# Changes in cyanobacterial density due to application of Artificial Floating Island model with macrophytes: an experimental case study in a tropical reservoir

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## ABSTRACT

The Valle de Bravo (VB) reservoir is part of an important hydraulic system that provides about 40% of potable water to 21.5 million inhabitants of the Metropolitan Zone of Mexico City (Mexico). This reservoir shows deterioration in water quality due to its current eutrophic condition, which favors the recurring of cyanobacterial blooms. To date, there are no restoration strategies for this reservoir, so the use of eco-technologies such as Artificial Floating Islands (AFI) is proposed for the removal of nutrients and the improvement of water quality. Therefore, in this work AFIs have been implemented using two macrophytes (*Phragmites australis* (AFI-P) and *Schoenoplectus* sp. (AFI-S)) to evaluate the presence and distribution of potentially toxic cyanobacteria in relation to physicochemical variations at the AFI sites. The study was carried out over a period of 24 months (October 2016 -September 2018) divided into two cycles (C-I and C-II) with a dry and rainy season each. Cyanobacteria were the dominant group in the phytoplankton during all the study period. Nine potentially toxic cyanobacterial species were detected, with the predominance of *Microcystis aeruginosa*, *Aphanizomenon yezoense*, *Pseudanabaena mucicola*, *Anabaena planctonica* and *Planktothrix agardhii*. In this work, AFIs increased nitrates and had no effect on phosphates. Cyanobacteria were not reduced at AFI sites, however in rainy season in the second annual cycle (C-II) the concentrations of extracellular microcystins in the AFI-P and AFI-S were decreased while intracellular toxins were more strongly reduced only in the AFI-S. Each AFI had a specific effect on four out of five potentially toxic cyanobacteria. Thus, AFI-P promoted the increase of *M. aeruginosa* but reduced *A. planctonica*, while AFI-S promoted both *A. yezoense* and *P. mucicola*. The AFIs modified the dynamics among cyanobacteria particularly diazotrophic *A. yezoense* which was favored by nitrates and the other three species maintained their presence by the phosphates. *M. aeruginosa*, non-diazotrophic, responded to nitrates only in the absence of *A. yezoense*. Finally, in VB reservoir we found a mutually exclusive relationship between *M. aeruginosa* and *A. yezoense* likewise between *A. planctonica* and *P. mucicola*.

## INTRODUCTION

Currently most aquatic systems in the world present a eutrophic condition due to high internal nutrient loads (*e.g.*, sediment nutrient recycling), or excessive external loads that come from human activities (Paerl, 2009). Valle de Bravo (VB) is the main water reservoir of Cutzamala hydraulic system and presents these eutrophication characteristics. It supplies about 40% of drinking water to the Metropolitan Zone of Mexico City (Carnero-Bravo *et al.*, 2015). The trophic state of VB began in 1980 as oligotrophic, changed to mesotrophic in 1987 (Olvera-Viascán *et al.*, 1998) and became eutrophic in 2008 to date (Merino-Ibarra *et al.*, 2008).

Water quality deterioration has favored cyanobacterial blooms recurrence (Schindler *et al.*, 2016). This causes high densities of cyanobacteria triggering odor problems, decreasing aquatic biota due to the release of toxins, low dissolved oxygen and when cells collapse by senescence, the concentrations of nitrogen (N) and inorganic phosphorus (P) increase significantly (Zhu *et al.*, 2013; Wang *et al.*, 2016; Nandini *et al.*, 2019). The cyanobacteria species in VB reservoir identified as toxin-producing and bloom-forming harmful taxa include *Microcystis aeruginosa* (Kützing) Kützing 1846, *Microcystis flos-aquae* (Wittrock) Kirchner 1898, *Planktothrix agardhii* (Gomont) Anagnostidis, and Komárek 1988, *Woronichinia naegeliana* (Unger) Elenkin 1933, *Anabaena planctonica* Brunnthaler 1903, *Aphanizomenon yezoense* M. Watanabe 1991, *Lyngbya birgei* GMSmith 1916, *Pseudanabaena mucicola* (Naumann and Huber-Pestalozzi) Schwabe 1964. Despite this, a permanent monitoring of the concentration of microcystins in this reservoir is not done (Gaytan-Herrera *et al.*, 2001; Alillo-Sanchez *et al.*, 2014). The ecological studies in VB have focused mainly on assessing changes in trophic status regarding to climatic conditions, evaporation versus rainfall, water level fluctuations, wind regime and dissolved oxygen depletion, all

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of these associated to the presence of cyanobacteria (Merino-Ibarra *et al.*, 2008; Ramírez-Zierold *et al.*, 2010). However, there are no restoration projects in the reservoir, except for a study that addresses the removal of internal P loads using modified bentonite (Phoslock®) (Márquez-Pacheco *et al.*, 2013).

Based on the foregoing, the current problems that present the eutrophic aquatic systems have prompted the application of ecological rehabilitation methods to increase water quality using floating treatment wetlands (Borne *et al.*, 2013; Wang *et al.* 2015) or also called Artificial Floating Islands (AFI; Nakamura and Mueller, 2008; Yeh *et al.*, 2015). In some experimental trials with AFI, models use controlled loads of nutrients, mainly N and P in concentrations similar to those found in natural waterbodies. The purpose of these studies is to determine the maximum removal efficiency of the AFI models for the above-mentioned nutrients (N and P) (Abed *et al.*, 2017; Mayo and Hanai, 2017; Wang *et al.*, 2019). In other types of tests, the AFIs can also be evaluated when they are introduced into natural eutrophic systems, where nutrient concentrations vary seasonally and modify the availability of N and P. Likewise, these two nutrients alter the performance of plant species for the removal of nutrients, registering efficiency values different from those determined in studies with controlled variables (Nakamura and Mueller, 2008; Fang *et al.*, 2016; Olguín and Sánchez-Galván, 2017). Depending on the size, contact time and type of macrophytes used, the AFI models, in addition to increasing their efficiency of removal, can also extend their ecological restoration capacity by decreasing the algal biomass and in particular of some species of cyanobacteria (Park *et al.*, 2018; Wang *et al.*, 2019). These AFIs are low-cost biological alternatives that have been used internationally for more than two decades and are currently in full swing by retaking the capacities of emerging macrophytes used in constructed wetlands (Nakamura and Mueller, 2008; Yeh *et al.*, 2015; Shahid *et al.*, 2018). The efficiency of AFIs is based on the ability of roots to improve water quality through absorption of nutrients such as N and P and promoting flocculation and sedimentation of suspended matter. The release of organic and inorganic substances by roots can favor biofilm development (Headley and Tanner, 2012; Weragoda *et al.*, 2012). These macrophyte systems indirectly reduce algal density by nutrient competition (Pavlineri *et al.*, 2017; Park *et al.*, 2018) and in some cases due to an allelopathic effect (Nakai *et al.*, 2010; Takeda *et al.*, 2011; Zhou *et al.*, 2019). Macrophytes such as *Phragmites* and *Schoenoplectus* species are used in floating island systems for their wide distribution and adaptability in tropical and subtropical environments (Nakamura and Mueller, 2008; Pavlineri *et al.*, 2017). In field experiments *Phragmites australis* Cav. (Trin.) ex Steud. removes about 10 to 15 g $m^{-2}$ of total N, 1 to 3 g $m^{-2}$ of total P and reduces up to 45% the chlorophyll levels (Pavan *et al.*, 2015; Castro-Castellon *et al.*, 2016). *Schoenoplectus tabernaemontani* (C. C. Gmel.) Palla has a removal performance of 3.5 g $m^{-2}$ for P and 9.5 g $m^{-2}$ for N (Wang *et al.*, 2015; Choudhury *et al.*, 2019).

On the other hand, in studies in large aquatic systems, it has been difficult to clearly observe the ability of macrophytes to reduce the high densities of algae, which usually are quantified by indirect methods such as chlorophyll (Park *et al.*, 2018). Therefore, this type of works should also include direct methods to understand the interaction between macrophytes with each of the cyanobacterial species, since the macrophyte-cyanobacterium relationship could be specific and intensify the relationship between both groups (Šantrůčková *et al.*, 2010; Zhang *et al.*, 2014; Urakawa *et al.*, 2017; Gao *et al.*, 2019).

The study area in the VB reservoir was the water intake; in this site cyanobacteria are concentrated, including some toxic species, due to winds and surface currents. Its presence in large volumes alters the treatment of drinking water (Merel *et al.*, 2013). Based on this background, our work consisted in evaluating whether the implementation of an AFI model in the VB reservoir could reduce the density of cyanobacteria in the water column, due to the removal of nutrients by macrophytes, regardless of the existence of allelopathic processes associated with these macrophytes.

