Article

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Cyanobacterial bloom in the Chen Ha karst landscape

Bloom de cianobacterias en el paisaje Kárstico de Chen Ha

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Abstract

The Ejido of Kopomá with an extension of 963,000 hectares, participates in the environmental services program. In this property is located the Cenote Chen Ha. An open-air water body that is often affected by anthropogenic activities that put its conservation at risk. The present study aims to use environmental health indicators to determine the degree of deterioration it presents. Based on this, a conservation and sustainable management plan will be developed. Phytoplankton biodiversity monitoring was carried out in spring and summer. The physicochemical variables determined were pH, temperature, conductivity, dissolved oxygen. In spring greater diversity was observed and the dominant species were diatoms, however, in summer cyanobacteria, *Coelosphaerium, Woronichinia naegeliana and Microcystis predominated*. Alkaline pH in summer: 8.24 and dissolved oxygen: 3.38 ppm. Regarding species richness, the Shannon index was 2.748 for spring and 2.389 for summer. No similarity between populations was observed, the beta similarity index was 0.25. The Chen Ha cenote is highly vulnerable to environmental conditions, our results demonstrated the formation of a cyanobacteria bloom with toxic potential in summer.

Cyanobacteria, diversity index, *Coelosphaerium*

Resumen

El Ejido de Kopomá con una extensión de 963,000 hectáreas, participa en el programa de servicios ambientales. En dicho predio se localiza el Cenote Chen Ha. Un cuerpo de agua a cielo abierto que con frecuencia es afectado por actividades antropogénicas que ponen en riesgo su conservación. El presente estudio tiene por objetivo utilizar indicadores de salud ambiental, para determinar el grado de deterioro que presenta. Con base a ello se va a elaborar un plan de conservación y manejo sustentable. Se realizó un monitoreo de biodiversidad de fitoplancton en primavera y verano. Las variables fisicoquímicas determinadas fueron pH, temperatura, conductividad, oxígeno disuelto. En primavera se observó mayor diversidad y las especies dominantes fueron las diatomeas, sin embargo, en verano predominaron las cianobacterias, *Coelosphaerium, Woronichinia naegeliana y Microcystis*. El pH alcalino en verano: 8.24 y el oxígeno disuelto: 3.38 ppm. Respecto a la riqueza de especies el índice de Shannon fue 2.748 para primavera y 2.389 en verano. No se observó similitud entre poblaciones, el índice de similitud beta fue 0.25. El cenote Chen Ha es altamente vulnerable a las condiciones ambientales, nuestros resultados demostraron la formación de un Bloom de cianobacterias con potencial tóxico en verano.

Cianobacterias, índice de diversidad, *Coelosphaerium*

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Introduction

One of the main characteristics of the hydrology of Yucatan is the karst landscape, which originates from coral reefs and marine sediments that, when exposed to the surface, formed limestone rock. Limestone rocks ($CaCO_3$) react with surface or subway water (which penetrates the fissure of the rocks) in the presence of CO_2 dissolving with the formation of calcium bicarbonate ($Ca(CO_3H)_2$). The insoluble residue composed of clay minerals and iron oxides forms a reddish deposit, known as decalcification clay. The factors that determine the dissolution of the rock are: purity ($CaCO_3$ content greater than 70%), exposure time, thickness of the layers, mineralogical composition, degree of porosity and the fracturing of the rock, in addition to geohydrological and climatic conditions. (Estrada *et al*., 2019). For the particular case of Yucatan the purity of $CaCO_3$ is estimated at 89 and 99 %. Cenotes are bodies of water formed by dissolution and collapse of limestone rock. There are records of approximately 7000 to 8000 cenotes in the State of Yucatan.

The present study was conducted in the Cenote aguada type: Chen Há, which corresponds to a depression of impermeable soils, where surface runoff water accumulates and mixes with salt water due to the porosity of the karst system, which favors the formation of caverns and channels that conduct substantial amounts of water (Valadez, 2012).

Increased nutrients from agriculture or untreated water discharges promote the proliferation of phytoplankton, with a decrease in water quality, see Figure 1.

