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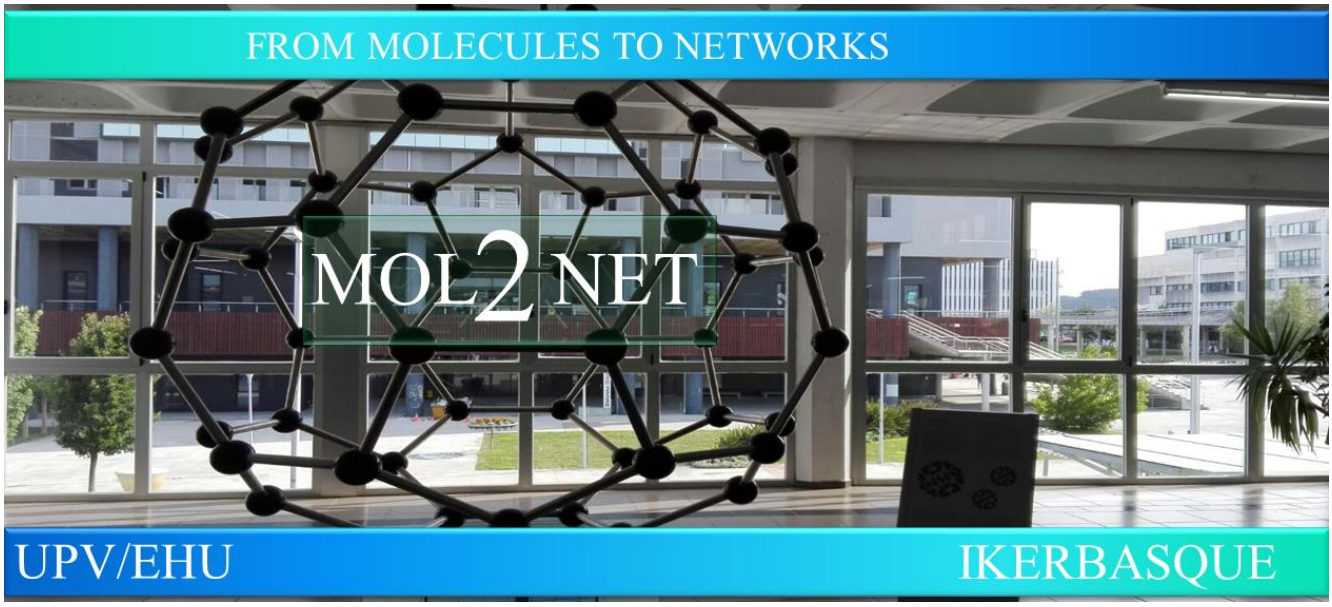

Non-specific cyanobacteria bloom and microcystin detection in Abreus reservoir, Cienfuegos, Cuba

