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Kinetics and diversity indices of phytoplankton in T´zonot type watery in Kopomá

Cinética e índices de diversidad de fitoplancton en T´zonot tipo aguada en Kopomá

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Abstract

The cenotes, made up of a mixture of karst soil, fresh and salt water, provide ecosystem services: water, climate, landscape, and headquarters for religious and recreational activities. They are niche species that are part of the trophic chains and conserve valuable genetic information product of their evolution and adaptation to the ecosystem. It is important to know them before they can be lost due to anthropogenic activities. The objective of this work was to study the spatiotemporal biodiversity of phytoplankton in the Chen Ha Cenote. Monitoring activities included spring, summer and winter seasons. Environmental variables were monitored with a multiparameter probe. Classification of microorganisms by microscopy. The pH was slightly alkaline in all monitoring results. Temperature varied from 28.7 to 32 ° C, dissolved oxygen ranged from 2.4 to 3.2 ppm. Conductivity was 2.962 during spring and 2650 microS.cm^2 in wintertime. 41 species were identified in spring, 27 in summer and 29 in winter. During the spring and winter seasons diatoms predominated. *Navícula*, *Coelosphaerium* and *Nitzschia* were the dominant species in spring, summer, and winter periods, respectively. In accordance with the Jackard similarity index, greater similarity was observed between spring and winter showing a value of 0.458, compared to summer with a value of 0.3877.

Biodiversity, Karst, Microorganisms

Resumen

Los cenotes, conformados de una mezcla de suelo kárstico, agua dulce y salada, aportan servicios ecosistémicos: agua, clima, paisaje, sede de actividades religiosas y recreativas. Son nicho de especies que forman parte de las cadenas tróficas y conservan valiosa información genética producto de su evolución y adaptación al ecosistema. Es importante conocerlas antes de que puedan llegar a perderse derivado de actividades antropogénicas. El objetivo del presente trabajo fue estudiar la biodiversidad espaciotemporal de fitoplancton, en el Cenote Chen Ha. El monitoreo incluyo primavera, verano e invierno. Las variables ambientales se monitorearon con sonda multiparamétrica. La clasificación de microorganismos mediante microscopia. El pH fue ligeramente alcalino en todos los monitoreos. La temperatura 28.7 a 32 ° C, el Oxígeno disuelto desde 2.4 hasta 3.2 ppm. Conductividad 2.962 en primavera a 2650 microS.cm^2 en invierno. Se identificaron 41 especies en primavera, 27 en verano y 29 en invierno. En primavera e invierno predominaron las diatomeas. *Navícula, Coelosphaerium* y *Nitzschi*a fueron las especies dominantes en primavera, verano e invierno, respectivamente. En conformidad con el índice de similitud de Jackard se observó mayor similitud entre primavera e invierno 0.458, respecto del verano con valor de 0.3877.

Biodiversidad, Karst, Microorganismos

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Introduction

Water is a finite, vulnerable and vital natural resource; however, its availability is not homogeneous at the national level (CONAGUA). It is worth mentioning that it is the duty of the environmental authorities to guarantee for the human population the availability of quality water and its treatment. For some, water is considered a global heritage inherent to the communities and ecosystems in which it is found, however, for others it is an economic resource with monetary value.

95% of the soil of the State of Yucatan is made up of $CaC0_3$ limestone rocks (Karst landscape) and groundwater. (Estrada-Medina et al., 2019). The dissolution of the rock favors the fragmentation of the surface soil, which collapses leading to formation of a great diversity of cenotes.

Cenotes are described as caves flooded with a mixture of fresh and salt water. They are classified as open when the wetland is exposed to the sun's rays, semi-open when part of the surface is open and closed when they are located in caverns with absence of light. The cenotes are a source of water for the human population, their geographical distribution in Yucatan includes the ejido and private lands. It is worth mentioning that the sale of these properties is associated with the sale of water (Cortés, 2018).

The tourist development of the region and the influx of visitors to the Peninsula encourages the privatization and exploitation of cenotes as a tourist attraction linked to archaeological sites. The packages include tourist guides as well as the commercialization of handicrafts of fabric, stones, wood, symbols of the region, pyramids, calendars, catrinas etc. Tour packages are marketed through websites or all-inclusive hotels (Jouault, 2021).

