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# Approach to a biotic integrity index of phycoperiphyton in the Colombian Zapatosa Swamp Complex

## Aproximación a un índice de integridad biótica del ficoperifiton en el Complejo Cenagoso de Zapatosa de Colombia

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### Abstract

Microalgae are producer organisms that have been used as bioindicators since the 19th century. Microalgae are characterized by their rapid reproduction, short life cycle, and easy collection. Although their identification is complex, other attributes have been evaluated, such as their morphological measurements and functional traits, which allow a quick and efficient assessment of these organisms and address some taxonomic inconveniences. In this work, an evaluation tool for the ecosystem health of the Zapatosa Swamp Complex (ZSC) was developed, based on the composition as well as biological and ecological characteristics of the periphytic algae community, to determine its biotic integrity and assess the ecological state of these swamps. To do so, the composition of algae in the ZSC was analyzed, some aspects of their functional morphology were evaluated, and the most appropriate functional variables were selected for the development of an index of biotic integrity (IBI) of this community. Mathematical and statistical analyses indicated that the selected attributes (maximum linear dimension, biovolume, surface area, silica exoskeleton, mucilage, colonies, aerotopes, and flagella) were suitable for developing the IBI and that they were correlated with some environmental variables. Using the IBI results, the ecological conditions of the different ZSC areas were discriminated. However, the proposed index is a first approximation that will need further development to become an effective management and prediction tool. It should also take into account seasonal changes in the hydrology of the ZSC.

*Keywords:* algal morphology, algal morphometry, ecosystem health, functional traits, periphyton

### Resumen

Las microalgas son organismos productores utilizados como bioindicadores desde el siglo XIX. Se caracterizan por su rápida reproducción, sus ciclos de vida cortos y la facilidad de su colecta, y aunque su identificación es complicada, se han evaluado otros atributos como sus medidas morfológicas y sus rasgos funcionales, los cuales permiten hacer una rápida y eficiente valoración de estos organismos, subsanando algunos inconvenientes taxonómicos. En este trabajo se desarrolló una herramienta de evaluación de la salud ecosistémica del Complejo Cenagoso de Zapatosa (CCZ), basada en la composición y en las características biológicas y ecológicas de la comunidad de algas perifíticas, para determinar su integridad biótica y valorar el estado ecológico de este conjunto

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de ciénagas. Para ello, se analizó la composición de dichas algas en el CCZ, se evaluaron algunos aspectos de su morfología funcional y se seleccionaron las variables funcionales más apropiadas para la elaboración de un índice de integridad biótica (IIB) de esta comunidad. Los análisis matemáticos y estadísticos mostraron que los atributos seleccionados (dimensión lineal máxima, biovolumen, área superficial, exoesqueleto de sílice, mucílago, colonias, aerotopos y flagelos) fueron razonablemente apropiados para el desarrollo del IIB y que se correlacionaron con algunas variables ambientales. Con los resultados del IIB se discriminaron las condiciones ecológicas de las distintas zonas del CCZ. Sin embargo, el índice propuesto es una primera aproximación, que deberá desarrollarse en el futuro para lograr una herramienta de gestión y predicción eficaz, teniendo en cuenta los cambios estacionales en la hidrología del CCZ.

*Palabras clave:* morfología algal, morfometría algal, perifiton, rasgos funcionales, salud ecosistémica

## INTRODUCTION

Bioindicators are living organisms that allow for the assessment of ecological health and environmental changes occurring in ecosystems (Parmar et al., 2016). Bioindicators make it possible to measure different aspects, from biochemical, physiological, or immunological markers at the cellular or tissue level (Peakall & Shugart, 1992) to population and community ecological variables, such as abundance, composition, and richness (Markert et al., 2003). Within this panorama, indices of biotic or biological integrity (IBI) consider a series of organismic and ecological attributes of individuals (Karr, 1991), using their biological characteristics to comprehensively and effectively assess the condition or health of ecosystems.

Biotic integrity is understood as the capacity of an organismal community to support and maintain a balanced, integrated, and adaptive condition, as well as having a compositional, diverse, and functional organization comparable to that of the natural regional habitats (Karr & Dudley, 1981). Human societies have strong effects on the natural environment, but ecosystems with high biotic integrity are presumed to be able to resist or recover quickly from most natural disturbances and overcome major disturbances of anthropogenic origin (Karr et al., 1986). Thus, a biological system is healthy when it can express its inherent potential, remain in a stable condition, retain its capacity for self-repair in the face of disturbances, and require minimal external support for management.

An IBI is based on multiple parameters, which is advantageous when the evaluated system is complex. In its construction, both expert judgment and quantitative criteria are used, which allows for the identification of what is in excellent or poor condition in an orderly and consistent manner (Karr et al., 1986; Kerans & Karr, 1994; Wolterbeek et al., 1996). The metrics or biological attributes selected for the final index should include those aspects that contain the most appropriate ecological information according to the objective pursued (Stribling et al., 1998). However, the actual structure and state of the biotic community can be misinterpreted (Barbour et al., 1999), which is why the attributes that compose the IBI should evaluate the factors that best correlate with biotic integrity. Individually, each attribute provides information about a specific characteristic of the sampling site, and, taken together, they characterize the underlying ecological integrity of that site (Karr et al., 1986). The final value of an IBI will allow us to measure the ecological function of the system. This involves steps of selection, weighting (valuation), scaling (transformation of indicators into dimensionless measures), and mathematical manipulation (Markert et al., 2003).

The first IBI was proposed for the fish community by Karr (1981), from which numerous works have been done to develop IBIs in different biological groups and many regions of the world, as can be seen in the reviews made by Beck and Hatch (2009), Huang et al. (2022) and Vadas et al. (2022). In Colombia, the development of biotic integrity indices for communities of aquatic organisms has a recent history. The first study was elaborated by Solano-Figueroa et al. (2011), who developed an IBI for macroinvertebrates of Amazonian rivers in the Amacayacu National Natural Park. In another theoretical approach, Pinilla-Agudelo et al. (2014) proposed a series of IBIs for riparian vegetation, fish, macroinvertebrates, and periphyton as useful methods for environmental flow assessment in rivers licensed by environmental authorities. Other works based on the macroinvertebrate community have been carried out in the swamps of Cesar River (Martínez-Rodríguez & Pinilla-A, 2014), in fluvial systems of the Chicamocha River basin (Vera-Sánchez & Pinilla-Agudelo, 2020) and the Ánimas River in Boyacá Department (González-Tuta et al., 2023). For fish, there is an IBI of lotic systems of a dry forest in the Caldas Department (Bolívar-García et al., 2017) and another for the fish community of the Chato River (Chocó Department) (Murillo Asprilla et al., 2018). Currently, there is a knowledge gap in Colombia regarding the development of biotic integrity indices based on periphytic algal communities. This paper presents the results of the first research that proposes an IBI based specifically on periphytic algal communities, whose results can provide for better ecological, political, and social management of the water resources of the Zapatosa Swamp Complex (ZSC).

## METHODOLOGY

### Study area

The Zapatosa swamp is the largest freshwater reservoir in Colombia and acts as the sediment trap of the floodplain of the Cesar and Magdalena rivers and is the buffer zone of the Magdalena River (Rangel-Ch., 2012). The main tributary of the Zapatosa swamp is the Cesar River from the Sierra Nevada de Santa Marta. The Zapatosa swamp is responsible for much of the water dynamics of northeastern Colombia. In times of high flow, the Magdalena River enters the swamp and pushes the Cesar River back. In dry periods, the Magdalena River receives the water stored in the swamp complex. In addition to the large main body of water, there are many small lentic systems and communication channels that together make up the Zapatosa Swamp Complex (ZSC) (Rangel-Ch., 2012). The climate of the ZSC is bimodal-tetra-seasonal, with a period of higher precipitation starting in September and a subsequent increase in flow between October and December. Another period of high levels is observed between May and July, but it is notably lower. The average depth of the swamp complex is 4.15 m (Rangel-Ch., 2012).

### Fieldwork

Sampling was conducted between July 15 and 28, 2021, corresponding to the lowering of the flooding in the ZSC. To have different environmental and ecological health conditions for the phycoperiphyton community, a selection of sites was made according to the following criteria: areas of greater richness of fish species according to local fishermen; land use and state of vegetation cover analyzed with cartographic tools and the ArcGIS geographic information system; proximity or remoteness to human settlements. Nineteen sampling zones were considered within the ZSC, of which the Arroyo Alfaro and the Montecarlo stream behaved as lentic environments during the sampling period. The other 17 sites were littoral zones of lentic systems (Figure 1), whose coordinates and general characteristics are shown in Table 1.

