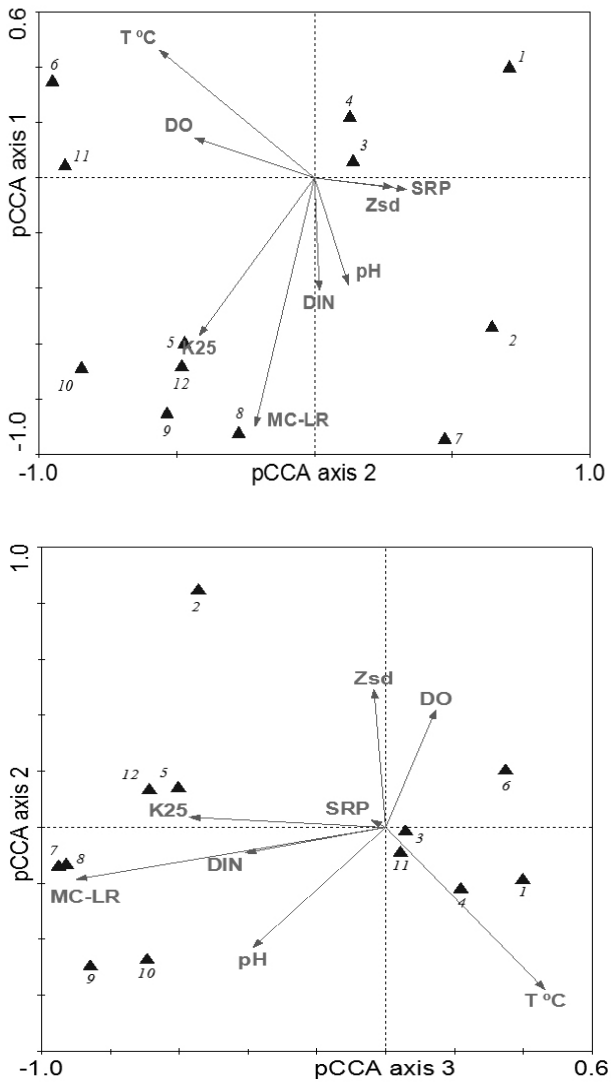

Fig. 7. (A) axis 1 and 2; and (B) axis 2 and 3. Biplot based on pCCA separated in variables and species. Abbreviations of species presented as numerals: 1 = *Merismopedia* sp., 2 = *Woronichinia naegeliana*, 3 = *Microcystis wesenbergii*, 4 = *Pseudanabaena mucicola*, 5 = *Planktothrix agardhii*, 6 = *Lyngbya birgei*, 7 = *Anabaena* sp., 8 = *Anabaena planctonica*, 9 = *Anabaena* aff. v*iguierii*, 10 = *Anabaena crassa*, 11 = *Cuspidothrix issatschenkoi*, 12 = *Aphanizomenon yezoense.*

Cyanotoxins

From February to June, the 4 µg L^{-1} mean toxin concentrations in VB exceeded the safety value established by WHO (1998) for drinking water (1 µg L^{-1}). A daily ingestion of 12.5, 50, and 150 µg L^{-1} respectively, of MC-LR by children weighing 5 and 20 kg and adults weighing 60 kg could have adverse effects on health (Dietrich and Hoeger 2005). A low probability of acute

health effects exists from the concentrations of MC-LR levels found in VB (Chorus and Bartram 1999), but exposure to chronic doses promotes liver tumours (Ito et al. 1997, WHO 1998, Rzymski et al. 2011).

The VB reservoir has a permanent wind pattern that blows mainly along the major northwest–southeast axis in the afternoon, reaching up to 7–8 m s^{-1}. At night and early morning, the wind is weak (~1.7 m s^{-1}), blowing in the opposite direction to the dam (Merino-Ibarra et al. 2008), which causes an accumulation of phytoplankton near the wall dam. Hence, accumulation of toxic species could explain the highest concentration of MC-LR in this area; in comparison, this accumulation does not occur in the center of the reservoir. A higher biovolume of cyanobacteria was observed in the Tizates River area (Fig. 5); however, the concentration of MC-LR at the Wall dam was higher, possibly due to the senescence of cyanobacteria that arrive by wind and water currents.

