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Cenote Chen ha, and water quality indicators

Cenote Chen ha, e indicadores de calidad de agua

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Abstract

The cenotes of the Yucatan Peninsula are characterized by karstic relief, warm temperatures, and transparency of their water bodies. The mixture of fresh water from infiltrations of rainwater and runoff coupled with underground rivers or marine intrusion, make up the habitat of aquatic, amphibian, and terrestrial species. The Chen ha cenote, located in Chocholá, was used for tourist purposes, however, the lack of an environmental culture and some anthropogenic activities cause deterioration and contamination of the wetland, which putting its conservation at risk. The objective of the present work was to carry out a limnological analysis in Cenote Chen ha, to determine the level of fragility of the ecosystem. The variables analyzed were pH, dissolved oxygen, temperature, conductivity and phytoplankton biodiversity. Results. 2600 µS/cm of conductivity, 8.25 pH and 2.8 ppm of dissolved Oxygen. Phytoplankton: Diatoms 46.2%, Cyanobacteria 38.1%, Chlorophytes 8.1%, Euglenas 5.5%, and Dinoflagellates 2.1% predominated. *Asterionella* was identified as an indicator species of environmental health. *Microcystis aureginosa*, *Planktothrix agardhii*, and *Cyanosarcina caribeana*, *Pseudanaena*, *Peridium* and *Gonyaulax* species are shown as indicators of environmental contamination. No dominance of any species was observed; however, frequent monitoring of indicator species is recommended.

Anthropogenic, Intrusión, Enviromental

Resumen

Los cenotes de la península de Yucatán se caracterizan por relieve cárstico, temperatura cálida y transparencia de sus cuerpos de agua. La mezcla de agua dulce procedente de infiltraciones de agua de lluvia y escorrentías con ríos subterráneos o intrusión marina, conforman el hábitat de especies acuáticas, anfibias y terrestres. El cenote Chen ha, localizado en Chocholá, se empleó con fines turísticos, sin embargo, la falta de cultura ambiental y actividades antropogénicas ocasionan deterioro y contaminación del humedal, ello pone en riesgo su conservación. El objetivo del presente trabajo fue realizar un análisis limnológico en el Cenote Chen ha, para determinar el nivel de fragilidad del ecosistema. Las variables analizadas fueron: pH, oxígeno disuelto, temperatura, conductividad y biodiversidad de fitoplancton. Resultados. 2600 µS/cm de conductividad, 8.25 de pH y 2.8 ppm de Oxígeno disuelto. Fitoplancton: predominaron las Diatomeas 46.2 %, Cianobacterias 38.1%, Clorofitas 8.1%, Euglenas 5.5 % y Dinoflagelados 2.1 %. Se identificó *Asterionella* como especie indicadora de salud ambiental. *Microcystis aureginosa*, *Planktothrix agardhii*, y *Cyanosarcina caribeana*, *Pseudanaena*, *Peridium* y *Gonyaulax* como indicadores de contaminación ambiental. No se observó dominancia de alguna especie, sin embargo, se recomienda monitorear con frecuencia las especies indicadoras.

Antropogénico, intrusión, ambiental

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Introduction

Freshwater water resources in the Yucatan Peninsula are limited. Devoid of rivers, lakes and lagoons, 97% of its freshwater is located underground and the surface blooms called cenotes are the main sources that provide the vital liquid to the population (Cortes Campos, 2018). However, the whole karst aquifer of the State has a positive balance, that is, the amount of water that is extracted, does not exceed the natural recharge (Estrada Medina, Jimenez Osornio, Alvarez Rivera , & Barrientos Medina, 2020).

The word "Cenote" comes from the Mayan word d'zoot, which means a natural well of water. In the Peninsula, limestone rocks are abundant, and the presence of a large number of fractures and faults favors a highly permeable surface with the formation of a huge hydraulic complex of caverns and underground rivers (Fragoso-Servon & Alberto Pereira , 2016).

Open-air cenotes receive water from rainfall, surface runoff and/or groundwater inputs. Although the influence of tides is associated with high levels of salinity and the halophilic species found in these environments, they can also be freshwater depending on the magnitude of the rainfall discharge or entry of seawater currents.