Dissolved oxygen, percent oxygen saturation, water temperature, pH, and electrical conductivity were recorded *in situ* with Hydrolab and Hanna multiparameter probes; transparency and depth were assessed with a Secchi disk. Measurements of the physical and chemical variables were made between 10 am and 2 pm. Water samples were taken to a certified laboratory for analysis of total organic carbon (TOC), orthophosphates, calcium, biological oxygen demand ($BOD_5$), chemical oxygen demand (COD), turbidity, iron, magnesium, silica, total nitrogen, nitrates, total phosphorus, phosphates, sulfates, total dissolved solids (TDS), total suspended solids (TSS), total hardness, alkalinity, and fecal and total coliforms. These samples corresponded to the integration of subsurface water (20 cm), water from the middle of the photic zone, and water from approximately 50 cm above the bottom.

The protocols established by IDEAM and INVEMAR (2021) for periphyton collection were followed. The most representative substrate was selected and fragments were taken for their preservation. Wood pieces were cut from plants attached to the substrate and immersed in the water body. These pieces were preserved in a plastic bottle containing o solution made of distilled water, 90% ethanol, and 40% formalin in a 6:3:1 ratio (Transeau solution). Occasionally,

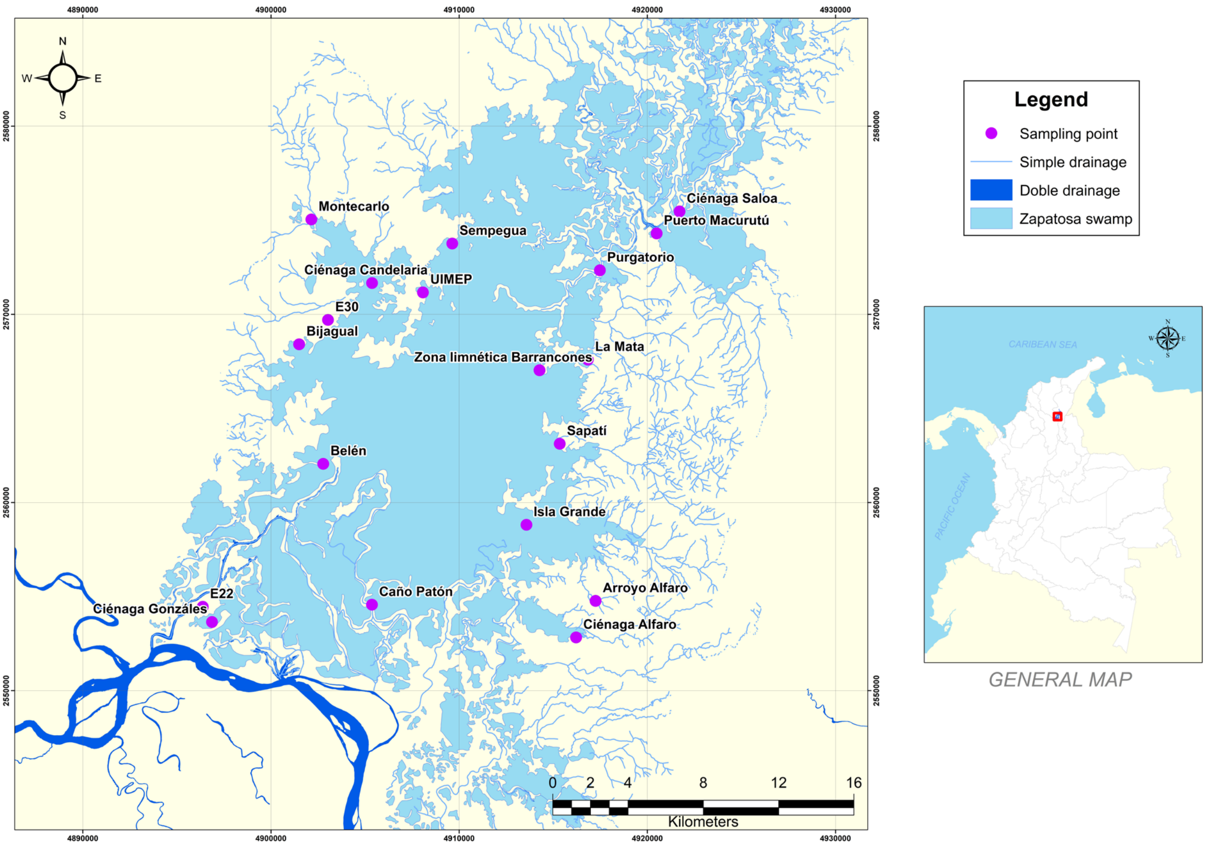


**Figure 1.** Map of the sampling points in the Zapatosa Swamp Complex.

leaves or stems were collected from floating plants (two sites), or rocks (one site), as these were the only substrates available.

## Laboratory analysis

Laboratory analyses were performed at the Biology Department of the Universidad Nacional de Colombia, Bogotá. Collected substrates were scraped and their areas were determined. Taxonomic identification was done with local and regional keys (Bicudo & Menezes, 2006; Dos Santos, 2016; Necchi, 2016) and other, more general keys (Bellinger & Sigee, 2015; Wehr et al., 2015). Four slides per site were evaluated using a microscope with an integrated camera, with photographs of the algae used to make morphometric measurements of maximum linear dimension, area, surface-to-volume ratio (S/V), and biovolume. The calculation of surface area, S/V, and biovolume was based on the works of Lewis (1976), Hillebrand et al. (1999), and Sun and Liu (2003). Morphometric measurements were performed with ImageJ software (Rueden et al., 2017) on at least 20 individuals of each taxon. The occurrence of some categorical or nominal traits (silica valvae or exoskeleton, organization into filaments, colony formation, and presence of mucilage, aerotopes, or flagella) was also determined for each taxon, following the recommendations of Biggs et al. (1998). To determine algae abundance, individuals of each taxon present were counted until at least 200 individuals of the most representative species were obtained, using an inverted microscope and the sedimentation technique in Utermöhl-type chambers (Lund et al., 1958). The results of algal abundance were expressed in individuals per $cm^2$.

## Construction of the Index of Biotic Integrity

The construction of the Index of Biotic Integrity of the Zapatosa Phycoperiphyton (IBI-ZP) began with a descriptive analysis of the environmental variables (box plots or box-and-whisker diagrams) and the selection of the reference sites, for which the principal component analysis (PCA) of the

**Table 1.** Location and general characteristics of sampling sites in the ZSC

| Location (site abbreviation) | System types | Coordinates | Altitude (m.a.s.l.) | Surrounding uses |
|---|---|---|---|---|
| Ciénaga González (CG) | Lentic | N 9°00'33.0"<br>W 73°56'19.2" | 28 | Natural coverage |
| Montecarlo (M) | Lotic (lentic at the time of sampling) | N 9°12'10.2"<br>W 73°53'28.0" | 22 | Livestock<br>Natural coverage |
| Belén (B) | Lentic | N 9°05'17.3"<br>W 73°53'18.7" | 26 | Natural coverage<br>Livestock |
| Bijagual (BJ) | Lentic | N 9°08'44.1"<br>W 73°54'01.1" | 26 | Livestock<br>Natural coverage |
| Ciénaga Candelaria (CC) | Lentic | N 9°10'20.4"<br>W 73°51'42.00" | 30 | Livestock<br>Natural coverage |
| Sempegua (S) | Lentic | N 9°11'29.1"<br>W 73°49'22.40" | 29 | Natural coverage |
| Ciénaga Saloa (CS) | Lentic | N 9°12'25.5"<br>W 73°42'46.5" | 26 | Natural coverage |
| Zona limnética - Barrancones (ZL-B) | Lentic | N 9°07'50.1"<br>W 73°46'49.9" | 33 | Livestock<br>Natural coverage |
| La Mata (LM) | Lentic | N 9°08'08.60"<br>W 73°45'25.9" | 23 | Livestock |
| Ciénaga Alfaro (C-AL) | Lentic | N 9°01'11.00"<br>W 73°45'11.4" | 35 | Natural coverage<br>Agriculture |
| Sapatí (SAP) | Lentic | N 9°05'42.91"<br>W 73°46'14.40" | 25 | Livestock<br>Natural coverage |
| E22 | Lentic | N 8°59'19.2"<br>W 73°58'08.3" | 28 | Natural coverage |
| E30 | Lentic | N 09°09'16.4"<br>W 73°52'58.5 | 26 | Natural coverage<br>Others: bathing place |
| Unidad Integral de Mejoramiento Pesquero (UIMEP) | Lentic | N 09°10'4.3"<br>W 73° 50'13.2" | 33 | Livestock<br>Agriculture<br>Natural coverage |
| Arroyo Alfaro (AA) | Lotic (lentic at the time of sampling) | N 9°00'07.70"<br>W 73°45'45.34" | 31 | Livestock |
| Puerto Macurutú (PM) | Lentic | N 09°11'47.4"<br>W 73°43'26.4" | 26 | Livestock<br>Urban settlement |
| Purgatorio (PU) | Lentic | N 09°10'43.4"<br>W 73°45'05.1" | 28 | Livestock<br>Agriculture<br>Natural coverage |
| Isla Grande (IG) | Lentic | N 9°03'22.5"<br>W 73°47'12.1" | 30 | Natural coverage |
| Caño Patón (CP) | Lentic | N 9°01'03.6"<br>W 73°51'40.8" | 28 | Livestock |