Figure 1 Cenote Chen Há, summer 2022
Source: Own elaboration

Algae are autotrophic organisms with simple levels of organization and structure; this group includes cyanophyceae or blue-green algae. Phytoplankton fix several thousand tons of carbon per year and constitute the first link in the food chain (Salas, 2009). Cyanobacteria use photosynthesis as their main source of energy. They are among the most primitive organisms on earth, their origin is estimated at 3.5 billion years ago. Their growth is favored with high concentrations of nutrients, however, some researchers report the feasibility of toxic bloom formation in oligotrophic water bodies. It is worth mentioning that it is estimated that more than 50% of these blooms are toxic. Toxins are usually grouped into neurotoxins and hepatotoxins, and their production is directly proportional to the increase in biomass (Roset, J., 2001).

Microalgae reflect the ecological state of surface waters; they are sensitive to physicochemical changes in the environment and are therefore considered biological indicators of water quality, productivity, eutrophication, acidification of the environment and contamination by heavy metals. Most frequently in freshwater bodies (Baylon, 2018).

Cenotes provide benefits in terms of biological diversity of aquatic species, contribution in terms of landscape and environmental protection as well as being outdoor recreational areas (Salas, 2009).

The objective of the present work was to study the changes in biodiversity related to spatio-temporal environmental conditions that describe the vulnerability of Cenote Chen há to environmental factors. The analyses were carried out in the spring and summer of 2022. The Shannon diversity index was determined, based on the richness of species and their components; the value obtained is used as an indicator of the state of contamination of the water body (del Río, 2003).

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Materials and methods

Limnological monitoring. The Chen Ha cenote is located in Chocholá, Yucatán with coordinates 20° 41' 22.3" N and 89 ° 52´ 33.8´ W. It is an open-air cenote 85 m in diameter and 27 m deep. Based on the accessibility to the water body, 2 monitoring stations were established and determinations were made in spring and summer 2022. Monitoring of environmental variables: temperature, conductivity, pH, dissolved oxygen, total suspended solids was carried out with a multiparameter probe.

Biodiversity study. Samples were collected by horizontal trawling with phytoplankton net of 40 µm pore size, 30 cm diameter for 60 seconds. Samples were fixed with 1% lugol solution and transferred to the microbiology laboratory of the Universidad Politécnica de la Zona Metropolitana de Guadalajara, based in Cajititlán Jalisco Mexico. For qualitative analysis, the species were recorded and a list of presence (1) and absence (0) was prepared. The analysis and classification was carried out by the traditional method, using a Leica microscope and 30 and 40 X objectives (Vizcaíno et al., 2021). The statistical software EstimateS 9.1.0 for Windows was used to calculate diversity indices. Shannon's alpha diversity index was performed based on incidence data (presence-absence). To determine beta diversity (abundance-based data), multivariate similarity analysis according to Shanon H (Baylon et al., 2018) was employed. Figure 2.

Figure 2 Limnological monitoring of Chen Há cenote (Summer 2022)
Source: Own elaboration

Phylogenetic analysis. It was performed with the sequences deposited in the NCBI database. BLAST (Basic Local Alignment Search Tool) sequence was used Coelosphaerium sp. S3C5 genes for 16rRNA, 16S ribosomal RNA intergenic spacer 23 SrRNA partial and complete sequence. Sequence cleanup was performed visually by removing sequences without conserved or repeated sites. NCBI diagrams were used.

Results

Phytoplankton was represented by 55 taxa belonging to 5 divisions: Bacillariophytes (14), Cyanobacteria (22), Chlorophytes (13), Euglenophytes (3) Dinophytes (3). Unlike those reported by Valadez et al. (2013) in which 63 taxa and six divisions were identified Bacillariophyta (28), Cyanobacteria (22), Dinophytes (six), Chlorophytes (three) Euglenophytes (two), Cryptophytes (two).

Figure 3 shows the morphology of some cyanobacteria and diatoms species identified in the Chen Há Cenote.