Special attention should be paid to *Lyngbya birgei* in VB because, rather than MCs, it produces lipopolysaccharide, also called dermotoxin or lyngbyatoxin (Ito and Nagai 1998), which causes severe contact dermatitis for people participating in water sports. When ingested, it causes severe oral and gastrointestinal inflammation leading to diarrhea and fever symptoms (Ito and Nagai 1998, Sivonen and Jones 1999).

Woronichinia naegeliana was an important species (third in abundance) in this study, mainly from February to May. Despite reports of this species producing MC-LR (Santos et al. 2012), we found low correlation with MC-LR levels (r = 0.21; Table 2).

Planktothrix agardhii had a maximum abundance in May (0.2×10^4 cells mL^{-1}), but high abundance has also been reported in December (0.3×10^4 cells mL^{-1}; Gaytán et al. 2011), likely because the content of phycoerythrin in this taxon increases its tolerance to low light incidence, as occurs at the end of the year (Scheffer et al. 1997). This taxon is a potential producer of different isoforms of MCs (Christiansen et al. 2003, Pawlik-Skowrońska et al. 2004) and is one of the species with the highest MC content in its cells (Fastner et al. 1999). This taxon also reached significant correlation values with MC-LR (r = 0.42; Table 2).

Other potentially toxic species were found in small concentrations from February to November in 2010 (Kurmayer et al. 2003, Via-Ordorika et al. 2004, Cronberg and Annadotter 2006) but during other periods reached significant levels of abundance. These species include *Anabaena* aff. *spiroides* in July–October 2000, *Microcystis botrys* and *M. flos-aquae* in October 2000 and March 2001 (Gaytán et al. 2011), and *M. aeruginosa* in September–November 2012 (CNA 2012).

Although *Microcystis wesenbergii* was the dominant species in the reservoir, and genus *Microcystis* is the leading producer of MCs (WHO 2003, Blahová et al. 2007), we found a low correlation with MC-LR (r = 0.24; Table 2). This taxon does not produce MCs (Xu et al. 2008) because it does not have the fraction *mcyE* gene (Watanabe 1996, Kurmayer et al. 2003, Via-Ordorika et al. 2004). For this reason, the *M. wesenbergii* bloom study by Vasconcelos et al. (2010) did not find MC-LR because samples were absent of MCs producers.

Anabaena biovolume presented the highest correlation with MC-LR equivalents. This genus produces mainly anatoxina-a (Sivonen and Jones 1999, Falconer and Humpage 2005), but some species also produce MCs (Sivonen and Jones 1999, Fujii et al. 2002, Rejmánková et al. 2011, O'Neil et al. 2012). Msagati (2006) reported the production of MC-LR in *Anabaena* sp. at temperatures <25 °C (a condition present from February to April in VB). In particular, *A. planctonicum* biovolume explained most of the pattern of MC-LR concentration observed in the early months of stratification in VB, a finding that agrees with the association of MC-LR with *A. planctonica* in pCCA (Fig. 7A axis 1 and 2; Fig. 7B, axis 2 and 3) and that reported by Bruno et al. (1994) in the same species.

Although we found no published information on the toxicity of *Anabaena* aff. *viguierii*, Lozano (2009) reported that a strain isolated from VB with the same characteristics as *Anabaena* aff. *viguierii* presented the fraction *mcyE* gene that codifies for MC, which could explain the relatively high correlation coefficient found and a similar behavior with *A. planctonica* in pCCA analysis.

One of the most interesting findings of this study was that minor components of the cyanobacterial biomass in the system produce relative high concentrations of MC-LR. This indicates that when these species become dominant under the right conditions, extremely high MC concentrations could be expected, and available data suggest DIN is an important factor promoting their growth.

The production of MC-LR by cyanobacteria populations reported in this study and the report of the toxin associated with the presence of *Microcystis* in 1999 and 2000 (Ramírez et al. 2004), as well as the abundance of the potentially toxic species, warrants the establishment of a permanent monitoring program for the eutrophic VB reservoir. Furthermore, isolation of the different species should be maintained, genetic testing should be used to identify toxic cyanobacteria, and chromatography methods should be used to establish the types of cyanotoxins produced. This information is currently almost nonexistent in Mexico, particularly in VB.

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