Surrounding use refers to the condition observed in the vicinity of the area at the time of sampling. Site abbreviations are included

abiotic and bacteriological variables was used to reveal the environmental gradients formed at the sampling sites. PCA is a robust technique for detecting such gradients in aquatic environments (Fathy et al., 2012) and has been successfully used for constructing indices of biotic integrity to select both the least impaired and most altered locations (Li et al., 2010; Rodríguez-Olarte et al., 2006; Whittier et al., 2007). For the present case, the PCA facilitated the identification of the least contaminated sites and their selection as the reference points. To choose the most representative features of the ZSC phycoperiphyton, the box plot technique was initially used based on the averages of these variables per site; Spearman correlations were also performed between the average values per site and the PCA ordination axes of the abiotic variables. It should be noted that attributes such as richness or abundance of taxa categorized as tolerant or intolerant were not utilized, as there is insufficient ecological information regarding these characteristics for the microalgae taxa present in the country's swamps.

Secondly, the signal-to-noise index (average of the attribute/individual attribute value at each site, Wolterbeek et al. 1996) of the morphometric and functional variables per site was used, and the results were plotted using box plots. Spearman correlations were performed between the signal-to-noise values and the PCA ordination axes of the abiotic variables.

To corroborate the selection of the biotic attributes that best expressed the stress gradient, the indications of Barbour et al. (1999) and Gerritsen et al. (2000) were followed, which consisted of comparing the variability of each attribute in the reference sites concerning the altered sites using the box plots technique, which made it possible to define the variables that showed changes between the two situations. The Discriminative Efficiency (DE) of each attribute was also calculated:

$$DE = 100x\frac{a}{b} \quad \text{(Equation 1)}$$

where *a* corresponds to the number of impacted sites with a value of the attribute below the 25th percentile, at reference locations if the attribute decreases with disturbance, or to the number of impacted sites with a value of the attribute above the 75th percentile, at reference locations if the attribute increases with disturbance, and *b* is the total number of impacted sites. ED values close to 100% indicate that this attribute is a suitable metric to integrate the IBI-ZP.

Standardization and scoring of the selected attributes were performed using the following equations:

For attributes that decrease with perturbation:

$$Score = \left(100 * \left(\frac{X}{X95 - Xmin}\right)\right) \quad \text{(Equation 2)}$$

where X is the value of the attribute at each site, X95 is the 95th percentile of the attribute values at all sites, and Xmin is the minimum recorded value of that attribute.

For attributes that increase with disturbance:

$$Score = \left(100 * \left(\frac{(Xmax - X)}{(Xmax - X5)}\right)\right) \quad \text{(Equation 3)}$$

where X is the value of the attribute at each site, X5 is the 5th percentile of the attribute values at all sites, and Xmax is the maximum value recorded for that attribute. The final value of the IBI-ZP for each site is the average sum of all attributes and varies between 0 and 100 (Gerritsen et al., 2000).

The IBI-ZP scales were established based on the 75% and 25% percentiles of the IBI-ZP of the reference localities, as follows: values higher than the 75% percentile correspond to sites with high biological integrity of the community; percentiles between 50% and 75%, sites with moderate biological integrity; percentiles between 25% and 50%, sites with poor biological integrity; and localities with values lower than the 25% percentile are of very poor biological integrity.

## RESULTS

### The physical, chemical, and microbiological environment of the swamp complex

The complete results of the 28 physical, chemical, and bacteriological variables measured in the ZSC are presented as supplementary material (Table S1). Figure 2, constructed with 9 of the 11 variables selected (with coefficients of variation greater than 20% and not covarying with other variables), shows that total hardness (25.7 to 76.1 mg/L $CaCO_3$), sulfates (10 to 20.3 mg/L $SO_4$), total organic carbon (3.6 to 25 mg/L), and silica (1.1 to 13.4 mg/L $SiO_2$) were relatively more variable in the swamp complex. The same was true for oxygen saturation (Table S1), which ranged from 2 to 99%. $BOD_5$ (2 to 9.5 mg/L $O_2$) fluctuated moderately; the other variables had averages with a tendency to be low, as was the case with Secchi transparency (0.62 m, Table S1), total phosphorus (0.1 mg/L P) and iron (0.48 mg/L Fe) (Figure 2A). Coliform bacteria showed moderate records, with averages of 1,209 NMP/100 mL for fecal and 30,266 NMP/100 mL for total bacteria (Figure 2B), but the latter reached high values (540,000 NMP/100 mL) in Ciénaga Alfaro.

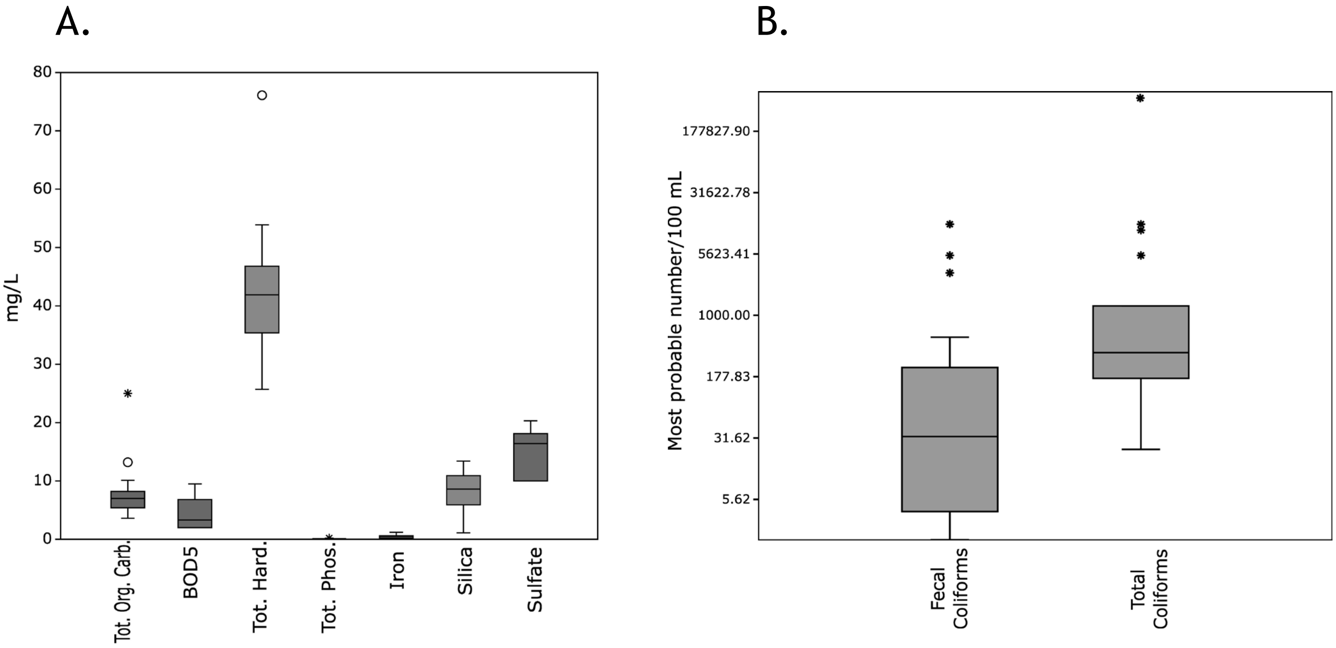


**Figure 2.** Box plots of the environmental variables selected in the ZSC. **A.** Physical and chemical variables. **B.** Bacteriological variables. The scale of the coliform bacteria data (total and fecal) is shown in logarithm to highlight the differences.

