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## Water quality assessment using biological indicators in Cane River - Colombia

### Evaluación de la calidad del agua mediante indicadores biológicos en el río Cane – Colombia

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#### KEYWORDS

Macroinvertebrates; water quality assessment; paramo ecosystems; biological monitoring; biotic indices  
Macroinvertebrados; evaluación de la calidad del agua; bioevaluación; monitoreo biológico; índices bióticos

#### ABSTRACT:

This study shows the results of a water quality assessment for the Basin of the Cane River, which belongs to Iguaque Flora and Fauna Sanctuary National Natural Park, a naturally protected area formed by the Andean Forest and paramo ecosystems in Colombia. The water quality of this river is impacted by agricultural runoff and domestic wastewater from human activities in the buffer area. This was assessed by using chemical, physical, and biological indexes. The IDEAM, NSF and water quality indexes were based on the European standards of measurement. Further to this, BMPW/Col and ETP biological indexes were analyzed and, amongst these, IDEAM WQI (Water quality index) and ETP showed a good correlation. A similar trend was found between the IDEAM water quality index and BMWP/Col index, showing their potential as a good tool for water catchment, diagnosis, assessment, and management in Andean paramo basins. In total, 537 specimens of macroinvertebrates were collected, identifying 8 taxonomic orders, 16 families and 18 genera. Predominant orders were Trichoptera, Ephemeroptera, and Diptera. Baetidae and Planariidae taxa were found in the three water quality levels, showing a decrease in number with a decrease in water quality. Thus, both species have shown sensibility to water quality parameters and also to riverine habitat quality, being then interesting species to determine water quality, deserving more detailed studies for similar cases in high Andean forests and paramo basins.



**RESUMEN:** Este estudio analizó la cuenca media del río Cane, que en su parte alta pertenece al Parque Nacional Natural Santuario de Flora y Fauna Iguaque, un área natural protegida formada por ecosistemas de bosque andino y páramo en Colombia. La calidad del agua de este río está impactada por la escorrentía agrícola y las aguas residuales domésticas y se evaluó mediante indicadores químicos, físicos y biológicos. Se aplicaron los índices del IDEAM, NSF y un índice de calidad del agua basado en normas europeas. También se analizaron los índices biológicos BMPW/Col y ETP. Se encontró una buena correlación entre el índice nacional de calidad del agua (IDEAM) y el índice ETP, mostrando su potencialidad como una buena herramienta para el diagnóstico y manejo de captaciones de agua en cuencas forestales andinas. Se colectaron 537 especímenes, en los cuales se identificaron 8 órdenes, 16 familias y 18 géneros. Los órdenes predominantes fueron Trichoptera, Ephemeroptera y Diptera. Los taxones Baetidae y Planariidae se encontraron en los tres niveles de calidad del agua mostrando una disminución en el número con una disminución en la calidad del agua. Así, ambas especies han mostrado sensibilidad a los parámetros de calidad del agua y también a la calidad del hábitat ribereño, siendo entonces especies interesantes para determinar la calidad del agua, ameritando estudios más detallados para casos similares en cuencas de bosques altoandinos y páramos.

## 1. Introduction

Physicochemical and bacteriological parameters are the main indicators used to determine the state of a water body. However, they have some limitations since, in general, they only allow us to easily know the variation in time of water quality conditions and the relationships between the different environmental factors if in-depth analyses are made. The measurement of these parameters consumes time and important economic resources, because chemical analyses are needed for several samples [1]. For this reason, biological indicators based on the use of living organisms, such as macroinvertebrates, are currently being used in addition to conventional water quality parameters and indicators [2]. When the habitat is altered, aquatic systems get stressed, which is reflected in the biodiversity. Biological indexes based on the population of macroinvertebrates indicate this general condition of the aquatic ecosystem and, therefore, the water quality of streams. Additionally, physico-chemical conditions can be momentarily altered by climatic conditions and water discharges, among other factors [2].

These indicators are based on the tolerance, presence, abundance, and richness of the macroinvertebrates present in the body of water. Some of the most used biological indicators in water quality studies are the Biological Monitoring Working Party (BMWP), the Average Score Per Taxon (ASPT), the EPT index (Ephemeroptera, Plecoptera, and Trichoptera), and the SIGNAL-2 [1], [3]–[5]. This study used the first two indices. The application of these indices requires the analysis of the characteristics of each monitored place, as well as the comparison with the physicochemical parameters to verify their validity.

In Colombia, one of the first studies of bioindicators was carried out by [6], who studied the fauna of macroinvertebrates as indicators of the degree of pollution in the Medellín River. In this way, [7] published the first guide for studying aquatic macroinvertebrates in the department of Antioquia, which years later would be adapted for the different bodies of water in Colombia BMWP/Col.



Some studies developed worldwide tried to find the relationship between biological and physicochemical indices for water quality assessment. One of the most complete approaches in this sense was the study made by [10], who studied 49 different water, pollution, and sediment types in 15 rivers in China. Their analysis included 5 biological indices: taxa richness, BMWQ, t-BMWQ, a-BMWQ, and density. In this study, no direct relationship was found between these indices and State Environmental Protection Administration types of water established for China for medium coarse and fine substrates. On the other hand, [5] evaluated 20 sites in the Richmond River catchment in Australia, finding that the most sensitive index was SIGNAL 2, and the most robust one was the Family Richness percentage. They concluded that the EPT index showed the response to habitat qualities and water quality at the same time. They also concluded that the EPT index was significantly affected by the riparian vegetation at the river borders, which is highly related to land use. Similarly, a study by [45] in the Otun River in Colombia found a relationship between biological and chemical indexes. They concluded that the BMWP/Col and ICOMO index were related to rainy and dry seasons. A similar result was found by [46], who found that BMWP/Col values and the ICOMI, ICOMO and ICOSUS indicators coincided for the Cauca River in the urban area of tPopayan, Colombia. Another example of this relation is the case of the study made by [8], which showed that BMWP for rivers in Thailand was related to water quality established by chemical methods. In the same way, [9] figured that there was a good correlation between physicochemical parameters such as temperature, electrical conductivity (EC), and BOD (Biological Oxygen Demand); and the presence or absence of certain species of macroinvertebrates at Moiben River, Kenya [10]. Besides these results, most of these methods and their comparisons showed that it is necessary to analyze, complement, and develop regional indices further to understand the freshwater ecosystems in a holistic way [8], [10], [11]. These studies also concluded that as macroinvertebrate classification at the species level could be difficult, the selection of a few indicator species in each ecosystem could be useful, and the classification to the trophic guilds approach could give more accurate results [10], [12], [13]. To our knowledge, many of the high Andean Catchment biomonitoring assessments by macroinvertebrates use adapted indexes or general indexes to qualify water quality. Still, they do not correlate them with water quality physicochemical indexes [14]–[17].

Therefore, this study uses physical and chemical parameters to determine the water quality of the Cane River in its middle basin. It also analyzes its relationship with the presence of bioindicator organisms and biological indicators. As mentioned earlier, few of the previous studies made in Colombia, analyzed biological indices and physicochemical indices and their relationship. The study developed by [18] for the Frio River, located in Cundinamarca, Colombia, an ecosystem located starting at 3.749 masl, reported that the ecosystem state of this river uses both physicochemical and biological indices. [19] studied biological and physicochemical indicators for the Negro River in Antioquia, comparing its quality in 2002 and 2007, finding a water quality improvement in this period, and that there was not a correlation between chemical and biological indices. On the other hand, most of the Colombian studies in this area use the biological indicators themselves to determine water or ecosystem quality. The study made by [20] for coffee farms in Cundinamarca and Santander, Colombia, shows a correlation between the BMWP Index and the presence or absence of coffee farms certified by environmental criteria close to the monitoring point, showing a clear relationship among them. [21] studied the Garagoa River in



Boyacá, Colombia, in 2013, establishing the water quality using some bioindicators such as BMWP/Col, and analyzing the ecosystem state in two different climatic conditions for this basin. [22] studied Guachicos River in Pitalito, Colombia, using the BMWP/Col Index, finding that waters ranged from non-polluted to slightly polluted, and the dominant genera was Trichoptera for monitoring made from March to September 2018. In addition, [23] studied the Santo Tomas stream in Pitalito, Colombia, applying biological indices, such as BMWP/Col, EPT, and ASPT, finding very clean waters to slightly polluted for this basin.

