

Biodiversity and bioindicators of eutrophication in the Basilio Badillo dam**Biodiversidad y bioindicadores de eutrofización en la presa Basilio Badillo**

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Abstract

The Basilio Badillo dam is a habitat for terrestrial and aquatic species and site for recreational and economic activities. Currently, there exist studies related to the chemistry of the stored water however this work is the first directed to describe its biodiversity. The relative abundance of aquatic species depends on their ability to adapt to climate, nutrients, light intensity, oxygen, pollution and residence time of the water in the dam. The objective of this work is to determine the diversity of phytoplankton. Six monitoring stations were established for the analyzed variables: pH, conductivity, temperature and dissolved oxygen with a multiparameter probe. Phytoplankton samples were collected by surface trawling. The Shannon index of the dam was 2.52 (light contamination), it is worth mentioning that 37.8% of microalgae was identified as Chlorophytes, 34.1% corresponded to Cyanobacteria, 27.3% diatoms, 0.3% Cryptophytes, 0.3% Euglenophytes and 0.15% Dinoflagellates. The dominant species were cyanobacteria: *Microcystis novacekii*, *M. aureginosa* and *Fragilaria crotonensis* (diatom), all of them bioindicator species of eutrophicated water bodies, it is worth mentioning that cyanobacteria have toxic potential. The station with the lowest diversity index 1.23 was station one. Conclusions: based on biodiversity, the body of water was classified as slightly polluted.

Cyanobacteria, diatoms, phytoplankton, bioindicators

Resumen

La presa Basilio Badillo, es hábitat de especies terrestres y acuáticas, sede de actividades recreativas y económicas. Actualmente se cuenta con estudios de la química del agua almacenada, sin embargo el presente trabajo es el primero en describir su biodiversidad. La abundancia relativa de especies depende de su capacidad para adaptarse al clima, nutrientes, intensidad luminosa, oxígeno, contaminación y tiempo de residencia del agua en la presa. El presente trabajo tiene por objetivo determinar la diversidad del fitoplancton. Se establecieron seis estaciones de monitoreo, las variables analizadas: pH, conductividad, temperatura y oxígeno disuelto con sonda multiparamétrica. Las muestras de fitoplancton se recolectaron por arrastre superficial. El índice de Shannon de la presa fue 2.52, cabe mencionar que 37.8% de microalgas se identificó como Clorofitas, el 34.1% correspondió a Cianobacterias, 27.3% diatomeas, 0.3% Criptofitas, 0.3% Euglenofitas y 0.15 % Dinoflagelados. Las especies dominantes fueron cianobacterias: *Microcystis novacekii*, *M. aureginosa* y *Fragilaria crotonensis* (diatomea), bioindicadoras de cuerpos de agua eutrofizados, cabe mencionar que las cianobacterias poseen potencial tóxico. La estación con menor índice de diversidad 1.23 fue la estación uno. Conclusiones: con base en la biodiversidad el cuerpo de agua se clasificó con contaminación leve.

Cianobacterias, Diatomeas, fitoplancton, bioindicadores

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Introduction

“Las Piedras” or “Basilio Badillo” dam is located near Ejutla, Jalisco, in the Administrative Hydrological Region VIII Lerma-Santiago-Pacific and the hydrological region 16 Armería-Coahuayana which includes the hydrological basins: Las Piedras, Canoas and El Rosario. It borders the following municipalities: Juchitlán and Chiquilistlán to the north, El Grullo and El Limón to the south; Tonaya to the east and Unión de Tula to the west (Jalisco State Water Commission, 2015).

The Basilio Badillo dam captures permanent effluents from the Tuxcacuesco River, intermittent tributary streams and rainstorms. With an extension of 472.21 square kilometers and a height of 900 m above sea level its total capacity is 182'100,000 cubic meters, with an average length of 18 kilometers, about 0.5 km of average width and a 75 m depth in the deepest areas.

The (NAMO) capacity is 146 (hm³) and the 2021 stored volume was 125 (hm³) (sina.conagua, 2021). The dam provides ecosystem services such as temperature regulation, landscape, water recharge and is habitat for numerous species. Associated economic activities are fishing, tourism and agricultural irrigation for the states of Colima and Jalisco. The fishing activity is carried out by the cooperative society “Basilio Badillo” which is made up of 35 users. The average tilapia fry catch is 500 kg, in proportion to the amount of annual stocking (Michel et al., 2012).