It is worth mentioning that the biodiversity of the existing species and their distribution change with the reigning environmental conditions, trophics variables throughout the year, nutrient limitations, or water circulation patterns.

The cenotes provide the Mayan community not only water, but also a revered space for the celebration of myths, beliefs and religious rituals. Currently they are of great economic value for the establishment of irrigation systems and the selling of tourist services due to the beauty of their waters, their cavernous formations and their relationship with archaeological sites (Fragoso-Servon & Alberto Pereira , 2016).

According to the related literature and interviews conducted with users, the main contaminants of these bodies of water come from activities like pig farming, poultry farming, corn nixtamalization, fertilizer residues, open dumps, septic tanks leaks, among others (Cervantes-Martinez, Mezeta-Barrera, & Gutierrez-Aguirre, 2009).

In accordance with the regulatory framework of the National Water Commission (CONAGUA), cenotes are treated as groundwater resources. In the State of Yucatan, the selling of ejido lands is feasible and therefore the commercialization of these bodies of water is also possible. It is worth mentioning that many cenotes are owned by individuals or of an ejido or communal social character, such is the case of the Chen há cenote. (Cortes Campos, 2018).

This wetland was used for recreational purposes, and it was the headquarter of ecotourism activities, such as camping and swimming; however, the lack of an environmental culture added to the use of bad practices caused environmental deterioration and put at risk the loss of biodiversity of the body of water.

Chen ha is an open cenote type waterbody with an 85 m diameter and 27 m depth. It belongs to the municipality of Chocholá, and is located about 40 minutes from Mérida, Yucatan, with coordinates 20º 41 '22.3" N and 89º 52' 33.8" W.

The present work is part of a monitoring study of water quality and biodiversity of phytoplankton that will be carried out during the different seasons of 2022 and 2023.

Its purpose is to seek indicators of environmental pollution, directed to take actions that cap help to restore the environmental balance of the ecosystem, as well as the sustainable use of the ecosystem services provided by the waterbody such as recreational, cultural, hiking, habitat for species, protection and conservation of the body of water among others.

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The first monitoring event was carried out during the spring seson (May 2022) and the present work describes the results found. Diatoms are the dominant species, however, a great diversity of cyanophytes characteristic forms of the Mexican Caribbean were observed, as well as the presence of dinoflagellates with bioluminescence capacity are some of the species identified. The diversity of species that cohabit these environments are usually tolerant to high concentrations of salts and temperature close to 30 º C.

Methodology

This document includes the results of the analysis conducted during the spring 2022 season. Continuity will be given in summer, autumn and winter periods.

With the help of a multiparameter probe, the folowing environmental variables were recorded: Temperature, pH, conductivity, total suspended solids, and % of dissolved oxygen.

For the study of biodiversity, samples were recovered from the coastal zone, using the technique of horizontal dragging for 1 minute with a phytoplankton net of 30 cm in diameter, 50 cm in length and 20 micrometers thinck mesh.

The samples were fixed with 1% lugol solution and transferred to the Microbiology laboratory of the Polytechnic University of the Metropolitan Area of Guadalajara.

The morphological characterization was carried out with a Leica compound microscope and 10x and 40x lenses. The literature consulted for the identification of the species was based on studies by different authors cited in the bibliographic references.

Plylogenetic analysis

The search for gene and protein sequences was carried out based on NCBI data. Blast (Basic Local Alignment Search Tool) was used using 18s ribosomal RNA gene secuence de *Navicula viridula* for diatomeas and *Gloeothece* 16r RNA gene for cianobacterias. The cleasing Secuence was visually performer, removing the sequences that did not contain conserved sites or the repeated ones. NCBI diagrams were used.

Results

The Chen ha cenote is characterized by calm and transparent waters, with emerging vegetation such as *Phragmites autralis* and floating species within which *Nymphaea ampla* stands out.

Figure 1. Limnological monitoring, biodiversity of phytoplankton Cenote Chen Ha. May 11, 2022
Source: Vizcaino et al., 2022

The pH values recorded in May ranged from 7.65 to 8.58; similar to those reported for the cenotes of Cholul, Seminario, Vergel, X'caamal, Xoclán and Variance locations, with values of 7, 7.3, 8.2, 8.0 and 7.0, respectively during the dry season (Tavera, Novelo, & Lopez, 2013). It is worth mentioning that according to the literature the pH value is related to the dissolution of calcite from rocks and when it has values of 6.53 to 7.56 soluble forms of bicarbonate (HCO_3^-) predominate.