Figure 3 *Coelosphaerium*, *Microcystis novacekki*, *Chroococcus turgidus*, *Aulacoseira granulata*, *Diatoma vulgaris*, *Microcystis wesenbergii*, *Mougeotia*, *Pleurotaenium*, *Nägeli*, identified in Cenote Chen Ha, in the monitoring conducted in summer 2022
Source: Own elaboration

Regarding relative abundance, diatoms dominated in spring and cyanophytes in summer, probably favored by environmental variables Our results agree with the researchers regarding the relative abundance of the divisions since cyanophytes were more abundant during summer 143, with respect to 117 in spring. (See graphic 1).

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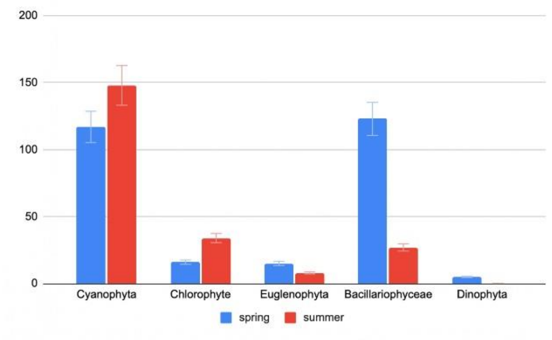


Graphic 1 Phytoplankton diversity in the Chen-Ha cenote, according to monitoring conducted in spring and summer 2022
Source: Own elaboration

Figure 4 shows the diversity of species identified in the limnological monitoring carried out in both spring and summer. The species identified in both climatic seasons correspond to *Coelosphaerium*, *Microcystis aureginosa*, *Botryococcus*, *Pseudanabaena limnetica*, *Oscillatoria* sp., *Merismopedia tenuissima*, *Crucigenia cuadrata*, *Volvox*, *Aulacoseira granulata*, *Aulacoseira italica* and *Navicula*. Greater species diversity was observed in spring, in that season the dominant species was *Navicula* (diatom) and in summer the dominant species was *Coelosphaerium* (cyanophyte).

In summer, *Woronichinia naegeliana*, *Microcystis novacekki*, *Aphanocapsa elachista*, *Nodularia*, *Rhabdoglea yucatanensis*, *Mougeogia viridis*, *Mesotenium macrococcum*, *Colacium vesiculosum*, *Eptitemia argus* and *Nitzschia* were also detected.

In spring the diversity was higher, species such as *Microcystis wesenbergii*, *Microcystis flos-aquae*, *Microcystis incerta*, *Planktothrix agardhii*, *Lyngbya*, *Chroococcus turgidus*, *Planktolingbia limnetica*, *Sinechocystis*, *Gloethece magna* were identified, *Pandorina Charkowiensis*, *Mallomonas*, *Closterium*, *Staurodesmus extensus*, *Tetrastrum*, *Chlorella*, *Selenastrum gracile*, *Kirchneriella*, *Euglena Spirogyra*, *Euglena geitieri*, *Surirella*, *Gomponema angustatum*, *Asterionella*, *Peridium Cinctum*, *Gonyaulax* sp.

Cyanobacteria are known as blue-green algae, they present a cellular organization similar to bacteria due to their great diversity of forms and/or specialized structures, however their metabolism is similar to plants due to their ability to perform photosynthesis (Arana et al., 2019). It should be noted that for the same species there are toxin-producing and non-producing strains.

Toxins are considered to be secondary metabolites obtained as by-products of metabolic pathways associated with photopigment synthesis. They accumulate in the cytoplasm and are released when cell lysis occurs. (Roset, 2001).

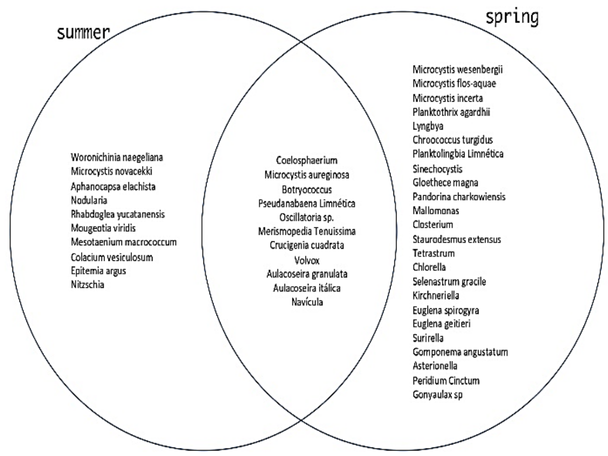
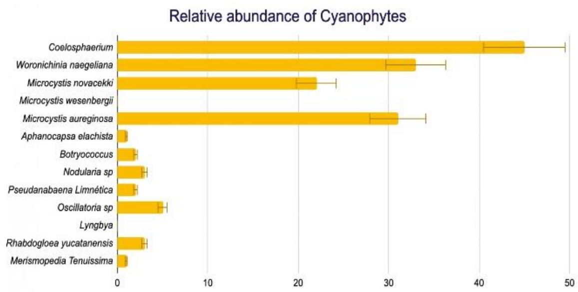


