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Articles

Epilithic diatoms as bioindicators of water quality in the Pachanlica River, Ecuador

Diatomeas epilíticas como bioindicadores de calidad hídrica en el río Pachanlica, Ecuador

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Abstract

Diatoms have been recognized as effective bioindicators of water quality due to their sensitivity to physicochemical changes and rapid response to environmental perturbations. In Europe, their use is standardized for the ecological evaluation of rivers, and in South America they have been applied in Andean rivers in Colombia and Peru. However, in Ecuador, their study is still limited, which generates the need to investigate their applicability in Andean ecosystems. The objective of this study was to analyze the use of epilithic diatoms as bioindicators of water quality in the Pachanlica River in Ecuador, selecting 14 sampling points distributed altitudinally in high, medium and low zones. Physicochemical, chemical and microbiological parameters were evaluated using multiparameter probes and spectrophotometry, following standardized protocols for the determination of nutrients, chlorophyll a and diatom communities. The results showed oligotrophic conditions in the upper zone and mesotrophic conditions in the middle and lower zones of the sub-basin, with a decrease in water quality in the lower zone due to wastewater discharges and agricultural activities. The index of specific sensitivity to pollution showed the tolerance of the dominant diatom species to pollution, while the low diversity at critical points indicated a progressive ecological deterioration. Although diatoms reflect the biological quality of water, they can overestimate its general quality from an ecological approach, generating contradictions with chemical indicators and limiting their use in the evaluation of quality under public health criteria in highly intervened systems.

Keywords: Water quality, water pollution, algae, eutrophication, rivers, Pachanlica, Ecuador.

Resumen

Las diatomeas han sido reconocidas como bioindicadores efectivos de la calidad del agua debido a su sensibilidad a cambios fisicoquímicos y rápida respuesta a perturbaciones ambientales. En Europa, su uso está estandarizado para la evaluación ecológica de ríos, y en Sudamérica han sido aplicadas en ríos andinos de Colombia y Perú. Sin embargo, en Ecuador su estudio es aún limitado, lo que genera la necesidad de investigar su aplicabilidad en ecosistemas andinos. Este estudio tuvo como objetivo analizar el uso de diatomeas epilíticas como bioindicadores de calidad del agua en el río Pachanlica en Ecuador; se seleccionaron 14 puntos de muestreo distribuidos altitudinalmente en zonas alta, media y baja. Se evaluaron parámetros fisicoquímicos, químicos y microbiológicos mediante sondas multiparamétricas y espectrofotometría, siguiendo protocolos estandarizados para la determinación de nutrientes, clorofila a y comunidades de diatomeas. Los resultados evidenciaron condiciones oligotróficas en la zona alta y mesotróficas en las zonas media y baja de la subcuenca, con una disminución de la calidad del agua en la zona baja debido a descargas de aguas residuales y actividades agrícolas. El índice de sensibilidad específica a la polución evidenció la tolerancia de las especies dominantes de diatomeas a la contaminación, mientras que la baja diversidad en puntos críticos indicó un deterioro ecológico progresivo. Aunque las diatomeas reflejan la calidad biológica del agua pueden sobreestimar su calidad general desde un enfoque ecológico, lo cual genera contrariedades con los indicadores químicos y limita su uso en la evaluación de la calidad bajo criterios de salud pública en sistemas altamente intervenidos.

Palabras clave: calidad del agua, contaminación del agua, algas, eutroficación, ríos, Pachanlica, Ecuador.

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Introduction

Water quality is critical to ecosystems and public health, as it directly influences the environment and people. Physicochemical, biological, and microbiological assessments provide a detailed view of the state of water bodies, accurately identifying contaminants. However, many contaminants go undetected using traditional methods, highlighting the need for new alternatives (Pauta *et al.*, 2019). In this context, the use of diatoms is presented as an advanced methodology, complementing conventional techniques and providing essential tools for sustainable management of water resources (Castillejo *et al*., 2024).

Various methods are applied for the analysis of water quality, including tools that reflect the ecological state and its variation in response to pollution, as well as standardized methods that allow the comparison of different ecosystems (Confederación Hidrográfica del Ebro, 2005). Bioindicators, like certain living organisms, can be related to the health of an ecosystem based on their presence (Díaz-Quirós & Rivera-Rondón, 2004). The alteration in the number of organisms, their disappearance or changes in their behavior, may be related to the effects of environmental stress on the ecosystem (Segura-García, Almanza, & Ponce-Saavedra, 2016). This relationship can be studied to explain the conditions of an aquatic ecosystem and to demonstrate pollution processes.

Epilithic diatoms have proven to be effective bioindicators due to their sensitivity to physicochemical changes, especially to the increase in nutrients such as nitrogen and phosphorus, responsible for organic pollution and eutrophication (Díaz-Quirós & Rivera-Rondón, 2004). These unicellular eukaryotic microalgae are abundant in lotic ecosystems and are part of the food chain, being food for numerous organisms (Céspedes-Vargas, Umaña-Villalobos, & Silva-Benavides, 2016). Their high renewal rate, ease of collection, handling and analysis make them good bioindicators of short-term disturbances (Pardo, García, Delgado, Noemi, & Abrain, 2010).

The present study aims to analyze the use of epilithic diatoms as bioindicators of water quality in the Pachanlica River, Ecuador.

Materials and methods

Study area

The Pachanlica River is located in the province of Tungurahua, beginning in the foothills of the Carihuairazo, Chimborazo, and Igualata knot volcanoes. It has an area of 397.60 km^2, a perimeter of 110.41 km, and a maximum length of 39.00 km. The source of the river occurs at the coordinates 1° 14' 8" S and 78° 32' 57" W, to flow into the Ambato River at 1° 27' 1" S and 78° 44' 52" W, contains the Mocha River, which later forms the Quero River and later takes the name of Pachanlica River, a total stretch of 49.23 km.

The microbasin has elevations ranging between 2 200 and 6 200 meters above sea level (m.a.s.l.) and is characterized by a temperate-humid climate, with an average temperature of 15°C and an annual rainfall ranging between 400 and 600 mm (HGPT, 2019). Figure 1. The river receives both domestic and industrial wastewater discharges from the cantons of Mocha, Quero, Cevallos, Ambato and Pelileo, which together are home to more than 71 266 inhabitants (INEC, 2022).