The deterioration of water resources is reflected on the water quality of surface runoff. Pollution from the watershed approach goes beyond administrative limits (Hernández et al., 2020). The micro-watershed faces several problems such as deforestation, erosion and land use change, all of which favors the formation and increase of solids into the runoff that enters the body of water. The diverse origin of runoff includes water from populations with untreated water discharges. According to Hernández et al., 2020, the Tuxcacuesco river basin is contaminated with wastewater, industrial waste, dissolved salts and pathogenic microorganisms.



Figure 1 Basilio Vadillo Dam, Jalisco Mexico spring 2022

Source: Own elaboration

Traditional monitoring programs are limited to physical-chemical indicators, which, in addition to a high cost, reflects chemical conditions of the water at the time of sampling, unlike bioindicators. Microalgae are recommended as tools to assess the ecological status of surface waters and lotic ecosystems because they describe the biological integrity of an aquatic system, are highly sensitive to changes in water quality due to organic and inorganic pollutants, their colonization capacity and short life cycles (Sardi, 2018).

It is advisable to use methods based on the identification and classification of aquatic species richness in the community (Shannon Wiener Equity Index). The relative number or abundance of them describes the dominance or equity of a community based on its resilience or ability to adapt to the environmental conditions prevailing in the ecosystem (Teodor, 2019).

Phytoplankton is constituted by aquatic microorganisms, photosynthesizers with short life cycles that inhabit suspended in the water column and as well as continental, estuarine and marine environments" (Esqueda-Lara, 2016). The present study aims to study environmental factors, the diversity of existing fish and phytoplankton and to characterize bioindicators of environmental pollution to evaluate in a timely manner the chemistry and biology of the water, as well as the taking of actions that could mitigate the environmental impact caused.

Methodology to be developed

The present work was carried out in the Basilio Vadillo Dam located in the State of Jalisco, with coordinates 19° 15' N, 104° 04' W. The monitoring was carried out in April 2022 (I). Five sampling stations were established according to the coordinates of Table 1 and Figure 2.

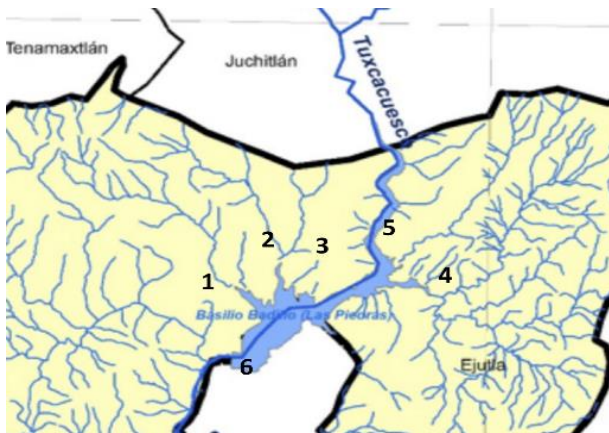


Figure 2 Monitoring stations at the Basilio Vadillo dam, located en Ejutla Jalisco, México. Abril 2022

Source: Modified from the Ejutla Municipal Hydrological Technical Sheet

Station	North latitude	West Latitude
Download Arroyo la Estancia	19° 55'53.2" N	104° 03'32.0" W
Cuamecate stream discharge	19° 56'14.9" N	104° 03'02.0" W
El tempisque stream discharge	19° 55'32.2" N	104° 02'52.8" W
La Parota	19° 55'02.1" N	104° 03'04.1" W
Embarcadero	19° 54'42.0" N	104° 03'24.1" W

Table 1 Monitoring stations, Atlangatepec Dam, Jalisco, Mexico

Source: Own elaboration

Environmental variables: a multiparameter probe was used, the variables analyzed include pH, dissolved oxygen, conductivity, and temperature. **Analysis of phytoplankton:** the samples were collected using an horizontal drag technique, with a duration of 1 minute, phytoplankton net of 30 cm in diameter, 50 cm in length and 20 micrometers of mesh width. The samples were fixed with formaldehyde, kept in refrigeration, and transferred for analysis to the Microbiology laboratory of the Polytechnic University of the Metropolitan Area of Guadalajara based in Cajititlán Jalisco, Mexico and in the Laboratory of multiple uses of the Technological Institute of Tlajomulco.