This study was conducted in the Villa de Leyva, sector of Boyacá, starting from the section that went out of a naturally preserved area. This section was chosen since the river is born in the National Natural Park Sanctuary of Flora and Fauna of Iguaque, a conservation area, and continues its course through an area where rural populations are developing different economic and social activities. In its course through this zone, the river suffers a gradual degradation by these activities. The objective was to evaluate the suitability of different water quality indexes based on biological and physicochemical parameters for this part of the basin and know its conditions.

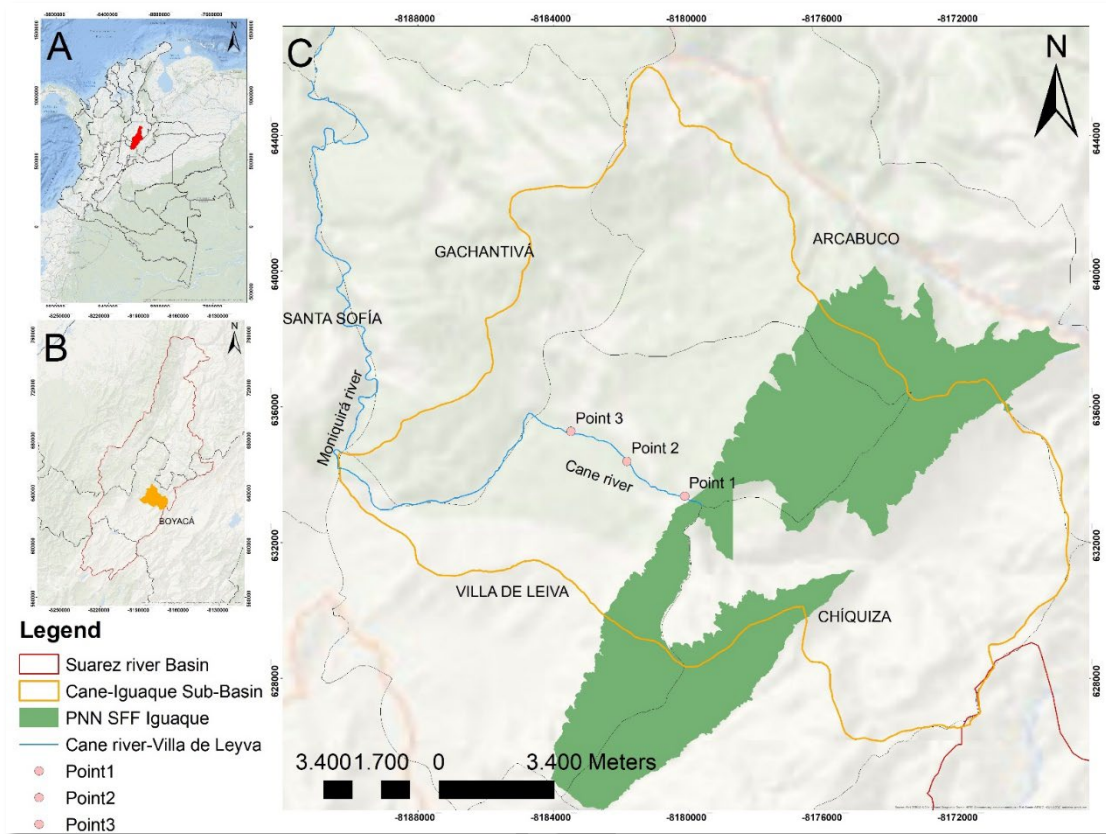
In this way, this study compares different characteristics of water quality in intervened ecosystems in areas near a paramo zone, a strategic ecosystem for the provision of water in Colombia. This study, as a few others made in high Andean rivers, attempts to understand the relation among usual biological indices such as BMWP/Col and ETP and some WQI, to check the suitability of already developed biological index adapted to a country level such as BMWP/Col for assessing water quality in a high Andean catchment. In particular, the relationship with IDEAM WQI, the most used water quality index in Colombia, is analyzed. In addition, this study aims to establish the relationship between the presence of certain macroinvertebrate species and water quality, comparing it with previous studies developed in the country and the world. In the same way, water quality and ecosystem characteristics are studied for this basin located in the surroundings of an important Andean Forest preserved area.

## 2. Methods

### 2.1. Study Area

The Cane-Iguaque River basin has five sub-basins: Roble, Cebada, Colorada, Iguaque, and Campohermoso. In the upper part is the Iguaque Flora and Fauna Sanctuary National Natural Park (SFF) which occupies 6,750 hectares, of which 53.9% are located within the basin[24]. The Cane River originates in the municipality of Chiquiza, has a total length of 29,7 km, crosses the SFF, and empties into the Moniquirá River. The study area is in the La Colorada sub-basin, municipality of Villa de Leyva, Boyacá, Colombia, with a length of 4,0381 km, presenting heights from 2200 to 3750 m.a.s.l and an annual rainfall of 1600 mm. Therefore, monitoring was carried out in 3 points: point 1 is in the upper part of the basin whose coordinates are N 05°40'49,3", W 73°29'0,25. This point borders the SFF. Point 2 is in the middle of the basin with coordinates N 05°41'22,4", W 73°29'55,4", and point 3 is in the lower part of the studied area with location N 05°41'50,8", W 73°30'48,6" as shown in figure 1.





**Figure 1** Location of the Cane-Iguaque Basin and study area. A. Suarez River Basin, B. Cane-Iguaque Rivers sub-basin. C. Monitoring points location

## 2.2. Sample Techniques

### 2.2.1 Water Quality Parameters

An integrated sample was taken at each point in the river. Monitoring was made for 8 hours during the day, on a normal day, considering there were normal climatic conditions, which means no rain before or during the sampling. Punctual samples were taken every hour at the three points according to Figure 1, and they were mixed in quantities proportional to water velocity at that point, to get a total volume of 1 liter for each point. Next, the sample was introduced into a previously sterilized container, refrigerated, and taken to the laboratory for posterior analysis. A multiparameter equipment from Hanna Instruments, reference HI 9829, was used for field measurements. Field parameters analyzed were pH, dissolved oxygen (DO), EC, water temperature, atmospheric pressure, oxidation-reduction potential (ORP), and total dissolved solids (TDS). The samplings were carried out in June, September, and December 2021. June and December are months with low precipitation levels and September has high precipitation levels. The following chemical parameters were measured in the laboratory: Chemical Oxygen Demand (COD), BOD, nitrates, nitrites, phosphates, ammonia, turbidity, suspended solids, and E. Coli. These parameters

were chosen along with field parameters because they are considered the most representative of the water quality and are needed to calculate the water quality indices proposed for this study. COD was measured using the adapted method EPA 410.4, and BOD was measured using the respirometric method. E. Coli was measured using a defined substrate technology, Colilert. Nitrates were measured by adapting the method of the Cadmium Reduction Method (4500-NO<sub>3</sub> Standard Methods for the Examination of Water and Wastewater). Additionally, nitrites were determined by an adaptation of the ferrous sulfate method, phosphates by an adaptation of the ascorbic acid method (4500-P Standard Methods), ammonia by the Nessler method. Turbidity was determined following Standard Methods 2130, and suspended solids by filtration and heating to dryness at 104-105°C [25].

### 2.1.2 Macroinvertebrates Sampling

The macroinvertebrates were sampled quantitatively, using the Kick sampling technique, in which a type D net was placed against the current, and the substrate was removed by hand for a certain period (5 to 10 minutes), separately sampling the different microhabitats present on the site. Subsequently, the samples were fixed with 70% alcohol in appropriate plastic containers.

Afterward, in the laboratory, each sample was observed with a stereoscope to separate the organisms and carry out their identification at the family and genus level through the guides by Roldan (1988) [7], Gonzalez et al. (2013) [26] and Dominguez and Fernandez (2009)[27]. Photographs were taken, and in addition, the relative abundance values were obtained for the different taxa found.