Figure 4 Ben's diagram for phytoplankton biodiversity monitored in Cenote Chen Há in spring and summer 2022
Source: Own elaboration

The relative abundance of cyanobacteria is shown in graph 2. The most abundant species in summer was *Coelosphaerium*, followed by *Woronichinia naegeliana*. The genus *Coelosphaerium* consists of globose colonies, covered with mucilaginous and colorless sheath and spherical or hemispherical cells located at the periphery of the colony, its cell division is in two planes, toxigenic species have been reported as *C. kuetzingianum*, *Nägeli* (Aguilera y Echenique).



Graphic 2 Relative abundance of cyanobacteria identified in Cenote Chen Ha in October 2022
Source: Own elaboration

Our results coincide with those reported for Lake Shinji, Japan, where in September 2009 the number of *Coelosphaerium* cells was 4.8 x 10 5 cells. L^{-1} and in October it increased to 6.12 x 10 7 cells. L^{-1} and in November the number of cells decreased to 4.64 x107 cells L^{-1}.

According to Godo et al, (2016) *Coelosphaerium* sp. has the ability to produce geosmin (E-1,10-dimethyl-E-9-decane). Said molecule contributes an intense aroma and unpleasant taste to water, with a decrease in its quality. The values of geosmin in this water body are directly proportional to the number of cells. The researchers reported ranges of 12 to 4 ng. L^{-1} in September, 640 to 20 ng. L^{-1} in October and 413 to 17 ng. L^{-1} in November. This molecule is also produced by cyanobacteria, oscillatoriales and nostococales. According to the author, the values of 4 to 10 ng. L^{-1} of geosmin provide taste and odor to the water.

There are reports of *Woronichinia naegeliana* in both eutrophic and oligotrophic lakes, so it is considered to be highly tolerable and characteristic of lentic ecosystems (Nowicka-Krawczyk & Zelazna-Wieczorek, 2017). For the particular case of de Woronichinia naegeliana, its ability to produce microginin FR3 was reported, with LC50 equal to 7.78 µg mL^{-1} with toxic effect to invertebrates, however, the mechanism of action is unknown (Bober & Bialczyk 2017).

Figure 5 shows the dendogram obtained regarding the biodiversity of cyanobacteria, identified in the month of October, in which these species were dominant. Greater similarity was observed between Woronichinia naegeliana and *Coelosphaerium* sp. with respect to *Microcystis* and *Chroococcus* sp.

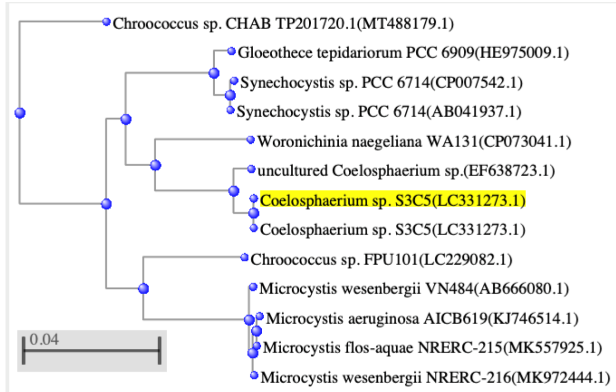


Figure 5 Diversity of cyanobacteria recovered in the Chen Ha cenote in October 2022
Source: Own elaboration

According to Mohan and Thomas, 2022, *Woronichinia naegeliana* has been observed associated with blooms of Microcystis in southern India and its toxic capacity is frequently recognized, and there are reports of its presence in freshwater ecosystems with temperatures close to 29°C and low levels of nitrates and phosphates.