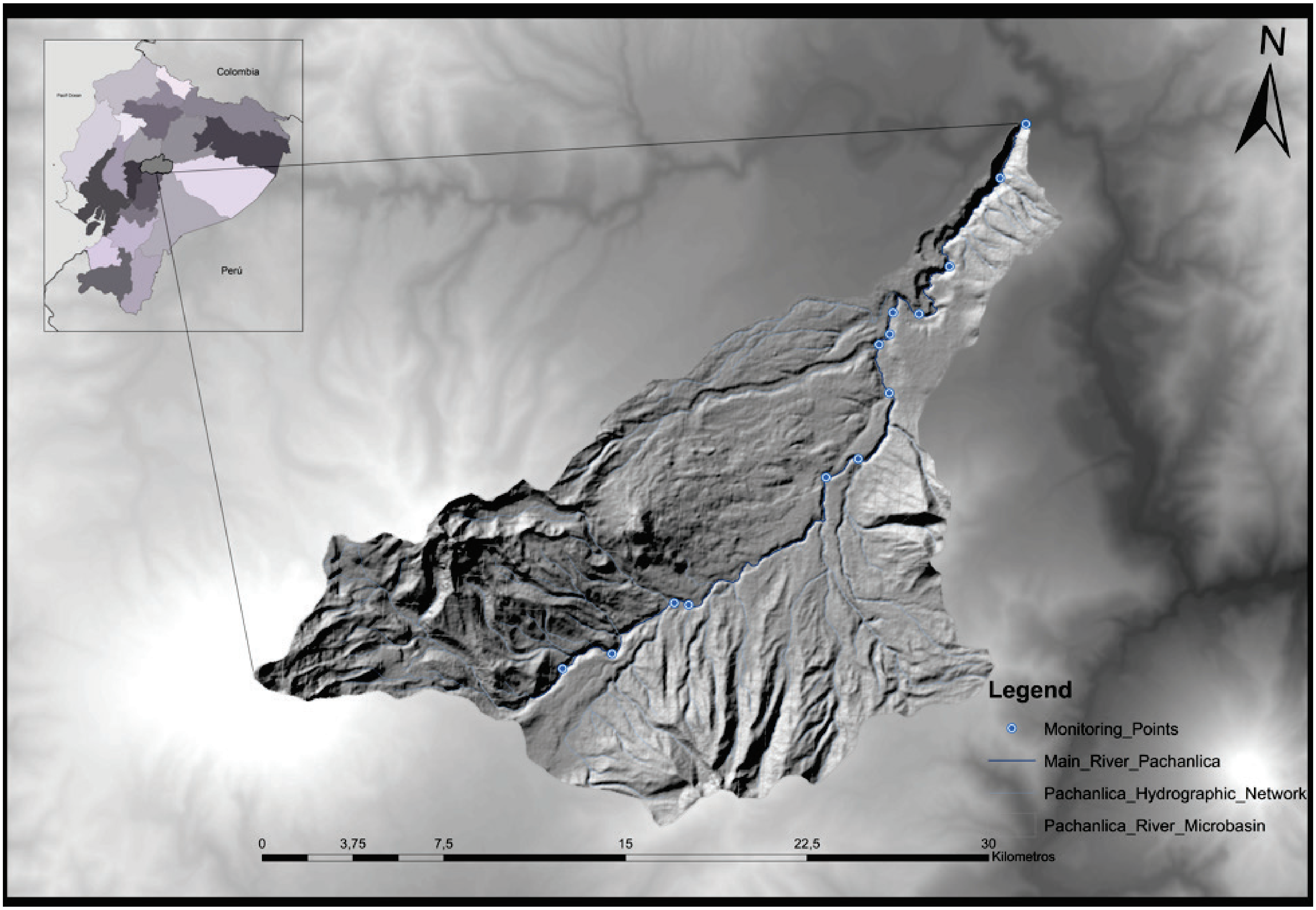

Figure 1. Monitoring points in the Pachanlica River microbasin. Source: Prepared by the authors using ArcGIS (version 10.4).

Collection of water and diatom samples

Fourteen monitoring points were randomly selected along the 37.73 km of the main channel of the Pachanlica River, segmenting the basin into the upper zone (4 points), the middle zone (3 points), and the lower zone (7 points). Factors such as accessibility, the presence of wastewater discharges, along with geomorphological and hydrological aspects, guided the selection to ensure adequate coverage (Larrea-Murrel, Romeu-Alvarez, Lugo-Moya, & Rojas-Badía, 2022).

Field sampling was carried out between November 2023 and April 2024, covering the transition and dry seasons (INAMHI, 2017). The sampling carried out followed excellent monitoring practice protocols established by the Instituto Ecuatoriano de Normalización (INEN, 2014) in the NTE INEN-ISO 5667-1 standard, recording the results in logbooks and chains of custody prior to the samples being submitted to the laboratory. During periods of transition and drought, river flow is usually lower, which facilitates the adhesion of epilithic diatoms to the rocks and minimizes their transport to other sites due to the decrease in water flow (Hurtado & Arias-Real, 2024).

In situ physicochemical parameters such as pH, temperature, electrical conductivity, and dissolved oxygen were measured in the field using HANNA multiparameter probes, model HI98199, after calibration. Flow rate was measured using a Global Water model FP11 flow probe. Diatom samples were collected at a depth of 20 to 50 cm by scraping the epilithic material from 4 to 5 rocks per site located on the riverbed with hard-bristled brushes and storing them in dark glass jars with 4 % formalin for preservation. Oxidation with 50 % hydrogen peroxide (H_2O_2) and controlled heating (70-90 °C) was then applied to remove organic matter and facilitate observation of valve ornamentation, following the protocol described by Céspedes-Vargas *et al*. (2016).

Water sample collection followed NTE-ISO 5667, which includes the use of 1 000 ml amber bottles, which were washed with distilled water and rinsed with river water during collection. The refrigerated samples were transported to the water resources laboratory for further analysis within 24 hours.

Nutrient and COD analysis

Nutrient and chemical oxygen demand (COD) analysis was performed spectrophotometrically using HACH model DR3900 equipment. Nutrient analysis used a 10 ml sample and HACH brand reagents for nitrates (Nitra Ver 5®), nitrites (Nitri Ver 3®), and phosphates (Phos Ver 5®). COD analysis used specific vials and a HACH DRB 200 digester. The sample was pre-digested for 2 hours before measurement. Spectrophotometric measurement absorbances for nitrates, nitrites, phosphates, and COD were 400, 507, 880 and 620 nm, respectively.

Determination of chlorophyll

The samples were transferred to filtration equipment with 0.47 µm glass microfiber filters. 200 ml of contaminated water and 2 000 ml of clean water were filtered until the filter was saturated. The folded filter was then placed in 15 ml Falcon tubes, 10 ml of 90 % acetone was added, the tube was sealed, and it was covered with aluminum foil. The tubes were refrigerated for 18 hours. Afterwards, the tubes were brought to room temperature and were centrifuged at 3 000 rpm for 20 minutes. Spectrophotometric measurements were performed on a Thermo Scientific Evolution 201 UV-Visible spectrophotometer (Madison, WI, USA), calibrated with 90 % acetone as a blank, where absorbances were read at 750, 664, 647, and 630 nm.