The morphological characterization was carried out with a Leica compound microscope and 10x and 40x objectives according to the methodology of Vizcaíno et al., 2021. The literature consulted for the identification of the species was based on studies by different authors cited in the bibliographic references. Diversity indices were based on the methodology of (Sepkoski, 1988).

Phylogenetic analysis. Sequences deposited in the NCBI database were used. Blast (Basic Local Alignment Search Tool). For the analysis of fish sequences, the *Cyprinus carpio* 18S ribosomal RNA gene, partial sequence was used. For diatoms, the 18s ribosomal sequence RNA was used sequence of *Navicula viridula*. In the case of cyanobacteria, a *Pseudanabaena limnetica* lim 1 gene for 16 s ribosomal RNA partial sequence was used. For algae, the partial sequence of *Aulacoseira granulata* voucher VILL20 18 S small ribosomal subunit RNA was used. Sequence cleaning was performed visually by removing sequences without preserved or repeated sites. NCBI diagrams were used. The biodiversity index was conducted according to Shannon-Wiener, 1949 according to which maximum diversity is reached when all species are equally present.

Results

The average pH value was: 8.8 +/- 0.5 units, a value that is classified as slightly calin waters. Similar to those obtained in the Tuxcacuesco River during the dry season with a value of 8.12. The average conductivity: 303 +/- 15 µS/cm, slightly lower than the value reported for the Tuxcacuesco River (electrical conductivity) 0.390 µS/cm, classified as low salinity (Hernández et al., 2020). The average dissolved oxygen was 2.4 +/-0.1 ppm. According to Berdeja (2022), the different organic or inorganic components dissolved or dispersed in water modify the physicochemical properties of it. In turn, the quality of surface or groundwater affects the growth and survival of all living beings present in the ecosystem, it is worth mentioning that those places near human settlements are more susceptible to contamination.

The average temperature recorded was 24.4 +/- 1 °C. According to the literature the average annual temperature in the municipality is 22.3 °C with a range of 33.4 and 11.3 °C. The highest temperatures are recorded in June and the lowest during January. The average annual rainfall is approximately 900 mm. The average annual potential evaporation is about 700 mm presenting the highest index during the months of March to June.

Regarding the biodiversity of fish, eight genera were identified, with species corresponding to *Cyprinus Carpio* (Common Carp), *Cyprinus Carpio var specularis* (Mirror carp), *Ictalurus ochoterenai* (Catfish, catfish), *Astyanax fasciatus* (Sardinita), *Poeciliopsis infans* (Fish), *M. salmoides* (Bass), *Lepomis rafinesque* (Blue-eared mojarra), *Lepomis macrochirus* (Blue-eared mojarra), *Oreochromis aureus* (Silver mojarra), *Oreochromis mossambicus* (Black mojarra), *Oreochromis nilotica* (Grated mojarra), *Cichlasoma beani* (Mojarra criolla).

Figure 3 corresponds to the phylogenetic tree of the identified fish species. It is divided into two clades, on one hand *Cyprinus carpio* (carp) and on the other *Oreochromis* (Mojarras) and *Micropterus salmoides* (Bass) species, which presented greater similarity between them. No similarity was observed with the loderos and sardinitas (endemic species).

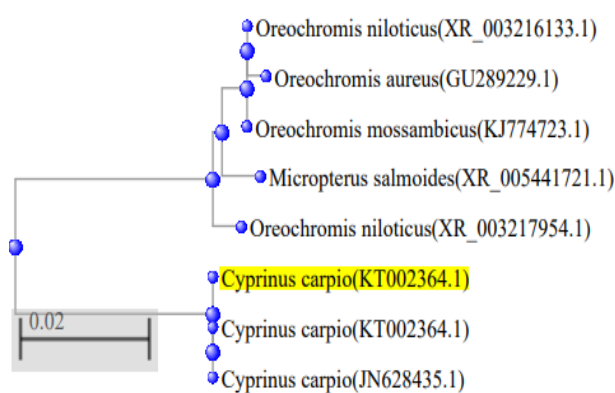
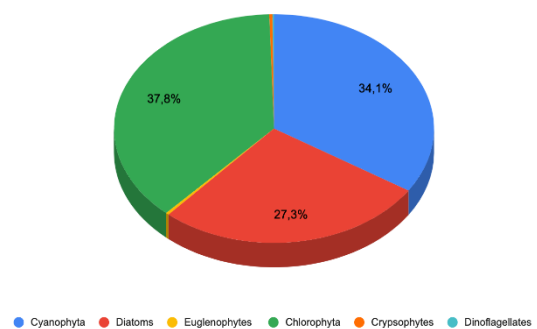


