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Planktonic cyanobacteria from the Abreus Reservoir, Cienfuegos, Cuba

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Abstract. The study of the cyanobacteria that make up the phytoplankton community of reservoirs is very important due to the production of toxins by some phytoplankton taxa. The composition and abundance of cyanobacteria and their relationship to physicochemical variables was determined during six months (March, April, June, September, November and December) in 2018 at five stations in the Abreus Reservoir, which is located in the south center of the Cienfuegos province (Cuba). Eleven new taxa were observed in the reservoir grouped into seven families, 14 genera, and 34 species. The toxigenic genera *Microcystis* and *Raphidiopsis* were observed at all collection points throughout the year, presenting a potentially persistent toxicity threat in this reservoir. Semi-accumulative blooms were reported in September. *Microcystis* sp. and *Raphidiopsis* sp. were the most abundant genera during observed blooms. The abundance of some cyanobacterial genera, including *Microcystis*, *Aphanocapsa*, *Raphidiopsis* and *Dolichospermum*, were strongly correlated with water temperature and transparency. Microcystin values are reported for the first time in Abreus Reservoir.

Key words: cyanobacteria, blooms, correlation, physicochemical variables, Cuba.

Resumen: Cianobacterias planctónicas del embalse Abreus, Cienfuegos, Cuba. El estudio de las cianobacterias que componen el fitoplancton de los embalses es muy importante debido a la producción de toxinas de algunas especies que forman este grupo. Durante seis campañas en 2018 se analizó composición y la abundancia de las cianobacterias y su relación con algunos parámetros físico-químicos de cinco estaciones en el embalse Abreus, en el centro sur de la provincia de Cienfuegos. Se obtuvieron 11 nuevos taxos para el embalse y un nuevo registro para Cuba, agrupados en siete familias, 14 géneros y 34 especies. Los géneros *Microcystis* y *Raphidiopsis* se reportaron en todos los puntos de colecta durante todo el año, por lo que representa una alerta frente al uso del embalse por su posible toxicidad. Se reportó un florecimiento semiacumulativo en una estación del mes de septiembre y en estaciones cercanas, concentraciones celulares también elevadas. *Microcystis* sp. y *Raphidiopsis* sp. fueron los géneros más abundantes durante la floración. Los géneros *Microcystis*, *Aphanocapsa*, *Raphidiopsis* y *Dolichospermum* mostraron una fuerte correlación con la temperatura y la transparencia. Se reporta por primera vez valores de toxinas en el embalse.

Palabras clave: cianobacterias, Florecimientos, correlación, variables físico-químicas, Cuba.

Introduction

From their origins to the present, cyanobacteria have successfully colonized diverse ecosystems, however cyanobacteria are most abundant and problematic in lentic freshwater ecosystems (Chorus 1999). In recent years, planktonic cyanobacteria have attracted significant attention due to their ability to form blooms, which can potentially affect human and environmental health through the production of varied toxic secondary metabolites (Fernández *et al.* 2005).

Past studies have documented cyanobacteria throughout Cuba (Komárek & Hindák 1975, Komárek 1984a, b, 1985, 1989a,b 1995, Komárek & Kovačik 1987, Hindák 1984, 1988). References to Cuban materials also appear in Lukávsky *et al.* 1992, Komárek & Novelo 1994, Komárkova-Legnerová & Tavera 1996, and Komárek & Anagnostidis 1999, 2005. The first and most complete information on the Cuban freshwater cyanobacterial species composition was published by Comas 2009a.

The Abreus Reservoir was built as a drinking water reservoir in 1985 near the municipality of Abreus, Cienfuegos, Cuba. The main tributary of the reservoir, the Damují River, collects and deposits nutrients from several sources that lead to an abundance of cyanobacteria. Several blooms of cyanobacteria have occurred in the reservoir. The most frequent species in these bloom have been *Anabaenopsis* sp., *Arthrospirakhannae* (Drouet & Stricklandin Drouet 1942), *Dolichospermum cf. floreaquae* (Lyngbye) (Wacklin *et al.* 2009), *D. cf. solitarium* (Klebahn) (Wacklin *et al.* 2009), *Microcystis panniformis*, *Raphidiopsis curvata* (Fritsch & Rich 1929), *R. gangetica* (Nair) (Aguilera, Berrendero, Kaštovský, Echenique & Salerno 2018) (sub. *R. curvispora* Watanabe 1995), *R. raciborskii* (Woloszinska) (Aguilera, Berrendero, Kaštovský, Echenique & Salerno 2018) and *R. curvata* (Fritsch *et al.* Rich 1929), reported by Comas *et al.* 2010 and Comas & Moreira 2013.

