

Benthic Macroinvertebrates as Bioindicators for Assessing Water Quality in the Cañipia River, Cusco, Peru.

Macroinvertebrados bentónicos como bioindicadores para evaluar la calidad del agua en el río Cañipia, Cusco, Perú.

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Highlights

- Biotic indices based on macroinvertebrates indicate a moderate impact on water quality.
- The families *Elmidae* and *Chironomidae* were the most abundant.
- The relationship between metal concentrations in water and sediment and the biotic indices varied depending on the season and monitoring stations.

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ORIGINAL RESEARCH

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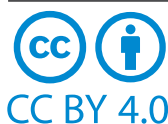
Bioindicadores; Calidad de agua; Índices bióticos; Índices de diversidad; Macroinvertebrados bentónicos; Metales totales

ABSTRACT

Introduction. Benthic macroinvertebrates (BMIs) are widely used as bioindicators of water quality due to their sensitivity to physicochemical alterations caused by pollution. Their presence and community composition provide a comprehensive assessment of the ecological status of aquatic ecosystems. **Objective.** This study aimed to assess the water quality of the Cañipia River, located in Cusco, Peru, using three biotic indices: BMWP/Col, ABI, and EPT. **Materials and Methods.** Sampling was conducted at four stations during the dry and wet seasons. BMIs were identified at the family level to calculate diversity and biotic indices. Additionally, physicochemical parameters were analyzed, and heavy metal concentrations in water and sediments were determined. **Results.** The families Elmidae and Chironomidae were the most abundant. According to the BMWP/Col index, water quality ranged from critical to doubtful, while the ABI index indicated a poor to good status. The EPT index classified water quality as poor to moderate. Simpson's index revealed fluctuating diversity, ranging from low to high during the dry season and from moderate to high during the wet season, whereas Shannon's index indicated low to moderate diversity. Margalef's index (DMg) was higher in the wet season. Regarding metal contamination, only station RCañi1, located upstream of the formal mining area, exceeded Peruvian regulatory limits for Ba, Cu, Mn, and Zn in water. Canonical Correspondence Analysis (CCA) showed seasonal relationships between metal concentrations and biotic indices. **Conclusions.** The three biotic indices suggest a moderate impact on the water quality of the Cañipia River, likely influenced by anthropogenic factors.

RESUMEN

Introducción. Los macroinvertebrados bentónicos (BMIs) son bioindicadores clave de la calidad del agua, ya que su sensibilidad a la contaminación permite evaluar integralmente el estado ecológico de los ecosistemas acuáticos. **Objetivo.** Evaluar la calidad del agua del río Cañipia, ubicado en Cusco, Perú, mediante la aplicación de tres índices bióticos: BMWP/Col, ABI y EPT. **Materiales y Métodos.** Se realizaron muestreos en cuatro estaciones durante las temporadas de estiaje y avenida. Los BMIs fueron identificados a nivel de familia para calcular índices de diversidad y bióticos. Adicionalmente, se analizaron parámetros fisicoquímicos y se determinaron las concentraciones de metales pesados en agua y sedimentos. **Resultados.** Las familias Elmidae y Chironomidae fueron las más abundantes. Según el índice BMWP/Col, la calidad del agua varió de crítica a dudosa, mientras que el índice ABI mostró valores de pobre a buena. Por su parte, el índice EPT indicó una calidad de agua de pobre a moderada. El índice de Simpson reflejó una diversidad fluctuante, con valores de baja a alta en estiaje y de moderada a alta en avenida, mientras que el índice de Shannon indicó una diversidad baja a moderada. El índice de Margalef (DMg) fue mayor durante la temporada de avenida. En cuanto a la contaminación por metales, solo la estación RCañi1, superó los límites permisibles peruanos para Ba, Cu, Mn y Zn en agua. **Conclusiones.** Los tres índices bióticos sugieren un impacto moderado en la calidad del agua del río Cañipia, probablemente influenciado por factores antropogénicos.



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INTRODUCCIÓN

Water resources are significantly impacted by anthropogenic activities such as mining and other industrial operations ⁽¹⁾. The discharge of effluents from mineral processing plants, among other sources, alters the physical and chemical composition of water, posing risks to both human health and aquatic ecosystems ^(2,3). In Peru, extensive mining activities and industries are concentrated in the Andean region ⁽²⁾, primarily in headwater areas and communal lands. This has led to socio-environmental conflicts between mining companies and local communities, affecting ecosystem services and generating significant economic implications ⁽⁴⁾. A notable example is the Andean city of Cerro de Pasco, where high concentrations of potentially toxic elements have been detected in children, water, and soil ⁽⁵⁾.

According to a nationwide water quality assessment conducted by the National Water Authority (ANA), 35 out of 159 hydrographic units in Peru exhibit elevated concentrations of potentially toxic elements, including arsenic (As), mercury (Hg), cadmium (Cd), lead (Pb), and iron (Fe), exceeding the permissible limits established by the national Environmental Quality Standards for Water (ECA) ⁽⁶⁾. The primary sources of contamination are informal mining operations and abandoned mining liabilities ⁽⁶⁾.

In the Espinar district of Cusco, Peru, environmental monitoring conducted by the Ministry of the Environment (MINAM) reported that metal concentrations in 15 communities exceeded the Maximum Permissible Limits for human consumption ^(7,8). Similarly, a study by the Environmental Assessment and Oversight Agency (OEFA, 2019) detected high levels of arsenic (As) and copper (Cu) in sediments from the Cañipia River microbasin, surpassing the thresholds established by the Interim Sediment Quality Guideline (ISQG) ⁽⁹⁾. These findings highlight a severe environmental and public health crisis in the region, exacerbated by mining activities and their impacts on local communities.

Benthic macroinvertebrates (BMIs) are widely recognized as bioindicators due to their sensitivity to changes in water quality ⁽¹⁰⁾. Several biotic indices, including the Biological Monitoring Working Party (BMWP/Col), the Andean Biotic Index (ABI), and the Ephemeroptera-Plecoptera-Trichoptera (EPT) index, are commonly used to assess aquatic ecosystem health ⁽¹¹⁾.

Given the lack of a regulatory framework in Peru for bioindicator-based water quality assessments, it is crucial to implement policies that promote their use. The BMWP/Col index ⁽¹²⁾ and the ABI, which has been adapted for high Andean rivers above 2,000 meters above sea level ⁽¹³⁾, serve as valuable references for the development of biological indices tailored to Peru's high-altitude aquatic ecosystems.

This study aimed to analyze BMIs as bioindicators to assess water quality in the Cañipia River, Cusco, Peru.

MATERIALS AND METHODS

Study Area. The Cañipia River basin is geographically located in the district of Espinar, province of Espinar, department of Cusco, Peru, at an altitude ranging from 3,000 to 5,000 meters above sea level ⁽¹⁴⁾. The river extends approximately 53 km, from its headwaters in the Putespunco ravine to its confluence with the Salado River ⁽¹⁵⁾. This area hosts mining operations, including the Tintaya mine, the Atalaya adit, and the Antapaccay Mining Company ⁽¹⁶⁾.