Regarding environmental variables, in spring the average temperature was 32.19 ºC, suspended solids: 1300 ppm and average conductivity 2603 µS/cm, i.e., they reached higher levels than in summer with values of 31.05 ºC for temperature, total suspended solids 1021 mg-L^{-1} and 2372 µS/cm, respectively (see Table 1). Temperate climates (25ºC) frequent in late spring, summer and autumn favor the formation of blooms. The ability of cyanobacteria to adapt to different light intensities is due to their ability to synthesize carotenoids and protective photopigments. (Forjan *et al*., 2007).

Variable	Spring	Summer
pH	7.65	8.24
ppm DO	2.7	3.38
µS/cm	2603	2373
ppm Tds	1300	1021.14
° C	32.19	30.92

Table 1 Average value of the environmental variables monitored in the Chen Ha cenote during the spring and summer of 2022
Source: Own elaboration

In spring the pH was slightly more acidic 7.65 with respect to the summer which was 8.24. According to Estrada et al. (2019), in karst landscapes, when the pH is in the range of 6 to 10 the predominant carbonate species in the water body is HC03-. The level of dissolved oxygen detected was lower 2.7 ppm with respect to the summer, 3.38 ppm.

In a study conducted in Lago Lagartos, Mexico, the temperature recorded in spring was 26.1 ºC and 27.8ºC in summer, respectively; the pH was 6.9 and 7. Conductivity values ranged from 13 to 18 mS/cm, higher values than those obtained in Chen Ha: 2.6 to 2.37 mS.cm (Valadez et al., 2012). According to Baylón (2018), electrical conductivity influences the distribution, composition, biomass and density of phytoplankton.

One of the characteristics that make cyanobacteria proliferate during summer blooms compared to other species is their tolerance to high temperatures and nutrient concentrations, which decreases vertical mixing in the water body and favors stratification. *Microcystis* is considered a species with adverse effects on aquatic ecosystems and the potential to produce microcystins, which are very stable in nature, producing intoxication in humans and fish mortality. It is worth mentioning that in a study conducted during the formation of a bloom of *Microcystis*, in the month of September, values of 5.5 to 6.5 dissolved oxygen, pH of 9 to 9.8 and temperature of 27.5 to 29º C were recorded (Mohan et al., 2020).

It is worth mentioning that when there is algae bloom production, the use of algaecides is not recommended, since they cause cell rupture and the release of toxins; the use of flocculants and activated carbon filters is recommended (Forján et al., 2008).

The Beta similarity index, calculated from the spring and summer identification, in accordance with the Jacquard model was 0.25. According to the scale, the closer to one the greater the similarity, the value obtained is very low, which indicates that the conditions changed due to the effect of the rainy season, runoff and groundwater mixing. See Figure 6.

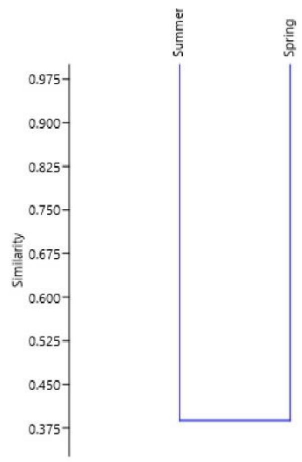


Figure 6 Index of similarity and distance between species identified in Cenote Chen Há, in spring and summer
Source: Own elaboration

Alpha diversity operates within the population at the spatiotemporal scale and beta diversity refers to the variation between populations (Salas, 2009). The scale of values from 0 to 4 bits.ind^{-1} (values greater than 3 bits.ind-1 indicate clean water, values of 1-3 bits.ind-1 indicate moderate pollution and values less than 1 bits.ind^{-1} are characterized as heavily polluted) according to Baylon (2018).