Dissolved oxygen was found in the range of 2.7 to 2.8 ppm. These results are low compared to those reported in Cholul, Seminario, Vergel, X'caamal, Xoclán and Variance sites, with values of 5, 7.2, 11, 6.8 and 7. mg.l^{-1}. As well as for those reported in 2005, for the Cenote Azul with values between 8 and 8.8 ppm, in the dry season.

The conductivity recorded was from 2594 to 2969 µ S.cm^{-1}. Similar values were reported for the Blue cenote: 2.4 to 2.6 mS.cm^{-1}. However, they are high compared to those reported for Cholul, Seminario, Vergel, X'caamal, Xoclán, Variance cenotes, with values of 1250, 1990, 1400, 1900, 1800 µ S.cm^{-1} respectively.

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The origin of high levels of conductivity are multifactorial or due to the presence of high levels of ions such as calcium, potassium, magnesium, sulfate, chloride, carbonate and bicarbonate, coming from the dissolution of rock calcites, and the infiltration of groundwater, seawater or wastewater leaks. It is worth mentioning that the ppm values of total suspended solids ranged from 2293 to 1306.

According to the literature, temperature values of 32 °C, 29 °C for Vergel, X'caamal, 30 °C in Xolul, respectively, and 28 in Seminario were reported for the Xoclán cenote. On the other hand in the Cenote Azul the average temperature was 29.2 +/- 0.9 ºC, which is classified as warm-tropical (Cervantes-Martinez, Mezeta-Barrera, & Gutierrez-Aguirre, 2009).

The temperature recorded in Chen Ha was 32.2 °C at 50 cm depth and 31.7 °C at 1 m depth. The decrease from 0.5 ºC to 1 m deep, the levels of detected oxygen and the conductivity values (higher than those reported) suggest low water mixture in the wetland. It is worth mentioning that the results obtained should be corroborated by making a vertical profile of temperature and salinity in future determinations.

The entry and exit of water from the wetland, regulates the variables of salinity concentrations, temperature, oxygenation, and this, together with the luminous intensity, determines the biological diversity (Pratolongo , Piovan, Zapperi , & L. Negrin, 2013). Inside the Cenote Chen ha the percentage of relative species abundance from highest to lowest was: Diatoms 46.2%, Cyanobacteria, 38.1%, Chlorophytes 8.1%, Euglenas 5.5% and Dinoflagellates 2.1%. See figure 2.

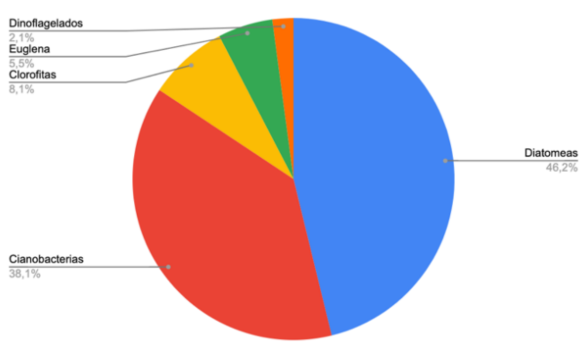

Figure 2 Relative abundance of phytoplankton detected in the Chen ha cenote, during the monitoring carried out in May 2022.
Source: Vizcaino et al., 2022

Primary productivity and phytoplanktonic populations change with environmental conditions and spatial-temporal variability (Obeso-Nieblas, Gaviño-Rodriguez, Obeso-Huerta, & Muñoz-Casillas, 2014). It is expected that in the rainy season the abundance and diversity of the reported species will decrease as well as the environmental variables, along with an increase in dissolved oxygen and a decrease in temperature values. Figure 3 shows some specimens of diatoms and cyanobacteria observed.

Regarding diatoms, *Surirella*, *Navicula viridula*, *Aulacoseira Italica*, *Aulacoseira sp*, *Gomponema angustatum*, *Melosira varians* and *Asterionella* were identified.