## The environmental gradient of the swamp complex

The arrangement of the 11 selected variables is illustrated in Figure 3. Iron and silica were the variables with the greatest weight in the analysis, which allows us to see several groupings of stations. In the first group, Caño Patón and Arroyo Alfaro were the most contaminated sites, having the highest amount of thermotolerant coliforms and iron. Stations UIMEP, Purgatorio, E22, Ciénaga Alfaro, Ciénaga González, Ciénaga Candelaria, Ciénaga Saloa, and Isla Grande, formed a second group characterized by more mineralized waters, all with higher total hardness, more silica and a moderate concentration of total coliforms. A third association corresponded to La Mata, Zona Litoral Barrancones and Sempegua, places that showed higher amounts of sulfates and $BOD_5$. Montecarlo, Sapatí, Puerto Macurutú, Belén, Bijagual, and E30 constituted the fourth grouping, with more transparent waters and lower concentrations of iron and fecal coliforms. It appears that this group had the cleanest waters. Thus, based on the PCA and field observations, four locations were selected as the best preserved: Puerto Macurutú, Belén, Bijagual, and E30. The four most degraded sites were Arroyo Alfaro, Caño Patón, Zona Litoral Barrancones, and Sempegua. The remaining sites (Ciénaga González, Ciénaga Candelaria, Ciénaga Saloa, La Mata, Sapatí, Ciénaga Alfaro, E22, UIMEP, Purgatorio, Isla Grande, and Montecarlo) had an intermediate degree of contamination.

## The ZSC phycoperiphyton and its functional traits on the deterioration gradient

The phycoperiphyton of the ZSC showed high heterogeneity and variability in structure. In total, 75 genera were found and 41 were present in both inverted microscope counts and morphological analysis photographs. The 35 unmeasured taxa belonged to genera that appeared only once or had abundances of less than 0.3%; the representation of these 35 taxa was only 1.46% in the total community. The most abundant phyla were Chlorophyta (green algae), Heterokontophyta (diatoms), and Cyanobacteriota (blue-green algae),

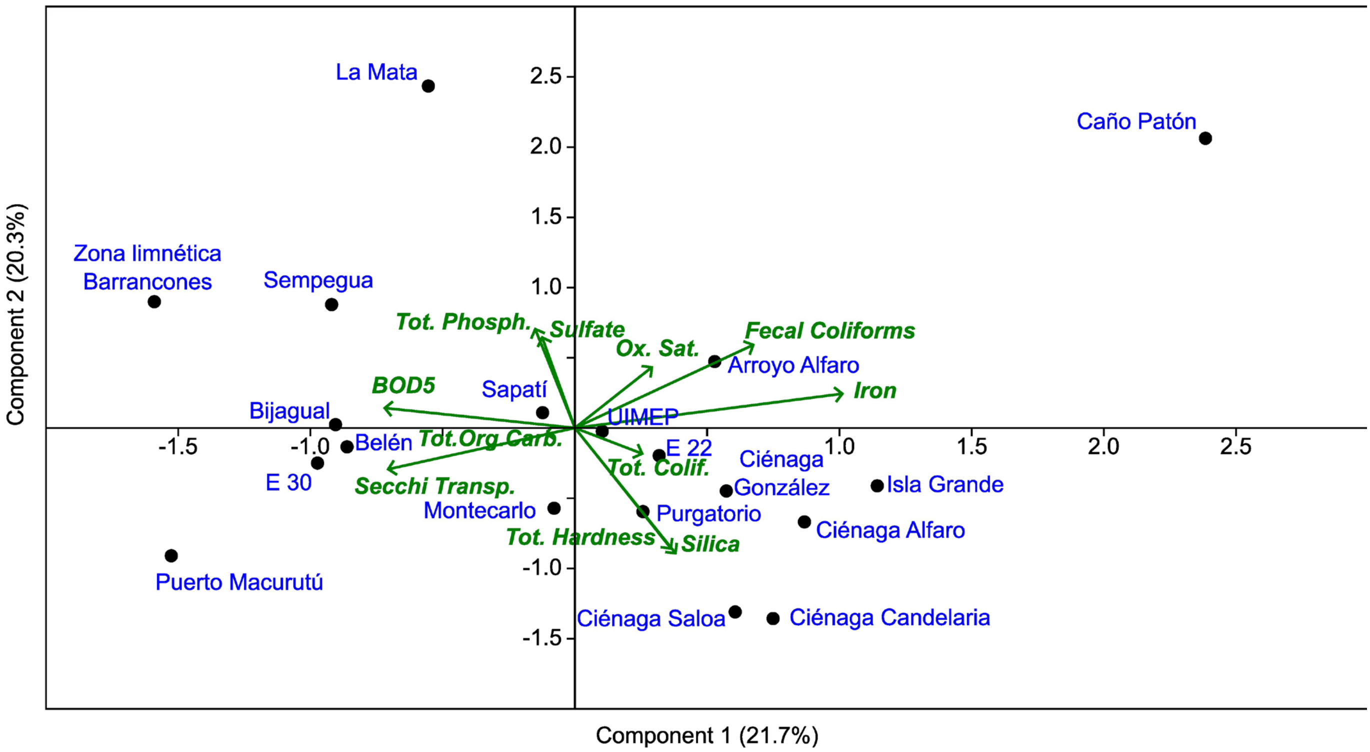


**Figure 3.** PCA of environmental variables. Eleven of the 28 variables evaluated were taken into account. The first three axes explained 59.4% of the variance. PC1: 21.7%; PC2: 20.3%; PC3: 17.4%.

with 53.6%, 24.4%, and 21.9%, respectively. The absolute abundances (ind/cm$^2$) of the 41 genera are presented as supplementary material (Table S2). The taxa *Aulacoseira* and *Oscillatoria* occurred at all sampling sites; *Aulacoseira*, *Cosmarium*, *Eunotia*, *Fragilaria*, *Nitzschia*, *Pinnularia*, and *Trachelomonas* were collected at more than 50% of the localities; *Ulnaria*, had highly variable abundances and was found at only six sites.

The comparison of the functional and categorical traits of the three sets of stations (reference, moderately polluted, and more degraded) for each attribute evaluated is presented in Figure 4. Maximum linear dimension (Figure 4A), biovolume (Figure 4B), and surface area (Figure 4C) tended to be lower at the reference sites, although without clear statistical differences. The surface area/biovolume ratio (Figure 4D) was similar in the three sets of stations. For their part, the presence of silica exoskeleton (Figure 4E), mucilage formation (Figure 4G), colony formation (Figure 4H), and the presence of aerotopes (Figure 4I) had lower medians at the reference sites, but without statistical differences with the other site groupings. However, the tendency for categorical traits to have higher and more variable values in the group of deteriorated sites was notable.

Spearman correlation analysis revealed only a statistically significant relationship between the presence of flagella and the second axis of the PCA (r= 0.5, *p*= 0.03) (Table 2). This axis was positively associated with fecal coliform concentration and iron (Figure 3). None of the other variables measured showed a significant correlation with the environmental axes evaluated. However, the presence of silica exoskeleton had a greater number of meaningful relationships with the other categorical attributes.