This work summarizes the results of the Cienfuegos reservoir phytoplankton monitoring program developed in 2018, including an update of the cyanobacteria present in the Abreus reservoir and the relationship between cyanobacterial abundance and physicochemical characteristics of the reservoir

Materials and methods

Study area: The Abreus reservoir is located in the municipality of the same name in the province of Cienfuegos, Cuba (Fig. 1). The reservoir was

originally used for agricultural purposes and currently supplies water to industries and a large portion of the population of Cienfuegos. The two main tributaries are the Damují and Jabacoa rivers (Fig. 1) and the reservoir receives almost all of the water that runs through the basin, as it is located on the last stretch of the Damují river. Its basin and geological formations are mainly composed of clays, marls and limestones, as well as basalts, epiclastic rocks and sandstones in smaller proportion (Laiz & Flores 2007). The region experiences two climatic seasons: a dry or less rainy season between November and April, with minimum accumulations in December and January, and a rainy season between May and October, with maximum precipitation between June and October (Caravaca 2011). Industrial, agricultural and human pollutants are partially treated before entering the reservoir but still negatively affect the water quality of the reservoir.

Sampling: Six monitoring campaigns were carried out in March, April, June, September, November and December of 2018 corresponding to each climatic period. Five monitoring points were determined (Fig. 1) after taking into account the physical-geographical characteristics and use of the reservoir, including (A1) entrance of the Damují river, (A2) reservoir center, (A3) entrance of the Limones river, (A4) drinking water facility intake, and (A5) Santa Ana river. For the qualitative analyses, plankton samples were collected using a plankton net (20 µm mesh) before being transferred to 250 mL bottles. They were then preserved with formaldehyde to obtain a final concentration of 3%. The quantitative samples were taken at a depth of 0.5m in 500mL flasks and preserved with acid Lugol's solution to achieve a final concentration of 1%.

Analysis of the samples: The samples were observed and studied in a Laborlux Leica-Leitz light microscope using bright field and phase contrast settings. For identification, specialized literature was consulted (UNESCO 2009) as well as expert knowledge. For cell counts, the samples fixed with Lugol's were allowed to settle in 25 mL chambers (Utermöhl 1958) for 24 h before 100 fields per sample were enumerated at 400x magnifications using a MOTIC inverted microscope.

Physical and chemical analyses: Physical variables measurements and chemical analyses were conducted in the Environmental Testing Laboratory of the Center of Environmental studies of Cienfuegos (CEAC) using the Manual of Instructions and Procedures L-SA-205. Dissolved