Unlike of the analyses in the horizontal flow wetlands with an input and output, our AFI model focused on the vertical study in an open system, considering the vertical migration capacity of cyanobacteria. Based on the above, it was implemented an AFI model using separately the native species *Phragmites australis* and *Schoenoplectus* sp., which due to their phytoremediation capabilities would achieve an effect related to the improvement of water quality in VB reservoir, and consequently modify the dynamic in the populations of cyanobacteria and as an extended effect the variation in the concentration of microcystin. Therefore, the objective of the present work was to evaluate if the proposed AFI models with macrophytes have the ability to decrease the density of cyanobacteria, including potentially toxic species and the presence of microcystins. To answer this question, we evaluated the effect of AFIs on the parameters that determine water quality and that are associated with the growth of cyanobacteria.

## METHODS

### Study area

The Valle de Bravo reservoir (State of Mexico, Mexico) is located at 19°11'50" N, l00°09'13" W at an altitude

of 1780 m asl (Fig. 1). This water body has an area of 18.55 km$^2$ with an average depth (Z) of 19.40 m and a maximum depth (Zmax) of 35 m (Olvera-Viascán *et al.*, 1998; Merino *et al.*, 2008 and Gaytan-Herrera *et al.*, 2011). The AFI model has a pentagonal shape of 4.5 m per side and a 3.85 m apothem that starts from the central area, each AFI covering an area of 43.31 m$^2$. The support structure was constructed with a galvanized tubular profile with a flotation system based on expanded polystyrene and a poly-aluminum and geomembrane coating (Fig. 2). We planted on the AFIs *P. australis* (haplotype I) and *Schoenoplectus* sp. (diploid karyotype 2 n=66) natives to Central Mexico in Cuitzeo, Michoacán, Mexico (Tena-Flores *et al.*, 2014; Colin and Eguiarte, 2016). Previous to planted on the AFIs we chose specimens of 1m height propagated in a greenhouse. In each AFI, around

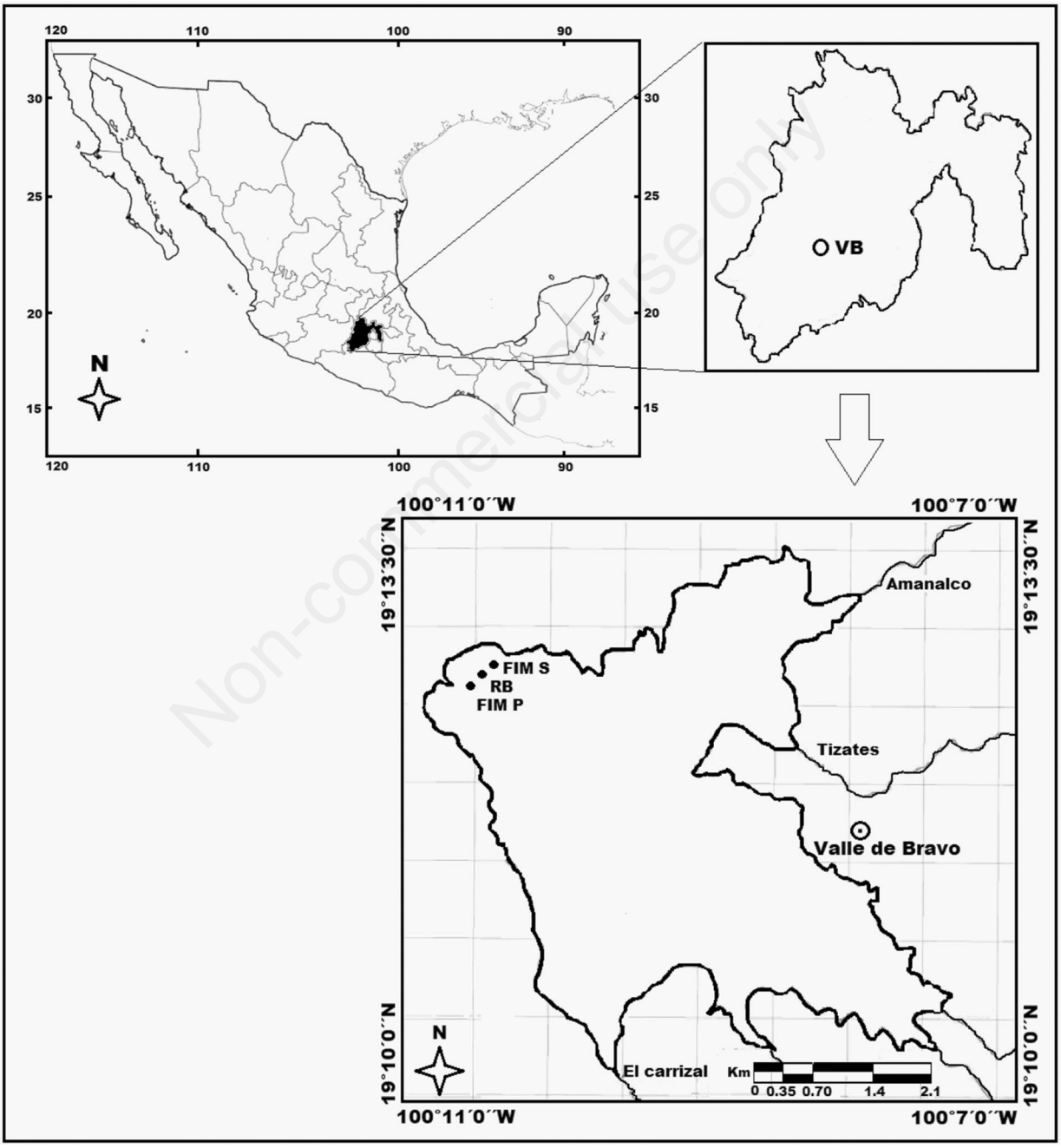


**Fig. 1.** Map of Mexico in the upper left box, in black Edo. Mex. Upper right box: in circle the municipality of Valle de Bravo. Lower right box: the Valle de Bravo reservoir, indicating the AFIs and Control location. The lines represent the rivers that converge with the reservoir.

400 culms of *P. australis* were placed, AFI-P (19°12'46" N, l00°10'74" W) and in the other, 50 m away the same number of plants but of *Schoenoplectus* sp., AFI-S (19°12'56" N, l00°10'77" W); as substrate for the macrophytes was used crushed coconut fiber. Given the large size of the VB reservoir, the study was in the area of water intake, where the Control (19°12'50" N, 100°10'74" W) was marked with a buoy without plants and the treatments applied were the AFI models (Fig. 1). The study of the water quality in the chosen points was made vertically according to the position of the water intake, which extracts the water from this area to send it to a drinking water treatment plant. So, there is not horizontal flow in and out to evaluate the efficiency of the AFIs. No repetitions of the AFI-P or AFI-S model were considered, since one of the initial purposes of the work was to determine the ecological restoration capacity of each structure as a pilot model tested in a large body of water and subsequently based on the results, their viability and scaling could be considered. As part of the maintenance of the AFI models, revision, repair or replacement procedures for the materials used in the structure were included. In the case of the macrophytes planted in the AFIs, for *P. australis* a pruning of the aerial part that protruded 1.5 m from the base, for *Schoenoplectus* sp. was made a flush pruning. This procedure was performed every six months to maintain uniform growth of all organisms. The plant biomass obtained from pruning was weighed dry in the laboratory to carry out a bromatological analysis of the amount of N and P contained in the biomass. The results were reported as g $m^{-2}$ for each AFI and their analysis was not part of the objectives of the work but was used to calculate and demonstrate the removal efficiency during 12 months. Due to the large number of specimens in each AFI and the disparity of growth between them, a monthly measurement of individuals was not carried out during this investigation. The study was carried out over a period of 24 months, which was divided into two annual cycles, denominated as C-I and C-II. Each cycle considered both the dry season (DS) and rainy season (RS), which are the two climatic seasons in the tropical and subtropical regions. The first annual cycle (C-I), DS was from October 2016 to March 2017 and RS from April to September 2017. For the second annual cycle (C-II), DS was from October 2017 to March 2018 and RS was from April to September 2018. The measurements of water physicochemical parameters and taking samples in situ were done at 1 and 2 m depth, considering the variations in the water column the values obtained were averaged for statistical analysis. All these measurements were made from 10:00 a.m. to 12:00 p.m. on the day 10 of each month. At the Control, AFI-P and AFI-S sites were carried out samplings included measurements *in situ* of pH, water temperature (portable potentiometer, Hanna HI 9126) and trans-