## Construction of the IBI-ZP: Selection of attributes using the Signal-to-Noise Index

The signal-to-noise index detects community attributes with the widest variations when

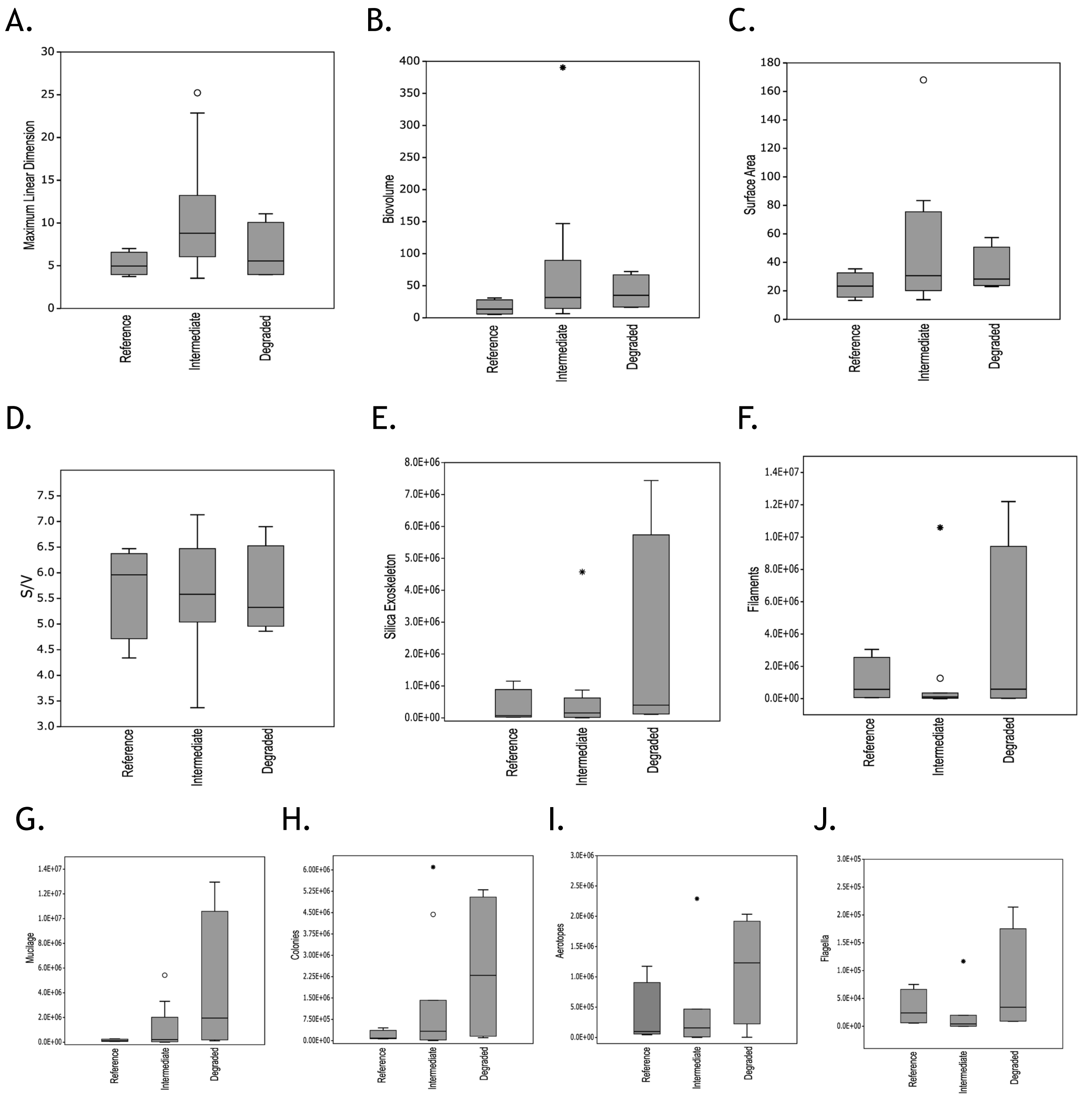


**Figure 4.** Comparative box plots between reference, intermediate, and degraded locations for morphological measurements and categorical traits. **A.** Maximum linear dimension (μm). **B.** Biovolume (μm$^3$). **C.** Surface area (μm$^2$). **D.** Surface area to biovolume ratio (μm$^{-1}$) **E.** Silica exoskeleton (n). **F.** Filament formation (n). **G.** Mucilage (n). **H.** Colony formation (n). **I.** Presence of aerotopes (n). **J.** Flagella (n).

compared. Biovolume was the morphological factor with the greatest variation, followed by maximum linear dimension and surface area (Figure 5A). The area/volume ratio presented minimal variability compared to the other attributes, making it impractical as a characteristic for the elaboration of the IBI-ZP. All the categorical variables presented sufficient variability to perform subsequent analyses (Figure 5B).

## Construction of the IBI-ZP: Discriminating Efficiency (DE) of candidate attributes

The discriminative efficiency was calculated for all

**Table 2.** Spearman correlations between functional traits and PCA ordination axes and between morphological and categorical traits

| | PC2 | MLD | Bv | Sa | Ex | Ff | Mu | Cf | Fl |
|---|---|---|---|---|---|---|---|---|---|
| PC2 | | 0.32 | 0.32 | 0.41 | 0.78 | 0.73 | 0.20 | 0.30 | 0.03* |
| Maximum linear dimension | -0.24 | | 1.16E-07* | 4.50E-08* | 0.39 | 0.57 | 0.47 | 0.20 | 0.81 |
| Biovolume | -0.24 | 0.90 | | 7.07E-13* | 0.23 | 0.82 | 0.57 | 0.30 | 0.48 |
| Surface Area | -0.20 | 0.91 | 0.98 | | 0.17 | 0.48 | 0.43 | 0.22 | 0.72 |
| Silica exoskeleton | 0.07 | 0.21 | 0.28 | 0.33 | | 0.0001* | 0.0005* | 0.0008* | 0.50 |
| Filament Formation | 0.08 | 0.14 | 0.05 | 0.17 | 0.76 | | 0.001* | 0.006* | 0.07 |
| Mucilago | 0.31 | 0.18 | 0.14 | 0.19 | 0.71 | 0.67 | | 1.72E-11* | 0.06 |
| Colony Formation | 0.25 | 0.30 | 0.25 | 0.30 | 0.70 | 0.60 | 0.97 | | 0.10 |
| Flagella | 0.50 | -0.06 | -0.17 | -0.09 | 0.16 | 0.41 | 0.44 | 0.38 | |

The upper triangle of data corresponds to the probability and the lower triangle to the correlation values. Significant values are highlighted with an asterisk (*$p \leq 0.05$). PC2: values of the second axis of the PCA, MLD: maximum linear dimension, Bv: biovolume, Sa: surface area, Ex: silica exoskeleton, Ff: filament formation, Mu: Mucilage, Cf: colony formation, Fl: flagella.

variables, except for the area/volume ratio, which was discarded due to its low variability (Figure 5A). As all attributes considered tended to increase as the pollution gradient increased (Figure 4), to calculate DE (Equation 1) the parameter *a* was assumed as the number of impacted sites with an attribute value above the 75th percentile in the reference localities and *b* correspond to the total number of impacted sites (Table 3).

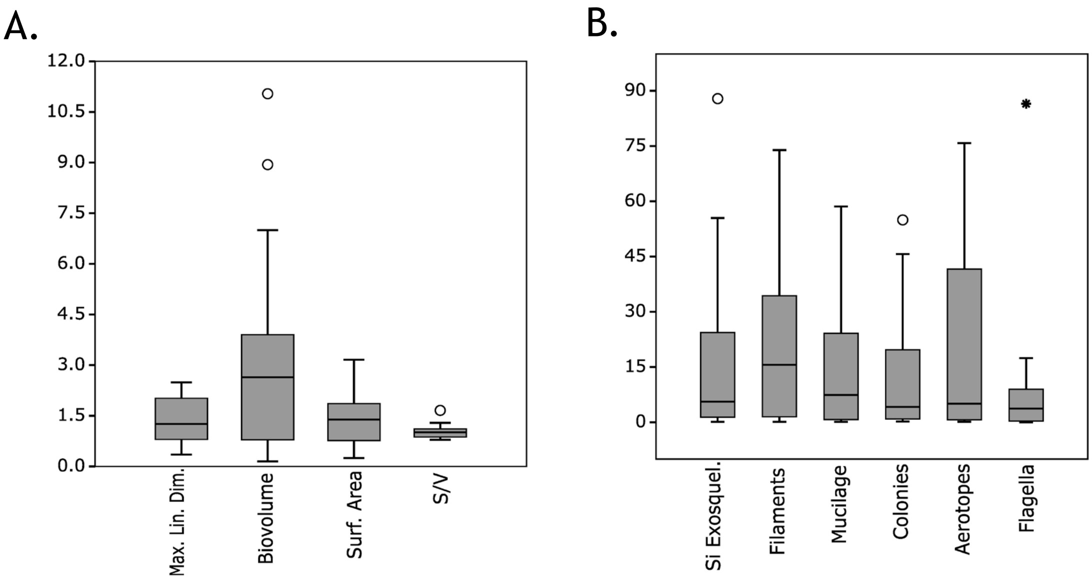


**Figure 5.** Comparative box plots of the signal-to-noise of functional features of ZSC phycoperiphyton. **A.** Morphological measurements. **B.** Categorical traits.