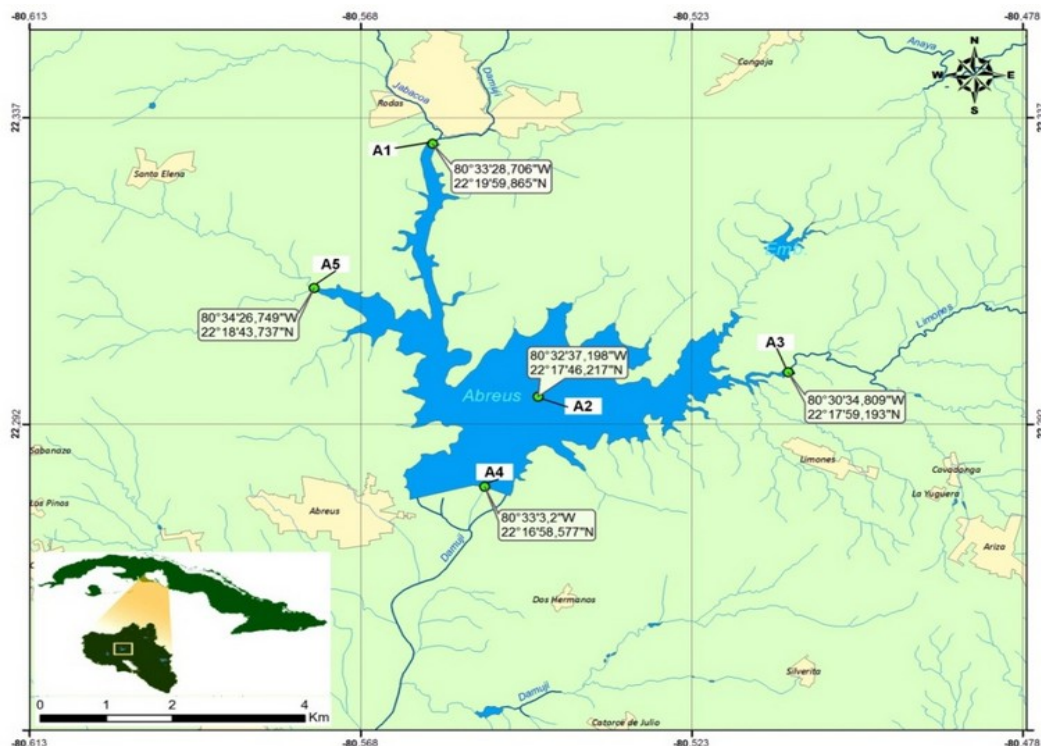


Figure 1. Study area including the Abreus Reservoir in the center of the province of Cienfuegos, Cuba.

oxygen *in situ* (DO) was performed by the Winkler Method and biological oxygen demand at 5 days by Winkler dilution. Nitrite-nitrogen was measured by spectrophotometry with blue indophenol. Magnesium (Mg) and chlorine (Cl) concentrations were determined via titration of EDTA and AgNO_3 , respectively. *In situ* water transparency (Tr) was measured with a Secchi disk. Chlorophyll-a (Clo a) samples were extracted with methanol and measured with a fluorometer (APHA 1998). The microcystin concentration was evaluated for the first time for the reservoir, in stations A2 and A4, these samples were collected only once in September 2018, without the presence of bloom. Particulate microcystin concentration was quantified for one sample using enzyme-linked immunosorbent assay after extraction using 75% aqueous methanol following the vendors directions (Abraxis PN 520011).

Statistical analysis: To summarize the relationship and patterns of the physicochemical variables and cyanobacterial abundance, a multivariate analysis with 10 pairs of data was applied to calculate each coefficient. Several descriptive statistics and Pearson product-moment correlations were calculated for each pair of variables. A cluster analysis by the nearest neighbor method was conducted to understand its spatio-temporal behavior, find the simplest link, and establish associations between

groups of phytoplankton species. The behavior of precipitation at points A1 and A4 was also analyzed because they have measurement stations as well as being the points with the highest water entry and exit from the reservoir, respectively.

Results

The three classical orders for the Cyanophyceae Class were reported, grouped into 7 families, 14 genera and 35 species (Table I). Of the total genera, 5 are considered to be toxin producers (*Microcystis*, *Planktothrix*, *Osillatoria*, *Raphidiopsis* and *Phormidium*) (Raymond2002).

In the quantitative analysis (Fig. 2a, b), we obtained a total of 17 in the dry and 21 species in the rainy season. In September, a cumulative or semi-accumulative massive bloom was reported on the surface at site A5 composed mostly by *Microcystis* sp., with 2.9×10^4 cells mL^{-1} , and *Raphidiopsis* sp. 2.2×10^4 cells mL^{-1} . The high abundance of *Pseudanabaena* sp. 7.2×10^3 cells mL^{-1} and *Aphanocapsa* sp. 7.9×10^3 cells mL^{-1} during this bloom indicates that they are accessory species to the bloom. There were also high concentrations of *R. gangetica* 1.4×10^4 cells mL^{-1} and *Microcystis* sp. 1.1×10^4 cells mL^{-1} reported in site A3 on the same date. In general, cyanobacterial abundance was

Table I. Composition of the cyanobacteria observed at each sampling point, with records for the reservoir compared to the previous studies of Comas, 2009 and Comas, *et al.* 2017 of the Abreus Reservoir, Cienfuegos, Cuba. * denotes a new record for the reservoir, ** denotes a new record for the country.