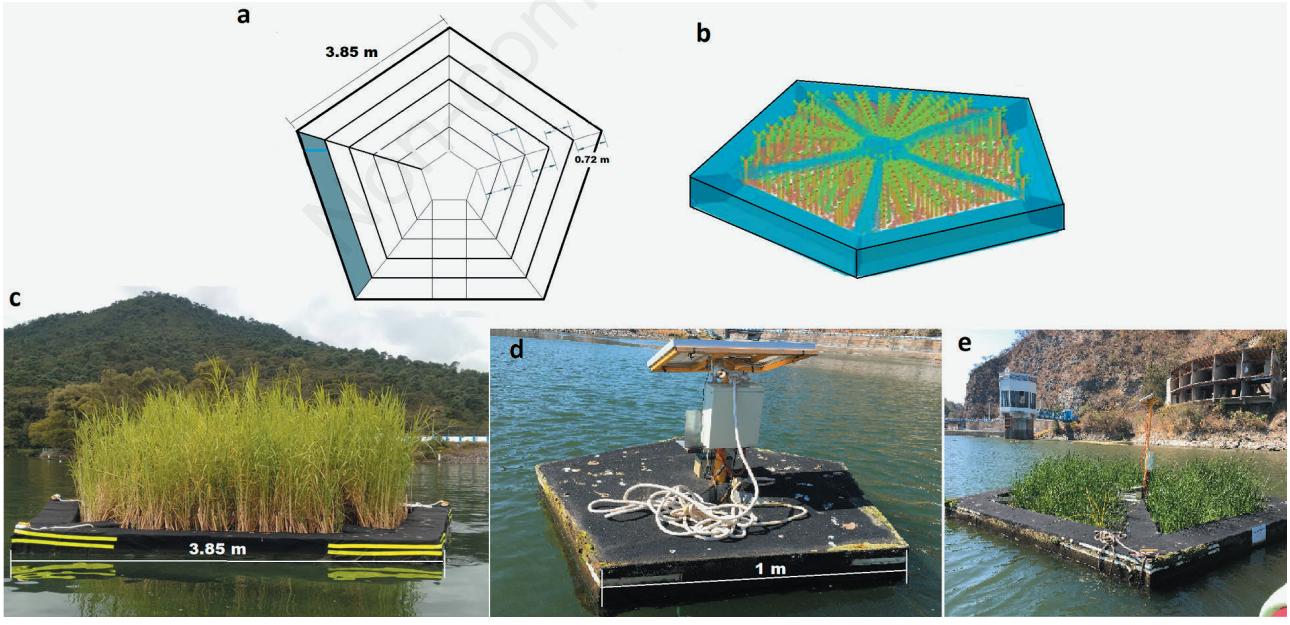


**Fig. 2.** Model of artificial floating island (AFI) with macrophytes placed in the reservoir VB. Pentagonal design of AFI (a). General projection of AFI with the macrophytes planted inside. The AFIs structures have five internal sections, divided by corridors without plants to access a central area, from where the water samples were taken (b). AFI planted with macrophyte *Phragmites australis*, AFI-P (c). Control, is a smaller structure due it is only a reference point for taking samples of water without treatment (d). AFI planted with macrophyte *Schoenoplectus* sp., AFI-S (e).

parency with Secchi disc. In the study area, 4.4 L aqueous samples were taken using a horizontal Van Dorn bottle (2.2 L, Watermark), the samples were deposited in amber glass bottles (1 L) and transported in refrigeration to the laboratory where the parameters, nitrates ($NO_3^-$), phosphates ($PO_4^{3-}$) and turbidity were analysed using a colorimetric method (Kit YSI 9500 with reagent system: Nitratest and Phosphate LR) based on Standard Methods (APHA, 2012). A review of the fluctuation in volume of water stored in the VB reservoir was carried out to determine its effect on the physicochemical parameters and cyanobacterial density. Volume changes was consulted in data base of the National Water Commission, CONAGUA, Mexico and the values recorded for the day, month and year in which this work was carried out were considered.

From the water samples, cell density of phytoplankton algal groups was directly quantified by the Utermöl method (Wetzel and Likens, 2000), using 50 mL sedimentation chambers and counted in D-Carl Zeiss inverted optic microscope with phases contrast and the results were expressed as cells $mL^{-1}$. Taxonomic identification was made based on Huber-Pestalozzi (1962, 1968), Prescott (1962), Komárek and Fott (1983), Tell and Conforti (1986), Popovsky and Pfiester (1990), Round *et al.* (1990), Comas (1996), Komárek and Anagnostidis (1999), Komárek (2003) and Cronberg and Annadotter (2006). The abundance of each species was determined according Dash (1993) as Relative abundance = (Number of cells by species / Total number cells) x 100. The biovolume for the cyanobacteria group was obtained based on Sun and Liu (2003).

The determination of biological parameters as cyanobacterial phycocyanin (PC) was made by fluores-

**Tab.1.** Species of phytoplankton encountered in the Valle de Bravo reservoir during the period from October 2016 to September 2018.

**Phylum Bacillariophyta**
Order Melosirales
1. *Melosira varians*
C.Agardh 1827
Order Fragilariales
2. *Fragilaria crotonensis*
Kitton 1869
Order Chaetocerotales
3. *Acanthoceras zachariasii*
(Brun) Simonsen 1979
Order Aulacoseirales
4. Aulacoseira *granulata*
(Ehrenberg) Simonsen 1979

**Phylum Chlorophyta**
Order Chlorellales
5. *Chlorella vulgaris*
Beyerinck [Beijerinck] 1890
6. *Oocystis marssonii*
Lemmermann 1898
7. *Oocystis pusilla*
Hansgirg 1890
Order Chlamydomonadales
8. Chlamydomonas globosa
J.W.Snow 1903
9. Pteromonas angulosa
(H.J.Carter) Lemmermann 1900
10. Chlorogonium minimum
Playfair 1918
Order Sphaeropleales
11. *Monoraphidium nanum*
(Ettl) Hindák 1980
12. *Monoraphidium arcuatum*
(Korshikov) Hindák 1970
13. *Pediastrum simplex*
Meyen 1829
14. *Monoraphidium irregulare*
(G.M.Smith) Komárková-Legnerová 1969

**Phylum Euglenozoa**
Order Euglenida
15. *Euglena viridis*
(O.F.Müller) Ehrenberg 1830
16. *Trachelomonas irregularis*
Svirenko 1914
17. *Trachelomonas ovata*
Y. V. Roll 1925

**Phylum Charophyta**
Order Desmidiales
18. *Staurastrum longiradiatum*
West and G. S. West 1896

**Phylum Cryptophyta**
Order Cryptomonadales
19. *Cryptomonas ovata*
Ehrenberg 1832

**Phylum Miozoa**
Order Gonyaulacales
20. *Ceratium hirundinella*
(O.F.Müller) Dujardin 1841

**Phylum Ochrophyta**
Order Chromulinales
21. *Ochromonas* sp.

**Phylum Cyanobacteria**
Order Oscillatoriales
22. *Planktothrix agardhii*
(Gomont) Anagnostidis and Komárek 1988
23. *Lyngbya birgei*
G.M.Smith 1916
24. *Arthrospira* sp.
25. *Arthrospira jenneri*
Stizenberger ex Gomont 1892
Order Chroococcales
26. *Microcystis wesenbergii*
(Komárek) Komárek ex Komárek in Joosen 2006
27. *Chroococcus limneticus* Lemmermann 1898
28. *Microcystis aeruginosa*
(Kützing) Kützing 1846
29. *Microcystis flos-aquae*
(Wittrock) Kirchner 1898
30. *Chroococcus dispersus*
(Keissler) Lemmermann 1904
Order Synechococcales
31. *Pseudanabaena mucicola*
(Naumann and Huber-Pestalozzi)
Schwabe 1964
32. *Merismopedia glauca*
(Ehrenberg) Kützing 1845
33. *Cyanodictyon* sp.
34. *Synechococcus subsalsus*
Skuja 1939
Order Nostocales
35. *Anabaena planctonica*
Brunnthaler 1903
36. *Aphanizomenon yezoense*
M. Watanabe 1991

cence analysis according the SOP 3 procedure of the Handbook of Cyanobacterial Monitoring and Cyanotoxin (Yéprémian *et al.*, 2017) using a Trilogy Turner Designs 7200 with a Fluorescence module: Phycocianin freshwater (7200-044).