In the related literature there are few records of the biodiversity of phytoplankton species in these bodies of water, which allow measuring or modeling, predictive patterns of diversity at the spatio-temporal scale. It is worth mentioning that some species disappear faster than those allowed by natural extinction processes associated with the accelerated transformation and degradation of natural ecosystems, which puts their conservation at risk.

According to Zuria et al., 2019, the spatial distribution of species is associated with environmental characteristics, the distribution and behavior of other species and the impact of humans. It is worth mentioning that each species houses into its genome the information of millions of years of evolutionary adaptations.

The present work is the first to report the incidence of phytoplankton present in the Cenote Chen ha during the spring, summer and winter seasons of 2022-2023.

Phytoplankton includes autotrophic organisms, with photosynthetic capacity, which considered initiators of trophic chains, and are distributed both in marine environments and fresh aquaculture waterbodies including lakes, rivers and dams. In the coastal environment, diatoms and dinoflagellates are abundant and although their distribution is not homogeneous under certain conditions, harmful algal blooms (HAB) can proliferate in both salt and fresh water (Moreno Diaz, 2015).

According to the literature, a niche is made up of the biotic and abiotic conditions in which a given species reaches its development and subsistence. These are described in a two-dimensional geographical space (latitude and longitude) and a multidimensional environmental ecological space (Mota Vargas et al., 2019). The study of alpha diversity allowed to monitor the richness of species in a community considered homogeneous and the effects on it due to changes in the environment.

Figure 1 demonstrates the changes in the ecosystem derived from the climatic seasons, associated with environmental variables such as light intensity, temperature, rainfall, as well as the impact on vegetation.

Figure 1 Image of the Chen Há cenote in spring, summer-autumn and winter
Source: Own elaboration

Methodology to be developed

For monitoring, 4 analysis stations were established. Environmental physicochemical parameters were determined with a multiparameter probe: pH, temperature, dissolved oxygen, conductivity, and total suspended solids.

Diversity of phytoplankton. It was determined by the dragging sampling technique carried out over 1 minute with a net of 40 micrometers pore diameter and 30 cm net diameter. The samples were analyzed by microscopy, with objectives lens 30 and 40 X. Diversity analysis was determined according to Shannom and Weber's methodology for alpha diversity and Jackard's methodology for beta diversity. (Vizcaíno-Rodríguez et al., 2021).

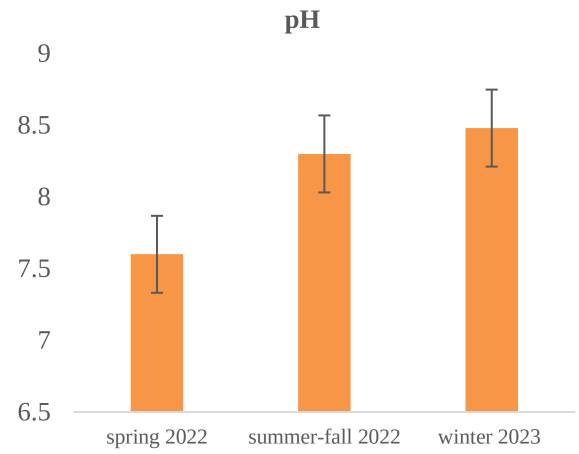
Phylogenetic analysis

An International Nucleotide Sequence Database Collaboration NCBI y Blast (Basic Local Alignment Search Tool) was used for cianobacterias, *Chroococcus turgidus* CCIBt3508 ribosomal RNA gene, partial sequence, 16S-23S ribosomal RNA intergenic spacer, complete sequence, and 23 S ribosomal RNA gene, partial sequence.

For the analysis of diatoms was used the sequence of *Navicula trivialis* 18SrRNA gene (partial), ITS1, 5,8S rRNA gene, ITS2 abd 28S rRNA gene (partial), strain 2-HV25, clone 2. The cleaning of sequences was carried out visually, in relation to the preserved sites. NCBI diagrams were used.