Division: Cyanophyta	Dry Season					Rainy Season				
	A1	A2	A3	A4	A5	A1	A2	A3	A4	A5
<i>Aphanocapsa delicatissima</i> West & G.S. West						+	+	+	+	
<i>Aphanocapsa holsatica</i> (Lemmermann) G. Cronberg & Komárek	+	+		+	+	+				
<i>Aphanocapsa</i> sp.		+	+	+	+				+	+
<i>Arthrospira</i> spp.							+	+	+	+
<i>Arthrospira fusiformis</i> (Voronich.) Komárek et Lund (*)						+				
<i>Arthrospira khannae</i> Drouet and Strickland								+		
<i>Chroococcus</i> sp.	+		+	+	+		+	+		+
<i>Chroococcus minutus</i> Keissler(*)		+		+		+			+	
<i>Coelomoron</i> sp.			+	+	+			+		
<i>Coelomoron microcystoides</i> Komárek	+	+	+	+		+		+	+	
<i>Coelomoron tropicale</i> P.A.C. Senna, A.C. Peres & Komárek	+	+	+	+	+	+		+		+
<i>Dolichospermum</i> sp.	+	+	+	+	+					
<i>Dolichospermum solitarium</i> (Klebahn) Wacklin, L. Hoffmann & Komárek				+	+					
<i>Merismopedia</i> sp.	+	+		+			+			+
<i>Merismopedia convoluta</i> Brébisson ex Kützing (*)						+		+		
<i>Merismopedia punctate</i> Meyen									+	
<i>Microcystis</i> sp.	+	+	+	+	+	+	+	+	+	+
<i>Microcystis brasiliensis</i> (Azevedo & C. L. Sant' Anna) Rigonato <i>et al.</i> , nom. inval.(*)										
<i>Microcystis cf. smithii</i> (Komárek). <i>et</i> Anagn.								+		
<i>Microcystis novacekii</i> (Komárek) Compère (**)		+	+	+					+	
<i>Microcystis panniformis</i> Komárek, Komárková- Legnerová, Sant' Anna, M.T.P. Azevedo, &P.A.C. Senna (*)	+	+							+	+
<i>Oscillatoria jenensis</i> G. Schmid (*)								+		
<i>Phormidium ambiguum</i> Gomont						+				
<i>Phormidium</i> sp.				+		+				
<i>Planktothrix isothrix</i> (Skuja) Komárek & Komárková	+	+	+	+	+	+	+	+		+
<i>Pseudanabaena</i> sp.							+	+	+	+
<i>Pseudanabaena catenate</i> Lauterborn						+		+		
<i>Pseudanabaena limnetica</i> (Lemmermann) Komárek (*)							+			
<i>Pseudanabaena mucicola</i> (Naumann & Huber-Pestalozzi) epiphyte on mucilaginous envelope of <i>Microcystis novacekii</i> (**)			+							
<i>Raphidiopsis</i> sp.	+	+	+	+	+	+	+	+	+	+
<i>Raphidiopsis philippinensis</i> (W.R. Taylor) Aguilera, Berrendero Gómez, Kaštovský, Echenique & Salerno (*)	+			+						
<i>Raphidiopsis gangetica</i> F.E.Fritsch & F.Rich								+		

Snowella sp. +

Snowella lacustris (Chodat) Komárek & Hindák + + + +

Sphaerospermopsis torques-reginae (Komárek) Werner, Laughinghouse IV, Fiord & Sant' Anna (*) + +