For the analysis of extracellular (E-MC) and intracellular (I-MC) microcystin, the biomass of the environmental samples was concentrated by centrifugation (13,000 rpm, 20 min). The supernatant was filtered through a Nylon Net Filter (HNWP04700, Merck Milllipore, Burlington, Massachusetts, USA) 0.45 µm pore-size membrane to obtain a cell-free aliquot and quantify the E-MC. The pellet was subjected to the freeze-sonication process to obtain an aqueous extract of the intracellular toxin (Triantis *et al.*, 2010; Merel *et al.*, 2013). Microcystins were quantified using an immunological kit based on the ELISA test (QuantiPlate™, EnviroLogix™). The results were reported as equivalents of LR microcystin. The seasonal production of microcystin per cell was calculated from the concentration of the I-MC divided by the total cells number of the nine toxic cyanobacteria species. Both the physicochemical measurements of the water in situ and the laboratory analyses were carried out in triplicate.

### Statistical analysis

The mean value of each of the physicochemical and biological parameters was calculated per season in C-I and C-II. A two-way ANOVA of repeated measures was applied with a Dunnett´s multiple comparison test, with α of 0.05 to determine the differences among the three sites. Relationships between the physicochemical parameters and the biovolume of the five dominant cyanobacterial species were calculated by Canonical Correspondence Analysis (CCA; Canoco for Windows 4.5) by season and site, taking the time (months) as covariate. The p values are presented in Supplementary Tab. 1 and correspond to the comparisons of Control *vs* AFIs and AFI-P *vs* AFI-S.

## RESULTS

The values recorded for $NO_3^-$ in the Control showed higher values in DS (C-I: 0.79±0.11 mg $L^{-1}$, C-II: 0.49±0.02 mg $L^{-1}$) than in RS (C-I: 0.33±<0.01 mg $L^{-1}$, C-II: 0.41±<0.01 mg $L^{-1}$). In DS of C-I the values were significantly higher in AFI-S and in AFI-P during RS, compared to the Control. In DS of C-II, AFI-P and AFI-S were significantly higher than Control but not between them, while that in RS the AFIs showed no significant difference with the Control (Fig. 3b). The $PO_4^{3-}$ in the Control, in DS (0.03±<0.01 mg $L^{-1}$) and RS (0.06±0.01 mg $L^{-1}$) of C-I showed lower values than the same seasons in C-II (DS: 0.09±<0.01 mg $L^{-1}$, RS: 0.09±<0.01 mg $L^{-1}$). AFI-P showed a significant increase with respect to Control in DS of C-I and significant decrease in RS of C-II. AFI-S showed a significant decrease during DS of C-II (Fig. 3d). pH in the Control showed in DS values lower (C-I: 7.3±0.3, C-II: 6.8±0.3) than in RS, where a tendency of increase (C-I: 7.8±0.2, C-II: 7.4±0.1) was observed. AFIs showed that pH values were significantly higher in C-I and in C-II during RS than Control. Between AFIs a significant difference was found during RS in C-I and DS in C-II (Fig. 3c). Temperature trends observed in the Control during DS presented lowest values (C-I: 20.1±0.3°C, C-II: 21.2±0.8°C) that in RS where the highest values were recorded (C-I: 23.3±0.3°C, C-II: 23.3±0.2°C). At the AFI sites no significant temperature variation were observed in both DS and RS with respect to Control. There was also no significant difference between floating islands (Fig. 3a). Turbidity in the Control showed lowest values during DS (C-I: 6.1±0.5 NTU, C-II: 9.8±0.8 NTU), while those values in RS increased (C-I: 19.3±0.2 NTU, C-II: 24.2±4.2 NTU). There was no significant difference between AFIs with respect to Control and between AFIs (Fig. 3e). Water storage in the VB reservoir showed that its volume during DS increased (C-I: 376 H $m^3$, C-II: 353 H $m^3$), while that during RS decrease (C-I: 316 H $m^3$, C-II 301 H $m^3$). Volume of water in the reservoir did not decrease during the study beyond 80% of its capacity. This trend of the seasonal variation in water fluctuation levels is similar to the trend of $NO_3^-$ and inversely to turbidity (Fig. 3f).

We identified 36 species of phytoplankton, of these the cyanobacteria were grouped into four orders (Oscillatoriales, Chroococcales, Synechococcales and Nostocales) represented by 15 species (Tab. 1). Biomass of phytoplankton in Control was mainly composed by two dominant taxonomic groups, diatoms, and cyanobacteria. In DS of C-I, the proportion between these two groups was of 49±2% for diatoms and 45±3% for cyanobacteria, while that in lower proportion were Chlorophyta 3±1%, Chrysophyta 1±1 % and <1% for the Euglenophyta, Dinophyta, Cryptophyta and Charophyta groups. During the following three seasons, cyanobacteria increased their biovolume (RS: 85±6% in C-I, DS: 90±10% and RS: 74±21% in C-II), but the diatoms decreased (Figure 4a). Cyanobacterial biomass at the AFI sites showed a greater trend of dominance than Control, but only in DS of C-I (AFI-P: 65±32%, AFI-S: 63±5%). After this season, the biovolume of cyanobacteria was similar among the three sites during the rest of the study (Fig. 4 b,c). The parameters associated with the presence of cyanobacteria were: transparency in the Control during the DS showed an increasing trend (C-I: 1.6±<0.1 m, C-II: 1.3±<0.1 m), while that in RS this trend decreased (C-I: 0.8±<0.1 m, C-II: 0.8±<0.1 m). This behavior of the transparency was similar at the AFI sites both in DS, and in RS. The AFI-S only in DS of C-I (1.8±<0.1 m) showed a significant increase compared to Control and AFI-P (Fig. 5a). Phycocyanin as

an indirect indicator of the cyanobacterial biomass during the study period showed a trend opposite to transparency. In the Control, phycocyanin in DS recorded low values (C-I: 4.8±0.7 µg $L^{-1}$, C-II: 7.5±1.1 µg $L^{-1}$) and in RS this increased (C-I: 17±0.6 µg $L^{-1}$, C-II: 16.3±3.4 µg $L^{-1}$). At the AFI sites, phycocyanin showed a seasonal variation such as the Control. However, in RS of C-II, the values of AFI-P were significantly lower than Control (Fig. 5b). E-MC values recorded were lower in DS (C-I: 1.4±0.1 µg $L^{-1}$, C-II: 0.4±0.1 µg L-1) than in RS (C-I: 2.3±0.1 µg $L^{-1}$, C-II: 1.5±0.1 µg $L^{-1}$). At the AFI sites in RS of C-I and C-II, E-MC values were significantly lower than Control and there was no significant difference between AFI-P and AFI-S (Fig. 5c). I-MC in the Control showed lower values in DS (C-I: 4.4±0.1 µg $L^{-1}$, C-II: 4.3±0.3 µg $L^{-1}$ than in RS (C-I: 4.9±0.1 µg $L^{-1}$, C-II: 3.9±0.1 µg $L^{-1}$). The AFIs