Results

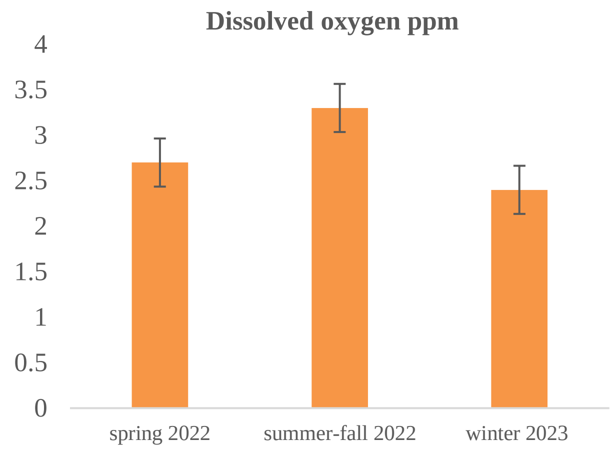
The pH of the water body changed in each season, according to Figure 1. The most acidic pH was obtained during spring, and the most alkaline in winter. Our results are slightly more alkaline than those reported for the cenotes of the municipalities of Yucatan: Tekit, Homun, Cuzamá and Sanahcat, with a range of 6.92 to 7.70. (Cruz et al., 2018).



Graphic 1 pH value in the Chen Ha cenote, spring, summer-autumn and winter 2023
Source: Own elaboration

The alkaline pH favors photosynthetic activity and high concentrations of dissolved oxygen which stimulates the growth of bacteria heterotrophs which are important in organic degradation (Sardi-Saavedra et al., 2016).

The range of dissolved oxygen was varied 2.4 mg/L to 3.3 mg/L, the highest value was detected in summer. See graph 2. The results obtained are lower than those reported for the blue cenote of Quintana Roo with values of 8.8 mg / L in dry and rainy season, and 6 mg / L in winter season, respectively. The authors reported the presence of oxygen in the entire water column, except for 50 m in the rainy season. (Cervantes-Matínez, 2009).



Graphic 2 Dissolved oxygen in the Chen Ha Cenote, monitored by multiparameter probe 2023
Source: Own elaboration

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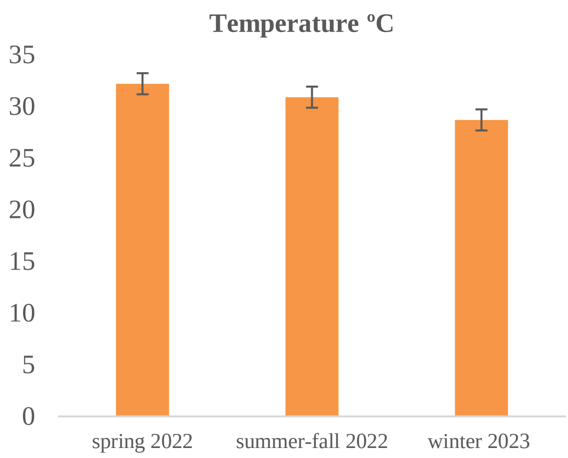
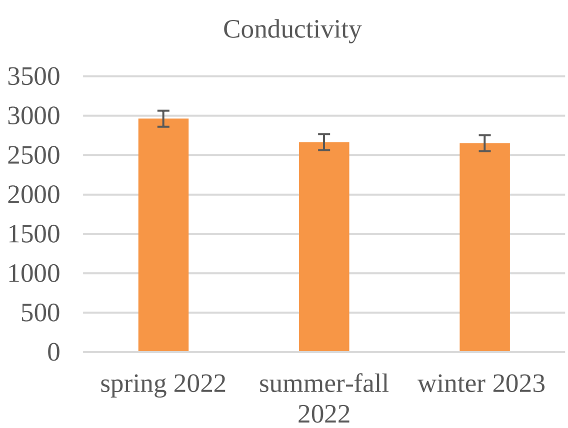
The highest temperature was detected in spring with a value of 32 º C and the lowest value in winter was 28.7 º C. The results are similar to those reported for the Blue Cenote, which presented an average annual temperature of 29.2 +/- 0.9 °C. The authors reported higher temperature values in dry and rainy seasons. The body of water was classified as warm-tropical (Cervantes-Matínez, 2009). Temperature influences the abundance of phytoplankton and bacteria (Abarzúa, 1995). (See graph 3).