To calculate chlorophyll a concentration in water, equation (1) by Jeffrey and Humphrey (1975) was used:

$$Cl\ a\ (mg/m3) = V_e * \frac{[(11.85 * (A_{664} - A_{750})) - (1.54 * (A_{647} - A_{750})) - (0.08 * - (A_{630} - A_{750}))]}{(V_f * L)} \quad (1)$$

Where:

V_e = volume of the acetone extract in ml

V_f = volume of filtered water in liters

A_{664} = optical density of the extract at 664 nm

A_{750} = optical density of the extract at 750 nm

A_{647} = optical density of the extract at 647 nm

A_{630} = optical density of the extract at 630 nm

Diatom identification

Diatom samples were prepared on permanent slides and observed with an AmScope T660 optical microscope with an 18 megapixel (MP) digital camera (AmScope MU1803). For diatom species identification and counting, parameters such as frustule count and community structure of diatoms were analyzed in 0.2 ml of sample (Krammer & Lange-Bertalot, 1986). Identification was performed primarily using the Duero Basin diatom guide (Blanco *et al*., 2011), validated by the Spanish Ministry of the Environment, and also integrated South American guides, such as the illustrated guide for Brazilian subtropical and temperate lotic systems (Lobo *et al*., 2016).

Ecological indices

The Shannon-Wiener Index was used to measure ecological diversity, and the Margalef Index was used to determine species richness. Both indices were calculated using PAST 4.03r® software (Hammer, Harper, & Ryan, 2001). The interpretation of the Shannon and Margalef Index was carried out according to the Moreno (2001) scale.

Evaluation of the IPS and TSI

The specific pollution sensitivity index (SPI) was calculated by the relationship between the relative abundance, sensitivity, and ecological tolerance of diatoms, using Equation (2), based on the calculation of Zelinka (1961), but proposed by Blanco *et al*. (2010):

$$IPS = \frac{S*(A_j*S_j*V_j)}{S*(A_j*V_j)} \quad (2)$$

Where:

IPS = specific pollution sensitivity index

S = sensitivity to pollution that maintains values between 1 and 5

V = ecological amplitude or tolerance maintains values between 1 and 3

A = relative abundance

To interpret the IPS values in relation to water quality, the scale proposed by Blanco *et al*. (2010) was used, in which a value between 17 and 20 indicates very good water quality, 13 to 17 indicates good quality, 9 to 13 suggests moderate quality, 5 to 9 indicates poor quality, and 1 to 5 indicates very poor quality.

To determine the trophic state index (TSI) in the Pachanlica River, the concentration of chlorophyll a was used, applying Equation (3) proposed by Carlson in 1977 and modified by Carlson-Aizaki (Moreno, Quintero, & López, 2010):

$$TSI_{Cla} = 10 * \left(2.46 + \frac{Ln(Cla)}{Ln(2.5)}\right) \quad (3)$$

TSI values were classified into four categories: oligotrophic (0-30), mesotrophic (30-60), eutrophic (60-90), and hypertrophic (90-100), each reflecting increasing levels of nutrients and phytoplankton, up to severe pollution.

Statistical analysis

To assess the influence of nutrients on the presence and abundance of certain diatoms, canonical correspondence analysis (CCA) was used. This analysis, recommended by Calizaya (2013), was calculated using Past 4.03® software. CCA is particularly useful for understanding how variations in nutrients affect the composition of diatom communities.

Additionally, Pearson's correlation coefficient was used to analyze the relationships between the Shannon and Margalef ecological indices and the nutrients present in the water. This analysis was performed using Statgraphics software version 19-X64® (Statgraphics Technologies, Inc., 2023).

Results

Physicochemical parameters

The river's pH ranged between 7.22 and 8.39, with a coefficient of variation (CV) of 3.71 %, suggesting high uniformity in this parameter along its course. The temperature ranged between 10.82 and 18.21 °C, with a mean of 14.69 °C and a CV of 12.58 %, indicating moderate thermal variability among the sampled areas.

Electrical conductivity, with a CV of 44.78 %, reflects greater variability and possible differences between zones, with values between 146 µS/cm for the higher zone and 684 µS/cm for the lower zone. Dissolved oxygen had a CV of 15.75 %, which also denotes variability between its values, 3.33 and 6.17 mg/l. Figure 2 shows the mean values and CV of the physicochemical parameters analyzed.

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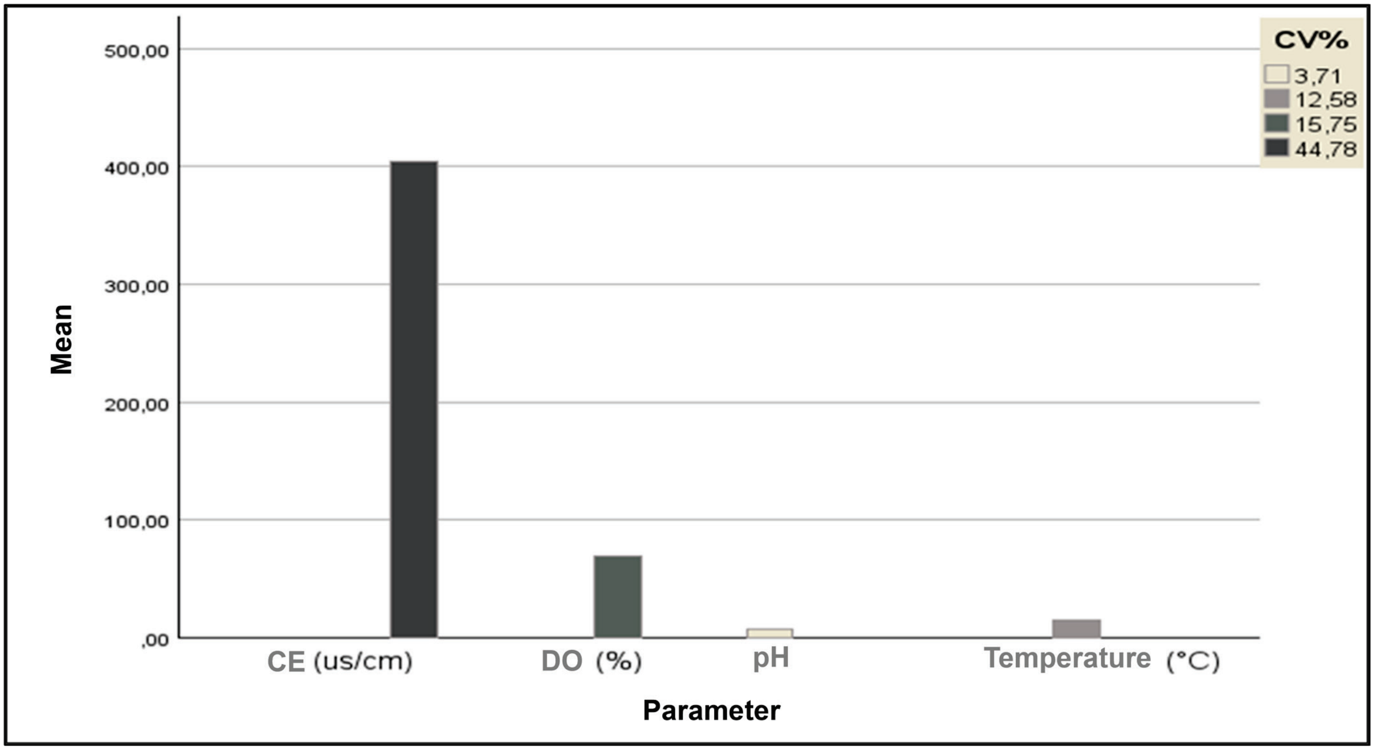

Figure 2. Mean and CV values of the physicochemical parameters analyzed. Note: Salinity is excluded due to its low magnitude. Source: Prepared by the authors using SPSS (version 29).