## 2.2. Water Quality and Biological Indices

### 2.2.1 Water Quality Indices

One of the water quality indexes used was the IDEAM WQI [28]. It includes the following parameters: DO, total suspended solids, COD, EC, Total nitrates/Total phosphates, and pH. The Institute of Environmental Studies in Colombia – IDEAM, uses this WQI to monitor the water quality of surface water bodies. The WQI proposed by [29] includes the parameters pH, EC, total settleable solids, ammonia, nitrite, nitrates, total phosphorus, COD, BOD<sub>5</sub>, (DO), and temperature. This WQI was proposed to evaluate watershed pollution in rivers, lakes, and Wastewater treatment plants in Spain. Finally, the National Sanitation Foundation Water Quality Index (NSF-WQI) was calculated using the parameters total solids, nitrates, phosphates, BOD, DO, temperature, turbidity, TS, pH, and fecal coliforms [30]. The rating scale for each index is shown in Table 1.

**Table 1** Water Quality Qualification according to IDEAM, NSF, and WQI

IDEAM WQI			Sanchez WQI			NSF WQI		
Description	WQI Range	Color	Description	WQI Range	Color	Description	WQI Range	Color
Good	0.91 – 1		Excellent	91-100	No categ	Excellent	91-100	No categ



Acceptable	0.71 – 0.9		Good	71-90	orized by colors	Good	71-90	orized by colors
Regular	0.51 – 0.7		Medium	51-70		Medium	51-70	
Bad	0.26 – 0.5		Bad	26-50		Bad	26-50	
Very Bad	0 – 0.25		Very bad	0-25		Very bad	0-25	

All the WQI above used the following empirical equation 1:

$$WQI = \frac{\sum_i C_i P_i}{\sum_i P_i} \quad (1)$$

Each WQI index proposes a normalized concentration value  $C_i$  with the respective weight  $P_i$  for each parameter.

### 2.2.1 Biological Indices

The Biological Monitoring Working Party (BMWP) index emerged in England in 1970, as a method for assessing water quality using macroinvertebrates as indicator organisms. This index is characterized by being simple and easy to apply. For its calculation, the presence or absence of macroinvertebrates must be stated by identifying their family. Each organism has a rating from 1 to 10 according to its level of tolerance to contamination, with 1 being the most tolerant and 10 the least tolerant [40]. The sum of the scores of the macroinvertebrates belonging to each family provides the total value of the BMWP, which indicates a state of water quality [31]. Due to the advantages in the application of this index, modifications of the BMWP scoring system have been developed for use in countries such as Colombia [7], [32], Spain [33], Poland [34], and Thailand [8], among others.

The rating for this index was adjusted for Colombia according to what is established by [31] and is shown in Table 2. Equation 2 is used to calculate the total value of the index at each monitoring point:

$$BMWP = NT_1 + NT_2 + NT_3 + \dots \dots \dots NT_n \quad (2)$$




Where,  $NT_n$  is the tolerance level corresponding to each family found, and the sum of these values indicates the state of the water quality (Table 2).

**Table 2** Classification of waters for the BMWP/Col index

Type	Value	Description	Color
I	>120	Clean Water	
	102-120	Waters not polluted or not sensitively altered	
II	61-100	Some evident pollution effects	





III	36-60	Polluted Waters	
IV	16-35	Very polluted waters	
V	<15	Heavily polluted waters	

The EPT index only considers the organisms of the order Ephemeroptera, Plecoptera, and Trichoptera. The species of these orders are characterized by being sensitive to pollution. The index is calculated by dividing the number of individuals found corresponding to these three orders by the total number of individuals collected, classifying the quality of the water source in different states according to this value [38] [41]. Equation 3 was used to obtain the value of the index, which is classified into different quality categories, as shown in Table 3.





$$ETP = \left( \frac{\sum_{i=1}^n T_i}{A} \right) * 100 \quad (3)$$

Where  $T_i$  is the total of the individuals present for each of the three orders Ephemeroptera, Plecoptera, and Trichoptera, and A is the abundance, which is calculated by the following equation:

$$A = \frac{E_i}{T_i}$$

Where A is the abundance of species;  $E_i$  is the number of individuals present from the three orders Ephemeroptera, Plecoptera, and Trichoptera, and  $T_i$  is the total number of individuals collected in the sample.

**Table 3** Classification of waters for the ETP index

Type	Range %	Description	Characteristics	Color
I	75-100	Very good	No impacts	
II	50-74	Good	Slightly impacted	
III	25-49	Fair	Moderately impacted	
IV	0-24	Poor	Severely impacted	

### 3. Results

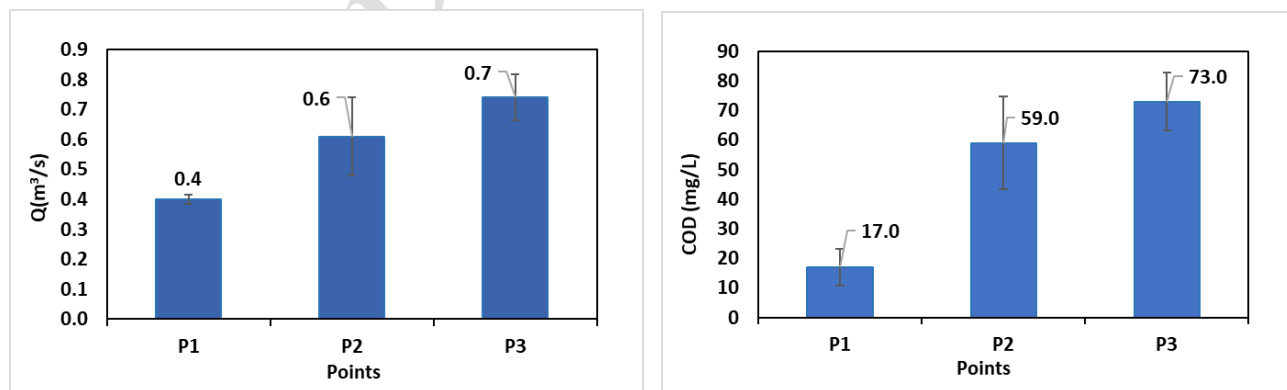
There was a difference in water quality parameters in time and space, because every point has its own characteristics related to surrounding activities and impacts. For COD, average values were 17 mg/L for point 1 (P1), 59 mg/L for point 2 (P2), and 73 mg/L for point 3 (P3). DO was 6 mg/L for p1, 4.8 mg/L for P2, and 3.7 for P3, showing a water quality diminishing downstream (Figure 2). P 1, located in the

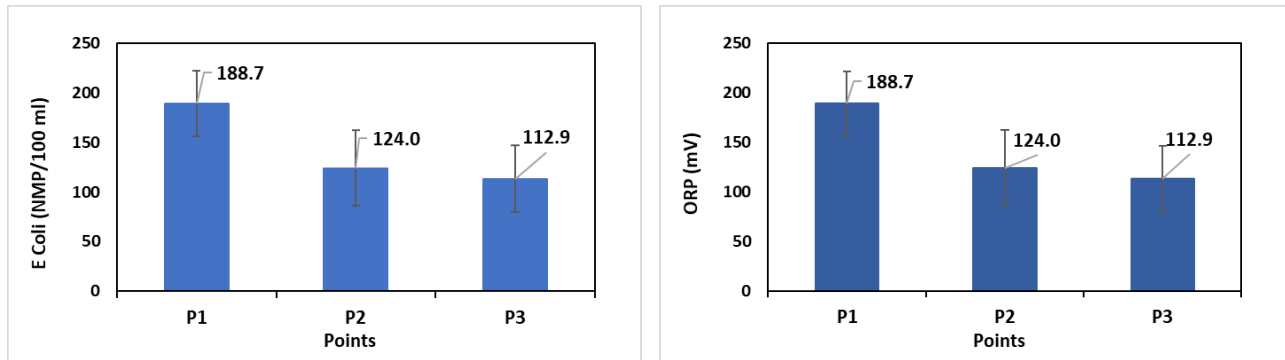


upper part of the sub-basin and being the closest to the nature reserve, presents less anthropic intervention. According to what was observed in the fieldwork, no residual water discharges were identified with the naked eye. In this place there is a water intake that supplies one of the village aqueducts in the municipality of Villa de Leyva. In the surroundings of P2, located in the middle part of the sub-basin and at 2118.2 meters from the first point, there is evidence of population growth and of the presence of economic activities such as cattle ranching and agriculture near the riparian buffer zone. Finally, P3, located in the lower part of the sub-basin at 1946.8 meters from the previous point, was characterized by having agricultural activities and rural housing close to the body of water.