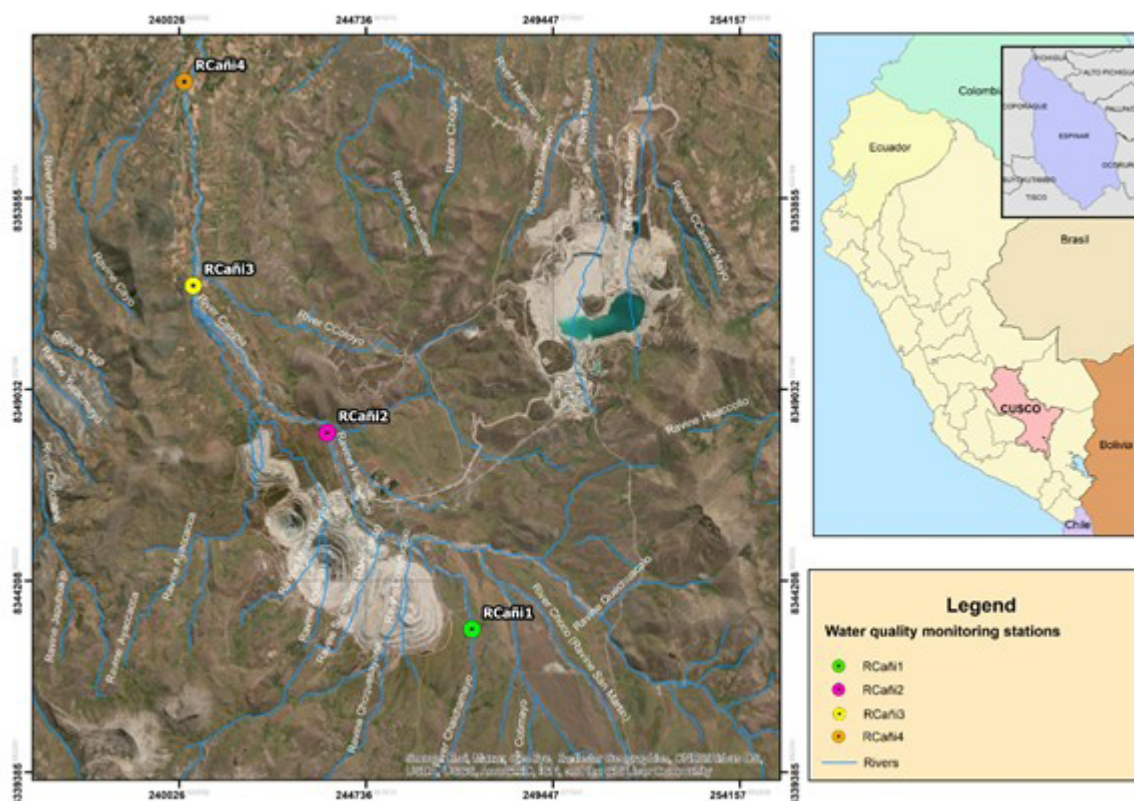


Figure 1. Cartographic map of the four monitoring stations in the study area.

Monitoring Stations. The selection of sampling sites was based on the natural characteristics of the river channel, turbulence, and accessibility for sampling (Figure 1) ⁽¹⁷⁾.

Four monitoring stations were established (Table 1) during both the high-flow season (February 2023) and the low-flow season (September 2022) ⁽¹⁸⁾. Station RCañi1 was designated as the control site, located upstream of formal mining operations. Stations RCañi2 and RCañi3 were positioned around the mining perimeter to assess potential environmental impacts on the water body. Meanwhile, station RCañi4 was situated near various anthropogenic activities.

Table 1. Monitoring stations for water quality in the Cañipia River, Cusco, Peru.

| Monitoring station | Location description | UTM coordinates | | Distance to pollution source (km) | Altitude (masl) |
|--------------------|--|-----------------|----------|-----------------------------------|-----------------|
| | | North (m) | East (m) | | |
| RCañi1 | Located in the Chalchamayo River, upstream of the Antapaccay Mining Company's operations. | 8342486 | 247412 | 8.78 | 4059 |
| RCañi2 | Situated outside the Antapaccay Mining Company's operational area, where tailings are discharged into the Huilcarani River. | 8347943 | 243766 | 6.23 | 3987 |
| RCañi3 | Located in the Huilcarani River, outside the Antapaccay Mining Company's operations, before its confluence with the Cañipia River. | 8351653 | 240387 | 8.30 | 3954 |
| RCañi4 | Located in the Cañipia River, downstream of the Ccoloyo River confluence. | 8356794 | 240162 | 10.16 | 3921 |

UTM coordinates WGS84, Zone 18L

Benthic Macroinvertebrates (BMIs). The collection of BMIs followed the methodology established by MINAM ⁽¹⁹⁾. The sampling effort covered a 100-meter stretch, using a Surber net with a 500 μm mesh aperture, positioned against the current for a 15-minute exposure period. The riverbed material was manually disturbed to capture organisms trapped in the net. This procedure was repeated three times at each monitoring station. The collected organisms were placed in airtight 200 mL plastic containers, properly labeled, and preserved in 70% ethanol for fixation and long-term conservation. The samples were then stored in a portable cooler at ambient temperature, protected from direct sunlight ⁽²⁰⁾.

Taxonomic identification at the family level was conducted at the Laboratory of Ecology and Animal Biodiversity (LEBA) of the Faculty of Natural Sciences and Mathematics at Universidad Nacional Federico Villarreal. A StereoBlue® stereomicroscope was used for the photographic documentation of specimens. Taxonomic classification at the family level was based on specialized identification guides ⁽²¹⁾⁽²²⁾⁽²³⁾⁽²⁴⁾, and expert taxonomists were consulted when necessary. The identified taxa were organized according to their phylogenetic classification, considering phylum, class, order, and family.

The specimens collected in this study were deposited in the Natural History Museum of the Faculty of Natural Sciences and Mathematics, under the catalog codes MUFV-ZOO HIDRO: 499 to 748. The BioDiversity Pro® software was used to calculate three alpha diversity indices at the family taxonomic level: Simpson's diversity index (1-D), Shannon's diversity index (H'), and Margalef's richness index (DMg) ⁽²⁵⁾.

Three biotic indices—BMWP/Col (Biological Monitoring Working Party adapted for Colombia), ABI

(Andean Biotic Index), and EPT (taxonomic richness of Ephemeroptera, Plecoptera, and Trichoptera groups)—were applied to assess water quality ⁽²²⁾⁽²⁶⁾⁽²⁷⁾. The BMWP/Col index was interpreted as >120 and 101–120 (Good), 61–100 (Acceptable), 36–60 (Doubtful), 16–35 (Critical), and <15 (Very Critical). The ABI index was categorized into four levels: Very Good (>74), Acceptable (45–74), Doubtful (27–44), and Critical (0–26). The EPT index was classified as Very Good (75–100), Good (50–75), Fair (25–50), and Poor (0–25).

Physicochemical Parameters. Four *in situ* parameters were measured to assess water quality: temperature (°C), pH (units), dissolved oxygen (DO, mg/L), and electrical conductivity (EC, $\mu\text{S}/\text{cm}$), using the HandyLab 680® multiparameter meter. Additionally, four *ex situ* parameters were analyzed: biochemical oxygen demand (BOD_5 , mg/L), sulfates (mg/L), ammoniacal nitrogen (mg/L), and total metals. The total metals analyzed included aluminum (Al), barium (Ba), bismuth (Bi), boron (B), calcium (Ca), copper (Cu), iron (Fe), mercury (Hg), nickel (Ni), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), and zinc (Zn), expressed in mg/L. Water samples were collected in 500 mL plastic containers with ethanol-based preservatives and stored in a portable cooler.

To preserve water samples for total metal analysis, they were maintained at a temperature between 4–10°C, with a pH < 2, and 10 drops of HNO_3 were added. Total metal concentrations in water were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) ⁽²⁸⁾. Biochemical oxygen demand (BOD_5), sulfates, and ammoniacal nitrogen were analyzed following standardized protocols ⁽²⁹⁾.

For sediment analysis, 200 g of samples were collected from a depth of 0–15 cm to determine total metal concentrations, following the United States Environmental Protection Agency (USEPA) protocol ⁽³⁰⁾. A total of 26 metals were analyzed, including aluminum (Al), barium (Ba), beryllium (Be), bismuth (Bi), boron (B), cadmium (Cd), calcium (Ca), cerium (Ce), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), mercury (Hg), nickel (Ni), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn), expressed in mg/kg.

Statistical Analysis. Data analysis was conducted using PAST software version 4.03®, considering each season separately (low-flow and high-flow periods). Pearson's correlation coefficient (r_p) was applied to evaluate the relationship between total metal concentrations (in water and sediment) and biotic indices (BMWP/Col, ABI, and EPT) based on the sampling period. In all cases, statistical significance was set at $p < 0.05$.