Salinity had a CV of 44.77 %, with larger fluctuations between 0.08 and 0.53 PSU. Finally, the flow parameter showed a CV of 44.54 %, reflecting high variability in flows, with values ranging from 0.37 in the upper zone, 0.17 in the middle zone, and 0.85 m^3/s in the lower zone.

Nutrient and COD analysis

Nitrite and nitrate concentrations increased in the middle and lower zones of the river, with a maximum of 1.72 mg/l at point P6 for nitrites and 28.00 mg/l for nitrates at point P13. Phosphates varied in the middle zone, with maximum concentrations of 47.90 and 74.70 mg/l observed at points P5 and P6, near agricultural and urban areas.

The COD increased throughout the sampling site transition. The upper zone (point 1) had a value of 19 mg/l, indicating good water quality and low industrial and urban intervention. In contrast, the lower zone obtained values of up to 100 mg/l at point P14, representing an area of high ecological risk (Emmanuel, Lacou, Balthazard-Accou, & Joseph, 2009). The analysis showed that points P5 and P6 had values between 116 and 155 mg/l (Figure 3).

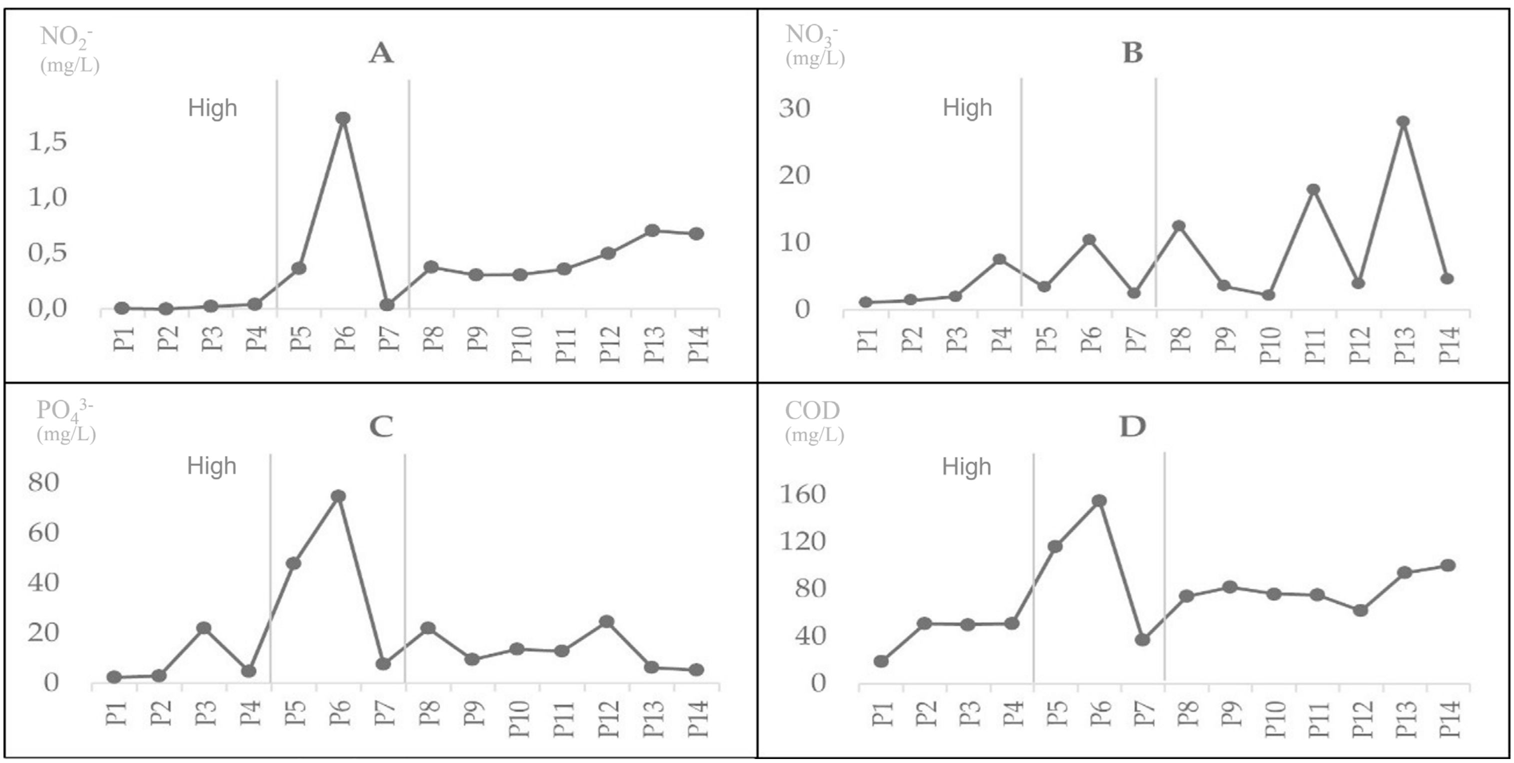

Figure 3. Chemical parameter values at 14 Pachanlica River monitoring points. A) Nitrites in mg/l, B) Nitrates in mg/l, C) Phosphates in mg/l, D) COD in mg/l. Source: Prepared by the authors using SPSS (version 29).

Determination of chlorophyll

Chlorophyll a concentration showed a progressive increase from the upper to the middle part of the MCP. At point P1, a concentration of 0.47 mg/m³ was recorded, increasing to 8.97 and 10.79 mg/m³ at points P5 and P6, respectively, associated with the influence of wastewater discharges. Subsequently, at point P7, a decrease to 1.30 mg/m³ was observed. However, in the middle-low zone, concentrations increased significantly, reaching values of 43.66 mg/m³ (P8), 46.84 mg/m³ (P9), and a maximum of 96.24 mg/m³ at point P10. Finally, at points P11 to P14, concentrations decreased to 9.29 mg/m³ and remained stable towards the mouth, as shown in Figure 4.