### 3.1. Water Quality Parameters

Figure 2 shows some of the most important parameters measured for the three monitoring points. Water flow shows that, as expected, point 1 has the lowest amount of water for being very close to the source of the Cane River, with an average of 0.4 m<sup>3</sup>/s, 0.6 m<sup>3</sup>/s at point 2, and 0.7 m<sup>3</sup>/s at point 3. COD values showed that besides having a higher flow, organic pollutant material grows along the monitoring area, being P3 more than 4 times more polluted than P1. The ORP is a parameter that indicates the oxidizing or reducing conditions present in the water body, showing a maximum of 188.7 average at P1, diminishing to 124 and 112.9 at P2 and P3, respectively. This value was concordant with the values found for nitrates form which were 0 at every point, but nitrites and ammonia were present at almost every point, reflecting middle-reducing conditions in these waters. DO levels were 6.0 mg/L at P1 and 80% of saturation levels at local conditions; 4.9 mg/L at P2; 62.9% of saturation level; and 3.7 mg/L at P3, 42% of saturation level. 1594 decree [42], the law that nowadays rules water quality in Colombia, has established a 6.5 - 9.0 mg/L level for OD for flora and fauna preservation in cold inland waters. Meanwhile, Ambient water criteria for fish life preservation in cold waters from EPA are 5-8 mg/L for early life stages and 4 mg/L for other life stages. Monitoring points P2 and P3 do not fulfil this standard, and point 3, is far from the recommended levels.





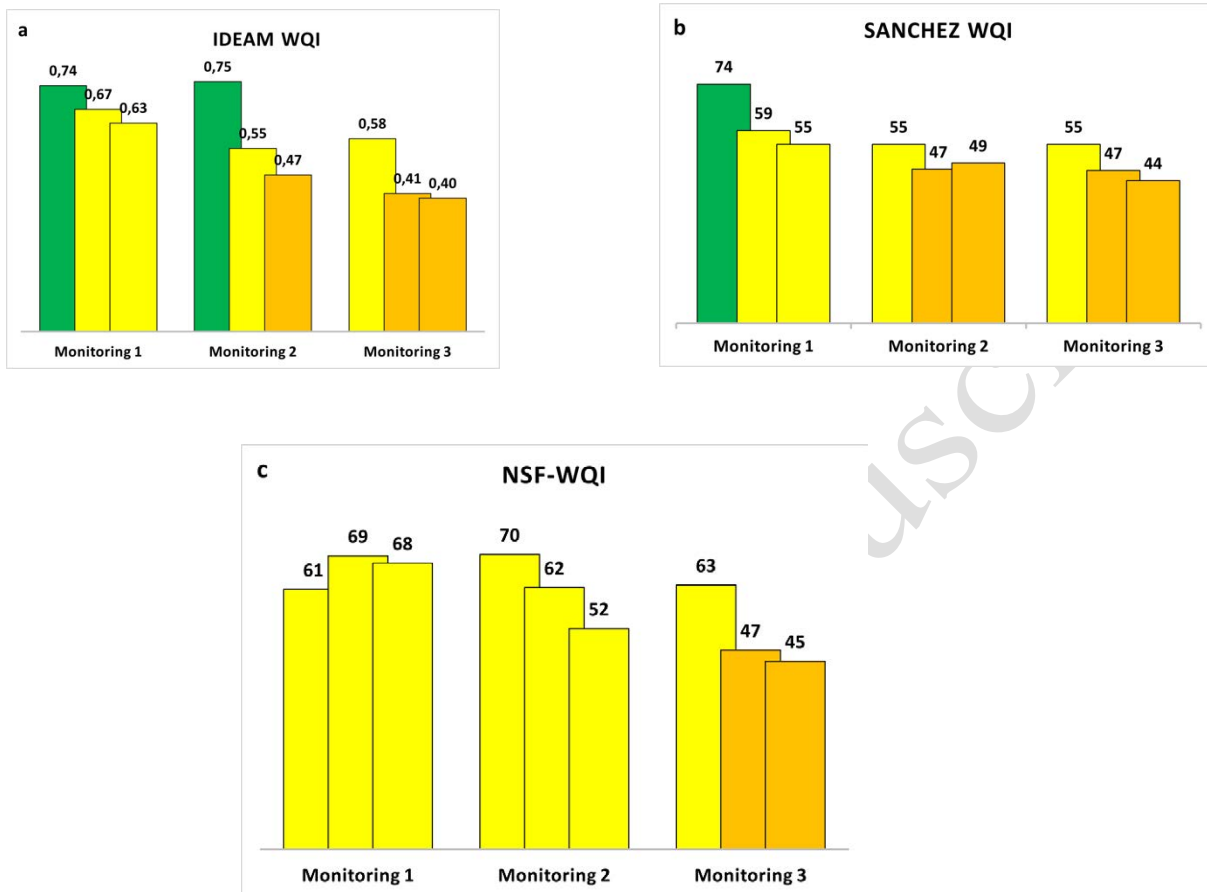
**Figure 2** Water Flow, COD, ORP, and E. Coli concentrations for three monitoring times. P1: Point 1, P2: Point 2; P3: Point 3

Results for parameters measured in the field and laboratory are shown in supplemental material (S1)

### 3.2. Water Quality Indices

The results obtained for water quality indices applied to the Cane River in the 3 points are shown in Figure 3. In the case of the IDEAM WQI, shown in Figure 3a; the first monitoring gave, as a result, an acceptable quality in the first point and regular for points 2 and 3. Second monitoring had, as a result, good in the first point, regular in the second point, and bad in the third point. The third monitoring had, as a result, regular in the first point, and bad quality in the last two points. The results for Sanchez WQI, based on European standards (figure 3b), were good water quality in P1, and medium for the other two points, and monitoring 2 and 3 gave medium quality in P1, and bad for P2 and P3. For NSF WQI in Figure 3c, three monitoring and three points resulted in slight variations with medium water quality in June and September monitoring, medium water quality at the first two sampling points and bad quality in P3. In general, it is concluded that in the first monitoring, water quality was better on average for the three points, being related to water flow that was  $0,73 \text{ m}^3/\text{s}$  on average in the three points compared to  $0.52$  and  $0.49 \text{ m}^3/\text{s}$  in the second and third monitoring, respectively. In addition, in this case, IDEAM and Sanchez WQI reflect, in a better way, the variations in water quality at Cane River than the NSF WQI. It can be concluded that the first point has a better water quality than the other points, being their quality in terms of those indexes is very close between points 2 and 3. No other studies have been found showing water quality for this river, despite its importance as a provider of environmental services in an area characterized by harboring a flora and fauna sanctuary[43]. However, [44] studied the Mamarramos brook microbasin, upstream of the area in this study, and in the section inside the preserved area and found excellent hydromorphological and very good BMWP/Col and Ecological Quality Index. It can be

concluded then that once the river goes out of the preserved area, its water quality deteriorates in a short section. Detailed data can be found in supplemental material S2.