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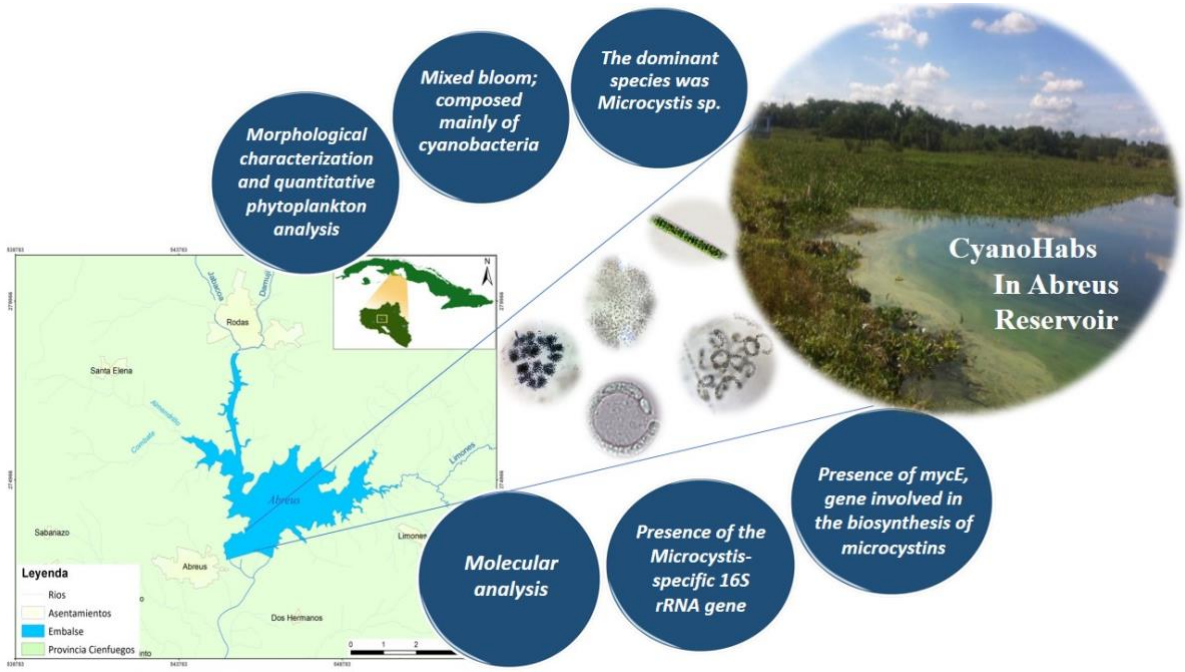
Graphical Abstract



Abstract: The reports of cyanobacterial blooms and their impact on ecosystems and human health have increased in the last two decades, becoming of emerging concern for the World Health Organization (WHO). Most of these blooms are non-specific, although a few species showed more dominance, frequently, toxin-producing strains of Microcystis. Likewise, in Cuba, some cyanobacterial water blooms have been published, mainly focused on species composition and abundance, but studies do not approach both morphological and molecular analyses. Herein, we performed the characterization of an unprecedented mixed bloom in the Abreus reservoir using morphological features and molecular biomarkers. We detected high concentrations of some species of cyanobacteria, in addition to some groups of phytoplankton. Our results revealed *Microcystis* sp. as dominant species, followed by *Sphaerospermopsis torques-reginae*, being confirmed through molecular biomarker analyses, the presence of the 16S rRNA gene and *Microcystis*-specific 16S rRNA gene. Besides, the screening on cyanotoxin genes, revealed the gene *mycE*, which is involved in the biosynthesis of microcystins. This study constitutes one the few records of non-specific Harmful Algal Blooms (HABs) in Cuba, based on both morphological and molecular level. Although this study shows unpublished data, we consider this work as an extended version of the article "Valle-Pombrol A., et al., Planktonic cyanobacteria from the Abreus Reservoir, Cienfuegos, Cuba. Pan-American Journal of Aquatic Sciences (2021), 16(1): 20-29; http://panamjas.org/pdf_artigos/PANAMJAS_16(1)_20-29.pdf". Indeed, our findings reinforce the importance of monitoring program of cyanobacteria in reservoir waters used for agricultural activities, animals, and human consumption.

The main bibliographic sources used in this paper are listed below [1-23].

Introduction

Cyanobacteria have been present in aquatic ecosystems about 2 billion years ago; however, in the middle of the last century, waterbody eutrophication started accelerating due to urbanization and industrialization. This together with climate change have led to harmful cyanobacterial blooms in lakes and reservoirs of most part of the world (Huisman et al., 2018).

Blooms of cyanobacteria have negative effects on aquatic organisms and even on human health, since many species can synthesize potent toxins. The most know cyanotoxins in freshwater are classified in: hepatotoxins

(microcystins, nodularins and cylindrospermopsins) that act mainly on liver, neurotoxins (anatoxins, saxitoxins, β-methylamino-L-alanine) that affect the nervous system, and dermatoxins (lipopolysaccharides) can cause allergenic, inflammatory and irritant responses on contact. The most frequently found toxins produced by cyanobacteria in freshwater ecosystems are the cyclic heptapeptide hepatotoxins of the microcystin group. *Microcystis*, *Planktothrix* and *Dolichospermum* are among the most frequent microcystins producer organisms (Codd et al., 2017).