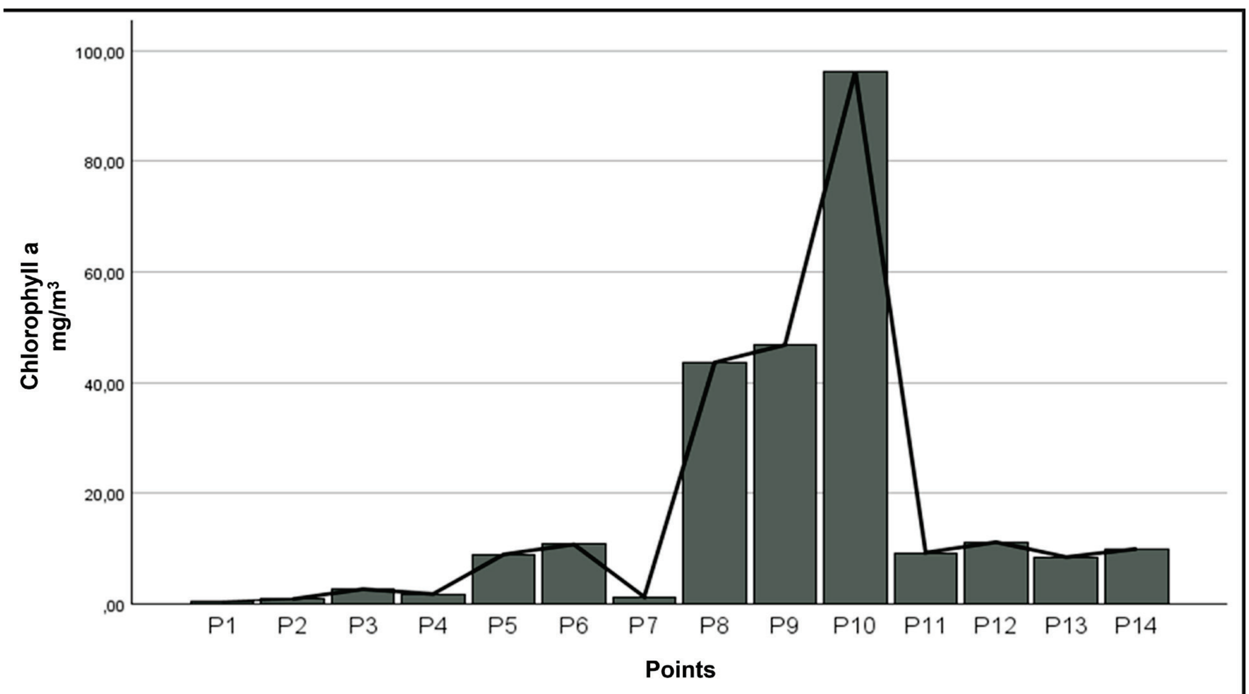

Figure 4. Chlorophyll a values at 14 monitoring points on the Pachanlica River. Source: Prepared by the authors using SPSS (version 29).

Diatom identification

A total of 3 900 diatoms were counted at the 14 sampling points in the Pachanlica River. A total of 44 diatom species were identified, of which the highest absolute abundances in the microbasin were *Cocconeis lineata* COLI (41.90 %), *Achnanthidium minutissimum* ACMI (15.72 %), *Pseudostaurosira brevistriata* PSBR (11.31 %), followed by *Nitzschia inconspicua* NIIN (4.62 %) and *Brachysira vitrea* BRVI (4.26 %). 15 species of diatoms showed a relative abundance greater than 5 %, representing 75.61 % of the community. These species included *Achnanthidium minutissimum* (ACMI), *Brachysira vitrea* (BRVI), *Cocconeis lineata* (COLI), *Encyonopsis subminuta* (ENSU), *Fragilaria rumpens* (FRRU), *Gomphonema lagenula* (GOLA), *Gomphonema pumilum* (GOPU), *Navicula erifuga* (NAER), *Navicula lanceolata* (NALA), *Nitzschia filiformis* (NIFI), *Nitzschia inconspicua* (NIIN), *Nitzschia palea sensu lato* (NITE), *Pseudostaurosira brevistriata* (PSBR), *Rhoicosphenia abbreviata* (RHAB) and *Nitzschia palea var. tenuirostris* (NIPA).

Using a CCA analysis, the affinity of diatoms with physicochemical gradients was sought. It was observed that the species NITE, NIPA, FRRU, and NIFI clustered with the parameters pH, COD, and phosphates, while NALA showed affinity for areas with higher phosphate concentrations. The species RHAB and ENSU were in the center of the graph, indicating tolerance to pollution. Meanwhile, the species PSBR, BRVI, NIIN, ACMI, and GOPU showed low affinity for eutrophic environments. In contrast, COLI was in the same quadrant as nitrites. Finally, GOLA and NAER were associated with high levels of chlorophyll a (Figure 5).