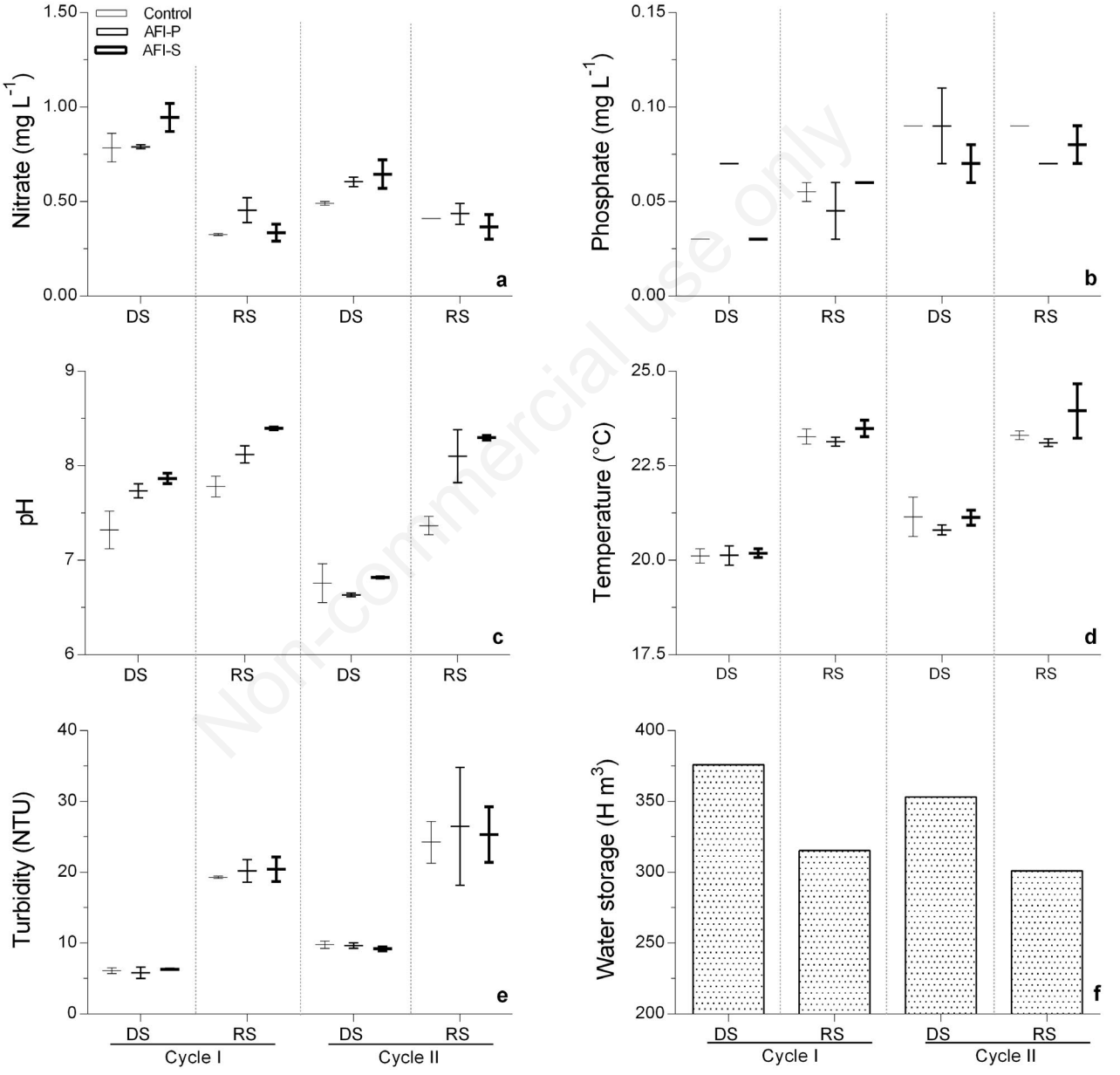




**Fig. 3.** Whiskers box of water quality hydrological descriptors related to the trophic state of the reservoir. The X axis represents the study period, from October 2016 to March 2017 dry season (DS), from April to September 2017 the rainy season (RS), which corresponds to the first annual cycle (C-I) and October 2017 to March 2018 the dry season and from April to September 2018 the rainy season, which corresponds to the second annual cycle (C-II). Average and standard deviation values from are at 1 and 2 m depth. Artificial floating island planted with *Phragmites australis* as AFI-P and artificial floating island planted with *Schoenoplectus* sp. as AFI-S.

showed a significant decrease in both seasons of C-II respect to Control. Particularly AFI-S had lower values than Control and AFI-P in RS of C-II (Fig. 5d). Microcystin production did not show seasonal variation and its concentration was significant higher in AFI-P than Control and AFI-S (Fig. 5e).

At the sampling points during the study, we identified nine species of potentially toxic cyanobacteria: *M. flos-aquae*, *M. botrys*, *Oscillatoria* sp., *P. planctonica*, *M. aeruginosa*, *A. yezoense*, *P. mucicola*, *A. planctonica* and *P. agardhii*. The first four were not considered for the analysis due to their low biovolume that did not exceed 1 $mm^3\ L^{-1}$. The other five species were found with higher biovolume and showed a seasonal behavior. *P. agardhii:* its presence was intermittent and it presented a maximum biovolume in RS of C-I in the Control (27±1 $mm^3\ L^{-1}$) and AFI-P while AFI-S was significantly lower than them (Fig. 6a). *P. mucicola*: in the Control the biovolume was higher in RS of C-I (13±5 $mm^3\ L^{-1}$). At the AFI sites in DS of C-I, a significant increase in biovolume was observed with respect to the Control, while that between the islands only in RS of C-I there was significant difference (Fig. 6b). *A. planctonica*: in the Control in DS of C-I it was scarce (21±14 $mm^3\ L^{-1}$), but it increased its biovolume in the following RS (609±401 $mm^3\ L^{-1}$). However, in C-II, a decrease was observed in DS (235±217 $mm^3\ L^{-1}$) and RS (217±42 $mm^3\ L^{-1}$). The AFIs not showed significant difference respect to Control during the period of study except AFI-S in RS of C-I (Fig. 6c). *A. yezoense*: in DS of C-I the Control was recorded highest biovolume (683±75 $mm^3\ L^{-1}$), while that in the following seasons had a sustained decrease. In the AFI-P in DS of C-II there was a significant increase while that in AFI-S it was in DS of C-I and C-II, both respect to the Control. Between islands there was a significant difference in DS and RS of C-I (Figure 6d). *M. aeruginosa*: The highest biovolume was recorded in the Control during RS of C-II (3265±640 $mm^3\ L^{-1}$). However, in AFI-P during DS and RS of C-I the biovolume was significantly higher than Control but not respect to AFI-S. In RS of C-II there was a significant difference between AFI-P and AFI-S, both sites were significant decrease compared to Control (Fig. 6e).

The results of the CCA of DS in the Control indicated that the biological parameters as phycocyanin (PC), extracellular microcystin (E-MC), intracellular microcystin (I-MC) and *A. yezoense* (A.y) showed a greater correlation with $NO_3^-$, while *P. mucicola* (P.m) was close to transparency (TRANS) (Fig. 7a). In RS the parameters as PC, I-MC, E-MC and *A. planctonica* (A.p) showed a similar association with temperature (TEMP) and $PO_4^{3-}$ and to a lesser extent with $NO_3^-$, turbidity (NTU), transparence (TRANS) and pH. *P. agrardhii* (P.a) was close to $NO_3^-$ while *M. aeruginosa* (M.a) was associated with TEMP and NTU (Fig. 7b). AFI-P in DS the parameters such as PC, I-MC, E-MC, A.p, P.m and P.a were associated with TEMP, NTU and pH, and to a greater extent with $PO_4^{3-}$ and not so with $NO_3^-$ which showed association with M.a (Fig. 7c). In RS, it was found that TEMP, $NO_3^-$ and $PO_4^{3-}$ were related to PC, I-MC, E-MC, M.a and A.p (Fig. 7d).

In DS at AFI-S site the parameters PC, E-MC, I-MC, A.y and P.m were close to $NO_3^-$ in a similar way to what was observed in the Control. M.a showed a closer correlation with TEMP, while P.a was with $PO_4^{3-}$ (Fig. 7e). In RS a close relationship of the variables environmental and biological, unlike of the Control and AFI-P (Fig. 7f).

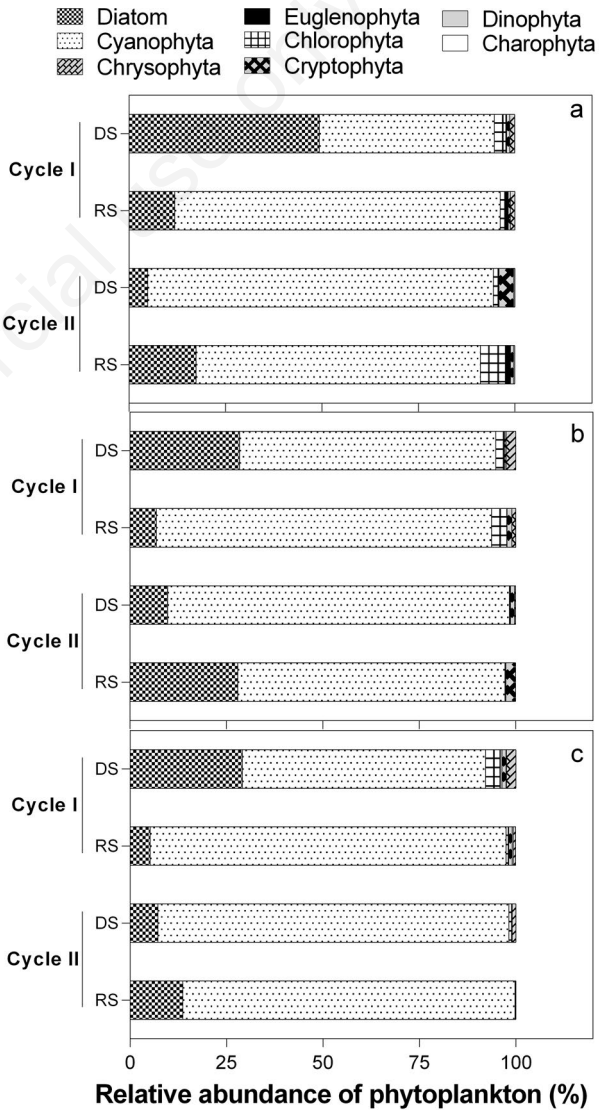


**Fig. 4.** Relative abundance of phytoplankton. The species were categorized in taxonomic phylum for the three sampling sites as Control, AFI-P and AFI-S. The cell density of the recorded species at 1 and 2 m deep was considered.