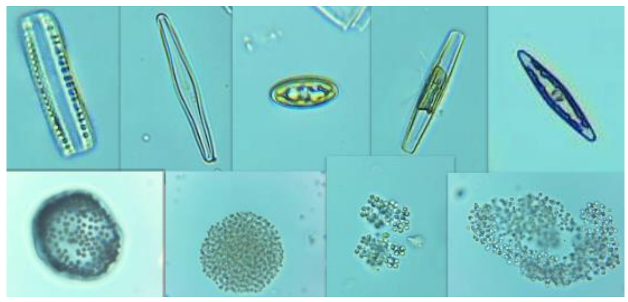

Figure 3 Diversity of diatoms and cyanobacteria observed in the Chen há cenote during May 2022. Mérida Yucatan
Source: Vizcaíno et al., 2022

Figure 4 shows the photograph of a *Melosira varians* specimen, an algae known as "water collars' ' in which the presence of chlorophylls and drops of oil provide the green and gold color.

Figure 4. *Melosira varians*, recovered from Cenote Chen ha, Yucatan Mexico, observed with optical microscope, 40X target
Source: Vizcaino et al., 2022

It is estimated that in Mexico, records from 5000 species of marine diatoms are found in waters of the Pacific and Gulf of Mexico.

Among the diatoms with environmental importance, it is worth mentioning *Asterionella* which is used as an excellent indicator of water, due to its ability to synthesize molecules with antimicrobial activity. Extracts prepared with acetone from their biomass have antimicrobial activity against *Staphylococcus aureus*, *P. aeruginosa and E. coli.* The phytochemical profile of such algae extracts includes essential oils, alkaloids and phenolic compounds (Najera Arce, Alvarez-Fitz, Perez-Castro, Toribio-Jimenez, & Castro Alarcon , 2018).

Regarding cyanobacteria, the following species were identified: *Coelosphaerium, Microcystis flos aquae, Plankthotrix agardhii, Pseudanabaena Limnética, Microcystis aureginosa, Oscillatoria Subtilissima, Lyngbia, Planktolyngbia Limnética, Merismopedia Tenuissima, Microcystis incerta, Sinechocystis, Chroococcus sp, Oscillatoria, Asteriocapsa xcaamalensis, Cyanosarcina Caribeana, Aphanocapsa holsática, Limnothrix borgertii, Microcystis wesenbergii and Gloeothece.*

According to studies carried out in lentic water bodies in Yucatan, 206 species of cyanoprocarionts classified in 84 genera, 31 families and 7 orders have been identified: *Synechococcales* (31.06%), *Chroococcales* (26.69%), *Oscillatorial* (26.69%), *Nostocales* (11.16%), *Spirulinales* (2.48%), *Pleurocapsales* (1.49%) and *Chroococcidiopsidales* (0.49%), respectively (Arana-Ravell, Barrientos-Medina, & Lopez-Adrian, 2019).

Some species of cyanobacteria have potential biotechnology applications, due to their nutritional properties. Some of them produce secondary metabolites with industrial application and others are used as indicators of environmental pollution.

In our work, most of the cyanobacteria observed are characteristic of freshwater bodies, however species characteristic of the Caribbean were also identified. For example, our results coincide with those recorded for the Xcaámal cenote: the *Cyanosarcina Caribeana* algae , was identified in the drought season but in the rainy season the authors report that they observed the massive proliferation of it in the Xoclán, X caámal and Seminario cenotes.

Records of the presence of *Asteriocapsa xcaamalensis* are found in the cenotes of Xoclán and X'caamal (Tavera, Novelo, Lopez, 2013)).

The species of chlorophytes detected were: *Crucigenia cuadrata*, *Staurodesmus extensus, Pandorina sp, Closterium, Tetrastrum, Kirchneirella, Botryococcus, Chlorella, Volvox sp, Selenastrum gracile.*

Chlorophytes species yield an ample diversity of bioactive compounds used for commercial purposes like proteins, lipids, carbohydrates, carotenoids, vitamins, cosmetics goods, and energy productions by-productos (Blanco, 2019).

The presence of *Euglena Spirogyra*, *Euglena geitieri, was observed.* Regarding the dinoflagellates, the following species were identified: *Peridium Cinctum* and *Gonyaulax sp.* They belong to toxic phytoplankton microalgae.