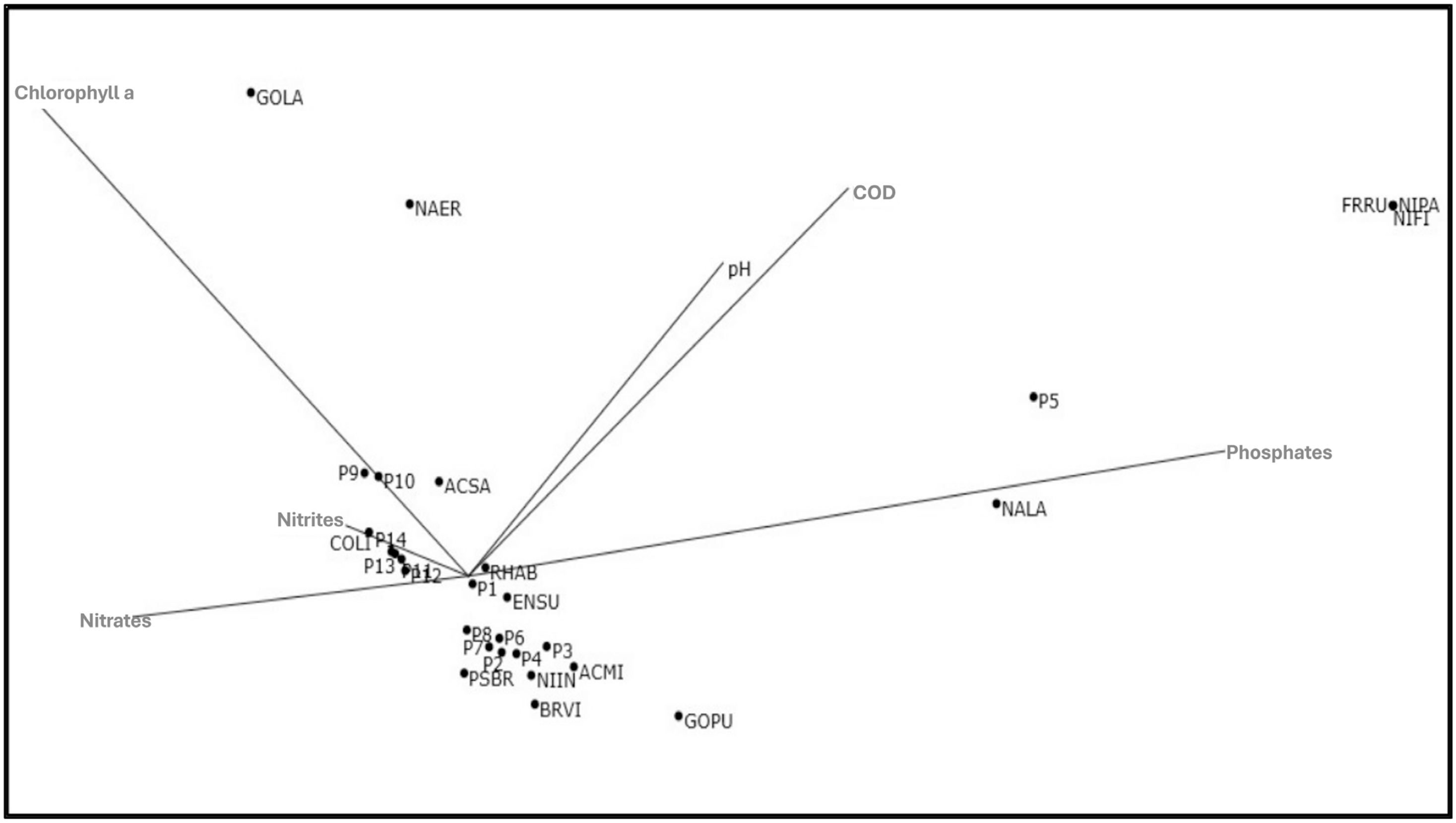

Figure 5. Canonical correspondence analysis for 14 sampling points, diatom species, and variables such as pH, phosphates, nitrates, nitrites, COD, and chlorophyll obtained in the Pachanlica River.

Ecological indices

The results of the Shannon index showed variability across the different sampling points. Low diversity was observed at points P1, P3, and P7, with values of 1.63, 1.93, and 1.91, respectively, and at points P9 to P14, whose values ranged from 0.73 to 1.55. On the other hand, points P2, P4, P5, P6, and P8 showed medium diversity, with values of 2.31, 2.07, 2.70, 2.22, and 2.07, respectively. Point P5 stands out with the highest value of 2.70, reflecting greater diversity compared to other points, while point P9 recorded the lowest value of 0.73, characterized by the predominance of the COLI species.

The results of the Margalef index reflected a variability in species diversity similar to that observed with the Shannon index. Low diversity was recorded at points P1, P7, and P9, with values of 1.71, 1.71, and 0.86, respectively. Similarly, at points P11 to P14, fluctuations were observed between 1.81 and 1.85. In contrast to these sampling points, points P2 to P6 and P8 and P10 showed medium diversity, with values between 2.01 and 3.72.

Table 1 shows that the Margalef index showed a moderate positive correlation with pH (0.40). In contrast, the Shannon index showed weak negative correlations with chlorophyll a (-0.34), COD (-0.11), nitrite (-0.23), and nitrate (-0.28). The positive correlation between chlorophyll a and COD (0.60), as well as nitrite (0.59) and phosphate (0.58), indicates elevated nutrient levels. The high positive correlation between nitrite and nitrate (0.77), as well as between nitrite and COD (0.80), shows that these nutrients have a limited influence on diatom diversity, as evidenced by the low correlations with the Shannon and Margalef indices.

Table 1. Correlation analysis of the Shannon and Margalef ecological indices.

	Shannon	Margalef	Chlorophyll a	pH	COD	Nitrites	Nitrates	Phosphates
Shannon		0.86	-0.34	0.25	-0.11	-0.23	-0.28	0.24
Margalef	0.86		-0.08	0.40	0.16	-0.05	-0.17	0.37
Chlorophyll a	-0.34	-0.08		0.00	0.60	0.59	0.38	0.58
pH	0.25	0.40	0.00		-0.15	-0.44	-0.42	-0.07
COD	-0.11	0.16	0.60	-0.15		0.80	0.54	0.47
Nitrites	-0.23	-0.05	0.59	-0.44	0.80		0.77	0.51
Nitrates	-0.28	-0.17	0.38	-0.42	0.54	0.77		0.24
Phophates	0.24	0.37	0.58	-0.07	0.47	0.51	0.24	

High correlation (0.70 to 0.89 or -0.70 to -0.89)

Moderate correlation (0.40 to 0.69 or -0.40 to -0.69)

Low correlation (0.01 to 0.39 or -0.01 to -0.39)

Specific pollution sensitivity index and trophic state index

The IPS for points P1, P2, P3, P4 in the upper zone of the micro-basin, point P7 in the middle zone and points P13 and P14 in the lower zone showed values between 14.38 and 16.08, indicating the presence of highly sensitive diatoms, classifying the water quality as good. For points P5, P8-P12, values between 10.90 and 12.79 were recorded, which are indicative of moderate water quality. Overall, the average IPS in the micro-basin was 13.60, near the lower limit of the good quality category.

The TSI reflected an oligotrophic state at points P1, P2, and P7, with values of 16.45, 23.62, and 27.43, respectively. Between points P3 and

P6 and P11 to P14, the TSI values indicated a mesotrophic state with values between 31.20 and 50.97, while points P8, P9, and P10 reached values of 65.81, 66.58, and 74.44, indicating a eutrophic state. Figure 6 showed an inverse relationship between the IPS and the TSI.