Total 11 13 12 18 11 13 10 16 11 10

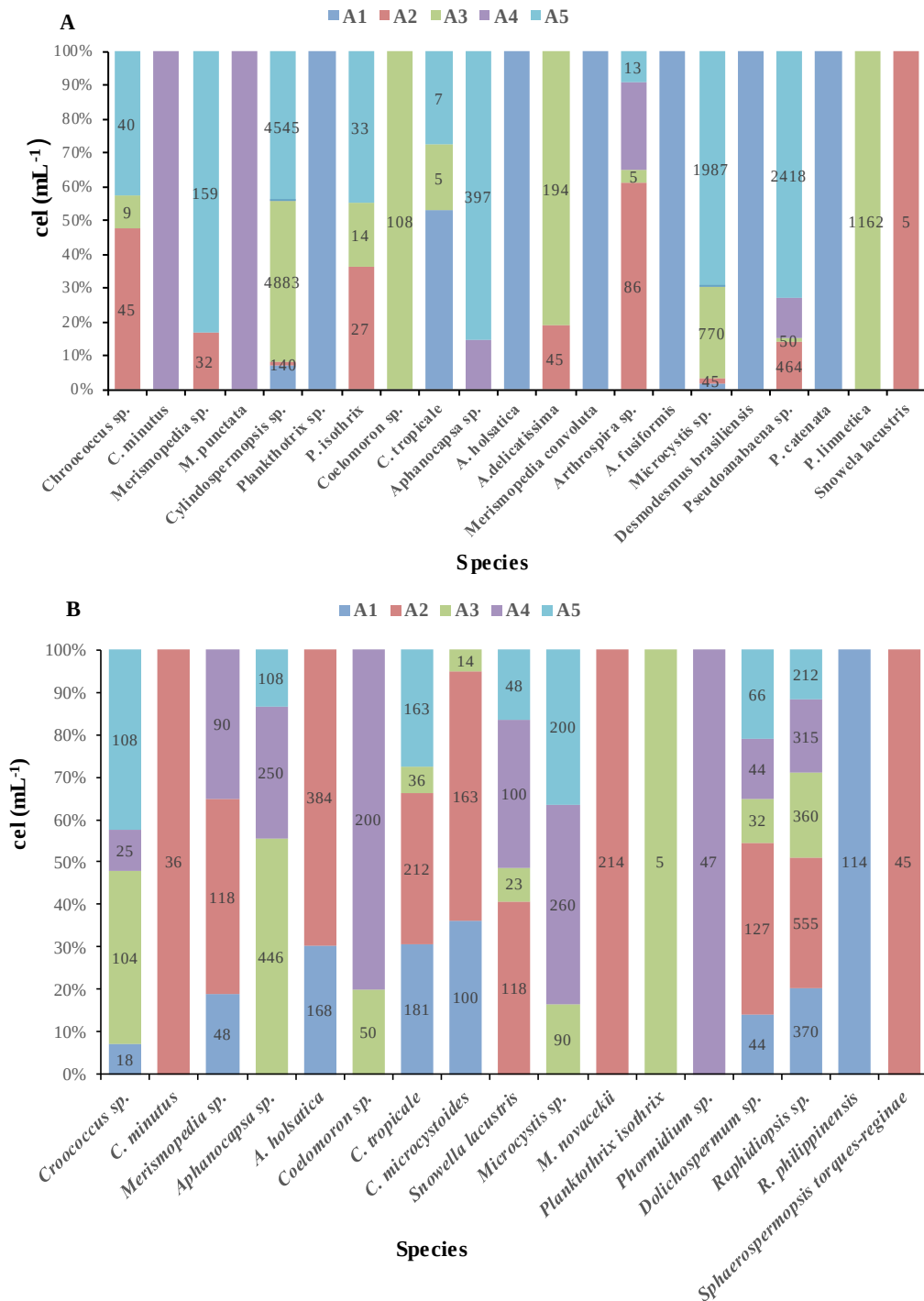
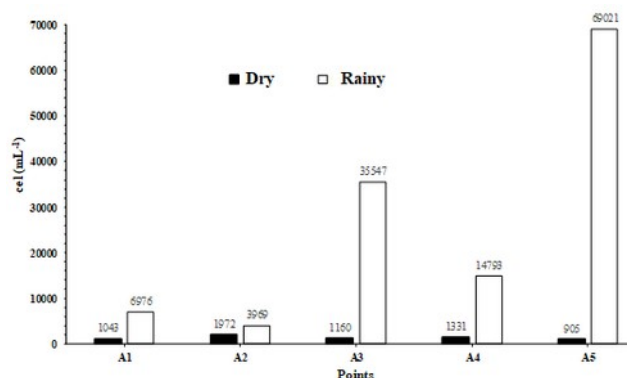


Figure 2. (A) Abundance of cyanobacteria (cells mL⁻¹) of specific species, corresponding to the rainy season and (B) in corresponding to the dry period in the Abreus reservoir, Cienfuegos, Cuba, during 2018.

Table II. Physicochemical variables measured at Abreus reservoir between March and December 2018.

Variables	Dry Season					Rainy Season				
	A1	A2	A3	A4	A5	A1	A2	A3	A4	A5
OD (mg/L)	7,83	8,62	8,48	8,62	7,97	5,65	7,68	7,90	7,25	7,75
Cl ⁻ (mg/l)	34	33	28,5	31,5	31,5	17,99	18,49	19,49	18,49	21
N-NO ₂ ⁻ (mg/l)	0,00079	0,00092	0,00132	0,00158	0,00276	0,03199	0,01916	0,01685	0,01172	0,00606
N-NO ₃ ⁻ (mg/l)	<0,0016	<0,0016	<0,0016	<0,0016	0,0028	0,0822	0,118	0,0375	0,042	0,1314
N-NH ₄ ⁺ (mg/l)	<0,0086	<0,0086	<0,0086	<0,0086	<0,0086	<0,01	<0,01	0,0203	0,047	0,0161
P-PO ₄ ³⁻ (mg/l)	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Mg ²⁺ (mg/l)	27,7	25,5	45,9	49,3	53	9,23	8,75	10,21	11,18	7,29
DBO ₅ (mg/l)	4,6	3,4	3,1	4,9	4,6	3,4	3,1	3,4	3,4	2,86
Clo "a" (µg/l)	3,19	2,98	2,8	3,19	2,51	4,42	3,12	1,8	3,52	3,47
Tr (%)	51,28	34,72	34,72	41,67	60,61	10,77	11,43	16,04	17,02	15,05
T (°C)	28,6	27,5	27,3	27,5	27,4	28	28	29	29	30
Precipitation (mm)	50	50,7	50,7	51,3	50,7	284	267	267	249,9	267