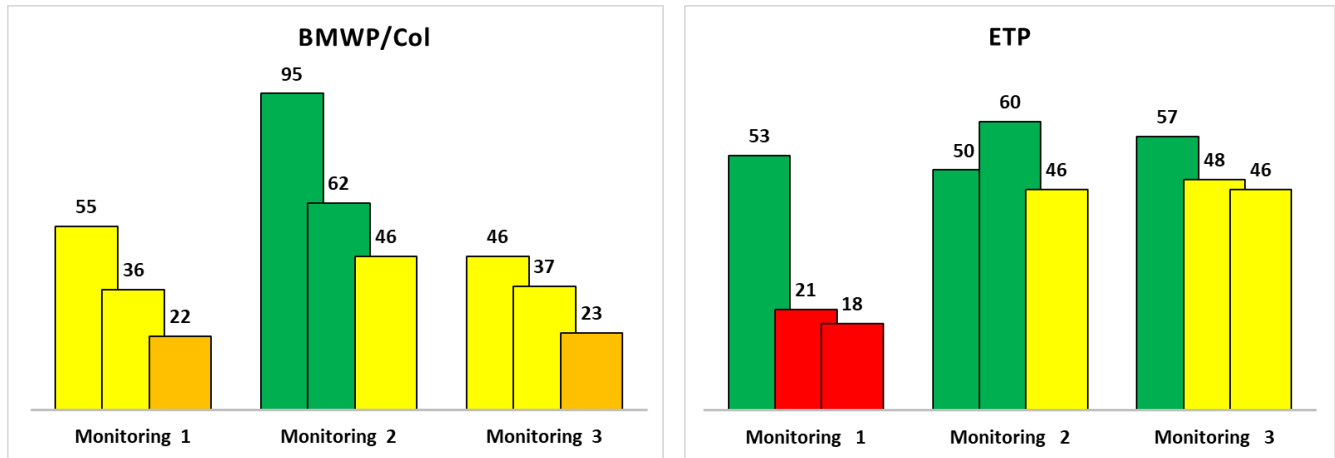


**Figure 3** Water Quality Indexes at Cane River Middle Basin – 2021

### 3.3. Biological indices

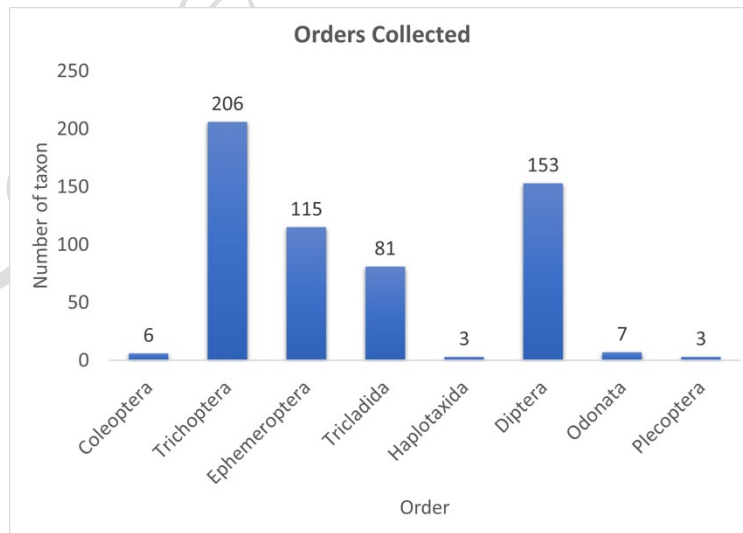
The aquatic macroinvertebrates found in the Cane River are classified by order, family, and gender and shown in supplemental material S3. 8 orders, 16 families and 18 genera were identified. The highest number of individuals was recorded in the September monitoring.

The results of the calculated biological indices are shown in Figure 4 (Values in supplemental material S4):



**Figure 4** Water Quality Biological Indexes at Cane River Middle Basin. 2021

According to the BMWP / COL index, points 1 and 2 were classified as contaminated waters or with some effects of pollution, while point 3 was classified as highly contaminated or contaminated waters. The second monitoring with the lowest level of contamination found was conducted in September. However, it should be noticed that this was also the monitoring in which a greater effort was made to sample macroinvertebrates, finding species that were not found in the other two monitoring dates. On the other hand, the ETP index is required to classify the collected specimens according to the order level, and quantitative data on the number of individuals per order is required. It only considers three orders, two of which are dominant in the study, with 206 individuals of the order Trichoptera and 115 of the order Ephemeroptera (figure 5).

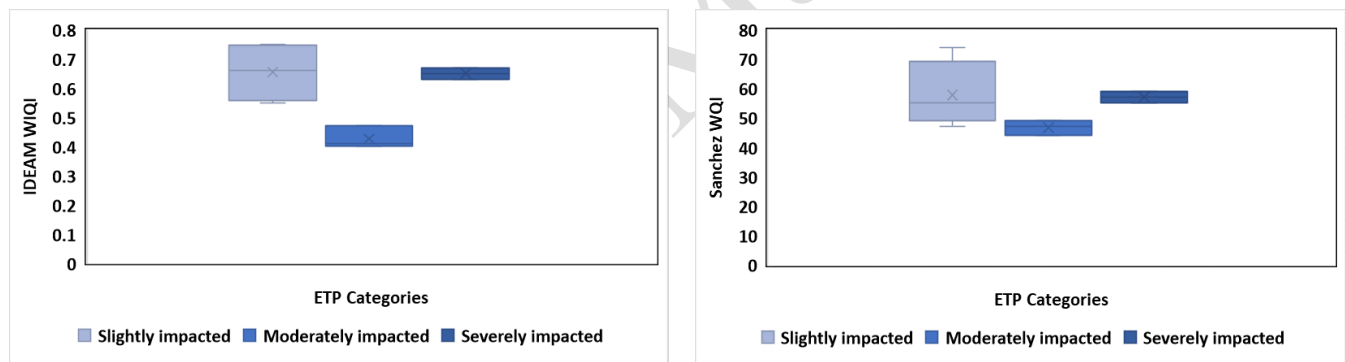


**Figure 5** Orders Collected. 2021

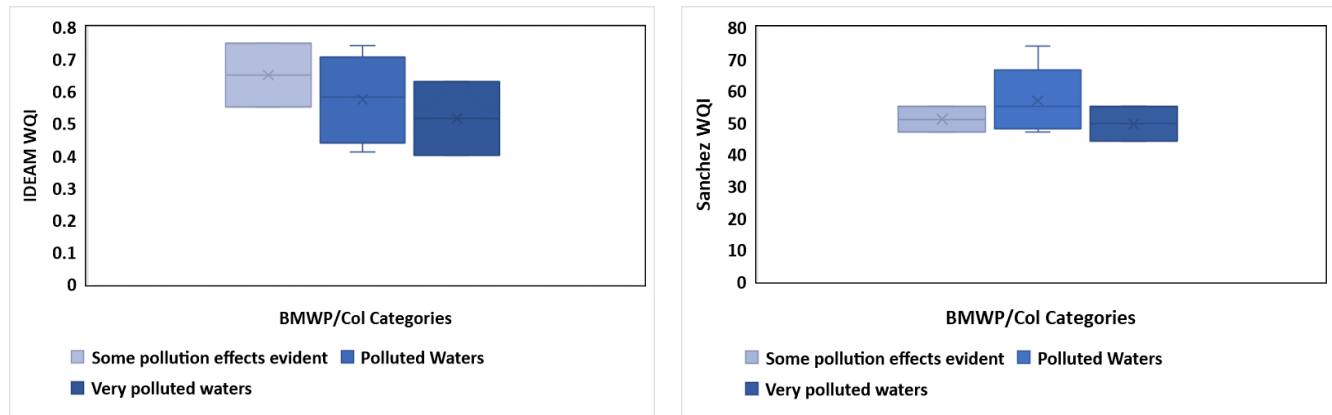
Results for the ETP Index indicated good water quality in the first point for the three monitoring dates, bad for point 3 on all 3 monitoring dates, and bad for point 2 for the 1st and 3rd monitoring dates, while the second resulted in poor water quality. In general, these results are more consistent with the quality indices obtained through physicochemical parameters.

### 3.3. Physicochemical vs Biological Indices

Figure 6 shows how ETP water quality categories are related to IDEAM WQI and Sanchez WQI water quality scores in the Cane River Basin, according to data found in this study. For slightly impacted waters found at P1, where little pollution was found, IDEAM WQI and Sanchez WQI had high values, as expected. The tendency for P2 was decreasing, but P3 gave a high bioindicators value again, showing a low relation between these indexes. As mentioned earlier and found by several researchers, a further study including variables related to habitat regional biodiversity, or trophic guilds, around the river in the 3 points could help to improve the explanation for these results and to confirm, as happened with all the high Andean catchments studied previously, that biological indicators based on macroinvertebrate classification at the family level are not a very accurate water quality indicator by itself in many cases, and that local conditions require scores for some species at a different level [12], [47].