Additionally, Canonical Correspondence Analysis (CCA) was used to determine the association between physicochemical variables, heavy metal concentrations, and the abundance of BMI families at the monitoring stations. The interpretation of CCA results was based on the distribution of variables within each quadrant of the graphical representation, considering axes 1, 2, and 3.

Data Availability. The dataset supporting the findings of this study has been deposited in Mendeley Data and is publicly accessible under the title “Benthic Macroinvertebrates as Bioindicators for Assessing Water Quality in the Cañipia River, Cusco, Peru” (Iannacone, 2025), available at: <https://doi.org/10.17632/fpzvxxvk6sm.1>.

Ethical Considerations. Authorization for BMI sampling in water resources was obtained from the Ministry of Production, in compliance with Article 21 of Supreme Decree No. 004-2020-PRODUCE (Ministry of Production). Additionally, the study was approved by the Institutional Committee on Ethics in Research with Animals and Biodiversity of Universidad Científica del Sur, under Approval Certificate No. 019-CIEI-AB-CIENTÍFICA-2022.

RESULTS

Benthic Macroinvertebrates (BMIs). A total of 7,564 BMI individuals were recorded, belonging to three phyla, four classes, nine orders, and 24 families during both the low-flow and high-flow seasons in the Cañipia River, Cusco, Peru. The Elmidae family exhibited the highest abundance, with 2,990 individuals (39.53%), followed by Chironomidae with 1,979 individuals (26.16%) (**Table 2**).

The average BMI abundance was 509.17 individuals during the low-flow season and 242.33 during the high-flow season, with no significant differences between seasons ($t = 1.66$; $p = 0.10$). However, when comparing BMI abundance among monitoring stations during the low-flow season, a significant difference was observed between RCañi1 and RCañi4 ($F = 6.25$; $p = 0.01$). No significant differences were found among stations during the high-flow season ($p > 0.05$).

When comparing BMI abundance between high-flow and low-flow seasons at each monitoring station, significant differences were observed at RCañi2 ($t = 8.85$; $p = 0.0008$) and RCañi4 ($t = 3.56$; $p = 0.02$), while no significant differences were found at the other stations ($p > 0.05$).

Table 2. Abundance of benthic macroinvertebrate (BMI) families during dry and wet seasons in the Cañipia River, Cusco, Peru.

| Phylum | Clase | Orden | Monitoring Stations | Seasons | | | | | | | | |
|------------------------|------------|----------------|---------------------|-----------------|--------|--------|--------|------------|--------|--------|--------|----|
| | | | | Dry season | | | | Wet season | | | | |
| | | | | RCañi1 | RCañi2 | RCañi3 | RCañi4 | RCañi1 | RCañi2 | RCañi3 | RCañi4 | |
| Overall Total–Families | | | | 69 | 1819 | 1722 | 2500 | 219 | 121 | 860 | 254 | |
| Arthropoda | Insecta | Ephemeroptera | Baetidae | 0 | 9 | 0 | 138 | 30 | 21 | 50 | 24 | |
| | | Plecoptera | Gripopterygidae | 0 | 0 | 0 | 5 | 0 | 0 | 2 | 0 | |
| | | Hemiptera | Corixidae | 0 | 143 | 49 | 46 | 2 | 3 | 14 | 4 | |
| | | | Gelastocoridae | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | |
| | | Coleoptera | Dytiscidae | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | |
| | | | Elmidae | 0 | 907 | 1053 | 643 | 91 | 40 | 173 | 83 | |
| | | | Psephenidae | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | |
| | | | Staphylinidae | 3 | 385 | 261 | 176 | 0 | 4 | 22 | 24 | |
| | | | Hydrophilidae | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | | Trichoptera | Hydropsychidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 |
| | | | | Hydroptilidae | 0 | 50 | 12 | 680 | 43 | 13 | 109 | 7 |
| | | | | Ceratopogonidae | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | Diptera | Chironomidae | 65 | 284 | 280 | 747 | 52 | 37 | 484 | 30 |
| | | | | Culicidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 |
| | | Dolichopodidae | | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | |
| | | Ephydriidae | | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | |
| | | Muscidae | | 0 | 1 | 3 | 6 | 0 | 0 | 0 | 0 | |
| | | Simuliidae | | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | Tabanidae | | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | | Tipulidae | 0 | 0 | 29 | 0 | 0 | 1 | 0 | 1 | | |
| Malacostraca | Amphipoda | Hyaellidae | 0 | 0 | 23 | 30 | 0 | 0 | 3 | 0 | | |
| Mollusca | Gastropoda | Basommatophora | Physidae | 0 | 17 | 0 | 16 | 0 | 0 | 0 | 1 | |
| | | Planorbidae | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Annelida | Clitellata | Oligochaeta | U/F | 0 | 0 | 0 | 9 | 0 | 0 | 0 | | |

U/F = Unidentified families.

Physicochemical Parameters. During both the low-flow and high-flow seasons, pH values along the studied basin ranged from neutral to alkaline (7.10 to 9.16). Water temperature varied between 9.6 and 22.10°C, while ammoniacal nitrogen concentrations ranged from 0.014 to 0.1 mg/L. Biochemical oxygen demand (BOD₅) was below 2 mg/L at most sampling points, except at RCañi1, which recorded 12.5 mg/L. Electrical conductivity (EC) was higher during the low-flow season compared to the high-flow season. Dissolved oxygen (DO) at the control station (RCañi1) was 4.53 mg/L during the low-flow season, whereas RCañi4 recorded 9.55 mg/L during the high-flow season. Total metal concentrations in water were compared with the current Peruvian regulations established by MINAM ⁽³¹⁾ (Table 3). RCañi1 exhibited Ba, Cu, Mn, and Zn concentrations (mg/L) exceeding at least one of the Environmental Quality Standards (ECA) categories set by MINAM, as shown in (Table 3). Similarly, total metal concentrations in sediments were recorded; however, these values were not compared with any regulatory standards, as no official guidelines for total metal concentrations in sediments exist in Peru.

Table 3. Physicochemical parameters and total metals in water and sediment at each monitoring station during the dry and wet seasons in the Cañipia River, Cusco, Peru.