## DISCUSSION

In the Valle de Bravo reservoir the dominance of cyanobacteria was maintained during all the study period, with diatoms prevailing the other groups of recurrent phytoplankton in the system. The presence of both groups can be associated with co-evolutionary aspects, taking the advantage of environmental factors in a similar way, among which nutrient concentration and temperature are the main growth promoters (Janson, 2002; Lürling *et al*., 2013; Schindler *et al*., 2016).

From our Control data, it was observed that eutrophication conditions prevail in the reservoir with a seasonal variation during the dry season with the increase of $NO_3^-$ and lower temperature, while in the rainy season an opposite behavior was observed. $PO_4^{3-}$ did not show seasonal variation, but a trend of sustained increase was observed during the study period. We observed that $NO_3^-$

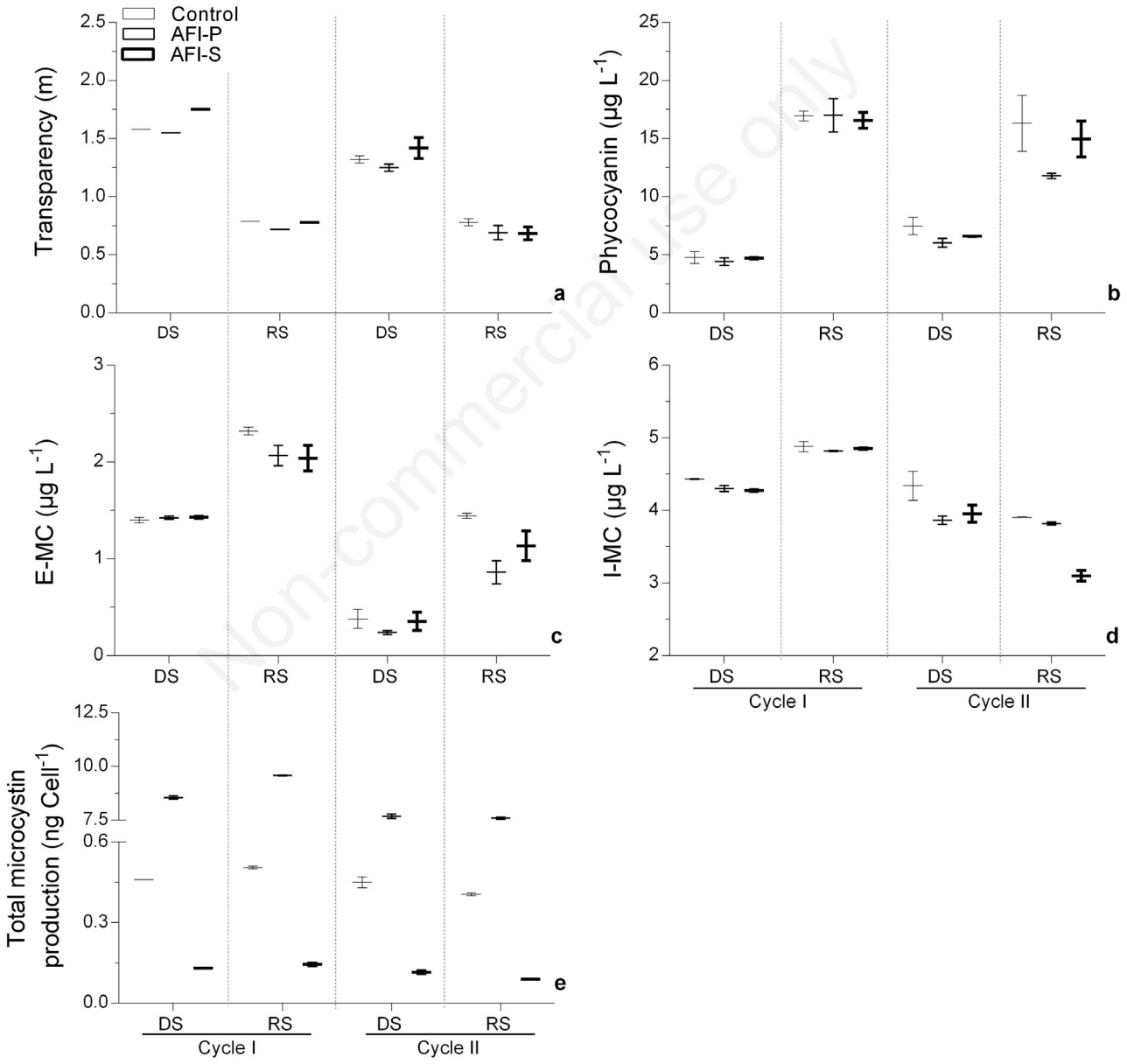




**Fig. 5.** Physicochemical parameters associated with the presence of cyanobacteria. On the X axis, the dry season as DS and the rainy season as RS. Both seasons represented during the study period in two annual cycles as C-I and C-II. Average and standard deviation values from are at 1 and 2 m depth.

were the limiting factor for diatoms, while $PO_4^{3-}$ and temperature for cyanobacteria. That is, when $NO_3^-$ concentrations were high and $PO_4^{3-}$ concentrations were low, diatoms were at their maximum densities, similar to the density of cyanobacteria. When $NO_3^-$ decreased, and $PO_4^{3-}$ and temperature increased, diatoms responded by decreasing their abundance, allowing an increase in cyanobacteria. In the reservoir, water temperature was the factor that regulated the seasonal dynamics between these two groups, since cyanobacteria are favored by warm temperatures while diatoms occur in temperate waters (van der Grinten *et al*., 2005; Mesquita *et al*., 2019).

The cyanobacterial biomass represented as phycocyanin showed a seasonal variation similar to pH and temperature but was opposite to the behavior of turbidity and $NO_3^-$. This response of cyanobacteria to the availability