Cyanobacteria surveillance is of major importance, and methods to achieve such require a prompt answer not only regarding the species that are producing the blooms but also the cyanotoxins that are being produced and/or released. The monitoring of cyanobacteria has so far been based on microscopic counting and the identification of single organisms, and using chemical/biochemical methods, and bioassays. Molecular methods represent complementary tools enabling detection of cyanotoxin genes, and also detecting potentially different toxin-producing strains (Baker et al., 2013).

Abreus reservoir is one of the most important freshwater ecosystems in the central southern region of Cuba. The reservoir has multiple uses including drinking water, irrigation and recreation. In the last years, some cyanobacterial blooms have been reported from this reservoir, being described only by morphological analysis (Comas et al., 2011, Comas & Moreira, 2013). Herein, we performed the characterization of an unprecedented cyanobacterial bloom in the Abreus reservoir using morphological features and molecular biomarkers.

Materials and Methods

Sampling site

Water was sampled at surface near the dam in the Abreus Reservoir (22°16'58,577"N; 80°33'3,2"W) (Fig. 1), Cienfuegos Province, southern-central region of Cuba. This was performed during an intense green water discoloration with approximately 1 Km of extension (December, 2017) (Fig. 2). Water samples (two 500 mL-replicates) were collected to phytoplankton occurrence and molecular analysis. Some physiochemical parameters (temperature, pH) were measured *in situ* by a HI9894 Hanna sonde. Phytoplankton samples for quantitative analysis were immediately fixed with acid Lugol solution; and another was preserved with formaldehyde (3%) for qualitative analysis. For molecular assays, one aliquot of 10 ml was filtered through Millipore 0.8 μm to collect cells and the filter was kept at -20°C until further analysis.

Phytoplankton analysis

Identification of phytoplankton species in the bloom was done with a binocular light microscope Laborlux Leica-Leitz equipped with an Axaer, Carl Zeiss digital camera. Microalgal enumeration was performed according to Utermöhl (1958) using 10 mL sedimentation chamber under an inverted microscope (MOTIC).

DNA extraction

Filters were defrosted and scraped with a sterile scalpel to remove the filtered cell biomass and placed in a sterile Eppendorf microtube. DNA was extracted with the PureLink™ Genomic DNA Mini Kit (Invitrogen, USA) following the protocol for Gram-negative bacteria in accordance with the manufacturer recommendations. A 50 μL of eluted DNA was stored at -20ºC before PCR amplification.

PCR amplification

PCR reactions were made in a final volume of 20 μL, containing 1 x PCR buffer, 2.5 mM MgCl2, 250 μM of each deoxynucleotide triphosphate, 10 pmol of each primer and 0.5 U of Taq DNA polymerase (Bioline, Luckenwalde, Germany). Presence of total cyanobacteria were detected initially through PCR amplification of the 16S rRNA and Phycocyanin operon marker genes followed by the specific amplification of different marker genes for cyanobacterial taxa (*Microcystis* sp. 16S rRNA, *Microcystis aeruginosa gyrB*, *Cylindrospermopsis raciborskii rpoC1*, *Planktothrix agardhii rpoC1*); and gene clusters involved in toxin biosynthesis such as microcystins (*mcyA*, *mcyB*, *mcyC*, *mcyD*, *mcyE*, *mcyG*), cylindrospermopsins (*CyrA*, *CyrB*, *CyrC*, *CyrJ*), saxitoxins (*sxtA*, *sxtG*, *sxtI*) and anatoxin-a (*anaC*). PCR assays for detection of marker genes were run either in the Biometra T-Professional Standard Gradient Thermal cycler (Germany) or in the Veriti 96-well Thermal Cycler, utilizing the primers and following the protocols reported previously (Neilan et al., 1995, 1997; Wilson et al., 2000; Schembri et al., 2001; Tillett et al., 2001; Fergusson et al., 2003; Hisbergues et al., 2003; Jungblut et al., 2005; Kellmann et al., 2006; Tanabe et al., 2007; Mihali et al., 2008; Rantala-Yilmen et al., 2011; Savela et al., 2015). The PCR products were

analysed by electrophoresis on 1% agarose gels stained with SYBRsafe (Invitrogen, USA); and visualized and photographed in the transilluminator Molecular Imager® GEL DOC™.