As shown in Table 3, filament formation had a low ED value (25%). For this reason, this characteristic was not taken into account for IBI-ZP.

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**Table 3.** Discriminative efficiency (DE) calculation of the morphological and categorical attributes of the ZSC phycoperiphyton community.

| Attribute name | Attribute behavior with increasing impairment | 75th percentile at reference locations | Equation used to calculate DE | Discrimination Efficiency (%) |
|---|---|---|---|---|
| Maximum linear dimension | Increase | 5.69 | (2/4)*100 | 50 |
| Biovolume | Increase | 21.92 | (2/4)*100 | 50 |
| Surface Area | Increase | 26.91 | (2/4)*100 | 50 |
| Silica exoskeleton | Increase | 356440.93 | (2/4)*100 | 50 |
| Filament Formation | Increase | 1562621.63 | (1/4)*100 | 25 |
| Mucilage | Increase | 195868.19 | (3/4)*100 | 75 |
| Colony Formation | Increase | 187280.82 | (3/4)*100 | 75 |
| Aerotopos | Increase | 368544.43 | (3/4)*100 | 75 |
| Flagella | Increase | 48660.36 | (2/4)*100 | 50 |

### Construction of the IBI-ZP: Standardization and scoring of selected attributes

According to the previous analyses, the attributes selected for the index were maximum linear dimension, biovolume, surface area, silica exoskeleton, presence of mucilage, colony formation, occurrence of aerotopes, and presence of flagella. As contamination increases, the functional traits also increase; so, to standardize these results, we used equation 3. The attributes were transformed to a 0-100 scale (Table 4).

**Table 4.** Standardized attributes by site. Averages of the set of attributes by sampling site are included

| Location | MLD | Bv | Sa | Ex | Mu | Cf | A | Fl | Average |
|---|---|---|---|---|---|---|---|---|---|
| Belén | 84.74 | 93.59 | 85.92 | 84.64 | 98.06 | 92.94 | 78.62 | 90.64 | 88.65 |
| Bijagual | 99.91 | 100.28 | 100.28 | 99.41 | 99.69 | 99.28 | 98.26 | 98.98 | 99.51 |
| E30 | 95.57 | 96.70 | 93.32 | 98.88 | 99.62 | 98.97 | 99.26 | 99.30 | 97.70 |
| Puerto Macurutú | 92.92 | 99.51 | 94.26 | 99.85 | 98.88 | 98.71 | 98.23 | 95.03 | 97.17 |
| Ciénaga González | 60.52 | 63.38 | 60.00 | 91.73 | 97.81 | 94.33 | 97.17 | 100.00 | 83.12 |
| Ciénaga Candelaria | 97.07 | 97.82 | 95.83 | 96.13 | 98.56 | 94.88 | 94.20 | 100.00 | 96.81 |
| Ciénaga Saloa | 55.84 | 82.64 | 73.31 | 88.41 | 84.60 | 76.98 | 58.28 | 0.00 | 65.01 |
| La Mata | 89.15 | 99.97 | 99.97 | 98.03 | 74.61 | 27.37 | 0.00 | 85.44 | 71.82 |
| Sapatí | 88.64 | 96.01 | 90.60 | 99.99 | 96.26 | 89.45 | 91.52 | 97.53 | 93.75 |
| Ciénaga Alfaro | 0.00 | 78.28 | 54.88 | 38.56 | 58.25 | 0.00 | 93.87 | 100.00 | 52.98 |
| E22 | 100.00 | 99.01 | 98.29 | 100.00 | 100.00 | 100.00 | 100.05 | 99.92 | 99.84 |
| UIMEP | 70.27 | 87.20 | 79.08 | 97.17 | 98.87 | 97.63 | 99.70 | 98.70 | 91.08 |
| Purgatorio | 79.85 | 96.10 | 89.28 | 99.91 | 99.92 | 99.96 | 99.85 | 98.16 | 95.38 |
| Isla Grande | 76.40 | 93.37 | 89.04 | 99.64 | 99.98 | 99.89 | 99.86 | 99.48 | 94.71 |
| Montecarlo | 11.05 | 0.00 | 0.00 | 99.44 | 99.92 | 99.28 | 98.25 | 99.89 | 63.48 |
| Sempegua | 98.38 | 96.85 | 94.02 | 91.71 | 96.99 | 95.28 | 83.84 | 98.90 | 94.50 |
| Zona limnética - Barrancones | 65.82 | 82.85 | 71.72 | 97.77 | 73.15 | 13.11 | 71.36 | 92.73 | 71.06 |
| Caño Patón | 84.56 | 88.16 | 89.12 | 98.71 | 99.30 | 98.61 | 99.99 | 98.74 | 94.65 |
| Arroyo Alfaro | 98.97 | 97.36 | 92.01 | 0.00 | 0.00 | 29.90 | 62.96 | 73.29 | 56.81 |

MLD: Maximum linear dimension, Bv: Biovolume, Sa: Surface area, Ex: Silica exoskeleton, Mu: Mucilage, Cf: Colony formation, A: Aerotopes, Fl: Flagella.

### Construction of the IBI-ZP: Establishment of rating scales

As suggested by Gerritsen et al. (2000), and taking into account the standardized attributes for each sampling site, the 25%, 50%, and 75% percentiles were calculated to obtain the IBI-ZP classification scales. Based on these data, four integrity classes were established for the index values (Table 5).

**Table 5.** IBI-ZP rating scale

| Value range | Color | Integrity classification |
|---|---|---|
| 0 ≤ IBI ≥71.43 | Red | Low biotic integrity |
| 71.43 < IBI ≤ 93.75 | Yellow | Regular biotic integrity |
| 93.75 < IBI ≤ 96.09 | Green | Moderate biotic integrity |
| IBI > 96.09 | Blue | High biotic integrity |

### Application of IBI-ZP to sites sampled in the ZSC

According to the classifications obtained (Table 5), we assigned the categories of biotic integrity of the phycoperiphyton to the sampled sites (Table 6).

The IBI-ZP enabled the classification of ZSC locations in a gradient, ranging from the most conserved sites with better ecosystem health to those where pollution implies greater deterioration, resulting in poorer ecosystem

**Table 6.** Index of Biotic Integrity of the phycoperiphyton community in the different sites of the ZSC according to the scales established in Table 5.

| Location | IBI-ZP | Interpretation of the IBI-ZP | Implications for ecosystem health |
|---|---|---|---|
| Ciénaga Saloa | 65.01 | Sites with LOW biological integrity of the phycoperiphyton community. | The poor physicochemical quality of the water makes it difficult and unfavorable for the periphytic algae community to develop well. The ecosystemic health of these sites is deficient. |
| Ciénaga Alfaro | 52.98 | | |
| Montecarlo | 63.48 | | |
| Zona limnética - Barrancones | 71.06 | | |
| Arroyo Alfaro | 56.81 | | |
| Belén | 88.65 | Sites with REGULAR biological integrity of the phycoperiphyton community. | The degree of water pollution causes a notable deterioration in the conditions for the development of periphytic algae. The ecosystemic health of these sites is insufficient. |
| Ciénaga González | 83.12 | | |
| La Mata | 71.82 | | |
| UIMEP | 91.08 | | |
| Sapatí | 93.75 | Sites with MODERATE biological integrity of the phycoperiphyton community. | The moderately polluted water conditions allow the periphytic algal community to develop. The health of the ecosystem at these sites is good. |
| Purgatorio | 95.38 | | |
| Isla Grande | 94.71 | | |
| Sempegua | 94.50 | | |
| Caño Patón | 94.65 | | |
| Bijagual | 99.51 | Sites with HIGH biological integrity of the phycoperiphyton community. | The high physicochemical water quality conditions favor an optimal development of the periphytic algae community. The health of the ecosystem at these sites is very good. |
| E30 | 97.70 | | |
| Puerto Macurutú | 97.17 | | |
| Ciénaga Candelaria | 96.81 | | |
| E22 | 99.84 | | |

health (Table 6). The sites with high biological integrity of phycoperiphyton were Bijagual, E30, E22, Puerto Macurutú, and Ciénaga Candelaria, characterized by more mineralized waters and natural zones around the water body. In contrast, in Ciénaga Saloa, Ciénaga Alfaro, Montecarlo, Zona Limnética Barrancones, and Arroyo Alfaro, the biological integrity of the phycoperiphyton was low. Spatially, it is notable that at least four of the sites with the highest IBI-ZP are located in the western sector, while stations with low and regular biotic integrity ratings are located in the eastern zone (Figure 6).