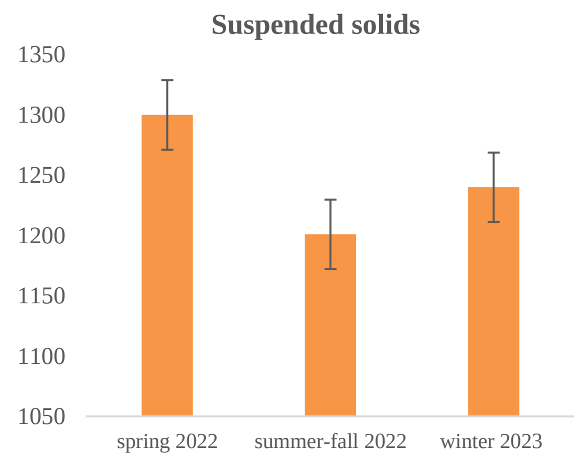
Graphic 3 Temperature recorded in the Cenote Chen-Ha in the spring-winter cycle 2022 – 2023
Source: Own elaboration

The highest conductivity values were observed during spring with a maximum value of 2962 microS.cm^{-2} and the lowest value during the winter with 2650 microS.cm^{-2}. These results coincide with what is reported in the literature for water samples from cenotes in the State of Yucatan with a minimum value of 688.0 micro–S.cm and a maximum value of 2381.0 micro S.cm with an average of 1175.78 micro S.cm. (Cruz- Sánchez et al., 2018) Figure 4.



Graphic 4 Conductivity recorded in the Chen Há cenote, April 2022- January 2023
Source: Own elaboration

Suspended solids were found in the range of 1300 to 1200 ppm the highest values were reached during spring (graph 5). The results are higher compared to those reported with a range of 515.77 ppm as a minimum value and 2381.0 ppm as a maximum value. For cenotes of the State of Yucatán (Cruz-Sánchez et al., 2018). The values exceed the values allowed by the Official Mexican Standard NOM-127-SSA1-2021 Water for human use and consumption, whose limit of total dissolved solids was 1000 mg.L.



Graphic 5 Content of suspended solids in the water body of the Chen-ha spring-winter 2022-2023 cenote
Source: Own elaboration

The diversity of phytoplankton species changed as a function of the climatic season shown in Figure 2. The present species at the moment were *Coelosphaerium*, *Microcystis wesenbergii*, *Microcystis aureginosa*, *Botryococcus*, *Chroococcus turgidus*, *Mougeotia Scalaris*, *Aulacoseira granulate*, *Pinularia accurans*, *Nitzschia*, *Navicula*, *Gomphonema angustatum* var *sarcophagus*, *Prorocentrum micans* Ehrenberg for all monitoring they are in the center of the diagram. The greatest diversity was observed in spring time with 41 species, 27 in summer-autumn and 29 in winter time.

In the biodiversity analyses, 20 species of cyanophytes were identified: *Coelosphaerium*, *Woronichinia naegeliana*, *Microcystis novacekki*, *Microcystis wesenbergii*, *Microcystis aureginosa*, *Microcystis flos-aquae*, *Microcystis incerta*, *Aphanocapsa elachista*, *Botryococcus*, *Nodularia* sp., *Planktothrix agardhii*, *Anabaena affinis*, *Pseudanabaena Limnética*, *Oscillatoria* sp., *Lyngbya*, *Microcystis wesenbergii*, *Chroococcus turgidus*, *Rhabdogloea yucatanensis*, *Merismopedia Tenuissima*, *Sinechocystis*.

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11 Chlorophytes species were identified: *Pandorina charkowiensis*, *Crucigenia cuadrata*, *Mougeotia Scalaris*, *Closterium Mesotaenium macrococcum*, *Staurodesmus extensus*, *Tetrastrum*, *Pleurotaenium Nägeli*, *Chlorella*, *Selenastrum gracile*, *Kirchneriella*. Two Euglenophytes: *Euglena spirogyra*, *Colacium vesiculosum.* 16 diatoms: *Aulacoseira granulata*, *Pinularia accurans*, *Nitzschia*, *Navicula*, *Surirella*, *Gomponema*, *Gomponema acuminatum*, *Gomphonema angustatum var sarcophagus*, *Skeletonema costatum* (Greville), *Diatoma vulgaris*, *Entomoneis alata*, *Asterionella*, *Cymbella auxospore*, *Craticula*, *Tabellaria ventricosa*, *Synedra.* Three dinoflagellates: *Prorocentrum micans* Ehrenberg, *Peridinium Cinctum*, *Gonyaulax* spp.

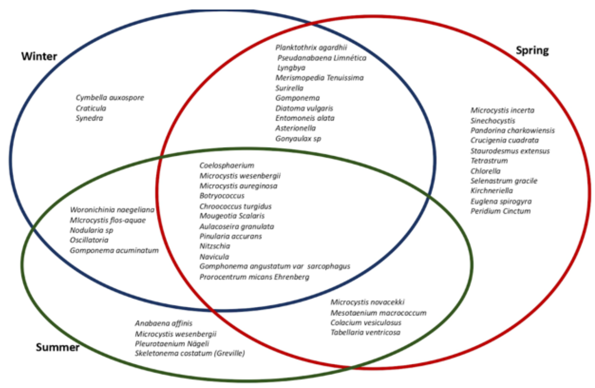

Figure 2 Diversity of phytoplankton species detected in the Chen Ha Cenote, during spring, summer and winter.
Elaboration: own.