**Figure 3.** Abundance of cyanobacteria (mL⁻¹ cells) at five sampling sites in dry and rainy season, in the Abreus reservoir, Cienfuegos, Cuba, during 2018.

higher in the rainy season than the dry season (Fig. 3).

The physical-chemical characteristics of the study area are shown in Table II. These parameters reflect the influence that the two climatic periods have on the hydrodynamic regime of the reservoir. The temperature was maintained between 27-30°C, an optimum range for cyanobacterial growth (Reynolds, 2006). Light availability in the water column, measured by transparency (%) Mg²⁺ levels (mgL⁻¹) were highest during the dry season. Consistently low concentrations of N-NO₂⁻, N-NO₃⁻, N-NH₄⁺ and P-PO₄³⁻ were reported during the sampling period, with several samples below the

limit of detection. Biological oxygen demand at 5 days ranged between 3.1- 4.9 (mg/L). Chlorophyll-a concentrations were low in comparison with the values detected by Betancourt *et al.* (2010) in this reservoir(31-42µgL⁻¹). The highest rainfall was in the rainy season, mainly at point A1 that is at the entrance to the reservoir, so the contribution of rain was big.

Precipitation in stations A1 and A4 had a similar behavior. The highest value in the period studied was May 2018 with 798 and 719mm respectively, associated with the tropical depression Alberto (Fig. 4), which caused the reservoir to discharge to its maximum capacity.

Discussion

The cyanobacterial composition and abundance reflect the hydrodynamic conditions of the study area. The abundance increased towards the confluence zone of the different tributaries of the reservoir. The main nutrient sources for the reservoir are the Damují and Jabacoa Rivers, which surround the city of Rodas that contributes anthropogenic nutrients that would otherwise not reach the reservoir. Increased precipitation during the rainy season led to a higher amount of nutrient inputs, which likely promoted cyanobacterial growth. Although the highest abundance (Fig. 3) was reported at points A3 and A5 related to a bloom, point A4 had a high abundance. Moreover,

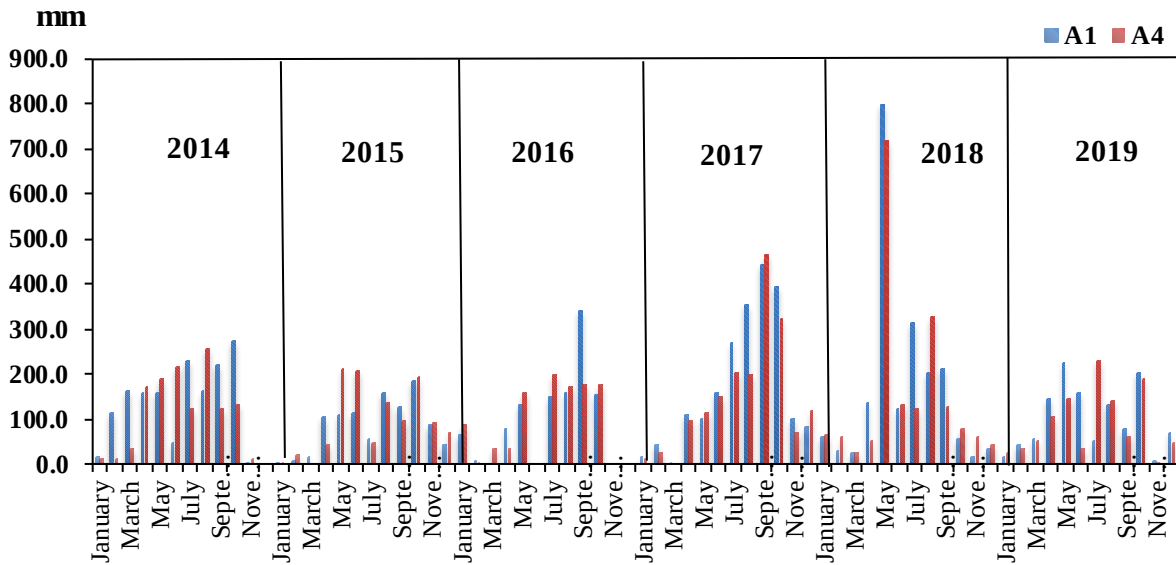


Figure 4. Behavior of precipitation in a period of 6 years in the Abreus reservoir.