Figure 3 Phylogenetic tree of species identified in the Basilio Vadillo dam, spring 2022
Source: Own elaboration

It is worth mentioning that *Oreochromis*, *Cyprinus* and *Micropterus salmoides* are species introduced in the Dam for commercial purposes and therefore are nonnative species. Regarding phytoplankton species, 37.8% corresponded to chlorophytes, 34.1% to cyanobacteria. Diatoms accounted for 27.3% and the rest corresponded to Euglenophytes, Cripsophytes and Dinoflagellates. (see Graphic 1).

Our results differ from those reported for the Mbói Ka'e Subreservoir in which 51.2% were classified as Crysophyta, 30.4% Chlorophyta, 12.8% Cyanophyta and 5.6% Euglenophyta (Albrecht & Albrecht, 2022). Regarding the diversity of chlorophytes, *Coelastrum*, *Staurastrum gracile*, *Binuclearia eriensis*, *Chlorella*, *Monorapidium griffithii*, *Monorapidium sp*, *Pediastrum clatharamun*, *Pediastrum duplex* and *Volvox aureus* were identified.



Graphic 1 Relative abundance of phytoplankton at the Basilio Badillo Dam, April 2022
Source: Own elaboration

A great diversity of cyanobacteria was observed: *Microcystis novacekii*, *Microcystis aureginosa*, *Anabaena Planktonica*, *Synechocystis*, *Pseudanabaena*, *Aphanizomenom issatschenkoi*, *Anabaena Contorta*, *Lyngbya*, *Limnothrix redekei*, *Planktolynghia Limnetica*, *Planktothrix agardhii*, *Anabaena espiroides* and *Synechocystis*.

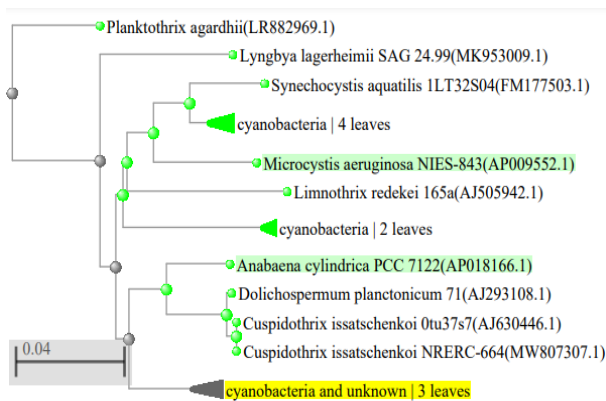


Figure 4 Phylogenetic tree of cyanobacteria. Identified, at the Basilio Vadillo dam, spring 2022
Source: Own elaboration

Regarding diatoms, *Aulacoseira granulata*, *Fragilaria crotonensis*, *Surirella nervosa* and *Tabellaria flocculosa* were identified. And with less diversity, the Euglenophytes: *Phacus* and *Trachelomonas intermedia*. Chrysophyte: *Mallomonas caudata*. Dinoflagellates: *Ceratium hirundinella*. (See Figure 5).

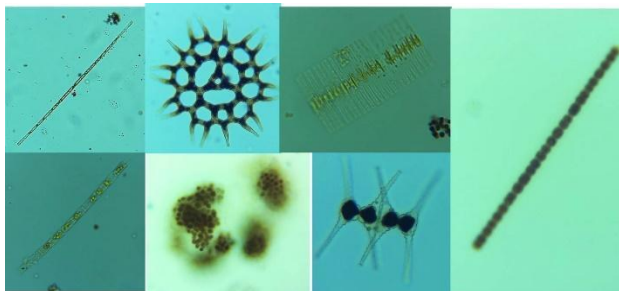


Figure 5 Phytoplankton species identified *Pseudanabaena limnetica*, *Pediastrum duplex*, *Fragilaria crotonensis*, *Aulacoseira granulata*, *Microcystis novacekii*, *Staurastrum gracile*, *Anabaena*. Identified in the Basilio Vadillo dam, Ejutla Jalisco
Source: Vizcaíno, 2022