Peridium is a freshwater dinoflagellate, it is an alga that has two flagella, and its morphological classification is not always adequate due to the great similarity between species, for example *P. gatunense* and *P. cinctum* which have been reported in the Caribbean. In Argentina P. *gatunense* was reported as responsible for the formation of blooms in lakes (Boltovskoy, 1983).

Gonyalaux is confused with another species of the same genus, within which *Lingulodinium polyedrum is a* cosmopolitan species, frequent in coastal areas with bioluminescent capacity. Records are found regarding its ability to produce Yesotoxins (YTX) with cytotoxic and cardiotoxic effects. However, under current conditions there were no mass proliferation events (Maciel-Baltazar, 2015).

Figure 4 shows the evolution of cyanobacteria according to the genome data obtained in the Genbank. Because no significant similarity was found between the different groups, 2 dendograms were elaborated, one for cyanophytes and one for diatoms (the most abundant species).

The phylogenetic tree of cyanophytes is classified into 2 clades, the first allows to distinguish Lyngbya from the rest of organisms. There is greater similarity between *Synechocystis* and *Microcystis*, with respect to *Gloeothece* which is classified in another clade.

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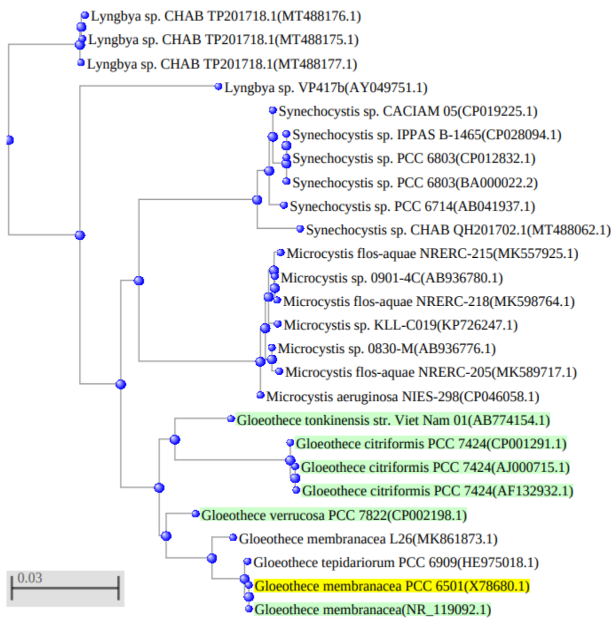

Figure 4 Phylogenetic tree of cyanobacteria identified in limnological monitoring in the Chen Ha cenote, Yucatan México, 2022.
Source: Vizcaino et al., 2022

According to the phylogenetic tree obtained for diatoms, these are classified into 3 main clades, in the first of *Aulacoseir*a and in the second asterionella. The third is subdivided into 3 clades: greater similarity was observed between *Surirell*a and *Gomphonema*, with respect to Navicula. See Figure 5.

The different types of algae did not evolve from a common ancestor, so there is no single basic structural pattern. Phytoplankton diversity is observed as a taxonomic and evolutionary unit from different ancestors with levels of organization determined by responses to natural selection pressures (Gonzalez, 1978).

When the cell density of a particular species is equal to or greater than 10^6 cells per liter, nutrient availability, temperature, oxygenation and luminous intensity are triggering factors that allow an algae bloom to develop form. However, a bloom can be harmless, harmful, or toxic.

There is a great diversity of toxins produced by algae as an adaptive response to the environment, such as ichthyotoxins with hemolytic activity, which cause the death of fish and marine organisms. As well as those that are accumulated and transferred through the trophic chain. All have an impact on health and the economy.