**Figure 6** IDEAM WQI and Sanchez WQI values (y) vs. ETP index categories (x) for the Cane River. 2021



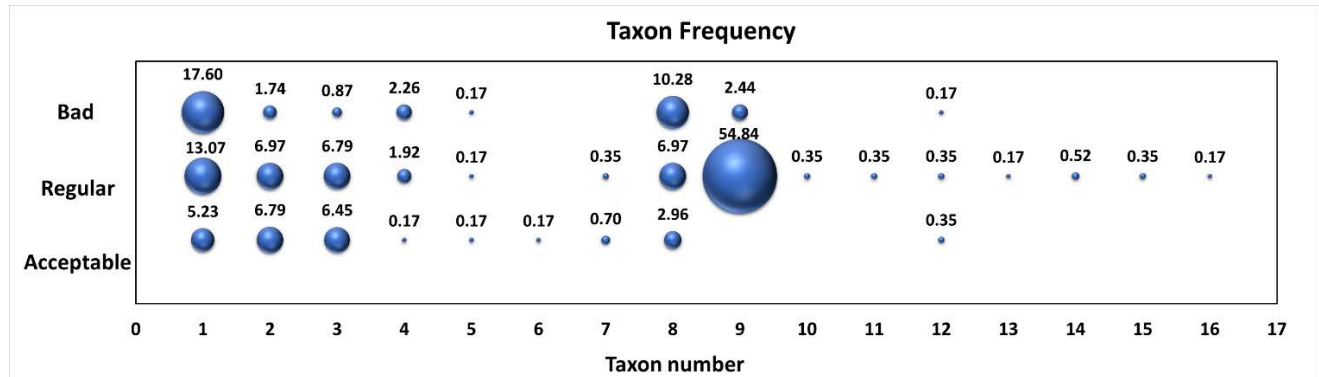
**Figure 7** IDEAM WQI and Sanchez WQI values (y) vs BMWP/Col index categories (x) for Cane River. 2021

On the other hand, figure 7 shows how BMWP/Col water quality categories are related to IDEAM WQI and Sanchez WQI water quality scores in Cane River Basin, according to monitoring made in June, September, and December 2021. A good relation was found between BMWP categories and IDEAM WQI values. IDEAM WQI [28] has 5 water quality categories based on parameters: DO, TSS, CDO, conductivity, and pH, with ponderations after being normalized under established relationships. This WQI is broadly used for assessing water quality in Colombia, using parameters measured in 150 stations along the country 4 times per year (one per trimester), and accurately shows the water quality in the country. In this case, it showed a clear trend of decreasing quality along the Cane River. Additionally, the BMWP/Col WQI [31] is an index adapted from BMWP developed for the UK to the macroinvertebrates present in Colombia. Its author explains how a defined BMWP/Col index should be adapted to each region depending on local geological conditions, showing that certain geological or local conditions could not permit a higher organism's diversity, although water quality is good, and that is why methodology must be adapted to regional conditions. However, according to results found in this study, the general BMWP/Col index has very good performance determining water quality in this river according to the IDEAM index, and consequently, could be used as a replacement for physicochemical-based indices to establish water quality for this basin. This can be related to the relatively good conservation state of the surrounding habitat due to the presence of a conservation area at the proximities. However, studies with longer monitoring times for each period and throughout the whole year (four monitoring per year, one per trimester) are necessary for having a definite conclusion about this, as well as studies comparing two very similar areas in terms of geological and pollution conditions, but with different habitat states.

### 3.5. Biotic Indices



A total of 537 specimens were analyzed, the most common being the hydropsychidae (1), with 28.36 % of the total, followed by Simuliidae (8) (17.12%), Baetidae (2) (12.47%), Planariidae (3) (11.64%), Blepharoceridae (9) (5.62%) and Leptohiphidae (4) (4.11%). Other taxas had less than 3% in frequency each (See Figure 8).



**Figure 8** Taxon Frequency for each water quality category according to IDEAM WQI, for Cane River. 2021 Taxon numbers: 1. Hydropsychidae, 2. Baetidae, 3. Planariidae, 4. Leptohiphidae, 5. Haplotaxidae, 6. Chrysomelidae, 7. Ortorapha Nematócera Tipulidae, 8. Simuliidae, 9. Blepharoceridae, 10. Dryopidae, 11. Calopterygidae, 12. Lestidae, 13. Leptophlebiae, 14. Perlidae, 15. Ptilodactylidae, 16. Scirtidae

The Families Hydropsychidae, Simuliidae, Baetidae, Planariidae, and Leptohiphidae were found in the three categories of acceptable, regular, and bad water quality calculated by the WQI-IDEAM. The family Hydropsychidae (Number 1 in Figure 8) of the order Trichoptera are among the most diverse macroinvertebrates and inhabit lotic ecosystems with high oxygen levels. They are insects living in riverine habitats all around the world [48]. The presence of this family has been reported in Colombian rivers where the water quality is between good, and regular [26]. The conclusion has been made that this species' survival in rivers depends not only on good habitat conditions but also on chemical conditions and factors such as DO and stream velocities. Studies have found that low oxygen concentrations do not affect their survival at levels as low as 2 mg/L, but in combination with toxic substances such as pesticides and changes in water flow due to rain and other conditions, can be detrimental to their survival [49]. Further analysis of the surrounding habitat for each point should be considered in future studies to understand its higher number for bad than that for acceptable water quality conditions.

Taxa Simuliidae (8), with more than one hundred species, are common flies, and their larval stadiums are aquatic; they are fixed over stones and plants, usually in clean and oxygenated waters. In this study, they were found in acceptable, regular, and bad-quality waters. It has been used in several studies as water quality indicators [50]–[52], and it is known that they react to physical and chemical impacts. Still, as larvae can live in anoxic conditions, they can be found in different environments [50]. Turbidity, water flow, and pH have also been found to be important parameters affecting its presence. Also, they live in





a broad spectrum of BOD values, but ammonium values above 1.45 mg/L have been shown to be detrimental to their survival in European rivers [50], [51] concluded that these taxa were present in streams with moderate urban impacts on water quality, but absent in heavily impacted waters, and made some correlations between each species and water quality chemical parameters. However, some authors consider that this species is not suitable as an indicator as a whole for a streams' water quality because each species has different conditions appropriate for their survival, and they differ considerably between them [50]. In this study, this taxon as a whole did not show its suitability as a biotic indicator, having more individuals in more polluted waters.

The taxa Baetidae (2), from the order Ephemeroptera, are very common invertebrates living in running waters and have more than one thousand species distributed worldwide and have been reported in several Colombian rivers [26], [53]. Many species prefer the stone substrate, and its pollution tolerance goes from very sensitive to tolerant, depending on the species. In general, for this order, biological diversity indexes between the species are the most accurate index to relate to water quality rather than the total number itself [54]. Correspondence with this was found in this study, in which the number of macroinvertebrates found for bad water quality was considerably lower than for acceptable water quality, being a potential bioindicator for water quality in this river.

Taxa Planariidae (3), from the order Tricladida, previously used as a biological indicator for polluted waters in Colombia [55], showed a decreasing number as water quality in the Cane River decreased. This order is at a high level in the macrobenthic food web because they are zoophagous, and are related to the whole ecosystem's health. Thus, the human impact on the riverine zones has an essential effect on their presence in the ecosystems [56]. Its presence along the whole study area and its sensitivity to pollution make it a good biotic indicator for water quality in this basin.

Taxa Leptohiphidae (4 in Figure 8) has also been reported in several Colombian rivers [53], [57], showing tolerance to vast changes in temperature and pollution conditions [58], explaining its presence in the different types of water quality in Cane River. However, it was more frequently found in point 3, than in point 1, and it did not have a direct relationship with WQI.

Between the less frequent taxa found in this study, the Chrysomelidae taxa (6) was found only in waters with acceptable conditions. In contrast, the Dryopidae, Calopterygidae, Leptophlebiidae, Perlidae, Ptilodactylidae, and Scirtidae (10, 11, 13, 14, 15, and 16) taxa predominated in waters with regular quality. Chrysomelidae, of the order Coleoptera, has been found in low quantities in waters in Australia and Iran, among others [39], [59], showing as in this study that it is not widely distributed in water basins, and its presence as a single macroinvertebrate order (taxa) for water quality in different conditions is not high. A similar result was found for the Calopterygidae family (11), which has previously been reported in Colombian rivers with regular water quality, and in this study were only found in water with regular conditions. The Ptilodactylidae taxa (15), previously found in waters with acceptable and regular conditions [26], were found only in regular conditions for water quality in this study. The Tipulidae (7)



and Blepharoceridae (9) families were present in waters with acceptable and regular quality and in higher quantities for acceptable quality. Tipulidae family was reported in other studies where the water was in the same quality conditions [60]. In general, classification to the family taxonomic level has shown in some studies for Andean Mountain catchments not being a very suitable approach to understanding water quality. High Andean stream biodiversity and community composition databases are necessary to better understand of water quality monitoring [61]. Also, using adapted Indices such as those made from the trophic guilds approach and regional adapted biotic indexes is a more accurate way to include ecosystem variations and their relationship with water quality [12].