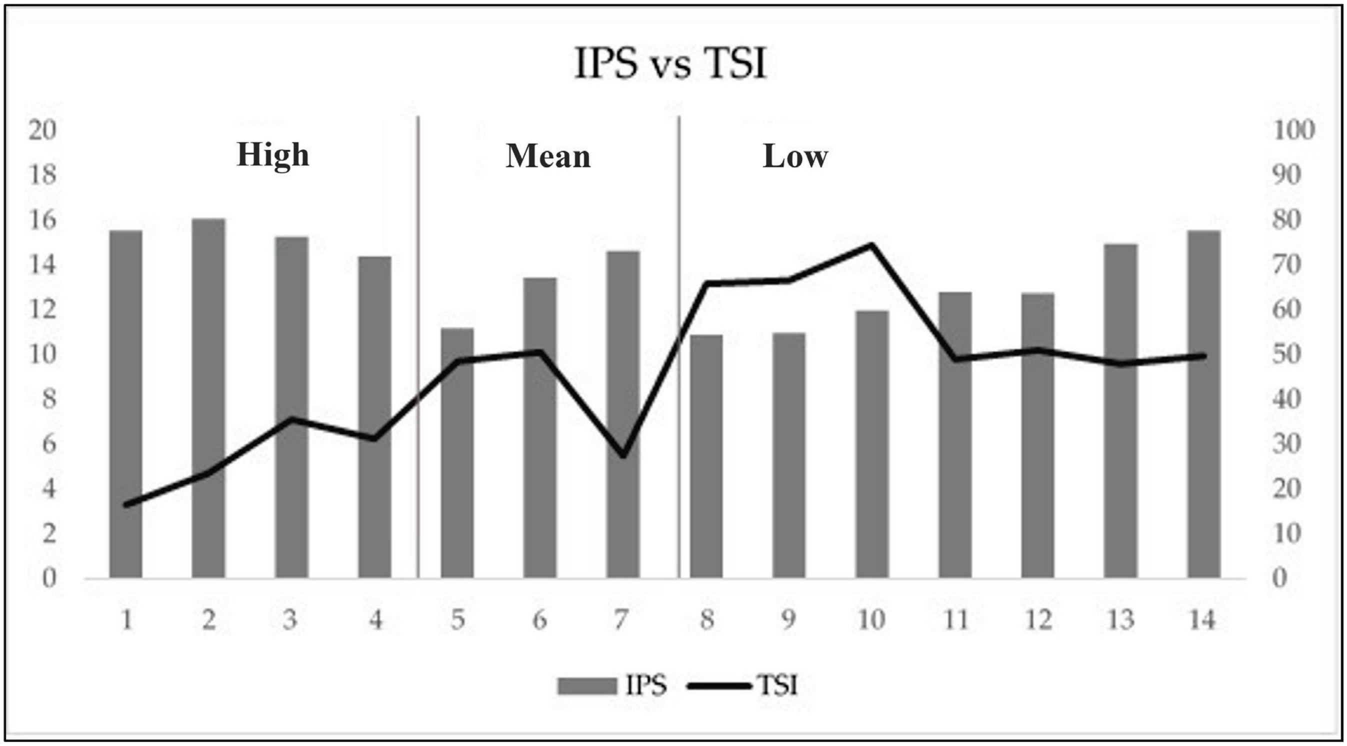

Figure 6. IPS and TSI scales. The IPS classifies water quality as good (13-17) and moderate (9-13). The TSI defines trophic status as oligotrophic (<30), mesotrophic (30-60), and eutrophic (60-90).

Discussion

Physicochemical parameters

The physicochemical pH condition of the Pachanlica River maintains homogeneous conditions throughout its micro-basin, presenting values close to neutrality, characteristic of freshwater Andean rivers (Bendezu-Bendezu & Bendezú-Hernández, 2021; Villamizar-Mendoza, Ramón-Valencia, & López-Areniz, 2020). The high variability of electrical conductivity and salinity is associated with direct and indirect wastewater discharges derived from agricultural activities in the upper and middle zones and industrial activities in the lower zone, with a progressive increase up to values of 554 and 0.28 mg/l, the same situation as, according to Buenaño, Vásquez, Zurita-Vásquez, Parra and Pérez (2018) associated with poor wastewater treatment or clandestine discharges.

Oxygen saturation conditions show a variability between 3.33 and 6.17 mg/l, maintaining higher values in the upper zone of the micro-basin. Similar to that reported by Plata-Díaz and Núñez-Avellaneda (2020) in Andean rivers of Colombia, mentioning that Andean rivers above 2 400 m.a.s.l. maintain oxygen concentrations between 7.5 and 8.6 mg/l. This behavior has been associated with the low temperatures and high turbulence present in these mountain ecosystems (Plata-Díaz & Núñez-Avellaneda, 2020). The lowest oxygen saturation concentrations are associated with wastewater discharge areas, which increases the pollutant reduction capacity and the oxygen demand for their purification (Villamizar-Mendoza *et al*., 2020; Barrios-Mendoza, Córdova-Mendoza, Córdova-Barrios, Argota-Pérez, & Iannacone, 2019).

Point P5, corresponding to the political boundary between the Quero and Cevallos cantons, whose flow rates are less than 0.17 m^3/s, reflects hypoxic conditions with a decrease in the self-purification capacity of organic matter. This suggests the production of toxic compounds such as hydrogen sulfide derived from the direct discharge of domestic wastewater from the Quero canton.

Nutrient, chlorophyll and COD analysis

The analysis of nutrients such as nitrates, nitrites, and phosphates in the Pachanlica River micro-basin provides a quantifiable basis for assessing water quality and trophic status (Benjumea-Hoyos, Suárez-Segura, & Villabona-González, 2018), since increases in the values of any of them lead to eutrophication processes (García-Miranda & Miranda-Rosales, 2018). Nitrite concentrations remain high, exceeding the guideline value of 1.0 mg/l suggested by Ayers and Wescot (1985) as a limit for agricultural water use, with maximum concentrations of 1.72 mg/l for point P6 and between 3.30 and 0.70 mg/l for the points corresponding to the middle and lower zones. These values are associated with poor wastewater treatment (Raffo-Lecca & Ruiz-Lizama, 2014), which causes a loss of aquatic life, mainly in fish (Téllez-Luna & Ospino-Jiménez, 2022; Bennett *et al*., 2021). In this context, microbial activity favors the conversion of high concentrations of nitrates to nitrites, generating a reduced environment that fosters favorable conditions for the development of COLI, a diatom reported to be tolerant to pollution (Wang *et al*., 2024; Lebkuecher, Atma, Conn, & Redwine, 2023).

In the case of nitrates, the Pachanlica River micro-basin concentrations, although considered low to moderate in the present study, with values between 1.10 and 28 mg/l, may be relevant from an ecological point of view. This is related to what was reported by Téllez-Luna and Ospino-Jiménez (2022, who, taking up the analysis carried out by Camargo, Alonso and Salamanca (2005), warn that even moderate concentrations of nitrates can generate toxic effects on aquatic organisms in freshwater bodies. The opposite situation was observed with phosphates; their values presented very high concentrations, exceeding

0.5 mg/l (Téllez-Luna & Ospino-Jiménez, 2022) throughout the micro-basin, and the highest were at points P5 and P6, with concentrations of 47.90 and 74.70 mg/l, respectively. These values are associated with direct discharges of domestic wastewater and runoff from agricultural activities, where the use of phosphate fertilizers stands out (Badamasi, Yaro, Ibrahim, & Bashir, 2019).

The high concentrations of phosphates in the micro basin maintain a moderate correlation with chlorophyll a, demonstrating favorable conditions for the presence of algae and a medium diversity of diatoms (García-Miranda & Miranda-Rosales, 2018; Pardo *et al*., 2010). Areas with a higher concentration of chlorophyll a have a lower diversity of diatom species. This situation was reflected in the middle and lower micro basin, contradicting the assertion that in an Andean River system, species diversity depends on primary production associated with chlorophyll (Donato-Rondón, 2019).

The COD showed a progressive increase in the different study areas. This situation represents a high ecological risk due to the low primary production of aquatic organisms (Misra & Chaturvedi, 2016), causing oxygen demands in the river system for the biogeochemical processes of organic matter decomposition and self-purification (González, Almeida, Calderón, Mallea, & González, 2014). Concentrations greater than 150 mg/l present at point P6 are associated with the total loss of aquatic biodiversity of larger species such as fish and smaller species such as macroinvertebrates, which affects the health and functionality of the aquatic ecosystem (Mohapatra *et al*., 2023; Misra & Chaturvedi, 2016).