Diatoms are organisms that are usually preserved as fossils in lake sediments; their study allows historical reconstruction, since sediments accumulate chemical and ecological information, which is used to infer trophic changes in water bodies. *Aulacoseira granulata* (fossil diatom) is characteristic of Eutrophicated water with high levels of nutrients and there are records of its presence in inland waters of Mexico according to Gabito (2010). Figure seven shows the phylogenetic tree of the identified algal species, based on the sequences available in the Genetic Resources Bank.

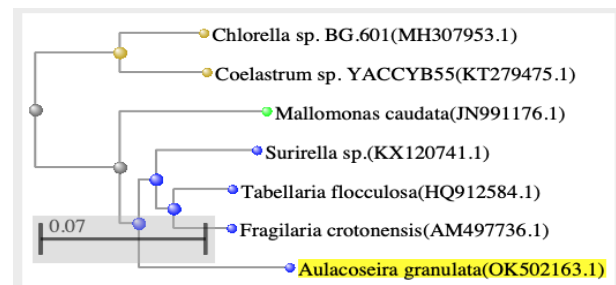


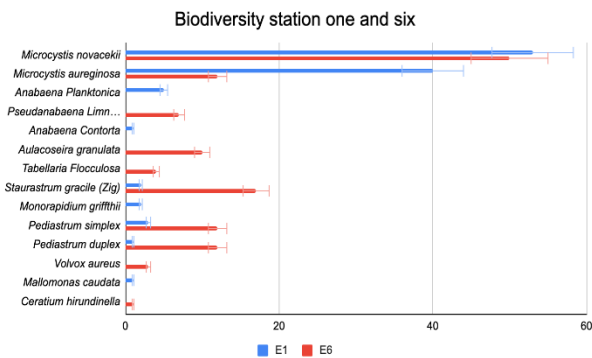
Figure 6 Phylogenetic tree of algae species identified in the Basilio Vadillo dam. April, 2022
Source: Own elaboration

Greater similarity was observed between *Mallomonas* (brown algae) and diatoms. The main fork allows to distinguish the Chlorophytes. The Shannon diversity index calculated at the dam was: 2.52. According to the literature, the index is directly proportional to biodiversity, the higher the value the greater the diversity, the normal range ranges between 1.5 and 3.5.

The dominant species were *microcystis novacekii*, *microcystis aureginosa* both cyanobacteria and *Fragilaria crotonensis* diatom algae. However, the distribution of species changed depending on the monitoring station. The rivers travel large areas of territory and along its way they are affected to a greater or lesser extent by anthropogenic activities. The pollution of rivers changes depending on the activities near the watercourse, for example the lack of treatment of discharges provides a large amount of organic matter facilitating the growth of pathogens, industrial activities promote the accumulation of heavy metals and tourism activities generate single-use plastic pollutants (Sardi, 2018).

In station one (discharge of the Arroyo la Estancia) *Microcystis novacekii*, *M. aeruginosa* and *Anabaena Planktonica* were the dominant species and the massive cyanobacteria in the water tributary translates into the low Shannon Index obtained: 1.23. This value classifies the tributary on the scale of moderate contamination, however there is a potential greater risk due to the dominance of cyanobacteria with added potential toxicity.

The increase in agricultural and livestock activities has a negative effect on water bodies, due to the dragging of solid and liquid waste that alters the community of microalgae species and induces the displacement, death and disintegration of species with the release of phosphate and macronutrients, by those that tolerate pollution levels (See Graphic 2).