Regarding the most abundant species in spring and winter seasons were diatoms. In spring the dominant species were *Navícula*, Surirella *and Pseudanabaena Limnética*, in summer (cyanobacteria) *Coelosphaerium*, Woronichinia naegeliana and *Microcystis aeruginosa* and in winter: *Nitzschia*, *Navicula*, *Entomoneis alata* and *Mycrocystis Flos-aquae* (cyanobacteria) (See Figure 3).

Figure 3 Diversity of phytoplankton present in the cenote Chen Ha, *Coelosphaerium, Entomoneis a., Botryococcus, Surirella, Merismopedia tenuissima, Navícula, Aulacoseira granulate*
Source: Own elaboration

According to Riquelme and Avendaño-Herrera (2023), the formation of a diatom bloom is related to the microbial community and its biomolecules that are released into the environment such as vitamins and growth factors. The bacteria-microalgae interaction is highly specific, bacteria use their ability to absorb, recycle nutrients and organic substances produced by phytoplankton in a symbiosis process.

It has also been demonstrated the presence of algae antagonist bacteria, with the ability to cause inhibition and lysis of microalgal cells and are a key factor for the decay of phytoplankton blooms associated with a rapid bacterial colonization of microalgal cells causing phytoplankton cell death. This fact provides continuity to the flow of carbon in the aquatic environment.

Phylogenetic analyses favor studies of evolutionary relationships between organisms, their distribution and role in the environment and trophic web. Microalgae are both prokaryotic and eukaryotic photosynthetic cells.

In photosynthetic eukaryotes, the 18 S gene, located in the small subunit of the ribosome, contains phylogenetic information with sufficient variability to distinguish between different taxa. The amplification of the 18 S gene by PCR with specific oligonucleotides allows the identification of classes or different phyla in eukaryotes as well as the creation of databases to search for coincidences or phylogenetic relationships (Andrade, 2017).

Figure 4 represents the phylogenetic tree of Diatoms. They are classified into two main groups in the first *Gomponema* is separated from the rest of organisms. The second group is bifurcated into two subgroups, there is greater similarity between *Asterionella* sp. and *Cratícula Pseudocitrus* with respect *to Nitzschia anatoliensis*. In the last group, greater similarity was observed between *Entomoneis* cf. *Alata* y *Surirella* con respecto de *Navícula trivialis*.

Diatoms form one of the most diverse groups of phytoplankton, they are fundamental participants in the recycling of carbon and silicates, and their silica wall is characteristic of the group.

Their origin is estimated in the Jurassic period, they have a high potential for adaptation, they are mostly photosynthetic and it is estimated that in the oceans they represent 50% of the biomass (Lora-Vilchis, 2020).

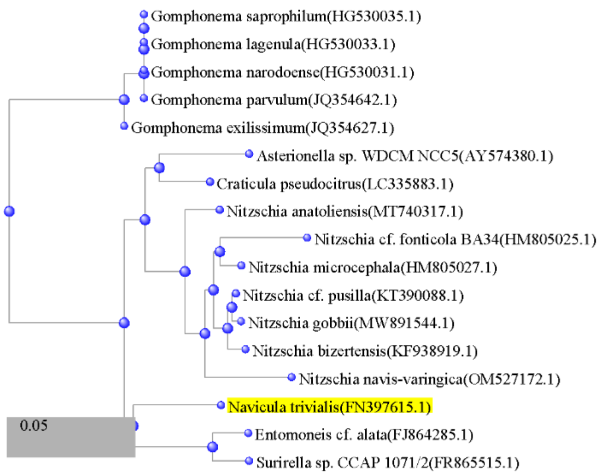

Figure 4. Phylogenetic tree of diatom algae identified in the different climatic seasons in the Cenote Chen Há, in the period April (2022) – March (2023)
Source: Own elaboration

In the case of prokaryotes, 16 S ribosomal RNA (rRNA) is the most widely used macromolecule in studies of bacterial phylogenesis and taxonomy. The 16S rRNA molecule contains highly variable regions, and includes regions of 20 or 30 bases that are completely exclusive to a single prokaryotic species (Andrade, 2017).