Applicability of ecological indices in water quality assessment

Ecological indices were found to be closely related to the context and type of ecosystem where the study was conducted, as they respond differentially to environmental gradients and levels of ecological disturbance (González-Tuta, Gil-Padilla, & Pinilla-Agudelo, 2023). Only 15 species represent 75.61 % of the total diatom community, which is lower than the diatom diversity found in the Andean Tota River, Boyacá Department, where 118 diatom species were identified (Donato-Rondón, 2019). This significant reduction in diatom abundance is explained by the fact that the Pachanlica River is a sink for wastewater discharges from domestic and industrial agricultural activities (Donato-Rondón, 2019).

The predominance of COLI at 41.90 %, followed by ACMI at 15.72 % and PSBR at 11.31 %, indicates tolerance to variable pH, nutrient, and temperature conditions, a characteristic that gives them a great capacity for adaptation. Céspedes-Vargas *et al*. (2016) and Taxagua (2013) highlight them as colonizing species, present in rivers with wastewater discharges.

Pearson correlation and CCA showed complex interactions between diatom species and environmental variables in the Pachanlica River. The NIPA, FRRU, and NIFI species clustered together with parameters such as slightly alkaline pH, high COD, and phosphate levels, consistent with other studies (Kopáček *et al*., 2020; Oña & Tonato, 2017). These species, considered indicators of poor water quality, are associated with eutrophic environments and show tolerance to pollution, as suggested by previous studies (Cantonati *et al*., 2021; Segura-García *et al*., 2016; Hernández-González, 2012).

Species such as RHAB and ENSU demonstrated a broad tolerance to contamination, corroborating their presence at points P5 and P8 with characteristics of mesotrophic environments with high concentrations of phosphates (Olszyński, Szczepocka, & Żelazna-Wieczorek, 2019; Bey & Ector, 2014; Calizaya, 2013). The BRVI species, considered highly sensitive to pollution (Bere, 2014), was distributed in the upper area of the micro-basin, an area that showed lower concentrations of nutrients and, therefore, a lower degree of anthropic intervention (Donato-Rondón, 2019). COLI, GOLA, and NAER species were associated with high levels of chlorophyll a and nitrites, suggesting a preference for eutrophic environments (Cantonati *et al*., 2021; Segura-García *et al*., 2016; Hernández-González, 2012).

The Shannon ecological index revealed a notable variability in diatom diversity across all sampling points in the Pachanlica River, suggesting that this diversity responds to both environmental conditions and human influence (Valdez-Marroquín *et al*., 2018). The Shannon index values indicated low diversity at points P1, P3, P7, and P9-P14, values reflected in a community dominated by species with greater tolerance to contamination, such as the case of COLI at point P9, a cosmopolitan species, indicative of poor water quality (Lebkuecher *et al*., 2023; Donato-Rondón, 2019).

The highest contaminant presence at point P5 is related to the highest diatom diversity. The presence of the species FRRU, NIFI, NIIN, NITE, and NIPA, which are tolerant to high concentrations of nutrients and organic pollution (Castillejo *et al*., 2018), in addition to the presence of the genus Nitzschia, are indicative of poor water quality (Castillejo *et al*., 2018).

Pollution sensitivity index (PSI) and trophic state index (TSI)

The IPS was a decisive tool in qualifying the ecological status (Blanco, 2024) of the 14 monitoring points in the Pachanlica River micro-basin, showing good quality in several sections. However, in the middle and lower basins, the IPS overestimated quality, since high nutrient levels evidenced pollution conditions compared to the guideline values of Ayers and Wescot (1985), suggesting the need to complement the IPS with the analysis of physicochemical parameters.

According to the results of the IPS, the Pachanlica River micro-basin generally presented a water quality classified as good, with an average of 13.60, although close to the lower limit of this category (Blanco, 2024). The high and low zones showed a predominance of sensitive species, while in the middle zone, the lower values suggest moderate anthropogenic pressure.

The relative abundance of diatom species is crucial for assessing IPS (Blanco, 2024). This index can overestimate water quality when 41.7 to 80.3 % of diatoms are highly sensitive to contaminants (Blanco, 2024; Keck, Bouchez, Franc, & Rimet, 2016), giving a good quality rating even though the complete biodiversity and true ecological status of the water body are not reflected. In contrast, when low-sensitivity species predominate, the IPS tends to underestimate the effects of nutrients and environmental stressors, resulting in a moderate quality estimate under potentially more deteriorated conditions.

The TSI analysis reflects points with oligotrophic conditions, characteristics of areas with low nutrient levels (Naumoski & Mitreski, 2010), which, as mentioned in the study by Hama-Salih, Mohammad and Mohammed (2021), and Nugraha, Sarminingsih and Alfisya (2020), high concentrations of dissolved oxygen contribute to the self-purification processes of the river or the effluent. The places where eutrophication processes occur are directly associated with the concentrations of chlorophyll a and phosphates, similar to the data obtained in this study for points P8, P9 and P10, and other research carried out in other Andean rivers (Lebkuecher *et al*., 2023; Bennett *et al*., 2021).

In the lower basin, the mesotrophic conditions found, despite an increase in flow, tend to have high concentrations of nutrients, and conditions of greater oxygenation develop (Guerrero-Lizarazo, Pinilla-Agudelo, & Estrada-Galindo, 2021), high concentrations of chlorophyll a between 9.29 and 11.20 mg/m^3 (Brehob *et al*., 2024) and accelerated transport of pollutants (Wang *et al*., 2022).

The health status of the ecosystem throughout the micro-basin, according to the analysis of results, can be attributed to maintaining a mesotrophic state with high concentrations of phosphates, which are contributed by agricultural activities and wastewater discharges (Brehob *et al*., 2024; Bennett *et al*., 2021).

Despite the strong correlation between the TSI and the IPS, the study does not show a pattern of equality in the values of the different parameters studied in the Pachanlica River fluvial ecosystem. On the contrary, with higher TSI values, the IPS value tends to decrease; this relationship is justified by the fact that the first index assesses the immediate state of the river, while the second assesses the pre-existing ecological environment (Bonada *et al*., 2024; Zhikharev *et al*., 2022).

Conclusions

The use of epilithic diatoms as bioindicators allowed the characterization of water quality in the Pachanlica River microbasin as predominantly good to moderate, using the IPS index. However, in the lower-middle zone of the micro basin, which is under high anthropogenic pressure, the composition, dominated by resistant or colonizing diatom species, led to an overestimation of ecological quality compared to the results of nutrients, COD, and chlorophyll. This contradiction showed that, although diatoms are useful for assessing the ecological health of the ecosystem, their exclusive application is insufficient under public health criteria. Thus, the need for a complementary approach to the IPS that integrates trophic tools such as the TSI to improve the accuracy of assessing the ecological status of Andean rivers is evident.

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