Regarding alpha diversity, the highest species richness was obtained in spring with Shannon index of 2.748 and 2.389 in summer. Similar results 2.77, obtained Baylon et al. (2018) at Station L^{-1} of the high Andean lagoon in the Pasco Region (October 2015). It is worth mentioning that in accordance with the scale of values the contamination is mild, with greater deterioration in summer (See Figure 7).

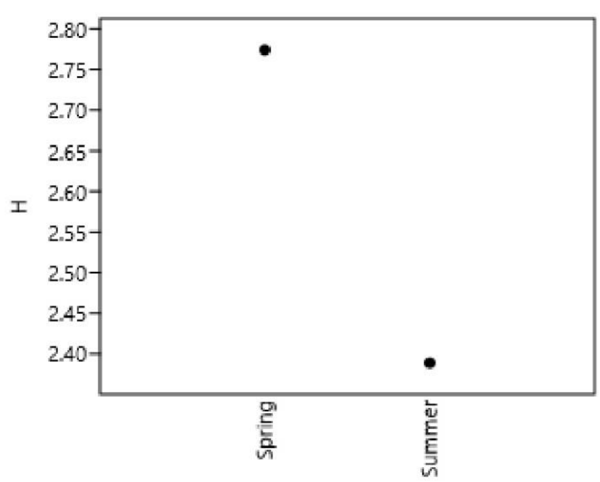


Figure 7 Alpha diversity index (Shannon H) calculated in spring and summer in the Chen Ha cenote, 2022
Source: Own elaboration

The available concentrations of nitrogen and phosphorus, as well as neutral and alkaline pH favor the growth and proliferation of cyanobacteria, many of which have low palatability due to their large size, the presence of mucilage and their toxic capacity, which favors their accumulation since they suffer less herbivory (de Oliveira *et al*., 2009).

Cyanobacteria are known for their ability to produce a great diversity of secondary metabolites with biological activity. Within which, they include methylisoborneol, geosmin, toxins type: alkaloids, cyclic peptides and oligopeptides, whose biological function is uncertain, as well as protease inhibitors (Bober & Bialcyk, 2017).

Blooms of cyanobacteria producing cyanotoxins in freshwater reservoirs expose the population because they are not removed by conventional treatments and filtration, causing serious public health problems (Lizarralde *et al*., 2020).

In 2012, Valadez reported the formation of blooms in the karst system caused by Microcystis panniformis in November 2007 and Oscillatoria princeps in April 2008. Worldwide, karst zones represent approximately 14.1% of the earth's surface, of which 12.2% is related to limestone. In Mexico, 2.21% of the world total is found, representing 8.93% of the total surface of the country. For the state of Yucatan, 95% of the state is karst landscape (Estrada *et al*., 2019). Karst systems are fragile and are contaminated by untreated discharges from pig farming, poultry farming, the nixtamalization of corn, fertilizers, pesticides, septic tanks, waste from the exploitation of limestone as raw material for construction, which eliminates vegetation and the rocky substrate until reaching the aquifer mantle, which favors the entry of contaminants (Estrada *et al*., 2019).

Our results demonstrate that environmental conditions influenced the biodiversity of the Chen Há cenote and that it is highly vulnerable to contamination. Since the diversity index between spring and summer populations was 0.25 indicating that there is no similarity between populations.

In summer, a bloom of cyanobacteria was observed whose dominant species was *Coelosphaerium,* followed by *Woronichinia naegeliana*, *Microcystis aeruginosa* and *Microcystis novacekki* with toxic potential and capacity to generate environmental impact. It is estimated that the temperature conditions associated with freshwater runoff from the rainy season as well as the probable dragging of fertilizers or agrochemicals as some of the probable sources of contamination favored the proliferation of cyanobacteria.

It is important to monitor the water quality of the cenotes that are used for tourism, since they are often used for recreational activities (diving, swimming, scenery, etc.) to avoid health problems and loss of biodiversity, since these bodies of water are interconnected by subway currents. Studies will continue in winter, in order to know its impact and establish if necessary a bioremediation, conservation and sustainable use plan for Cenote Chen Há.

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Conclusions

The Chen Há cenote experienced the formation of a cyanobacterial bloom in the summer of 2022. The dominant species was Coelosphaerium sp, the alpha diversity index (Shannon H) was 2.83.

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