Results and Discussion

Twenty-three microalgae species were identified in the bloom: seven cyanobacteria (*Coelomoron tropicale*, *Microcystis panniformis*, *Microcystis* cf. *smithii*, *Microcystis* sp., *Planktothrix isothrix*, *Raphidiopsis gangetica*, *Sphaerospermopsis torques-reginae*) (Fig. 3), seven chlorophytes (*Coenococcus tetrasporus*, *Coelastrum* sp., *Dictyosphaerium* sp., *Hariotina* sp., *Monoraphidium contortum*, *Pandorina morum*, *Pseudoschroederia antillarum*), two bacillariophytes (*Aulacoseira granulata* and *Nitzschia* sp.), one cryptophyte (*Cryptomonas marssonii*), one dinophyte (*Peridinium* sp.), one euglenophyte (*Trachelomonas bacillifera*), and one zygnematophyte (*Staurastrum* sp.).

The bloom was characterized by a clear dominance of cyanobacteria (99.9%). The other phytoplankton groups appeared in low concentrations. The bloom consisted of a mixed population, *Microcystis* sp. (31882 cells mL^{-1}, relative abundance 32%), *Sphaerospermopsis torques-reginae* (29673 cells mL^{-1}, RA 30%), *Microcystis panniformis* (18467 cells mL^{-1}, RA 19%) and *Planktothrix isothrix* (16624 cells mL^{-1}, RA 17%) were the predominant species (Fig. 4). *Microcystis* sp. is morphologically similar to *Microcystis novacekii*, however further detailed studies of morphology and molecular phylogeny are needed to confirm its identity. During the bloom, pH was 7.95 and temperature 26.3 ºC. In overall, climatological conditions such as prolonged droughts and high temperatures that concentrate nutrients as well, may have been the causes that triggered this unusual cyanobacterial mixed bloom.

According to previous studies, monospecific blooms of *Microcystis* spp. (*M. panniformis* and *Microcystis* sp. like in this study) as well as a mixed bloom of filamentous cyanobacteria (*Raphidiopsis gangetica*, *R. curvata*, *Dolichospermum* cf. *flos-aquae*, *D.* cf. *solitarium*, *Anabaenopsis* sp.) had occurred in the Abreus Basin (Comas et al., 2011; Comas & Moreira, 2013; Valle-Pombrol et al., 2019). However, this study shows the presence of *Sphaerospermopsis torques-reginae* in a mixed bloom for the first time in the Abreus reservoir. *S. torques-reginae* was originally described (as *Anabaena torques-reginae*) from Ciénaga de Zapata in central-southern Cuba (Komárek, 1984). A bloom of this species was linked to a massive fish death event in the Galindo reservoir (near Abreus Basin) in April 2017; without knowing if it was due to anoxic/hypoxic conditions given the high densities of *S. torques-reginae* or to the presence of toxic secondary metabolites (Comas et al., unpublished data).

Regarding the presence of Microcystis, was confirmed through molecular biomarker analyses for the general 16S rRNA gene and Microcystis-specific 16S rRNA, as previously reported (Neilan et al., 1997; Jungblut et al., 2005). Besides, the screening on cyanotoxin genes, revealed the presence of mycE gene, which is involved in the biosynthesis of microcystins. The detection of these genes in the Abreus sample can be related to the presence and predominance of *Microcystis* genera (*Microcystis* sp. + *M. panniformis*), well known to produce microcystins. Besides *Microcystis* spp., other subdominant cyanobacterial genera found in the sample can also able to produce microcystins, for example *Planktothrix* (Bernard et al., 2017).