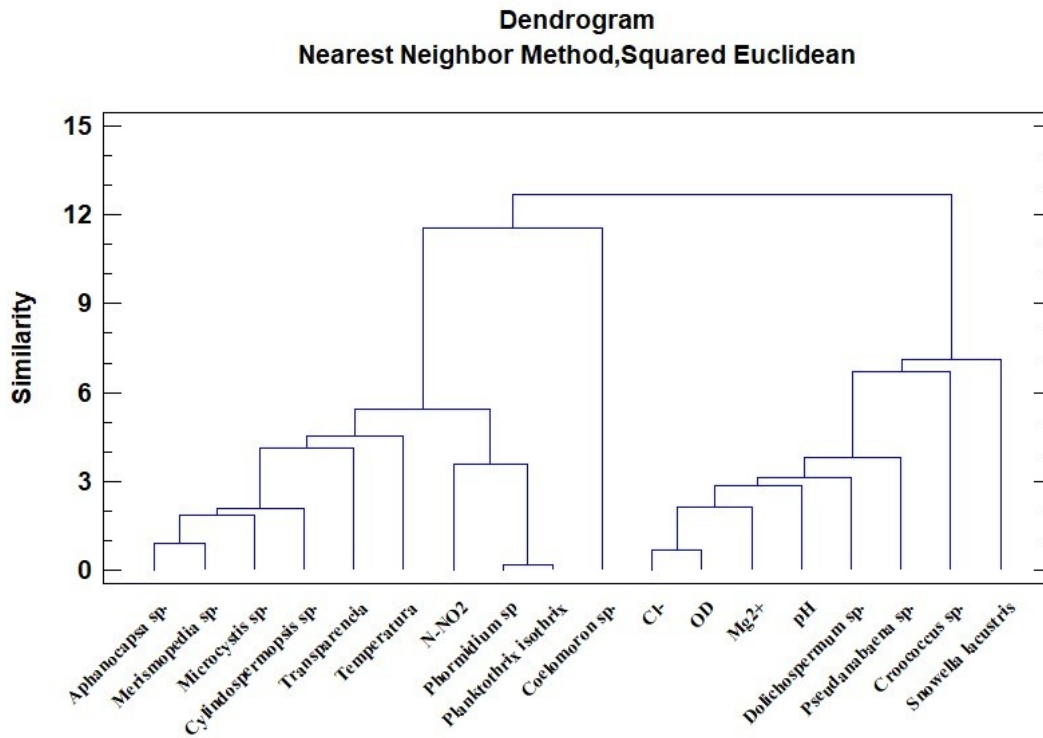


Figure 5. Dendrogram based on the nearest neighbor method. On the horizontal axis the interaction of species and physicochemical variables in 2018.

northeasterly winds (Morera-Gómez, *et al.* 2018) drag planktonic cyanobacteria towards the drinking water facility intake at site A4 that supplies drinking water to a greater part of the municipality of Cienfuegos. The potentiality of this point for the algal blooms that we researchers were seeing for some time was corroborated with a bloom in December 2017 of *Microcystis* sp., which is in the process of publication.

Interesting are the relationships found between the cyanobacterial species and the physicochemical variables (Fig. 5). The nearby neighborhood between *Aphanocapsa* and *Merismopedia* was observed in the samples throughout the year, their cell concentrations are very close, in addition to similar concentrations in the cases of blooms. In the same way there is a close neighborhood between *Microcystis*, *Raphidiopsis*

and the physical factors temperature and transparency, which were observed as key in the blooms. The genera *Snowella*, *Chroococcus*, *Pseudoanabaena*, *Dolichospermum* and *Coelomonon*, were rarely found in the samples, so their relationship is far away. This study shows a first approximation of the phycosociological behavior of these species in the reservoir. For which this statistical analysis based on abundance is a useful technique. An example of relationships between genders are *Microcystis*, *Raphidiopsis* and *Aphanocapsa* that remained in high concentrations throughout the year of study (Fig. 2). The abundance of species in the analysis with the dendrogram had no relationship per sampling station in either period. Here seems to be the key to their survival at any station of the year.