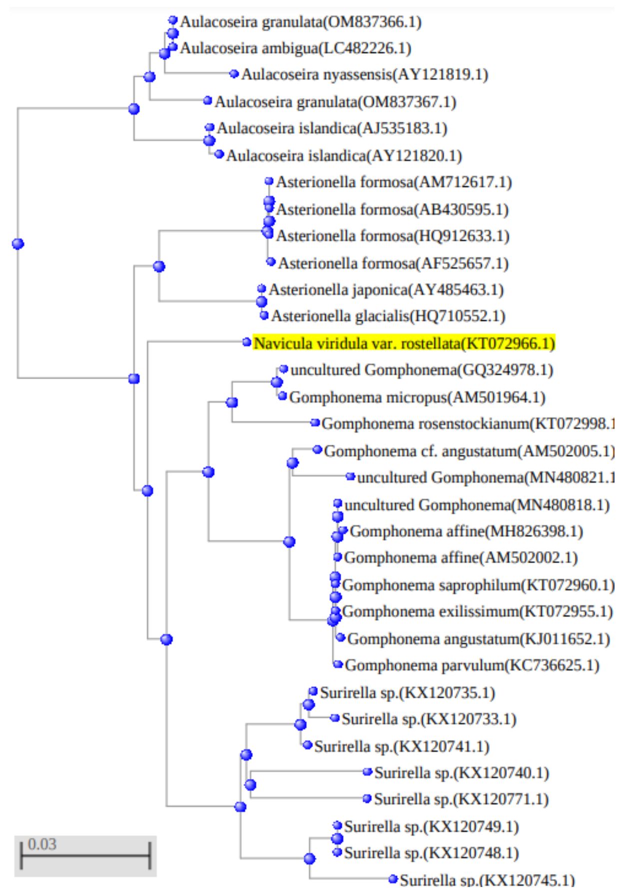

Figure 5 Phylogenetic tree of diatoms observed in the limnological monitoring of the Chen Há cenote during May 2022.
Source: Vizcaino et al., 2022

It is necessary to continue studies in different seasons, since physical phenomena such as rainfall, runoff and increase in sea level will change the availability of nutrients, water mixing and oxygenation.

The arrival of Sargassum to the Caribbean coasts generates a negative impact on tourism, its accumulation and degradation causes the production of unpleasant aromas such as methane as well as deterioration of the landscape. In response to this problem, several research centers developed a series of strategies to use it as a biomaterial with applications in the production of biofuels such as bioethanol; cellulose extraction for paper production, alginates, fucoxanthines, biostimulants or biofertilizers production, as well their use in the production of construction materials, among others. (Roussillon-Druker, J.; Calixto-Perez, E.; Escobar-Briones, E.; González-Cano, J.; Masiá-Nebot, L.; Córdova-Tapia, F. , 2022).

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The origin of the production of Sargassum is unknown for sure, however the most accepted theories include the eutrophication of runoff water from river discharges that reach the sea (which increase both organic and inorganic nutrients and favor the production of macroalgae in the ocean), as well as climate change, it is therefore important to know the state of the art of the water quality of the aquifers that communicate with the sea, for the taking of preventive or corrective actions.

The cenotes provide numerous ecosystem services, such as landscape, temperature regulation, habitat for aquatic, amphibians and birds species, so it is very important to conserve and make a sustainable use of them s in accordance with the indicators for a sustainable development: good quality water for all. It is worth mentioning that these bodies of water are intimately connected via groundwater and therefore a pollution source will have an impact on the entire basin, meanwhile a clean water point will have a positive impact.

Tourism development, as a strategy of economic growth of the Yucatan Peninsula in regions such as Cancun, Playa del Carmen and Bacalar increased the number of hotels and jobs, however, the economic spillover has made the gap between rich and poor larger. The wealth was accumulated in a few hands, investors and transnationals benefit from the possession of land and groundwater concessions, leaving communities and settlers in marginalization. (Angel, Reyes Maya, Barradas Miranda, & Castellanos Martinez, 2022) that is why this work is an integral part of a macroproject and aims to train the ejido community so that through the sustainable and sustainable use of its natural resources they develop tourist activities that allow them to benefit from this activity.

The results presented above are only the beginning of the activities necessary to integrate a network of collaboration among research, science, academic entities, and the general community, which will allow the taking of actions to mitigate the environmental deterioration product of anthropogenic activities carried out consciously or unconsciously and to preserve the legacy of humanity.

Annexes

Tables and suitable fonts.

Gratitude

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Conclusions

Chen Ha is the habitat of species that indicate environmental health conditions such as *Asterionella*, species with biotechnological applications and others with toxic potential such as cyanobacteria and dinoflagellates. It is important to value them and to find strategies for their conservation.

It is necessary to promote and start environmental culture campaigns directed to the users of the Cenote Chen há.

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