## DISCUSSION

### Environmental characteristics of the ZSC

The physicochemical and bacteriological conditions in the 19 zones of the ZSC were moderately

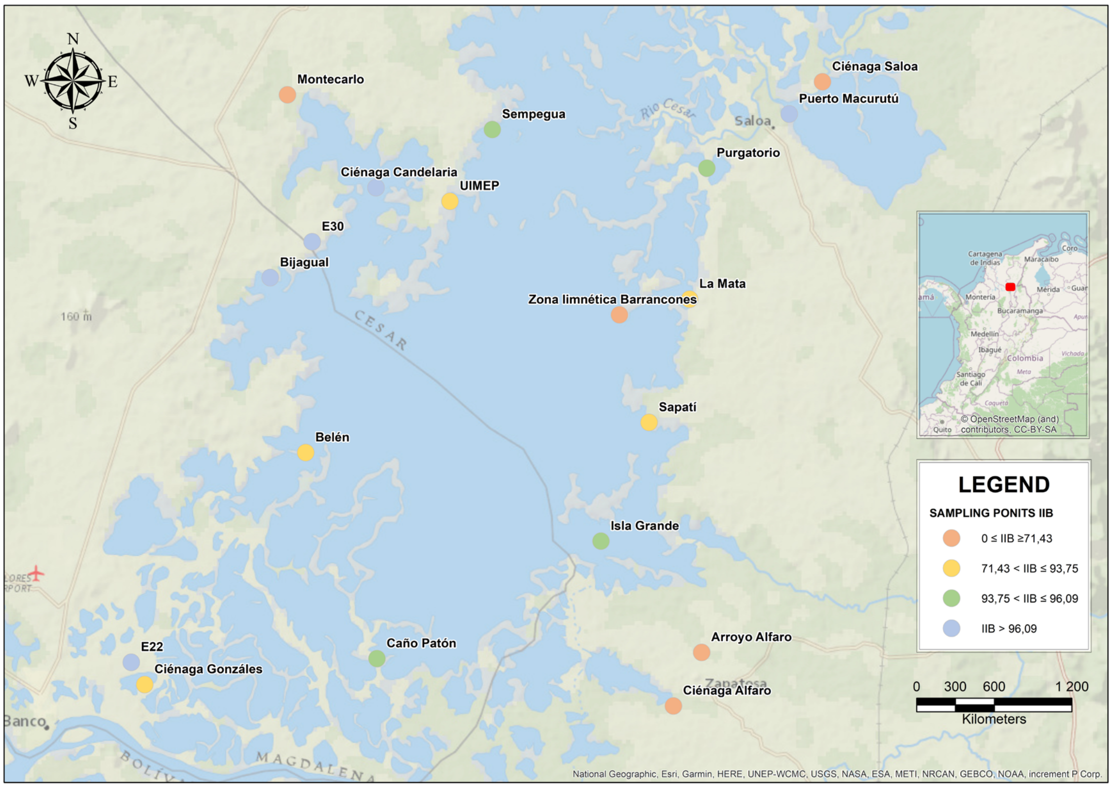


**Figure 6.** Classification map of the biotic integrity of phycoperiphyton in the ZSC. Color categories were assigned according to the scale established in Table 5. Base map: https://arcg.is/0kihb1.

heterogeneous. The results allow us to assume that there was a gradient of deterioration between the evaluated zones but without strong contrasts. This gradient was defined by the number of coliforms, including both thermotolerant and total, alongside the levels of oxygenation and mineralization of the water, primarily influenced by the concentration of iron, sulfates, and silica. The PCA results showed that the reference localities (Puerto Macurutú, Belén, Bijagual, and E30) exhibit better quality conditions. This can be attributed to their more natural land use, enhanced riparian vegetation cover, and lower levels of human activities. In contrast, in the most polluted areas (Arroyo Alfaro, Caño Patón, Zona Litoral Barrancones, and Sempegua), there is greater anthropic intervention focused on intensive cattle ranching and palm monocultures, as well as nearby urban centers. In these more degraded areas, larger mats of water hyacinth (*Eichhornia* spp.) were observed spread across a wider area on the water's surface. As is known, this genus of macrophytes is favored by a higher concentration of nutrients (Rodríguez-Lara et al., 2022).

### Functional features of ZSC phycoperiphyton

Periphytic algae of the ZSC tended to be very small, low in bulk, and small in surface area (Figures 4A, 4B, and 4C) at the better-preserved sites with superior-quality water. This trend has also been seen elsewhere, such as in oligotrophic lakes (AWWA, 2010; Kruk et al., 2017; Reynolds et al., 2002), and is a possible response to lower amounts of nutrients and more illuminated surface waters. Small algae grow faster, enabling better recovery from grazing. They also disperse more efficiently, aiding their movement within the periphyton matrix in search of nutrients (Dunck et al., 2015). At the most polluted sites, there was a greater abundance of algae with silica exoskeleton and genera that produce mucilage or form colonies. In the first case (siliceous exoskeleton), a significant number of diatom taxa, such as *Pinnularia* and *Navicula*, are associated with conditions of organic pollution and abundant nutrients, especially in areas close to urban settlements or rivers that flow through agricultural areas (Silva et al., 2022). In turn, specimens that produce mucilage and form colonies tend to thrive in saturated environments with sufficient resources (light and nutrients) and use these morphological adaptations to reduce or avoid predation (Kruk et al., 2010). The predominance of these colonial and mucilage-coated groups in the most degraded sites of the ZSC may be because many benthic diatoms attach to the substrate via mucilaginous stems (e.g., *Gomphonema*), produce gelatinous matrices (e.g., *Ulnaria*), and form colonies that attach to submerged surfaces (e.g., *Cymbella*) (Moresco & Rodrigues, 2010; Rimet & Bouchez, 2012). Some Chlorophyceae and cyanobacteria from eutrophic environments have the same strategies, as is the case with *Eudorina*, *Botryococcus*, *Dictyosphaerium*, *Aphanocapsa*, *Gloeocystis*, and *Microcystis* (Janse van Vuuren et al., 2006).

The statistical relationship between the presence of flagella and the PCA ordination axis representing microbiological contamination is due to the abundance of some Euglenophyceae taxa (*Phacus*, *Trachelomonas*) at the sites with the highest coliform concentrations. In this regard, it is well known that Euglenophyceae are associated with environments enriched with organic matter (polysaprobic condition) (Palmer, 1977), given their mixotrophic capacity (Nezbrytska et al., 2022). The areas where these algae were present were likely zones of wastewater reception. The high correlation between silica exoskeleton and other categorical traits is primarily due to its presence in the most abundant and frequent group of algae in the ZSC, which are diatoms.

## The index of biotic integrity of ZSC phycoperiphyton

To date, there have been few studies in Colombia regarding the use of morphometric and morphofunctional variables to assess periphytic and planktonic algae. This approach does not require knowledge of physiological traits, environmental conditioning, or taxonomic affiliation of the algae (Kruk et al., 2010). Instead, easily observable attributes, such as volume and the presence of mucilage, among others, are used. One such study was the functional classification of phytoplankton developed by Hernández et al. (2020) in six lentic systems in the Caribbean, Andean, and Amazon regions; these authors utilized the proposal developed by Kruk et al. (2010), which suggests the conformation of functional groups based on their morphology (FGBM). Another study was conducted by Guerrero Lizarazo et al. (2021), in which a classification of the periphytic algae of some rivers of the biogeographic Choco was made based on morphological and functional traits. These works attempt to evaluate the environmental condition of aquatic systems of different types, origins, and morphology grounded on the morphofunctional traits of microalgae. The study of functional ecology explains variations in microalgal communities founded on ecosystem environmental characteristics while also revealing the functional patterns of this group of organisms (Hernández et al., 2020).