| Season | Dry Season | | | | Wet Season | | | | MINAM ⁽³¹⁾ | | |
|-------------------------------------|---------------------|---------|---------|---------|------------|---------|---------|---------|-----------------------|---------------|---------------|
| | Monitoring Stations | | | | | | | | | | |
| Parameters | RCañi1 | RCañi2 | RCañi3 | RCañi4 | RCañi1 | RCañi2 | RCañi3 | RCañi4 | Category 3-D1 | Category 3-D2 | Category 4-E2 |
| Physicochemical Parameters | | | | | | | | | | | |
| BOD ₅ (mg/L) | 12,5 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | 15 | 15 | 10 |
| Ammoniacal Nitrogen (mg/L) | 0.041 | 0.020 | 0.016 | 0.016 | 0.014 | 0.1 | 0.1 | 0.1 | - | - | - |
| Sulfato (mg/L) | 11.4 | 433.7 | 325.6 | 203.3 | 3.2 | 113.2 | 159 | 140.4 | 1000 | 1000 | - |
| Temperature (°C) | 19.80 | 22.10 | 19.70 | 13.30 | 9.6 | 14.1 | 20.2 | 20.2 | - | - | - |
| pH | 7.10 | 8.50 | 8.24 | 7.72 | 7.73 | 8.21 | 9.16 | 9.16 | 6.5-8.5 | 6.5-8.4 | 6.5-9.0 |
| Electrical Conductivity (µS/cm) | 101,50 | 1551 | 1363 | 928 | 51.9 | 481 | 641 | 607 | 2500 | 5000 | 1000 |
| Dissolved Oxygen (mg/L) | 4.63 | 8.26 | 7.46 | 5.08 | 7.04 | 6.62 | 7.48 | 9.55 | ≥ 4 | ≥ 5 | ≥ 5 |
| Total metals in water (mg/L) | | | | | | | | | | | |
| Aluminum | 46.728 | 0.088 | 0.108 | 0.082 | - | - | - | - | 5 | 5 | - |
| Barium | 0.878 | 0.076 | 0.184 | 0.135 | 0.036 | 0.049 | 0.078 | 0.084 | 0.7 | - | 0.7 |
| Bismuth | 0.014 | 0.010 | 0.010 | 0.010 | - | - | - | - | - | - | - |
| Boron | 0.032 | 0.461 | 0.396 | 0.221 | 0.052 | 0.155 | 0.215 | 0.186 | 1 | 5 | - |
| Calcium | 34.98 | 147.6 | 137.4 | 95.77 | 13.79 | 59.86 | 84.91 | 80.09 | - | - | - |
| Copper | 0.118 | 0.050 | 0.043 | 0.036 | - | - | - | - | 0.2 | 0.5 | 0,1 |
| Strontium | 0.44 | 2.24 | 1.81 | 1.05 | 0.08 | 0.65 | 0.91 | 0.81 | - | - | - |
| Phosphorus | 2.592 | <0.006 | <0.006 | <0.006 | 0.07 | 0.06 | 0.06 | 0.09 | - | - | - |
| Irons | 42.52 | 0.02 | 0.04 | 0.02 | 0,10 | 0.03 | 0,02 | 0.02 | 5 | - | - |
| Lithium | 0.013 | 0.16 | 0.15 | 0.07 | - | - | - | - | 2.5 | 2.5 | - |
| Magnesium | 11.70 | 26.41 | 26.88 | 17.26 | 3.01 | 10.01 | 14.22 | 13.61 | - | 250 | - |
| Manganese | 3.41 | 0.003 | 0.005 | 0.003 | 0.004 | 0.019 | 0.006 | 0.012 | 0.2 | 0.2 | - |
| Mercury | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.001 | 0.01 | 0.0001 |
| Nickel | 0.019 | 0.0004 | 0.0004 | 0.0004 | - | - | - | - | 0.2 | 1 | 0.052 |
| Potassium | 10.71 | 18.22 | 13.83 | 9.45 | 4.11 | 5.60 | 8.09 | 7.93 | - | - | - |
| Silica | 137.53 | 13.86 | 22.12 | 27.19 | 25.53 | 21.78 | 20.58 | 21.00 | - | - | - |
| Silicon | 64.18 | 6.47 | 10.32 | 12.69 | 11.91 | 10.16 | 9.61 | 9.80 | - | - | - |
| Sodium | 8.86 | 156.9 | 123.8 | 67.20 | 4.11 | 35.21 | 53.48 | 48.86 | - | - | - |
| | | 5 | 3 | | | | | | | | |
| Titanium | 0.32 | 0.001 | 0.001 | 0.001 | - | - | - | - | - | - | - |
| Zinc | 0.14 | 0.042 | 0.095 | 0.028 | 0.031 | 0.046 | 0.027 | 0.032 | 2 | 24 | 0.12 |

mg/L - BOD₅ = Biochemical Oxygen Demand. **Category 3-D1** = Risk for Vegetation. **Category 3-D2** = Animal Drinking Water. **Category 4-E2** = Rivers, Coast, and Highlands.

Table 4. Total metals in sediment at each monitoring station during the dry and wet seasons in the Cañipia River, Cusco, Peru.

| Season | Dry Season | | | | Wet Season | | | |
|-----------------------------------|---------------------|--------|--------|--------|------------|--------|--------|--------|
| | Monitoring Stations | | | | | | | |
| Paramentes | RCañi1 | RCañi2 | RCañi3 | RCañi4 | RCañi1 | RCañi2 | RCañi3 | RCañi4 |
| Total metals in sediments (mg/kg) | | | | | | | | |
| Aluminum | 3550 | 3011 | 3027 | 3345 | 5501 | 4243 | 4014 | 4468 |
| Barium | 68.35 | 243.6 | 46.22 | 72.22 | 75.77 | 68.79 | 54.63 | 47.11 |
| Beryllium | 0.55 | 0.48 | 0.56 | 0.57 | - | - | - | - |
| Bismuth | 15.63 | 9.16 | 11.66 | 11.41 | 41.12 | 36.10 | 31.87 | 25.02 |
| Boron | - | - | - | - | 26.43 | 26.95 | 27.15 | 23.09 |
| Cadmium | 0.020 | 0.30 | 0.40 | 0.42 | 1.87 | 1.65 | 1.62 | 1.57 |
| Calcium | 3212 | 3156 | 3220 | 6874 | 3778 | 3194 | 3495 | 3755 |
| Cerium | 32.57 | 29.33 | 26.00 | 29.02 | 31.27 | 27.23 | 28.38 | 26.27 |
| Cobalt | 4.46 | 4.45 | 3.62 | 4.45 | 7.32 | 7.45 | 6.75 | 5.57 |
| Copper | 7.45 | 10.69 | 11.34 | 17.71 | 10.91 | 18.27 | 14.37 | 19.91 |
| Chromium | 0.97 | 0.59 | 1.26 | 1.33 | 9.46 | 7.94 | 9.26 | 6.87 |
| Strontium | 21.15 | 31.76 | 27.85 | 41.65 | 27.93 | 28.52 | 29.44 | 29.98 |
| Phosphorus | 778.68 | 669 | 626.4 | 593.6 | 794.4 | 672.6 | 741.3 | 671.2 |
| Iron | 8906 | 7809 | 8650 | 7963 | 18216 | 17415 | 17708 | 15139 |
| Lithium | 2.56 | 3.70 | 3.88 | 4.41 | - | - | - | - |
| Magnesium | 1299 | 1379 | 1318 | 1366 | 2211 | 1662 | 1883 | 1747 |
| Manganese | 313.17 | 1312 | 317.0 | 362.4 | 315.5 | 590.8 | 482.1 | 282.2 |
| Mercury | < 0.04 | < 0.04 | < 0.04 | < 0.04 | < 0.04 | < 0.04 | < 0.04 | < 0.04 |
| Nickel | 4.37 | 5.57 | 4.27 | 4.60 | 7.93 | 6.57 | 8.06 | 6.7 |
| Lead | 0.20 | 57.47 | 18.13 | 25.23 | 3.23 | 37.04 | 23.82 | 21.82 |
| Potassium | 434.28 | 447.6 | 421.1 | 506.6 | 770.9 | 540.9 | 535.8 | 543.3 |
| Silicon | 132.80 | 111.1 | 120.3 | 118.6 | 367.9 | 290.9 | 272.1 | 280.2 |
| Sodium | 166.98 | 259.7 | 272.0 | 283.7 | 263.8 | 256.3 | 316.3 | 311.6 |
| Titanium | 329.89 | 193.4 | 248.2 | 247.8 | 921.2 | 784.4 | 694.4 | 548.7 |
| Vanadium | 16.29 | 14.34 | 15.97 | 15.57 | 48.51 | 55.9 | 55.99 | 42.87 |
| Zinc | 21.49 | 33.73 | 38.18 | 37.26 | 33.18 | 53.44 | 46.66 | 41.75 |

Diversity Indices. The 1-D, H', and DMg indices recorded during the low-flow and high-flow seasons in the Cañipia River, Cusco, Peru, are presented in [Table 5](#). The Margalef richness index (DMg) ranged from 0.47 to 1.66, while Simpson's diversity index (1-D) varied between 0.11 and 0.80. Lastly, Shannon's diversity index (H') showed values ranging from 0.13 to 1.84 ([Table 5](#)).