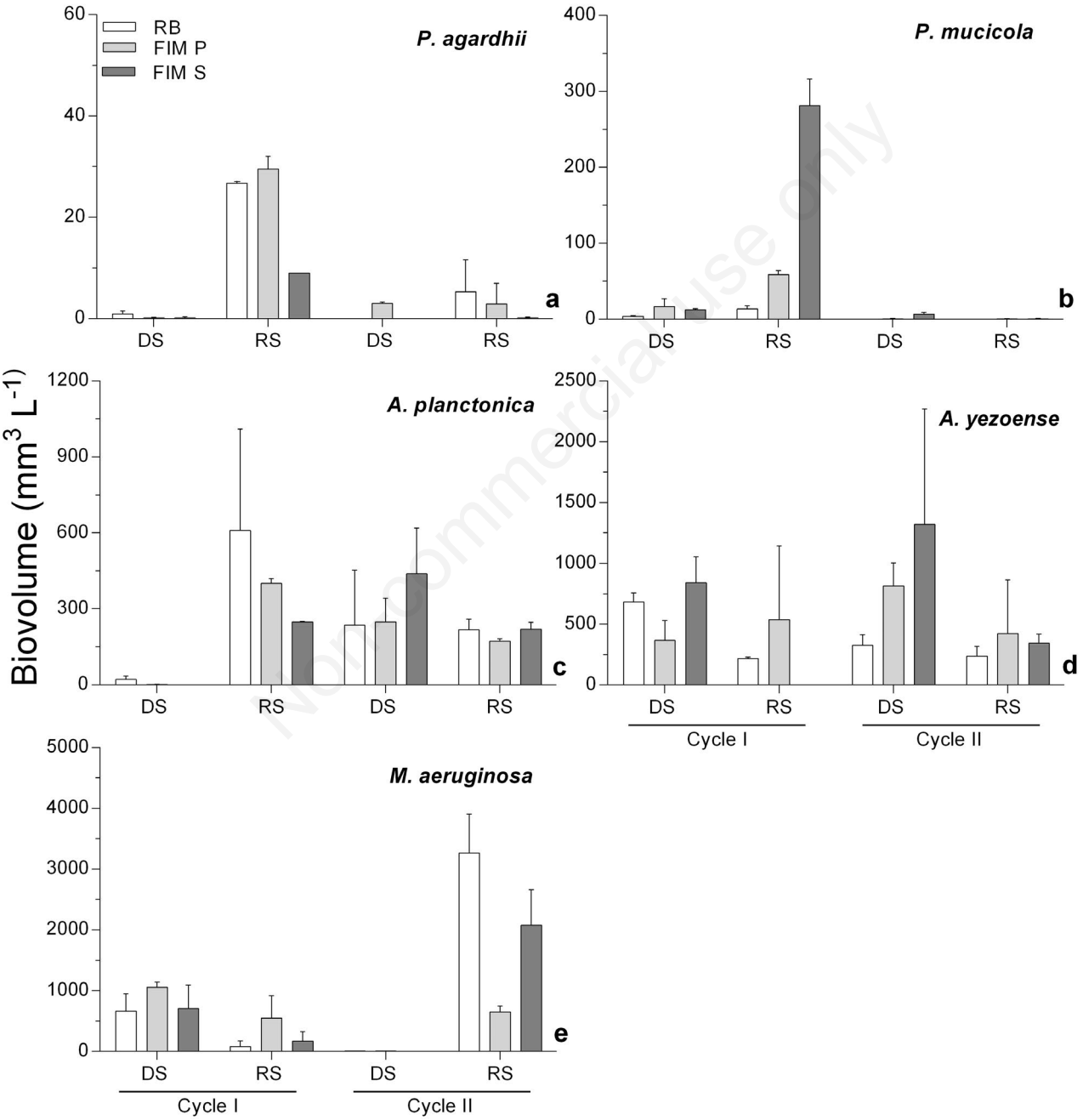


**Fig. 6.** Seasonal cellular density of potentially toxic five taxa cyanobacteria with greater recurrence found at Control, AFI-P and AFI-S sites. Average and standard deviation values from are at 1 and 2 m depth during the annual cycles C-I and C-II.

of $NO_3^-$ at first glance would seem contradictory to the factors that promote its proliferation (Lürling *et al*., 2013; Schindler *et al*., 2016). However, in our case, the sustained increase in $PO_4^{3-}$ compensated for the low concentration of $NO_3^-$, so the combination of both nutrients were responsible for cyanobacterial growth, as has been observed in other aquatic systems where these nutrients are not independently exclusive to promote the presence of cyanobacterial blooms (Davis *et al*., 2010; Jankowiak, 2019). On the other hand, it was observed that $NO_3^-$ showed a seasonal trend in relation to the fluctuation of the volume of water due to the hydraulic management of the reservoir, this indicates that the entry of water brings in a greater quantity of $NO_3^-$ than of $PO_4^{3-}$ (Ramírez-Zierold *et al*., 2010). Our $PO_4^{3-}$ values did not exceed the concentration of 0.01 $mgL^{-1}$, being lower compared to those recorded in previous studies with values around 0.50 $mgL^{-1}$ (Ramírez-Zierold *et al*., 2010; Gaytan *et al*., 2011).

In response to the abundance of cyanobacteria, we found that the microcystins recorded as E-MC and I-MC showed a trend in seasonal variation similar to phycocyanin and temperature. This means a relationship with cell growth and the toxin biosynthesis (Neilan *et al*., 2013); however, the biological parameters of microcystin were opposite to the behavior of $NO_3^-$. This may be due to the role that N plays, which under limiting conditions, activates the genes (*mcy*) involved in the production of these toxins, increasing their concentration, while under conditions of high N availability these genes turn off, reducing the amount toxin (Flores *et al*., 2005; Gobler *et al*., 2016; Pimentel and Giani, 2014). The relationship between microcystins and phycocyanin indicates that the cyanobacterial biomass in the reservoir is proportional to the quantity of toxins, which



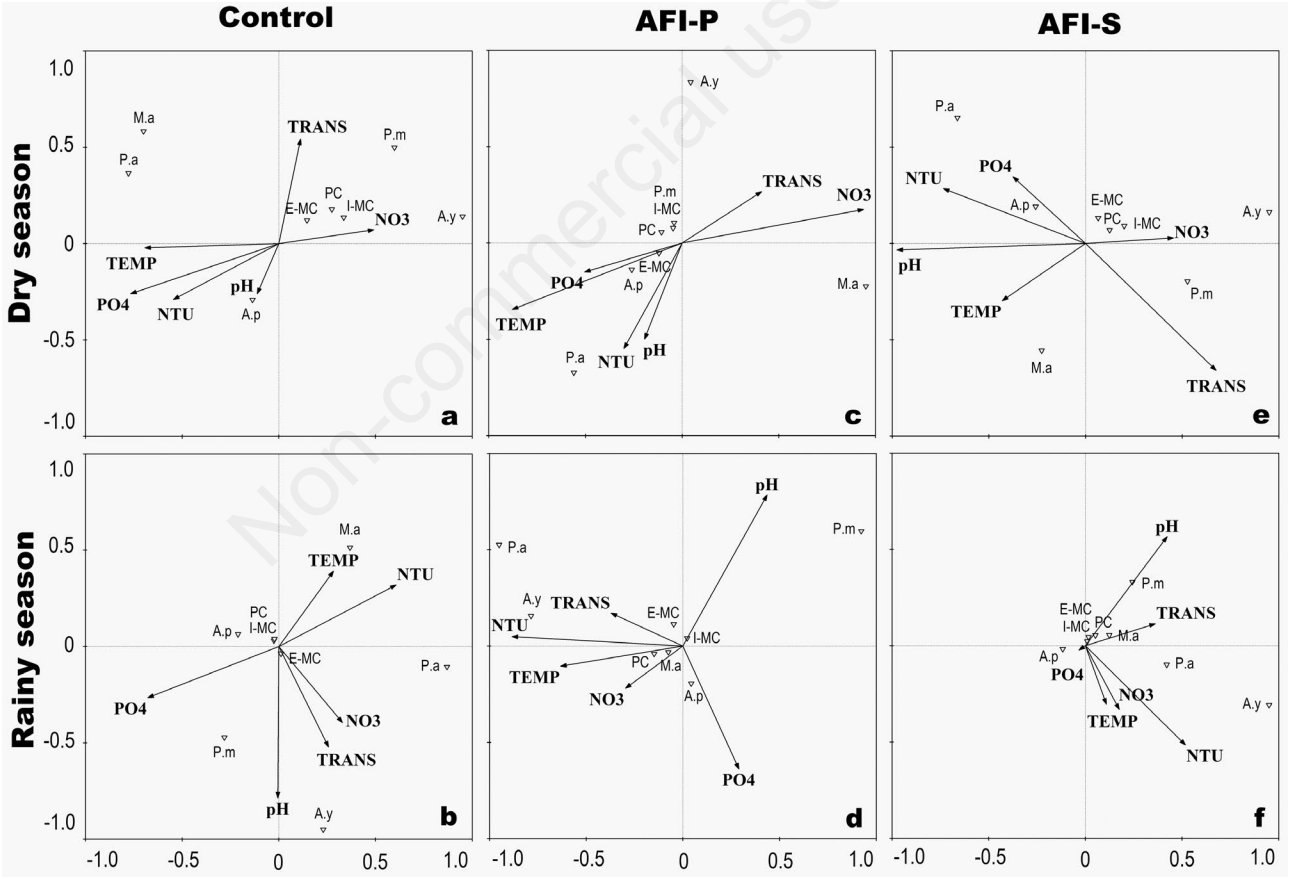


**Fig. 7.** Canonical Correspondence Analysis (CCA) at the Control, AFI-P and AFI S. Seasonal values of biovolume from the five cyanobacterial species with the highest occurrence, the data of the hydrological descriptors and the physicochemical parameters associated with cyanobacteria for the analysis were used. Biplot based on CCA. Control: dry season axis X 59.2, axis Y 29.6; rainy season axis X 56.0, axis Y 26.6. AFI-P: dry season axis X 52.5 axis, Y 34.9; rainy season axis X 58.5, axis Y 26.3. AFI-S: dry season axis X 62.3, axis Y 29.9; rainy season axis X 79.9, axis Y 17.6. TEMP, temperature; TRANS, transparency); NTU, turbidity; $NO_3^-$, nitrates; $PO_4^{3-}$, phosphates; P.a, *P. aeruginosa;* P.m, *P. mucicola;* A.p, *A. planticola;* A.y, *A. yezoense;* M.a, *M. aeruginosa;* I-MC, intracellular microcystin; E-MC, extracellular microcystin; PC, phycocyanin.

represents that during rainy season the potential risk due to the presence of cyanotoxin is greater for the use and management of reservoir water. However, the recorded levels both E-MC and I-MC did not exceed the WHO (1998) guidelines of 10 µg $L^{-1}$ for recreational water use.