Most of the algal morphofunctional traits selected for the construction of the IBI-ZP appear to be suitable for integrating the assessment of the biotic integrity of this community, as evidenced by the signal-to-noise index. Only the surface area/volume ratio (Figure 5) and filament formation (Table 3) were discarded; the first (S/V) had low variability, probably because algae with the largest surface area are also the most voluminous, so the S/V ratio tends to have low values close to unity. The presence of filaments appears to be a common characteristic among the periphytic algae of the ZSC. Many benthic taxa exhibit this behavior. Therefore, it is not a feature that distinguishes the different sites along the environmental gradient.

Three traits showed higher discriminatory efficiency (mucilage, colonies, and aerotopes) and four were moderate in their role in separating the most contaminated sites from the best-preserved ones (maximum dimension, biovolume, surface area, silica exoskeleton, and flagella) (Table 3). The environmental differences between the two groups of stations were not very marked, so the functional traits were also not too accurate in distinguishing the contamination gradient. Nevertheless, the results indicate that producing mucilage, forming colonies, and having aerotopes could be the most sensitive algal attributes to the deterioration gradient. The presence of mucilage increases with the chemical stress suffered by algae and constitutes a form of protection against higher degrees of mineralization (Nayaka et al., 2017). The formation of colonies and the appearance of aerotopes have more to do with buoyancy strategies, but the taxa that have these traits are also those that tolerate more polluted waters, as is the case of cyanobacteria such as *Aphanocapsa* and *Microcystis* (Sukharevich & Polyak, 2020).

According to Kerans and Karr (1994), to evaluate the attributes of a biological community and associate them with anthropogenic effects, it must be established whether these features vary in a statistically distinguishable way, whether they reflect aspects other than human impacts, and whether or not they are redundant. The high correlations between maximum linear dimension, biovolume, and surface area (Table 2) suggest a possible redundancy among these attributes; although they are related measures, each shows a different aspect of algal morphology. Thus, an alga may have a large linear dimension but a low biovolume (flattened cells). Similarly, spherical cells have a low surface area relative to biovolume, which may be high. Each of these morphological traits, in turn, is related to certain ecological processes, such as nutrient uptake, light uptake, buoyancy, and herbivory avoidance (Reynolds, 2006), all of which justify keeping these metrics in the analysis. The results of the present study show that some of the attributes selected for the IBI-ZP were statistically relevant but did not have sufficient discriminatory power. This was because the deterioration gradient was not as ostensible. The environmental conditions across the sampled sites in the swamp complex were generally similar, except for dissolved oxygen, water mineralization, and coliform bacteria number. Consequently, the reference sites were not sufficiently different from the impaired sites.

In the present study, reference sites were selected according to the "best available conditions" (Barbour et al., 1999) (i.e., those with the highest expected biological potential within the ZSC). Kerans and Karr (1994) state that in "less impacted" areas a significant difference in attribute distributions is expected when compared with impaired zones. Reference sites should provide a comparative basis for establishing changes in community structure relative to contaminated or stressed environments (Hellawell, 1986). Thus, reference conditions should accurately reflect the health of sustainable ecosystems (Gerritsen et al., 2000). However, in the ZSC it is practically impossible to find areas without anthropogenic intervention, so the reference sites established in our study did not have ideal ecosystem health conditions. This is one of the reasons why the ranking scales of the different degrees of biotic integrity of the phycoperiphyton had high values (e.g., the low integrity category reaches 71.43 points; Table 5). To solve this situation, it will be necessary to look for reference sites of better quality, even in marshes near or outside the ZSC, or to determine the reference conditions through consultations with experts to establish the optimal values of the physical and chemical variables of the water and the ideal characteristics of the functional traits of phycoperiphyton in a hypothetically pristine swamp ecosystem, within the studied region.

The mapping of the IBI-ZP results (Figure 6) shows that the swamp complex has two large sectors. The western region, with a certain tendency to show better ecosystemic health, is associated with self-consumption cattle ranching, has surrounding areas with less anthropic intervention, and presents more natural conditions. In contrast, the eastern zone is associated with a much greater anthropic influence, having several urban settlements with land used for intensive cattle ranching and palm monocultures.

According to the IBI-ZP developed, two-thirds of the sites evaluated were classified as areas with low biotic integrity. The limited range of the index's scales causes this. As stated above, this is probably because the reference sites were not sufficiently different in their environmental variables compared to the other sites in the swamp complex. Of course, the proposed IBI-ZP serves as a preliminary estimate based on limited data and simplifies the ecological complexity of the ZSC.

The hydrological cycle of the swamp complex was not fully considered, as samples were only collected during the falling water period. Future studies should include samples from all stages of the water cycle: low water, rising water, and high water. This approach will enhance our understanding of the environmental conditions in the ZSC, offering a clear picture of the deterioration phenomena that may be affecting the water body, as well as the overall functioning of the ecosystem. By implementing this monitoring, the IBI-ZP can be refined and tested, resulting in a more effective tool that offers greater assessment capabilities (Barbour et al., 1999). Such ecological valuation indices provide a rapid evaluation of the overall condition of an aquatic ecosystem, and their results should be easily understandable for water resource managers (Hill et al., 2000).

The IBI-ZP, like any other tool, must be employed appropriately. It is designed to be used only when the objective is to monitor biotic integrity at specific sites. It is effective for assessing numerous sites to identify those needing attention, evaluate trends over time at each site, and analyze the impact of specific human activities on water resources. These assessments should be complemented by bioassays and validations using additional data sets (Kerans & Karr, 1994). Similarly, tolerance and intolerance responses of periphytic algal taxa to factors such as the amount of organic matter, nutrient concentration, and acidity or basicity conditions need to be studied, as these characteristics could become metrics that could be integrated into the proposed biotic index.

Phycoperiphyton is a community of the first trophic level (primary producers) and, because of its short and rapid life cycles, often indicates early or recent effects on the aquatic system. Such effects are directly reflected in the algal response, unlike other consumer communities, such as macroinvertebrates or fish, for which environmental effects are delayed and mediated by other levels of the trophic structure of the ecosystem. Thus, the phycoperiphyton community reliably indicates short-term phenomena (weeks). To accurately assess deteriorating conditions over extended periods, such as months or years, it is essential to use suitable indicators for these time frames, including macroinvertebrates, fish, and macrophytes.

The proposed index was designed for the genus level, but it could be more effective if algae identification were conducted at the species level. However, there are significant difficulties in reaching this level, as expensive equipment (e.g., electron microscopes) and specialized taxonomic and technological knowledge (e.g., genetic identification) are required. Loss of information must be addressed when using fewer fine levels of classification (Barbour et al., 1999; Feio et al., 2009; Karr et al., 1986). It can be thought that the ecological preferences within the species of each genus are similar. While this may hold for some taxa, it does not apply universally (Feio et al., 2009). In this context, evaluating the functional and structural characteristics of algal communities, as proposed here, can help address some of the taxonomic challenges (Barbour et al., 1999).

## CONCLUSIONS

The IBI-ZP developed made it possible to determine the biotic integrity of the periphytic algal community of the ZSC and to preliminarily assess the ecological status of different zones of the Zapatosa swamp, but it needs to be complemented with continued studies in different periods of the hydrobiological cycle. In this way, it will be possible to make the necessary adjustments according to the different dynamics of the swamp complex.

To enhance the efficiency of the proposed IBI, it is essential to identify reference sites that possess more natural conditions. This involves exploring additional sampling locations not examined in this study, or considering nearby swamps more isolated from human activities with similar ecological characteristics.

The developed index is a tool designed to identify areas where ecosystem health is deteriorating. It can help in making timely and appropriate management decisions that benefit both the biological communities within the ecosystem and the human populations that rely on this vital water system in the country.

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## AUTHOR CONTRIBUTION

MCGL conducted the fieldwork and laboratory analyses. Both MCGL and GPA carried out the statistical analyses and contributed to drafting and critically revising the manuscript, enhancing its intellectual content.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## SUPPLEMENTARY MATERIAL

Available at: Supplementary Material

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