Table 5. Diversity indices at each monitoring station during the low-flow and high-flow periods in the Cañipia River, Cusco, Peru.

| Season | Low-water season | | | | High-flow season | | | |
|--------------|---------------------|--------|--------|--------|------------------|--------|--------|--------|
| Indices | Monitoring stations | | | | | | | |
| | RCañi1 | RCañi2 | RCañi3 | RCañi4 | RCañi1 | RCañi2 | RCañi3 | RCañi4 |
| Simpson 1-D | 0.11 | 0.67 | 0.57 | 0.76 | 0.71 | 0.75 | 0.62 | 0.80 |
| Shannon H' | 0.25 | 1.40 | 1.19 | 1.62 | 0.13 | 1.59 | 1.28 | 1.84 |
| Margalef DMg | 0.47 | 1.46 | 1.47 | 1.53 | 0.92 | 1.66 | 1.18 | 1.62 |

Biotic Indices. The BMWP/Col index classified water quality as doubtful (moderately polluted waters) at RCañi2, RCañi3, and RCañi4 during both low-flow and high-flow seasons, as well as at RCañi1 during the high-flow season. This classification was influenced by the presence of Glossiphoniidae, Tipulidae, and Tubificidae families, which are indicative of contaminated waters. In contrast, the ABI index classified water quality as ranging from poor to good, while the EPT index indicated water quality ranging from poor to fair (Table 6).

Table 6. Biotic indices at each monitoring station during the low-flow and high-flow periods in the Cañipia River, Cusco, Peru.

| Season | Low-flow period | | | | High-flow period | | | |
|------------|---------------------|--------|--------|--------|------------------|--------|--------|--------|
| Indices | Monitoring stations | | | | | | | |
| | RCañi1 | RCañi2 | RCañi3 | RCañi4 | RCañi1 | RCañi2 | RCañi3 | RCañi4 |
| (BMWP/Col) | 17C | 59D | 59D | 56D | 48D | 53D | 52D | 48D |
| ABI | 8P | 45A | 44A | 52A | 36D | 39D | 46A | 40D |
| EPT | 0P | 3.4P | 0.7P | 33G | 33G | 28F | 19P | 25P |

BMWP/Col = Biological Monitoring Working Party adapted to Colombia. **ABI** = Andean Biotic Index. **EPT** = Ephemeroptera, Plecoptera, and Trichoptera Index. **A**= Acceptable, **C**=Critical, **D**= Doubtful, **P**= Poor, **G**=Good, **F**= Fair.

Correlation Matrix. During the low-flow season, significant Pearson's correlation (r_p) values were observed between total metal concentrations in water and sediment and the three biotic indices (BMWP/Col, ABI, and EPT). A strong correlation was identified between BMWP/Col and metal concentrations in water, particularly Al, Ba, Bi, Cu, P, Fe, Mn, Ni, Se, Si, and Ti, as well as between ABI and Al, Ba, Bi, Cu, P, Fe, Mn, and Ni in water (Figure 2A). A positive correlation was found between BMWP/Col and Na and Zn in sediments, while ABI was positively correlated with Ca, Li, Na, and Zn, except for P, which showed a negative correlation with ABI. Additionally, EPT exhibited a positive correlation with Ca and P (Figure 2B).

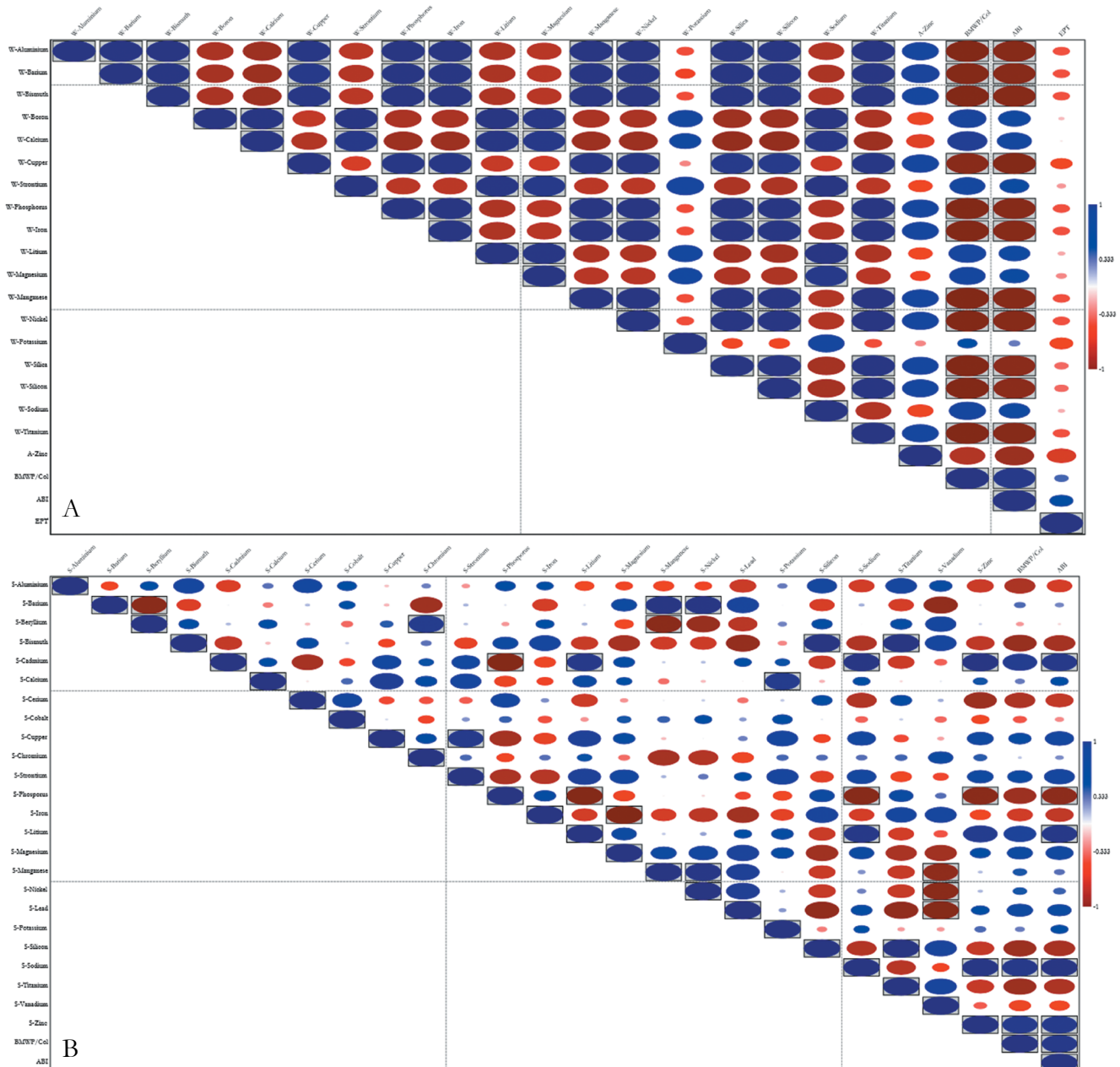


Figure 2. (A, top) Pearson correlation matrix between metals in water and biotic indices during the low-water season in the Cañipia River, Cusco, Peru. (B, bottom) Pearson correlation matrix between metals in sediments and biotic indices during the Low-flow season in the Cañipia River, Cusco, Peru. $p < 0.05$ indicates significant values, highlighted with a box. Blue represents positive correlations, while red represents negative correlations. W- refers to metals in water, and S- refers to metals in sediments.

During the high-flow season, several significant correlations ($p < 0.05$) were observed among total metal concentrations in water (Figure 3A), while only a few correlations were found with the biotic indices (BMWP/Col, ABI, and EPT). The BMWP/Col index exhibited a positive correlation with Mn and a negative correlation with Ca in sediments (Figure 3B).