These results highlight the presence of microcystin-producing genotypes in the Abreus reservoir which could represent a potential risk for animal and human health. Recently, microcystins were detected in a low concentration in samples from the Abreus reservoir through chemical methods (mass spectrometry), in a season without perceptible water bloom (Valle-Pombrol et al., 2021). This study constitutes one the few records of non-specific Harmful Algal Blooms (HABs) in Cuba, based on both taxonomic and molecular biomarker, including some for the detection of toxigenic cyanobacteria in waterbodies. However, further investigation may be necessary in order to clarify the identity and toxicity of cyanobacterial species; for example, combining morphological, molecular and chemical studies in multiple cyanobacteria cultured strains from this reservoir from central-southern Cuba.

Acknowledgments

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Figure captions

Figure 1. Study area: Abreus Reservoir located in Cienfuegos province, central-southern Cuba.

Figure 2. Cyanobacterial bloom in Abreus reservoir. A. Location near the Dam where the bloom occurred. B. Intense green water discolorations during the bloom.

Figure 3. Microscopic photographs of predominant and accessory cyanobacteria species found during the bloom. a. *Coelomoron tropicale*. b. *Microcystis* sp. c. *Microcystis panniformis*. d. *Raphidiopsis gangetica*. e. *Sphaerospermopsis torques-reginae*. f. *Planktothrix isothrix*. Scale bars = 10 µm.

Figure 4. Abundance (cells/mL) of principal phytoplankton species found during the bloom.