#### **4. Conclusions**

Water Quality in the Middle Basin of the Cane River ranged from acceptable to bad between June and November 2021, according to IDEAM WQI. The first monitoring water quality point was in an acceptable range, the second point was regular, and point 3 was in the bad quality range as an average for the 3 monitoring times. This clearly shows the degradation caused by anthropogenic sources. These include agricultural and livestock activities, water intake for 26 village aqueducts and 2 municipal aqueducts (Villa de Leyva and Gachantivá), and rural and urban wastewater discharges, among others. In the same way, biological indexes such as BMWP/col showed a decreasing quality for the 3 points at the 3 monitoring times, while ETP did not have a clear trend in water quality along the river course. WQI and biological indices were analyzed to check a possible relation, being BMWP/Col and IDEAM WQI values that showed the most similar trend. The main macroinvertebrate taxa found were, in its order: Hydropsychidae, Simuliidae, Baetidae, Planariidae, Blepharoceridae, and Leptohiphidae. Considering these, only Baetidae and Planariidae taxa were found in the three water quality levels, and their number showed a decrease in water quality. Consequently, its use for future water quality studies, including more detailed information, is recommended, as is the case with other high Andean Catchment studies. Some recommendations for future studies indicate the use of a diversity index for Baetidae rather than the total number, and for Planariidae, the recommendation is to include the impacts on the riverine habitats analysis to get a better knowledge of its suitability as a water quality indicator for this basin. In general, further analysis is recommended to validate these results to regional high Andean catchments based on additional surrounding habitat information and classification to the species level, for the most important taxa found in this study.

#### **5. Declaration of competing interest**

We declare that we have no significant competing interests, including financial or non-financial, professional, or personal interests interfering with the full and objective presentation of the work described in this manuscript.

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## 7. Author contributions

**Lina Patricia Vega-Garzon:** Methodology assessment, field work, redaction of introduction and results analysis

**Camilo Andres Pulido:** Field work, chemical analysis, redaction of introduction and results

**Sarai Sánchez Rodríguez:** Macroinvertebrates collection and identification, chemical analysis

**Geraldine Paola García:** Macroinvertebrates collection and identification, water sampling, chemical analysis

**Kellys Salcedo:** Methodology assessment, macroinvertebrates collection and identification

## 8. Data availability statement

where the data associated with a paper is available, and under what conditions the data can be accessed. They also include links (where applicable) to the data set

## References

- [1] N. Gil-Rodas *et al.*, “A comparative study of several types of indices for river quality assessment,” *Water Quality Research Journal*, vol. 58, no. 3, pp. 169–183, Aug. 2023, doi: 10.2166/wqrj.2023.029.
- [2] J. M. García, L. F. Sarmiento, M. Salvador, and L. Sabrina, “Uso de bioindicadores para la evaluación de la calidad del agua en ríos: aplicación en ríos tropicales de alta montaña . Revisión corta,” *UG Ciencia*, pp. 47–62, 2017.
- [3] J. V. A. da S. Santos *et al.*, “Assessing physical habitat structure and biological condition in eastern Amazonia stream sites,” *Water Biology and Security*, vol. 2, no. 2, Apr. 2023, doi: 10.1016/j.watbs.2022.100132.
- [4] B. Rimcheska and Y. Vidinova, “Ecological Status Assessment of Mountain and Semi-mountain Streams in the Aegean Watershed: Applicability of Biotic Indices BMWP and ASPT Based on Macroinvertebrates,” *Acta Zool Bulg*, vol. 72, no. 1, pp. 49–60, 2020, [Online]. Available: <http://www.acta-zoologica-bulgarica.eu/002326>
- [5] B. Cox, S. Oeding, and K. Taffs, “A comparison of macroinvertebrate-based indices for biological assessment of river health: A case example from the sub-tropical Richmond River Catchment in northeast New South Wales, Australia,” *Ecol Indic*, vol. 106, no. November 2018, 2019, doi: 10.1016/j.ecolind.2019.105479.
- [6] G. Roldán, J. Builes, M. Trujillo, and A. Suarez, “Efectos de la contaminación industrial y domestica sobre la fauna bentica del rio Medellín,” *Actualidades Biológicas*, vol. 7, no. 2, p. 11, 1973.
- [7] G. Roldan, *Guia para el estudio de los macroinvertebrados acuáticos del Departamento de Antioquia*. 1988.



- [8] S. E. Mustow, “Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score,” 2002.
- [9] F. O. Masese, M. Muchiri, and P. O. Raburu, “Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya,” *Afr J Aquat Sci*, vol. 34, no. 1, pp. 15–26, May 2009, doi: 10.2989/AJAS.2009.34.1.2.727.
- [10] M. Xu, Z. Wang, X. Duan, and B. Pan, “Effects of pollution on macroinvertebrates and water quality bio-assessment,” *Hydrobiologia*, vol. 729, no. 1, pp. 247–259, May 2014, doi: 10.1007/s10750-013-1504-y.
- [11] M. Custodio and E. Cavez, “Quality of the aquatic environment of high Andean rivers evaluated through environmental indicators: a case of the Cunas River, Peru,” *Revista chilena de ingeniería*, vol. 27, no. 3, pp. 396–409, 2019.
- [12] R. Jiménez, E. Barnuevo, B. Timbe, and P. X. Astudillo, “El uso de gremios tróficos en macroinvertebrados acuáticos como herramienta de monitoreo en los Altos Andes del Sur de Ecuador,” *Neotrop Biodivers*, vol. 7, no. 1, pp. 310–317, 2021, doi: 10.1080/23766808.2021.1953891.
- [13] A. Niba and S. Sakwe, “Turnover of benthic macroinvertebrates along the Mthatha River, Eastern Cape, South Africa: implications for water quality bio-monitoring using indicator species,” *J Freshw Ecol*, vol. 33, no. 1, pp. 157–171, Jan. 2018, doi: 10.1080/02705060.2018.1431969.
- [14] A. Cerón-Vivas, Y. Gamarra, M. Villamizar, R. Restrepo, and R. Arenas, “Water quality of Mamarramos stream. The sanctuary of fauna and Flora Iguaque, Colombia,” *Tecnología y Ciencias del Agua*, vol. 10, no. 6, pp. 90–116, 2019, doi: 10.24850/j-tyca-2019-06-04.
- [15] I. Liñero Arana *et al.*, “Calidad del agua de un río andino ecuatoriano a través del uso de macroinvertebrados,” 2016.
- [16] J. H. Pérez, L. C. Martínez-Romero, L. T. Castellanos-Guerrero, A. R. Mora-Parada, and Z. E. Rocha-Gil, “Macroinvertebrate bioindicators of water quality in artificial hydrological systems in the department of boyacá, colombia,” *Produccion y Limpia*, vol. 15, no. 1, pp. 35–48, Sep. 2020, doi: 10.22507/PML.V15N1A3.
- [17] M. Silva-Poma, L. Castillo, R. Elias, J. Peralta-Argomeda, and A. Huamantínco-Araujo, “Water Quality of the habitat of two endangered high-andean frogs using aquatic macroinvertebrates as bioindicators,” *International Journal of Conservation Science*, vol. 12, no. 3, pp. 1061–1076, Jul. 2021, [Online]. Available: [www.ijcs.ro](http://www.ijcs.ro)
- [18] S. Ayala Ramírez, W. A. Reinoso González, D. S. Calderón Rivera, Á. M. Jaramillo Londoño, and D. J. Mesa Fernández, “Determinación de la calidad del agua del río Frío (Cundinamarca, Colombia) a partir de macroinvertebrados bentónicos,” *Avances: Investigación en Ingeniería*, vol. 16, no. 1, pp. 49–65, 2019, doi: 10.18041/1794-4953/avances.1.5191.
- [19] Y. Montoya, Y. Acosta, and E. Zuluaga, “Evolución de la calidad del agua en el Río Negro y sus principales tributarios empleando como indicadores los índices ICA, BMWP/Col y ASPT,” *Caldasía*, vol. 33, no. 1, pp. 193–210, 2011.
- [20] L. Galindo-Leva *et al.*, “EVALUACIÓN DE MACROINVERTEBRADOS ACUÁTICOS Y CALIDAD DE AGUA EN QUEBRADAS DE FINCAS CAFETERAS DE CUNDINAMARCA Y SANTANDER, COLOMBIA,” 2012.
- [21] J. A. Gil, “Determinación de la calidad del agua mediante variables físico químicas, y la comunidad de macroinvertebrados como bioindicadores de calidad del agua en la cuenca del río Garagoa,” Universidad de Manizales, Manizales, 2014.