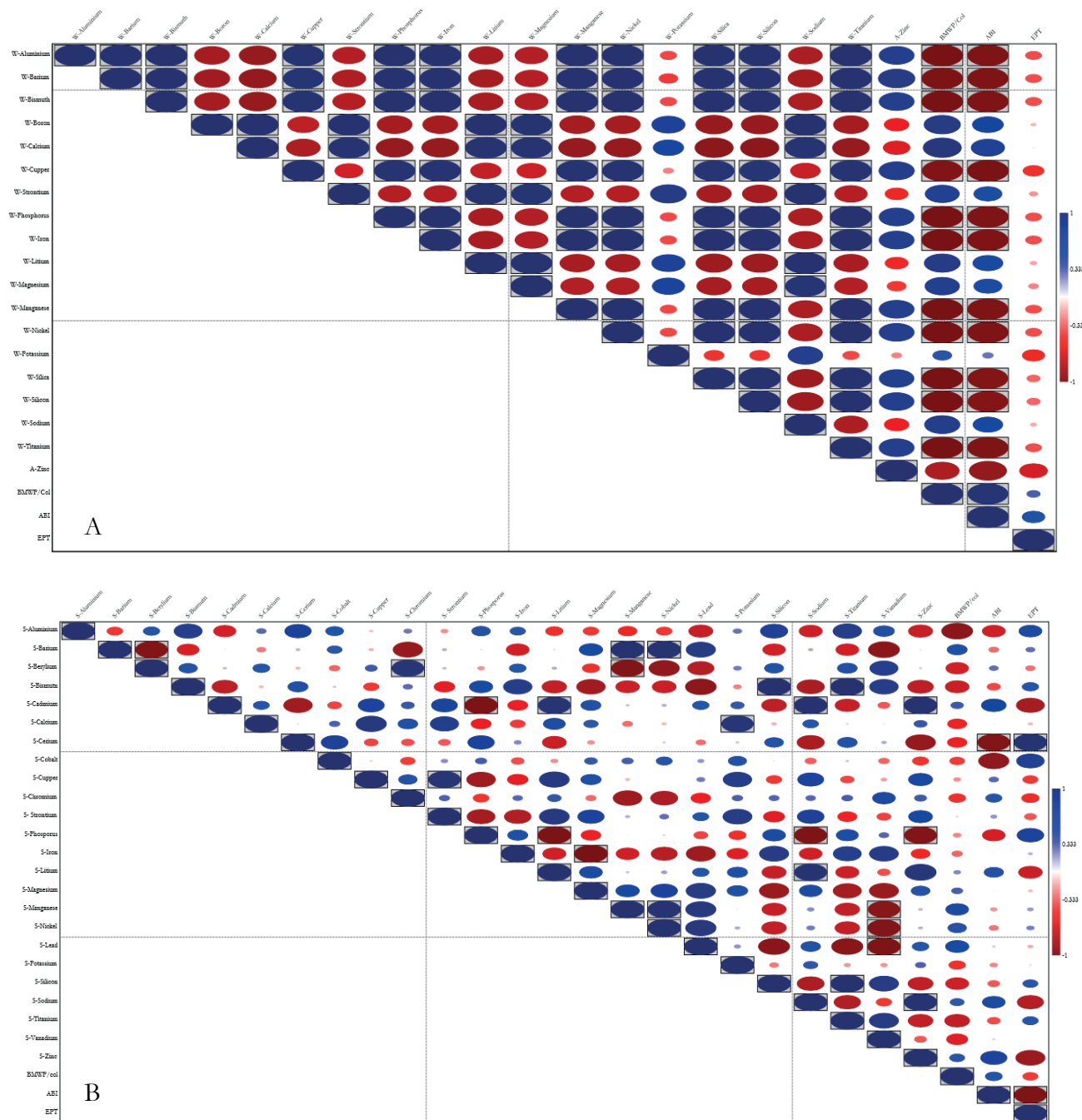


Figure 3. (A). Pearson correlation matrix between metals in water and biotic indices during the high-flow season in the Cañipia River, Cusco, Peru. (B). Pearson correlation matrix between metals in sediments and biotic indices during the low-flow season in the Cañipia River, Cusco, Peru. $p < 0.05$ = significant values highlighted with a “box.” Blue = positive correlation. Red = negative correlation. W- = metals in water. S- = metals in sediments.

Canonical Correspondence Analysis (CCA). The relationship between physicochemical parameters (ammoniacal nitrogen, sulfates, BOD₅, DO, temperature, EC, and pH) and BMI families during the low-flow season is detailed in (Figure 4), where the greatest variability is observed along CCA axes 1 and 2.

Regarding CCA axis 1, BOD₅ and ammoniacal nitrogen were the most strongly correlated variables, explaining 79.51% of the variance. In contrast, CCA axis 2 accounted for 12.05% of the variability, with pH and EC as the main associated variables. In RCañi2, a stronger correlation was observed between temperature, EC, pH, DO, and sulfates and the Staphylinidae family, whereas Corixidae, Simuliidae, Ceratopogonidae, and Hydrophilidae exhibited a weaker association. For RCañi3, temperature, EC, DO, and sulfates were associated with Elmidae and Planorbidae, while Gelastocoridae and Dolichopodidae were positioned farther from the axis, indicating a weaker correlation. Meanwhile, in RCañi1, during the low-flow season, BOD₅ and ammoniacal nitrogen showed elevated values and were strongly associated with Chironomidae and Muscidae. On the other hand, Hyalellidae, Dytiscidae, Physidae, Baetidae, Hydroptilidae, unidentified taxa (S/I), Gripopterygidae, and Ephydriidae exhibited a weaker association with these environmental variables (Figure 4).

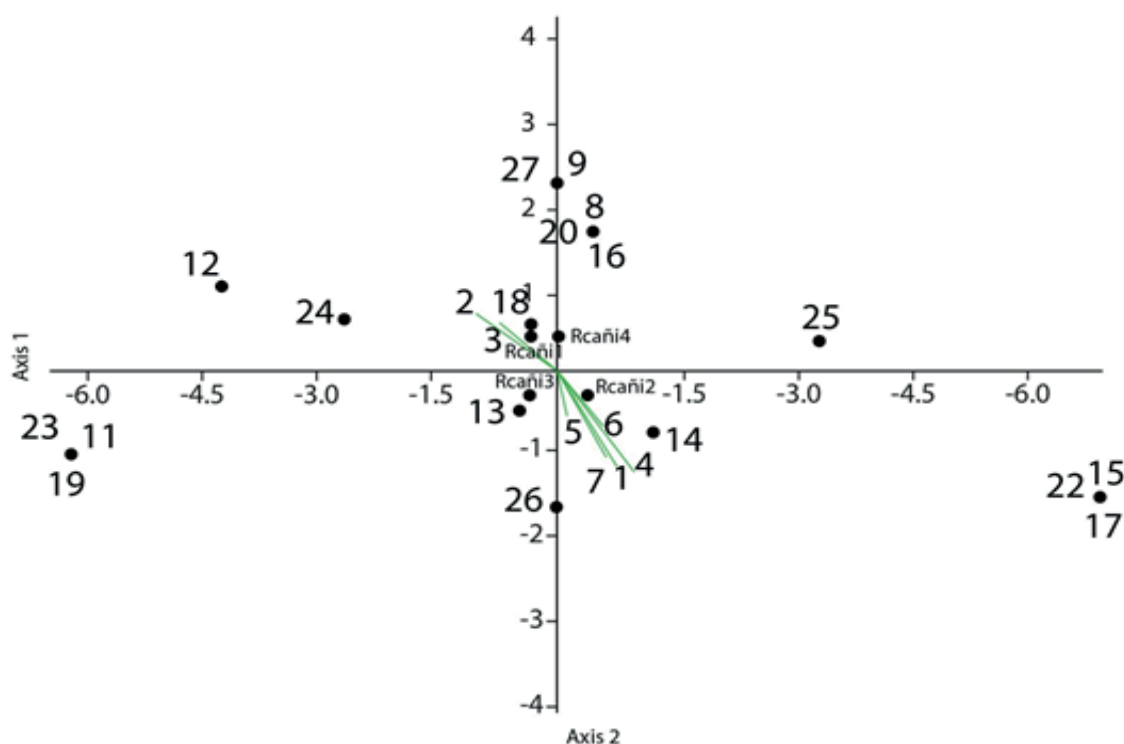


Figure 4. Canonical Correspondence Analysis of the relationship between physicochemical variables and benthic macroinvertebrate families during the low-flow period in the Cañipia River, Cusco, Peru. Axes 1 and 2 represent a two-dimensional plot. 1 = pH. 2 = Biochemical Oxygen Demand. 3 = Ammoniacal Nitrogen. 4 = Sulphate. 5 = Temperature. 6 = Electrical conductivity. 7 = Dissolved Oxygen. 8 = Baetidae. 9 = Gripopterygidae. 10 = Corixidae. 11 = Gelastocoridae. 12 = Dytiscidae. 13 = Elmidae. 14 = Staphylinidae. 15 = Hydrophilidae. 16 = Hydroptilidae. 17 = Ceratopogonidae. 18 = Chironomidae. 19 = Dolichopodidae. 20 = Ephydriidae. 21 = Muscidae. 22 = Simuliidae. 23 = Tipulidae. 24 = Hyalellidae. 25 = Physidae. 26 = Planorbidae. 27 = unidentified.