Figures

Figure 1

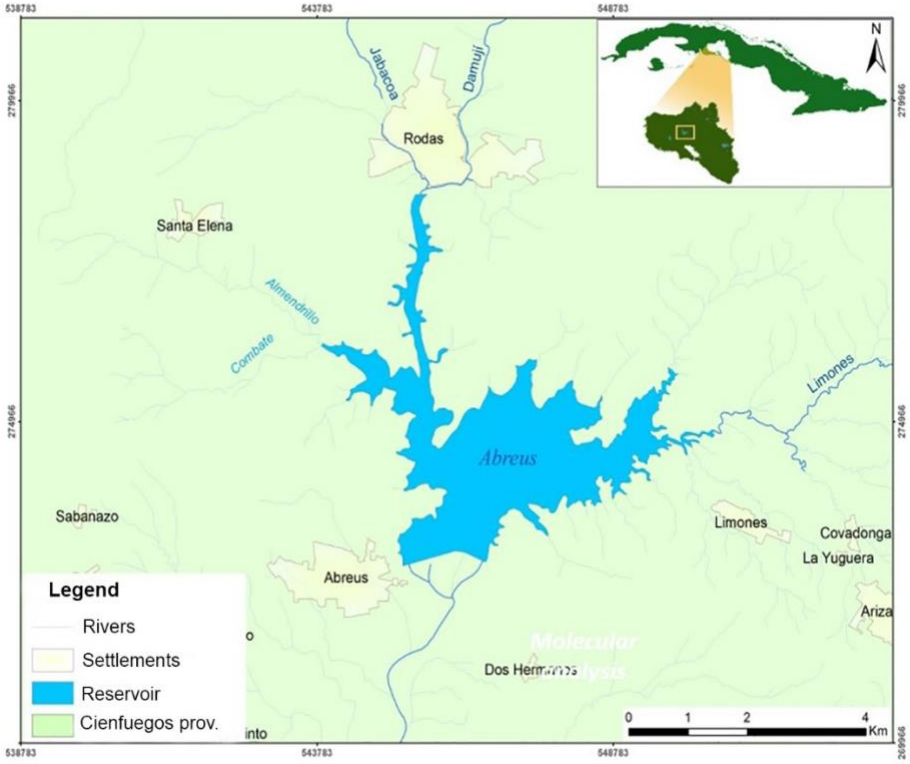


Figure 2

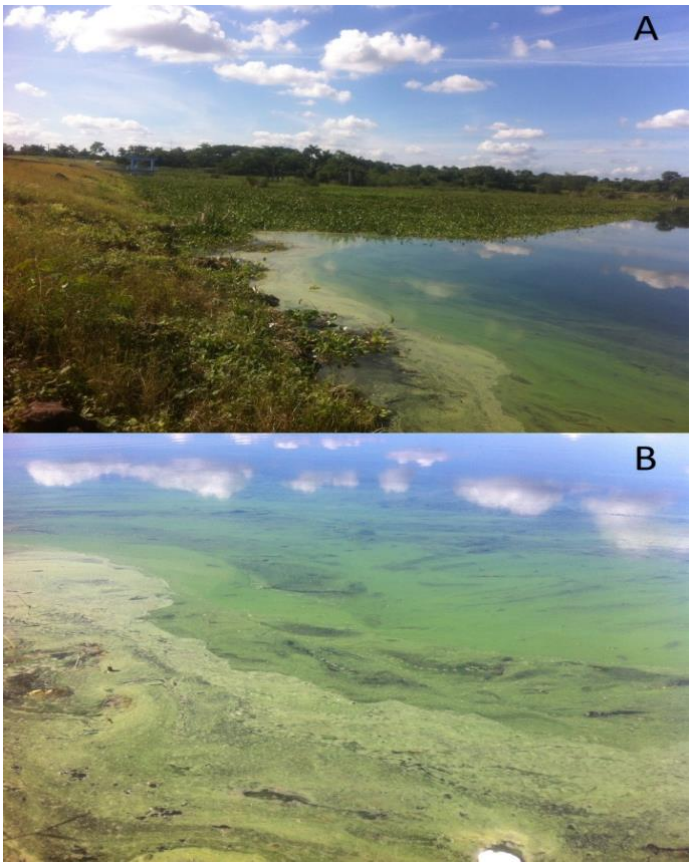


Figure 3

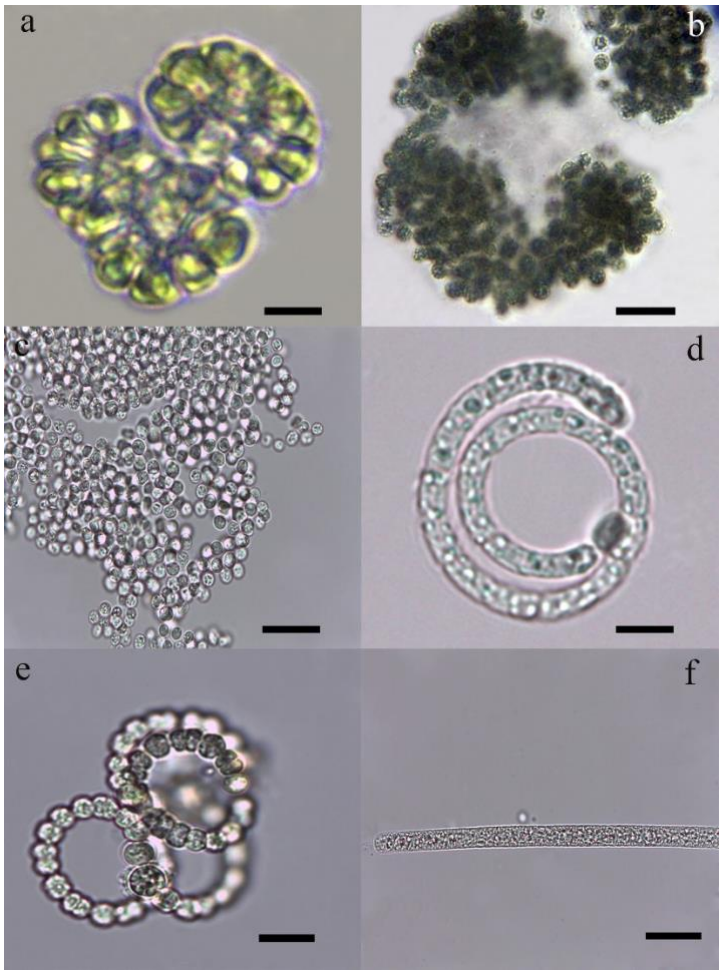
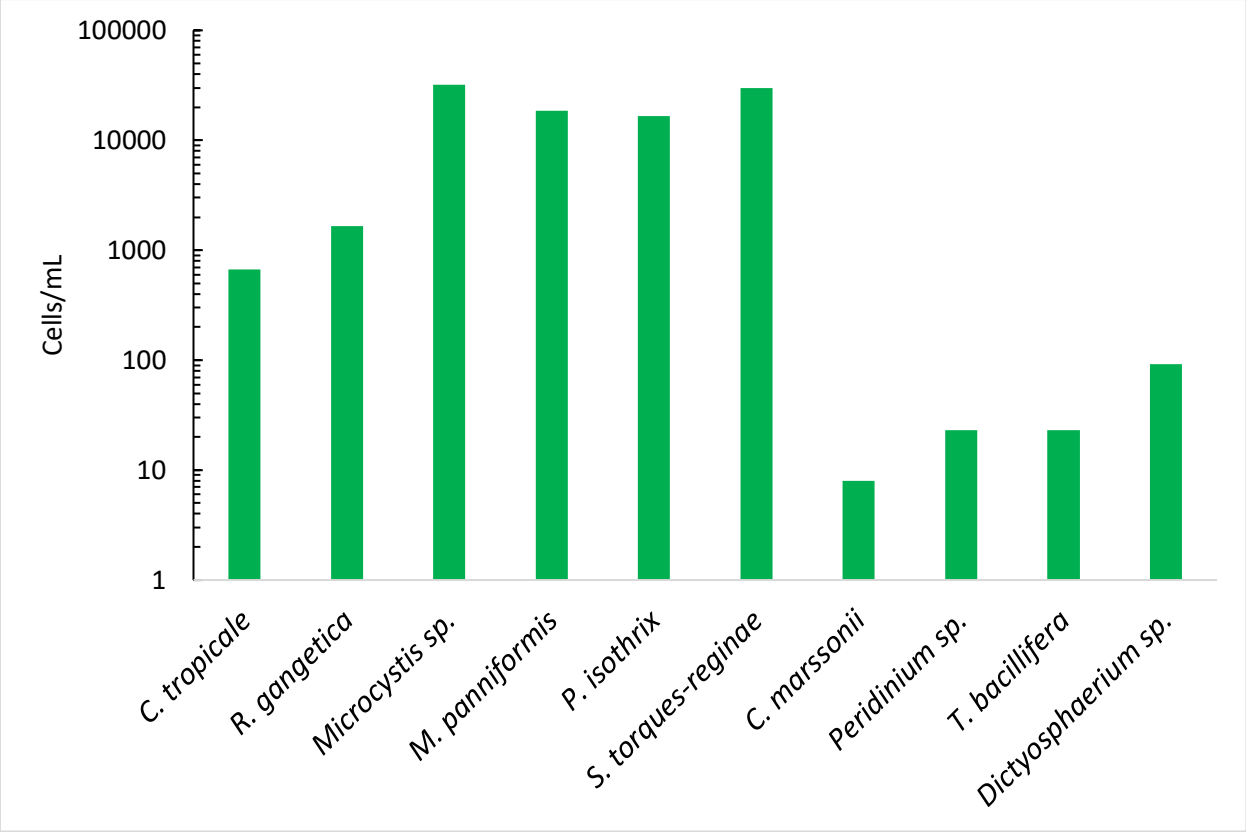


Figure 4


100000
10000
1000
100
10
1
Cells/mL
C. tropicale
R. gangetica
Microcystis sp.
M. pannijormis
P. isothrix
S. torques-reginae
C. marssonii
Peridinium sp.
T. bacillifera
Dictyosphaerium sp.