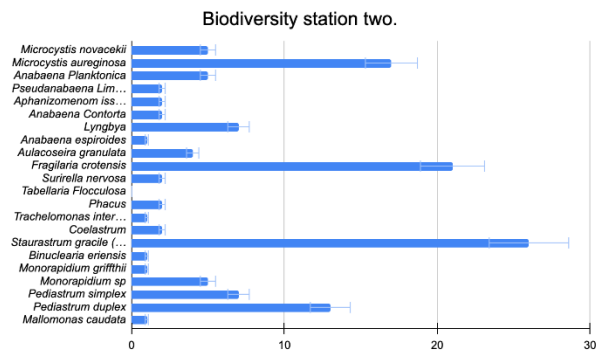
Graphic 2 Relative abundance of species in the Arroyo de la Estancia and Presa Basilio Vadillo stations. Jalisco Mexico

Source: Own elaboration

At station six, the Shannon index was 1.89, being the dominant species *Microcystis novacekii*. These conditions favored the indicator genus of algae in oligotrophic bodies: *Staurastrum gracile* (chlorophyte). These results coincide with the findings of Loaiza et al. (2011), who report that species characteristic of oligotrophic environments frequently cohabit eutrophic environments. This station does not include river discharge and its proximity to the station is suggested to be the main cause of its low diversity (Information shown in Graphic 3). At station two, mouth of the Cuamecate stream, the dominant species was *Staurastrum gracile* (chlorophyte), followed by *Fragilaria crotensis* (diatom), *Microcystis aeruginosa* (cyanobacteria), *Pediastrum simplex* and *P. duplex* (chlorophytes).

According to the literature, the 0.1 ppm concentration of nitrate and mercury chloride inhibits the growth of *F. crotonensis* *in vitro* cultures (Tompkins and Blinn, 1976). Studies carried out by Wallen (1996) with Chromium VI showed that *Fragilaria crotonensis* (Kitton) is sensitive to this metal, with respect to chlorophytes that demonstrated greater adaptability when a concentration 0.25 μ M was used.

Consequently, the presence of *Staurastrum gracile* and *Fragilaria Crotonensis* as dominant species, is related as a good indicator of environmental health (absence of heavy metals). It is worth mentioning that in this station the highest Shannon index 2.43 of the dam was recorded. This indicator reflects a mild level of contamination. (see Figure 9).



Graphic 3 Cyanobacteria Diversity in Station Two: Mouth of the Cuamecate Creek at the Basilio Vadillo Dam, Jalisco Mexico. Spring 2022

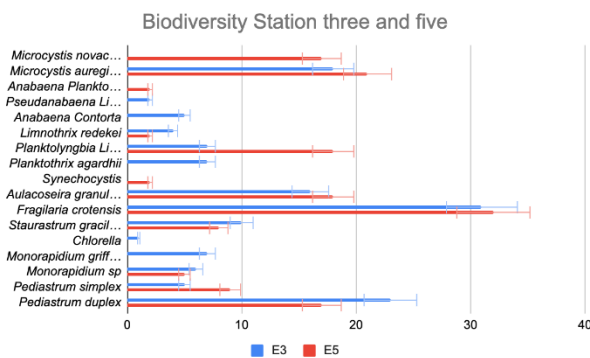
Source: Own elaboration

In station three the index was 2.33 (mild contamination), however, the dominant species was: *Fragilaria crotensis* and *Aulacoseira granulata* (both diatom algae). *Fragilaria crotensis* is considered an indicator species of eutrophication. *Aulacoseira granulata* is a cosmopolitan species, present in inland waters, associated with mixing in the water column. In Mexico it is frequent both in freshwater bodies to saline environments ranging from 100 to 1000 mg / L, pH from 7 to 9 and mesophilic to hypertrophic water bodies (Ramírez Nava M. and Caballero M., 2019).

At station five the index was 2.21 and the dominant species *Microcystis aeruginosa* (see figure 9). *Microcystis*, the best-known genus of cyanoprokaryotes, are indicators of environmental pollution in eutrophic water bodies with high availability of nutrients such as phosphate, nitrate and alkaline pH (Vizcaíno et al., 2017). They are microscopic colonial organisms and sometimes macroscopic, the division of their cells is along three planes perpendicular to each other, so their colonies are three-dimensional with the capacity to produce algal blooms.