Figure 5 illustrates the relationship between physicochemical parameters (ammoniacal nitrogen, sulfates, BOD₅, DO, temperature, EC, and pH) and BMI families during the high-flow season, showing greater variability along CCA axes 1 and 2. Regarding CCA axis 1, 79.26% of the variance in physicochemical variables was associated with sulfates, pH, temperature, EC, and ammoniacal nitrogen, while CCA axis 2 accounted for 16.59%, primarily correlated with DO. At RCañi4, a stronger association was observed between pH, temperature, EC, ammoniacal nitrogen, and DO and the Staphylinidae family. In contrast, Physidae, Hydropsychidae, and Culicidae, which are positioned farther from the axis, showed no association with these physicochemical parameters. For RCañi1 and RCañi2, CCA analysis revealed a very weak relationship between these physicochemical variables and the Tabanidae and Dytiscidae families. Meanwhile, at RCañi3, EC, pH, sulfates, and temperature were associated with Corixidae, Griptopterygidae, Chironomidae, and Psephenidae (**Figure 5**).

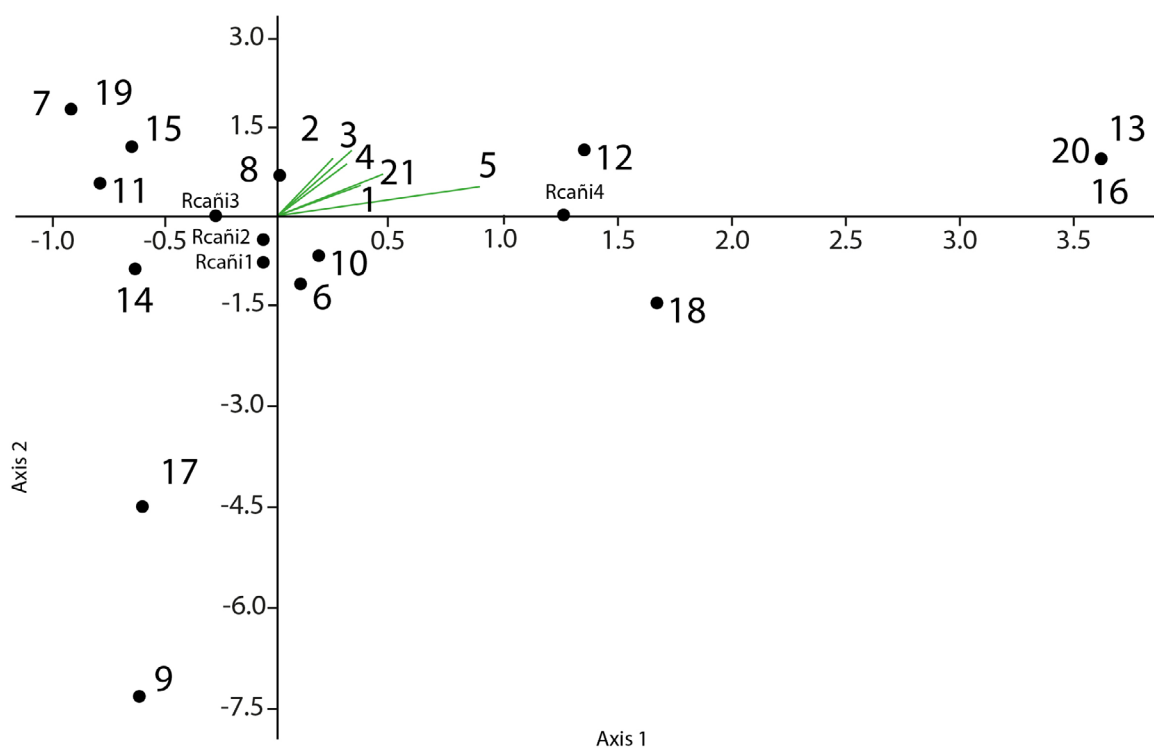


Figure 5. Canonical Correspondence Analysis of the relationship between physicochemical variables and benthic macroinvertebrate families during the high-flow period in the Cañipia River, Cusco, Peru. Axes 1 and 2 represent a two-dimensional plot. 1 = Ammoniacal Nitrogen. 2 = Sulphate. 3 = Temperature. 4 = Electrical conductivity. 5 = Dissolved Oxygen. 6 = Baetidae. 7 = Griptopterygidae. 8 = Corixidae. 9 = Dytiscidae. 10 = Elmidae. 11 = Psephenidae. 12 = Staphylinidae. 13 = Hydropsychidae. 14 = Hydroptilidae. 15 = Chironomidae. 16 = Culicidae. 17 = Tabanidae. 18 = Tipulidae. 19 = Hyalellidae. 20 = Physidae. 21 = pH.

DISCUSSION

The analysis of biotic indices revealed spatial and seasonal variations in water quality. Overall, the indices indicated poorer conditions during the low-flow season, with some stations classified as critical or very poor, while water quality tended to improve during high-flow periods (**Table 6, Figures 4 and 5**).

Conversely, in some monitoring stations, the Chironomidae family increased its populations during both the dry and flood seasons (**Table 2**), likely due to the rise in EC, although without exceeding national regulatory standards (**Table 3**)⁽³²⁾. This increase may be attributed to their use of organic matter as a food source, which can contribute salts or ions to EC⁽³³⁾. However, the highest EC values at the RCañi3 monitoring station during the dry season were associated with an increase in the abundance of the families Elmidae, Tipulidae, and Hyalellidae (**Table 2 and Figure 2**)⁽³⁴⁾.

The pH values recorded in this study fluctuated between 7.10 and 9.16, reflecting variations between neutral and alkaline conditions across monitoring stations and seasons (**Table 3**). Notably, some stations exhibited elevated pH levels, indicating alkaline conditions. This alkalinity suggests a geological influence, possibly associated with the presence of karstic limestone rocks⁽³⁵⁾, which can enhance the availability of potentially toxic metals under neutral to alkaline conditions⁽³⁶⁾. A pH above 9.0 can disrupt the physiological and reproductive development of aquatic organisms⁽³⁷⁾, particularly affecting the Baetidae family (order Ephemeroptera) during the high-flow season. These macroinvertebrates are highly sensitive to pH fluctuations and other environmental factors, which can lead to variations in their abundance⁽³⁸⁾. This pattern was observed at stations with alkaline pH levels (pH = 9.16) during the high-flow season, in contrast to the greater abundance of the Baetidae family in neutral pH conditions during the low-flow season (**Table 2, Table 3 and Figure 4**).