Considering the seasonal variation of the environmental parameters, these play a primary role in the proliferation of the five cyanobacterial species evaluated. In the case of *A. yezoense* it was present in both dry seasons, which seems to favor its growth due to the high concentrations of $NO_3^-$ even at the low temperatures of the season; unlike the low presence of *M. aeruginosa* and *P. agardhii*, mainly in the second dry season in C-II. Contrary to the rainy season, *A. yezoense* reduces its abundance, which is used by other cyanobacteria such as *A. planctonica*, *M. aeruginosa* and *P. agardhii* to increase their densities and not allowing the growth of *A. yezoense* again. Based on the above, $PO_4^{3-}$ also seem to participate specifically in the establishment of *A. planctonica* and *P. agardhii* when $NO_3^-$ decreases. Under these conditions, $PO_4^{3-}$ stimulates the proliferation of N-fixing cyanobacteria that complement its N requirements by fixing and transforming of atmospheric N and take advantage of the increase in P levels (Sabour *et al*., 2009; Herrero and Flores, 2019).

Four of the five species of cyanobacteria with the highest incidence are diazotrophic, so it is established that P regulates their appearance and permanence, while $NO_3^-$ contributes to maintaining their cell density. The results of the study showed the presence of dominant toxic groups such as *A. yezoense*, *A. planctonica*, *P. agardhii* and *P. mucicola* than compared to those registered in previous studies, such as *Nostoc* and *Oscillatoria* (García *et al*., 2002; Gaytan -Herrera et al., 2011; Alillo-Sánchez *et al*., 2015).

The theoretical basis of the presence of macrophytes in AFI aims at the elimination of nutrients in the aquatic system (Chang *et al*., 2017; Lucke *et al*., 2019) and consequently the reduction of phytoplankton (West *et al*., 2017; Park *et al*., 2018). In our AFI models, no differences were observed regarding the presence of the different taxonomic groups, particularly between diatoms and cyanobacteria, so the implementation of these models maintained the phytoplanktonic diversity in the study area.

Regarding the presence of cyanobacteria in the AFIs, it was observed in a timely manner that during the second rainy season in C-II, the AFI-P site showed a reduction in the presence of this group. However, this effect cannot be generalized because an increase in $NO_3^-$ was registered in these sites, which rather favors the growth of cyanobacteria, this increase in $NO_3^-$ occurred during the dry season at the AFI-S site, while in the rainy season at the AFI-P site. This effect of cyanobacteria-AFIs association was observed in the CCA results and can be explained because some biomass further provides P and N during senescence or when the cyanobacterial blooms collapse (Zhu *et al*., 2013; Chen *et al*., 2018).

Regarding the $PO_4^{3-}$ levels, these showed no change at AFIs with respect at Control, however the correlations show that this nutrient in AFI-S in rainy season was more directly associated with the PC, which also represents a factor that promotes cyanobacteria-macrophyte interaction at AFIs. The establishment of cyanobacteria in AFIs can be explained by the fact that root system provides oxygen and allowing nutrients vertical mobilization, modifying their solubility (Hubbard, 2010). On the other hand, the AFIs managed to reduce the concentration of the microcystins, particularly E-MC during the rainy season in C-II, and I-MC in dry season, and only AFI-S in rainy season C-II. This decrease is associated with the assimilation and transformation of microcystin that some macrophytes present and which has been also recorded for *P. australis* (Pflugmacher *et al*., 2001; Romero-Oliva *et al*., 2015; Cao *et al*., 2019).

The study shows during dry season, when $NO_3^-$ levels were higher in AFI-S, there was greater biovolume of *A. yezoense* than at Control site, which suggests that macrophytes concentrate these cyanobacteria in the rhizome area, which also acts as a biofilter that retains both particulate organic matter and the different forms of planktonic biomass (Ferdoushi *et al*., 2008; Castro-Castellon *et al*., 2016). In the case of at AFI-P site, there was a decrease with respect to the Control and, according to the CCA, this cyanobacterium was not correlated with $NO_3^-$.

In the case of *M. aeruginosa*, it was particularly observed at AFI-P site that the variation in its abundance had an annual behavior, since in C-I in the dry and rainy season there was a greater increase in biovolume than at Control. This means that the growth of *M. aeruginosa* as observed in the CCA, even its proximity to $NO_3^-$ does not stand out as the dominant species since it was observed that when it presented low densities, *A. yezoense* probably increases due to the fixation of atmospheric N when $NO_3^-$ concentrations are scarce (Ferber *et al*., 2004; Rolff *et al*., 2007). This suggests that the relationship between both species is mutually exclusive and regulated by cell density. Unlike what was described, AFI-P in the rainy season C-II showed a reduction of *M. aeruginosa* with respect to the Control, this reaffirms the type of relationship between both species and where again *A. yezoense* presented a higher density than *M. aeruginosa*. Another mutually exclusive relationship occurred between the species *A. planctonica* and *P. mucicola*, it was observed at AFI-P, when *A. planctonica* registered a lower biovolume *P. mucicola* increased its density. At AFI-S site the presence of a higher density of *P. mucicola* prevents *A. planctonica* from establishing itself. Therefore, it is observed that AFI-P reduced *A. planctonica* and AFI-S promotes the growth of *P. mucicola*, both in comparison with at the Control. In

the case of *P. agardhii* it was observed that when it is absent at Control and at AFI-S, it occurs in AFI-P.

Based on the above, we found that $NO_3^-$ increased in AFIs and had no effect on $PO_4^{3-}$, as has been recorded in *Phragmites*, which is that it is capable of enriching the medium due to its input of N (Ge *et al*., 2017). But that does not coincide with other proven models where these nutrients are removed (Abed *et al*., 2017; Wang *et al*., 2019). However the assimilation of nutrients in the water by the macrophytes was evidenced by the production of biomass with annual P values of 9 g P $m^{-2}$ at AFI-P and 5 g P $m^{-2}$ at AFI-S, while N values were 48 g N $m^{-2}$ at AFI-P and 32 g N $m^{-2}$ at AFI-S. These results exceed the values recorded in AFI models in which the same plant species were evaluated (Pavan *et al*., 2015; Castro-Castellon *et al*., 2016; Wang *et al*., 2015; Choudhury *et al*., 2019) and it could be explained due to the number of plants used in our models and the contact time with the water.

However, that kind of models evaluate this removal effect in systems with horizontal flow (Fang *et al*., 2016; Chang *et al*., 2017), not so in our study that was carried out in a reservoir where the AFIs were located at a site without water inlet and outlet. Furthermore, our purpose was to evaluate the changes in the water column, relating the physicochemical parameters with the presence of cyanobacteria and their vertical migration capacity in the presence of AFIs. Likewise, it was not observed that under these proven conditions, phytoplankton, particularly the group of cyanobacteria, decreased as it happens in the horizontal flow models (Park *et al*., 2018; Wang *et al*., 2019). What could be observed was that the AFIs presented specific-species effect, with the cyanobacteria, as has been observed in some works where particularly diazotrophic genera are related to macrophytes in models of floating islands in open systems (Urakawa *et al*., 2017).

## CONCLUSIONS

In our work we tested the effect of the AFIs in the VB reservoir, observing an increase in $NO_3^-$ and no effect on $PO_4^{3-}$, however, there were no variations in the presence of the different phytoplankton taxonomic groups. Likewise, the group of cyanobacteria was also not reduced due to the presence of AFIs, but the concentration of extracellular microcystin was lower in rainy season in the second annual cycle (C-II), while the intracellular toxin was in dry season C-II. It was also observed that each macrophyte had a specific effect on four cyanobacteria of the five selected potentially toxic. So AFI-P promotes the increase of *M. aeruginosa* and reduces *A. planctonica*, while AFI-S promotes *A. yezoense* and *P. mucicola*. It was determined that in the AFIs the dynamics between the cyanobacteria is regulated by the environmental factors in the following way: $NO_3^-$ favors the growth of *A. yezoense*, the temperature to *M. aeruginosa* and $PO_4^{3-}$ maintain the presence of the other three diazotrophic cyanobacteria. A mutually exclusive relationship was also observed on the one hand between *M. aeruginosa* and *A. yezoense* and on the other between *A. planctonica* and *P. mucicola*.

These results correspond to the vertical effect that the AFI pilot models had in a large eutrophic water system and so carrying out an ecological rehabilitation using these structures would imply increasing the number of floating islands and deepening the macrophyte-cyanobacteria interactions, also considering the allelopathic effect between species.

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