Figure 5 shows the phylogenetic tree of the cyanobacteria identified. They fall into two main groups. Greater similarity was observed between Microcystis *and* Synechocystis sp. with lesser similarity with respect *to Chroococcus turgidus*, *Gloeocapsa* is separated into a different group. According to the literature, the main causes that favor the development of these microorganisms include changes in salinity, contributions of fresh water from runoff with nutrient transport and temperature increases (Moreno, 2015).

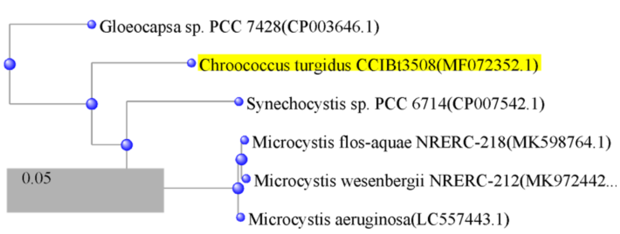

Figure 5 Phylogenetic tree of cyanobacteria identified in Chen Ha
Source: Own elaboration

These trees were selected for including the most abundant species in the water body. According to the literature, during the formation of a phytoplankton bloom in an aquatic environment the highest production of bacteria (3x107 bacteria per mL) is obtained after the maximum production of chlorophyll estimated at 80 mg.mL; it is worth mentioning that recent studies have shown a rapid increase of viral particles after the maximum concentration of bacteria (106 -109 particles x mL-1). These results have been corroborated in the laboratory. (Riquelme & Avendaño-Herrera, 2023).

It is worth mentioning that the summer period in which the pH increased, diversity decreased and cyanobacteria dominated, this allows to demonstrate that the body of water is very fragile and that the origin of runoff with high content of organic matter puts its preservation at risk. Our results coincide with those reported by Laura et al., (2018) who identified pH as one of the main environmental factors that determines the presence of cyanobacteria. They report that in slightly acidic pH (pH = 6) cyanophytes are scarce. In our study the pH was found slightly alkaline with values ranging from 7.5 to 8.5.

Species diversity is the result of ecological interactions between species and the environment, evolutionary and biogeographic history of regions. In particular, alpha diversity reflects the ecological relationships found in a specific space and time frame (Pérez-Hernández, 2015). There is currently great interest in the development of sensors that allow the composition of microalgae with the use of spatial sensors that interact with the properties of ultraviolet light and the reflection and refractive index, chlorophylls and accessory pigments of photosynthesis (Firme et al., 2023).

According to the Shannon-Wiener diversity index, an average level of diversity was observed in all seasons, the greatest diversity was detected in winter. With values of 2,809 for spring, 2,389 for summer-autumn and 2,946 for wintertime. See Figure 6. Less diversity was observed with respect to that reported for the month of August with a value of 3.01 for the Shannon Wiener index in Laguna La Viuda (Lima-Peru) in 2016. (Laura et al., 2018).

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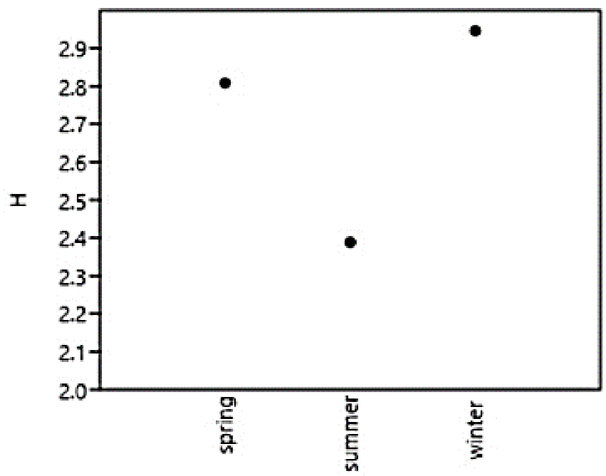

Figure 6 Shannon – Wiener biodiversity index determined for cyanobacteria and phytoplankton monitored in Chen Ha in spring, summer-autumn and winter.
Source: Own elaboration

The beta similarity index is defined as the variation or change in the identity of species between sites either by gain or loss of species (Perez-Hernández., 2015). According to the Jackard index, greater similarity was observed between the spring and winter seasons with a value of 0.458 with respect to summer – autumn with a value of 0.3877. (See Figure 7). Our results coincide with those reported by Huanaco et al., (2018) who mentions that in the study carried out by them, phytoplankton diversity is variable and is related to seasonality.