During the dry season, high EC concentrations were observed, ranging from 928 $\mu\text{S}/\text{cm}$ to 1551 $\mu\text{S}/\text{cm}$, while during the flood season, a concentration of 641 $\mu\text{S}/\text{cm}$ was recorded (**Table 3**). Simultaneously, low DO levels were evident in both seasons (**Table 3**). Under these conditions, the Chironomidae family was notably abundant (**Table 2**), as observed in the Huancabamba River in the El Limón dam section, Lambayeque, Peru, in Fish Cultivation Station in the Montelindo Farm of the Universidad de Caldas, Colombia⁽³⁹⁾⁽⁴⁰⁾⁽⁴¹⁾. Likewise, the order Plecoptera, particularly the family Gripopterygidae, was present during both the dry and flood seasons (**Table 2**), as observed in two urban monitoring sites of the river in the city of Quevedo, Ecuador⁽⁴²⁾.

DO availability is influenced by water turbulence and channel characteristics, which directly affect the distribution and richness of BMIs⁽⁴²⁾. Consequently, aquatic organisms in High-Andean regions have developed physiological and morphological adaptations to cope with low atmospheric pressure, which reduces DO solubility (**Table 3**)⁽⁴³⁾.

High concentrations of salts and nitrates negatively affect DO saturation levels due to chemical processes

occurring in the water ⁽³²⁾. However, precipitation serves as a natural source of DO ⁽⁴⁴⁾, facilitating the transport of sediments and organic matter ^(45,46). The elevated EC levels during the low-flow season contributed to a decline in taxonomic richness, particularly among BMIs sensitive to this parameter ^(33, 47, 48), as reflected in the values of the Margalef Index, as seen in the macroinvertebrate communities in southeastern Tibet and originating from the glacial Lake Cuomuliangla, west of the Mila Mountains, the Niyang River basin, China, also in three sectors of the Caney river, Restrepo – Meta, Colombia, and finally on a stretch of the Toribio River, Magdalena Swamp, Colombia (**Table 3, 4 and 5**).

A correlation between temperature, DO, and EC was evident ⁽⁴⁷⁾, as indicated by the CCA analysis (**Figures 2 and 3**). This analysis showed that at RCañi2, the Staphylinidae family was strongly associated with high temperature, EC, pH, DO, and sulfates, while Corixidae, Simuliidae, Ceratopogonidae, and Hydrophilidae exhibited a weaker association with these parameters (**Figures 2 and 3**). These findings align with the study by Chávez ⁽⁴⁸⁾, which highlights the influence of physicochemical factors such as temperature, pH, DO, and EC on BMI distribution, given that these arthropod families are sensitive to environmental conditions. In areas affected by mining and livestock grazing activities, water resource degradation is often linked to the presence of organic matter, creating anoxic conditions that favor the Chironomidae and Ceratopogonidae families ⁽⁴⁷⁾. Meanwhile, Elmidae accounted for 39.56% of the total BMI abundance (**Table 2**), yet its association with physicochemical parameters was relatively low, as indicated by CCA statistical analysis (**Figure 4 and 5**). This result contrasts with the findings of González-Córdoba et al. ⁽⁴⁹⁾, who reported a low correlation between temperature and pH and the Elmidae family.

In High-Andean rivers, the presence of BMIs is strongly influenced by altitudinal gradients, as different altitude ranges determine physicochemical conditions, including high dissolved oxygen (DO) concentrations ^(50,51), which promote greater organism richness. In this context, certain families exhibit higher diversity at lower altitudes, as they are better adapted to variations in temperature, organic matter availability (as a food source), and DO concentration, as seen in some families of caddisflies, that due to their great diversity and the fact that the larvae have different tolerance ranges, depending on the family or genus to which they belong, they are very useful as bioindicators of water quality and ecosystem health, also species susceptible to variation in physicochemical parameters, such as Perlidae and Ptilodactylidae in the Ecuadorian Amazon ⁽⁵²⁾⁽⁵³⁾⁽⁵⁴⁾⁽⁵⁵⁾⁽⁵⁶⁾.

A notable case is station RCañi1 during the low-flow season, where all indices agreed on severely degraded water quality. The BMWP/Col index classified it as critical, the ABI index indicated very poor conditions, and the EPT index rated it as poor (**Table 6**). This aligns with field observations, where reduced river flow was recorded, likely exacerbating pollutant concentration and limiting ecosystem resilience.

Water quality degradation was also evident at other stations, influenced by specific local stressors. RCañi2 was impacted by wastewater discharge from a pipeline, while RCañi3 and RCañi4 were affected by cattle excrement. According to Molina et al. ⁽⁵⁷⁾, ovine and bovine waste is associated with elevated sodium concentrations, which could further alter water quality.

These variations in water quality were reflected in macroinvertebrate assemblages, particularly in the significant abundance of the Elmidae family (**Table 2**). While their presence in this study was associated with degraded conditions, González-Córdoba et al. ⁽⁴⁹⁾ reported that Elmidae can inhabit a broad water quality gradient, ranging from moderately polluted to pristine environments. This suggests that their ecological role may be influenced by site-specific environmental factors and stressors.

Manganese (Mn) concentrations during the high-flow season showed a positive correlation with the BMWP/Col index (**Figure 3**), likely due to parent material erosion and mining activities ⁽⁵⁸⁾. Additionally, biotic indices increased with calcium concentrations (**Figure 3**), as calcium serves as an essential nutrient for these organisms ⁽⁵⁹⁾, supporting the observed positive correlation between EPT and calcium. Furthermore, the presence of organic matter, clay, and other particles ⁽⁶⁰⁾ facilitates metal accumulation in sediments, which explains the positive correlation of BMWP/Col and ABI with metal concentrations (**Figure 3**).

There is also a sensitivity gradient between BMI presence and metal concentrations (Mg and Na), where Coleoptera, Decapoda, Trichoptera, Diptera, and Ephemeroptera exhibit varying degrees of tolerance, from lowest to highest sensitivity (**Figures 2 and 3**) ⁽⁶¹⁾. Within Diptera, the Chironomidae family is recognized for its adaptability to organic matter and potentially toxic heavy metals ⁽⁶²⁾.

Flow velocity in each season significantly influenced the presence, absence, and abundance of BMIs. A study in Piburja Stream, Ecuador, found that during the high-flow season, hydrologically distinct habitats emerged, whereas during the low-flow season, macroinvertebrate diversity and density increased ⁽⁶³⁾. This pattern was reflected in the results of Simpson's Index (1-D) and Shannon's Index (H') across both seasons (**Table 5**).

It is recommended to use aquatic macroinvertebrates (MIB) as indicators of water ecological quality, as they provide information on the overall condition of the water body. This information is valuable for the implementation of citizen monitoring programs and projects aimed at conserving ecosystem services. Furthermore, it is suggested that MIB be included in environmental monitoring programs to track and control environmental impacts, thereby improving the effectiveness of environmental management policies and land-use planning.

It is important to note that global warming is gradually depleting river systems, leading to the local extinction of aquatic organisms ⁽⁵⁷⁾. This results in structural and functional changes in species, such as variations in distribution, population size, and decreased abundance, among other effects ⁽⁶⁴⁾. Likewise, climate change influences flow rate variations, which determine seasonal differences and, consequently, the presence of MIB communities ⁽⁶⁵⁾. Studies have shown that temperature increases beyond the tolerance range of aquatic species lead to changes in life cycles, growth rates, survival capacity, and the distribution of MIB species ⁽⁶⁶⁾.

CONCLUSIONS

The analysis of biotic indices revealed differences in water quality across stations and seasons, influenced by factors such as wastewater discharges and livestock activities. Variability in water quality was associated with the differential abundance of groups such as Elmidae and Chironomidae, highlighting the sensitivity of certain aquatic macroinvertebrates to environmental disturbances.

Additionally, a correlation was observed between metal concentrations and biological responses, underscoring the impact of parent material erosion and mining activities. These findings emphasize the need to integrate biomonitoring into environmental management and conservation programs for High-Andean aquatic ecosystems, particularly in the context of climate change, which affects water availability and the stability of biological communities.

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DECLARATION OF COMPETING INTEREST

The authors have declared no conflict of interest.

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