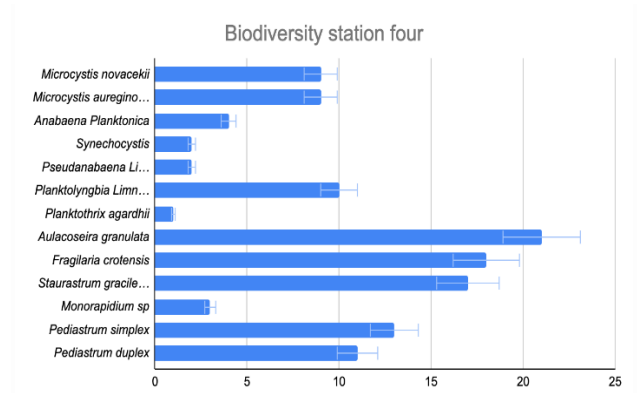
They are included in the catalog of potentially toxic cyanobacteria of inland waters for their ability to produce microcystins and endotoxins, which was confirmed in at least one strain worldwide. Within it we identify *Microcystis novacekii*, *Microcystis aureginosa*, *Microcystis flos-aquae*, *Microcystis wesenbergii*. (Ministry of Environment and Rural and Marine Affairs, 2011).

Eutrophication puts the recreational use of water resources at risk. (Gabito 2010), according to the literature, the Apulco tributary basin contributes poor quality water to the main channel of the Tuxcacuesco River, (Hernández 2020).



Graphic 4 Phytoplankton diversity at monitoring stations three (mouth of Jilgueros stream) and Station 5 (Fat stone stream station), Basilio Vadillo Dam, Jalisco Mexico
Source: Own elaboration

In station four: mouth of the stream of labor, greater diversity was observed: chlorophytes, diatoms and cyanobacteria. It had an index of 2.30 and the dominant species were *Fragilaria crotensis* and *Aulacoseira granulata*, see Graphic 5. Diatoms are part of plankton or periphyton, often attached to rocks or aquatic plants. They are considered indicators of organic pollution and eutrophication.



Graphic 5 Station 4. Phytoplankton diversity at the monitoring station: inflow of the work at the Basilio Vadillo dam, Jalisco Mexico. Spring 2022.
Source: Own elaboration

No significant difference was observed in the physicochemical variables monitored in the stations, however differences in biodiversity were observed. Our results coincide with Fernandez et al., 2022 who consider that the study of populations and their diversity is more representative and sensitive with respect to environmental variables, since the species present are the product of a process of natural selection.

The construction of dams at the national level, generates controversy. On one hand they provide benefits for the population such as: energy production, the availability of water for agricultural irrigation, flood prevention, however, there are few environmental impact studies downstream of them. They are considered hydraulic works that affect the morphology and channel of rivers and streams, representing a physical barrier for water and sediment dragging, with downstream impact by affecting the availability of water, loss of plant or animal biodiversity, economic activity, among others.

Based on the results of this work, the Basilio Badillo dam is a heterogeneous system that depends on the volumes of water discharges and pollution from various rivers or runoff, therefore, it is a fragile body of water.

The Shannon diversity index calculated in the dam was: 2.52, according to the scale it is classified as a level of mild contamination, however bioindicator species of eutrophicated water bodies were identified: *Fragilaria crotensis* and *Microcystis aureginosa* with the potential to cause impacts on the trophic chain and the aquatic environment. It is suggested to take corrective actions upstream of station 1 (discharge of the Arroyo la estancia). To avoid contamination of the dam since the environmental conditions in the path of such runoff favor the growth of cyanobacteria and the dominance of *Microcystis*.

It is worth mentioning that the results presented here correspond to the dry season, in which the volume of available water and temperature, as well as other environmental factors, heavily influences the relative abundance of the species. Knowing the history of the ecosystem is an important factor that allows the taking of actions required for the proper rehabilitation of the ecosystem. Increased phosphorus levels and eutrophication are common problems in water bodies. Derived from this, there is loss of transparency, increase in biomass, reduction of dissolved oxygen, etc. Frequently recommended actions are sediment dredging, nutrient supply reduction and manipulation of biological communities.

Microorganisms are sensitive to water fluctuations, which are not detected by intermittent chemical testing. They are considered biosensors because they allow quantifying and qualifying the level and evolution of pollution, present in an ecosystem thanks to its sensitivity to polluting substances.

Annexes

Appropriate tables and fonts.

Gratitude

This project was funded by the Polytechnic University of the Metropolitan Area of Guadalajara and the South University Center of the University of Guadalajara.

Conclusions

The Shannon diversity index calculated at the dam was 2.52, which corresponds to a mild contamination level. Bioindicator organisms of eutrophicated water bodies *Microcystis aureginosa* and *Fragilaria crotensis* were identified as dominant species of tributaries.

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