Using Pearson correlations (Table III), we found a linear relationship between the genera with greater dominance in all the periods, including *Aphanocapsa*, *Merismopedia*, *Raphidiopsis* and *Microcystis*. This relationship may be the cause of the presence of these species in all collection points throughout the year (Table II). On the other hand, physical variables, such as temperature and transparency, seem to be more related to the abundance of cyanobacteria than inorganic nutrients. These relationships may be the key to the permanence of cyanobacteria in all periods in the reservoir, since the temperature was maintained at more than 20°C throughout the year and the

transparency was good in the first meters of the water columns (Table I). These factors are conducive to the good growth of cyanobacteria in a reservoir (De Leon, 2002). Precipitation plays a key role in increasing the abundance of these species, this increase corresponding to the rainy season in the reservoir (fig. 3 and Table II). Therefore, for all the aforementioned, the key to the dominance of cyanobacteria is seasonal and due to its relationship with the main physical variables of the reservoir.

The potable water in the Abreus Reservoir is characterized by its humid earth flavor and therefore it is distinguished from other reservoirs in the province of Cienfuegos (Valle, pers. Pers.). Taste and odors of this type in drinking water are due to the production of volatile compounds, mainly geosmin and 2-methylisoborneol (2-MIB) (UNESCO 2009) and their most frequently cited source in freshwater are cyanobacteria (Suikkanen *et al.* 2005). Moreover, fifty-one cyanobacterial species have been reported to produce taste and odor compounds, such as 2-MIB and geosmin that can negatively affect the palatability of drinking water (Izaguirre & Taylor 1995, Suurnäki *et al.* 2014). Although the correlation between these compounds and the production of toxins has not been proven, many authors recommend it as a toxicity alert. They relate to *Microcystis* spp. and to *Raphidiopsis* spp. present in the Abreus reservoir with the production of these compounds (Bartram, 1999).

Table III. Paired Pearson moment correlations with P-values below 0.05, with a confidence level of 95%.

	<i>Raphidiopsis</i> sp.	<i>Merismopedia</i> sp.	<i>Microcystis</i> sp.	<i>Merismopedia</i> sp.	<i>Phormidium</i> sp.	<i>Dolichospermum</i> sp.	<i>Planktothrix isothrix</i>	Transparency	Temperature	OD	pH	Mg ²⁺
<i>Aphanocapsa</i> sp.	x	x	x					x				
<i>Raphidiopsis</i> sp.			x	x						x		
<i>Dolichospermum</i> sp.								x	x	x		
<i>Merismopedia</i> sp.			x					x				
<i>Planktothrix isothrix</i>					x							
<i>Pseudanabaena</i> sp.												x
OD								x		x		x
Cl ⁻						x		x	x	x		x
N-NO ₂					x		x	x	x	x		x
pH												x
Temperature								x		x		x

The presence of the genera, *Microcystis* and *Raphidiopsis*, at all the collection sites is a wake-up call to the use of the reservoir. The potential of these genera to produce toxic secondary metabolites, mainly microcystins and cylindrospermopsins, is always present. A study conducted in September of that same year in sites A2 and A4 found the presence of microcystin at 0.018 µg/L and 0.031 µg/L, respectively. Currently in Cuba there are no regulations for toxin levels, so the different international regulations that follow the proposal of the World Health Organization (1998) that set a value are useful provisional guideline limit 1 µg for microcystin-LR in drinking water, based on bioassays with mice. So, these values reported in the Abreus reservoir are low, but it is a warning sign because these values were taken at a time when the reservoir had not suffered disturbances in its environmental conditions.

Conclusions

Two new cyanobacterial taxa were observed in Cuba and eight for the Abreus Reservoir. The group with the highest composition and abundance was *Chroococcales*. In the month of September 2018, a cumulative or semi-cumulative bloom reported on the surface, composed of the genera *Microcystis* and *Raphidiopsis*. There was a higher abundance of cyanobacteria during the rainy season. The highest correlation was obtained between temperature, transparency and the genera *Microcystis*, *Aphanocapsa*, *Raphidiopsis* and *Dolichospermum*. We found relation between genera with greater dominance in all periods. Low levels of toxins were reported for the first time but they represent a sign of deterioration of the reservoir.

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