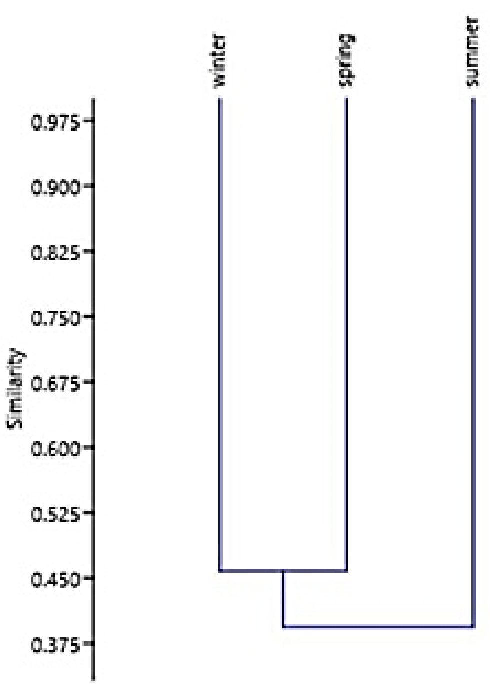

Figure 7 Beta similarity index, calculated from the biodiversity of cyanobacteria and phytoplankton monitored in the Chen Há cenote
Source: Own elaboration

Biological diversity includes the ecosystems in which species inhabit and interact and the genetic variability they possess. Biodiversity is valued from three biological, economic and cultural perspectives.

There is a significant degree of association between the abundance of phytoplankton and heterotrophic bacteria, with particular patterns of interdependence for each season of the year. The exudate of organic substances from active phytoplankton and the supply of carbon from dead phytoplankton are the main sources of organic matter for heterotrophic bacteria. (Abarzúa, 1995).

Each living being is a reservoir of irreplaceable evolutionary information with potential applications, is part of the trophic chains and is a source of inspiration. The Conservation of ecosystems has great value since they offer ecosystem services such as source of raw materials, climate regulation, erosion and diseases control and recreational activities (CONABIO), 2016. The identification of photosynthetic microorganisms by means rRNA sequencing is the method of choice for determining high taxonomic relationships. However, some organisms are known only by their molecular characteristics without having knowledge regarding their abundance, distribution or in situ role of their physiological characteristics (Andrade, 2017).

Bioremediation as a method for detoxification or removal of pollutants from the environment called high photosynthetic rate algal systems are recommended due to the high rate of algal biomass and oxygen. These systems provide a pleasant environment for bacteria to degrade organic matter (Sardi-Saavedra et al., 2016).

The knowledge of the genes and enzymes used in the metabolic process as well as the use of genetic engineering tools will allow the generation of microalgae species as industrial microorganisms and not only as laboratory models (Incera et al., 2022).

In matters of biodiversity, Mexico is recognized as megadiverse since it has about 70% of the known species on the planet, this is a privilege, however it also entails a commitment it is necessary to study the biodiversity associated with the different ecosystems, as well as sharing the results obtained that allow decisions to be made in favor of the environment, This is the main aim of this work to contribute to the knowledge of the richness of phytoplankton species that inhabit an open-air cenote in Chololá, Yucatán, Mexico, in which it was possible to demonstrate that diversity changes with environmental conditions throughout the year and that the alkaline pH that predominates in this body of water favors the development of cyanobacteria.

The cenotes are valuable because they are the niche of species related to the origin of life, but also for their economic value: tourism and recreation as well as cultural and religious values to which these resources are associated.

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Conclusions

The biodiversity of phytoplankton species changed depending on environmental variables, the most abundant species in both spring and winter seasons were diatoms and cyanobacteria in summertime. The dominant species were *Navícula*, *Coeloshaerium* and *Nictzschia*, for spring, summer and autumn seasons respectively. Regarding richness, average diversity was recognized in all seasons, with values of 2,809 in spring, 2,389 in summer-autumn and 2,946 for winter. Regarding alpha diversity, greater similarity was observed between the spring and winter seasons with a value of 0.458 with respect to summer – autumn with a value of 0.3877.

The main factors contributing to species diversity in the water column were identified as alkalinity, temperature and nutrients from rainwater runoff.

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