- [22] M. Sanchez and D. García, “Determinación del índice BMWP/Col, mediante la utilización de macroinvertebrados como bioindicadores de la calidad de agua, en el cauce del río Guachicos, que surte el acueducto del municipio de Pitalito,” Pitalito, 2018.
- [23] S. A. Murillo-Montoya, A. Mendoza-Mora, E. S. Restrepo-Bastidas, and M. Á. Rodríguez, “Utilización de macroinvertebrados acuáticos como herramienta para determinar la calidad del agua en la quebrada Santo Tomás, municipio de Pensilvania, Colombia,” *Rev Acad Colomb Cienc Exactas Fis Nat*, vol. 42, no. 164, p. 212, Oct. 2018, doi: 10.18257/raccefyn.655.
- [24] L. Castellanos, “Conocimiento etnobotánico, patrones de uso y manejo de plantas útiles en la cuenca del río cane-iguauque (Boyacá - Colombia); una aproximación desde los sistemas de uso de la biodiversidad,” *Ambiente e Sociedade*, vol. 14, no. 1, pp. 45–75, 2011.
- [25] Catalina Gómez-Castañeda, Diego Felipe García-Corredor, Gina Katerin Reyes-Garavito, Jaime Ricardo Lache-Aparicio, and Lina Patricia Vega-Garzon, “Analysis of space-time profiles of physical-chemical parameters of water quality In lake fúquene, Cundinamarca,” *Ingenio Magno*, vol. 11, pp. 8–28, 2020.
- [26] A. Gonzáles, A. Rivera, D. Granada, E. Contreras, and Y. Patiño, “Hidrobiologicos Jurisdiccion De Corantioquia,” *piragua-Programa Integral-Red Agua*, no. 90–100, 2013.
- [27] E. Domínguez and H. R. (Hugo R. Fernández, *Macroinvertebrados bentónicos sudamericanos : sistemática y biología*. Fundación Miguel Lillo, 2009.
- [28] IDEAM, “Hoja metodológica del indicador Índice de calidad del agua,” 2011. [Online]. Available: [www.ideam.gov.co](http://www.ideam.gov.co)
- [29] E. Sanchez *et al.*, “Use of the water quality index and dissolved oxygen deficit as simple indicators of watersheds pollution,” *Ecol Indic*, vol. 7, no. 2, pp. 315–328, 2007, doi: 10.1016/j.ecolind.2006.02.005.
- [30] H. Effendi, Romanto, and Y. Wardiatno, “Water Quality Status of Ciambulawung River, Banten Province, Based on Pollution Index and NSF-WQI,” *Procedia Environ Sci*, vol. 24, pp. 228–237, 2015, doi: 10.1016/j.proenv.2015.03.030.
- [31] G. A. Roldan, *Bioindicacion de la calidad del agua en Colombia*. Medellin, Colombia: Editorial Universidad de Antioquia, 2003.
- [32] G. Roldán-Pérez, “Los macroinvertebrados como bioindicadores de la calidad del agua: cuatro décadas de desarrollo en Colombia y Latinoamerica,” *Rev Acad Colomb Cienc Exactas Fis Nat*, vol. 40, no. 155, p. 254, Jul. 2016, doi: 10.18257/raccefyn.335.
- [33] Zamora-Muñoz and Alba-Tercero, “pubzamorac1996p332,” vol. 15, pp. 332–352, 1996.
- [34] Kownacki, Soszka, Fleituch, and Kudelska, “The ecological assessment of river quality in Poland on the basis of communities of benthic invertebrates,” *River biomonitoring and benthic invertebrate communities*, pp. 71–88, 2002, Accessed: Jan. 24, 2022. [Online]. Available: [https://scholar.google.com/scholar?hl=es&as\\_sdt=0%2C5&q=Kownacki+A%2C+Soszka+H%2C+Fleituch+T%2C+Kudelska+D.+The+ecological+assessment+of+river+quality+in+Poland+on+the+basis+of+communities+of+benthic+invertebrate&btnG=](https://scholar.google.com/scholar?hl=es&as_sdt=0%2C5&q=Kownacki+A%2C+Soszka+H%2C+Fleituch+T%2C+Kudelska+D.+The+ecological+assessment+of+river+quality+in+Poland+on+the+basis+of+communities+of+benthic+invertebrate&btnG=)
- [35] F. Mao *et al.*, “Revision of biological indices for aquatic systems: A ridge-regression solution,” *Ecol Indic*, vol. 106, Nov. 2019, doi: 10.1016/j.ecolind.2019.105478.
- [36] K. F. Roche, E. P. Queiroz, K. O. Righi, and G. M. de Souza, “Use of the BMWP and ASPT indexes for monitoring environmental quality in a neotropical stream,” *Acta Limnologica Brasiliensia*, vol. 22, no. 01, pp. 105–108, 2010, doi: 10.4322/actalb.02201010.
- [37] M. Zeybek, H. Kalyoncu, B. Karakaş, and S. Özgül, “The use of BMWP and ASPT indices for evaluation of water quality according to macroinvertebrates in Değirmendere Stream (Isparta,



- Turkey),” *Turkish Journal of Zoology*, vol. 38, no. 5, pp. 603–613, 2014, doi: 10.3906/zoo-1310-9.
- [38] J. P. Ramírez-Herrejón, N. Mercado-Silva, M. Medina-Nava, and O. Domínguez-Domínguez, “Validación de dos índices biológicos de integridad (IBI) en la subcuenca del río Angulo en el centro de México,” *Rev Biol Trop*, vol. 60, no. 4, pp. 1669–1685, 2012, doi: 10.15517/rbt.v60i4.2160.
- [39] B. Chessman, *SIGNAL 2.iv : a scoring system for macroinvertebrates (‘water bugs’) in Australian rivers : user manual*. Dept of the Environment and Heritage, 2003.
- [40] H. A. Hawkes, “ORIGIN AND DEVELOPMENT OF THE BIOLOGICAL MONITORING WORKING PARTY SCORE SYSTEM,” *Water Res*, vol. 32, no. 3, pp. 964–968, 1997.
- [41] S. Álvarez Carrión and L. Pérez Rivera, “Evaluación de la calidad de agua mediante la utilización de macroinvertebrados acuáticos en la subcuenca del Yegua, Honduras,” 2007.
- [42] Presidencia de la Republica, *Decreto 1594 de 1984*. 1984.
- [43] “Plan de ordenación y manejo de la cuenca del río Cane Iguaque.”
- [44] Y. Gamarra *et al.*, “Aplicación del protocolo CERA-S para determinar la calidad ecológica de la microcuenca Mamarramos (cuenca Cane-Iguaque), Santuario de Fauna y Flora Iguaque (Boyacá), Colombia ,” *Biota Colomb*, vol. 18, no. 2, pp. 11–30, Mar. 2018, doi: 10.21068/c2017.v18n02a02.
- [45] C. A. García-Alzate, C. Román-Velancia, D. X. Loera, M. Gonzalez, and M. Simunovic, “Physico-chemical and biological variables of San José Creek, Otún river drainage/upper Cauca, Colombia,” *Revista Investigación Universidad del Quindío*, vol. 20, pp. 38–48, 2008.
- [46] R. M. Padilla Moreno and C. A. Ospina Parra, “Biological and Physiochemical Conditions of the Surface Waters of the Cauca River in the Urban Area of Popayán, Colombia,” *Revista Ingenierías Universidad de Medellín*, vol. 20, no. 39, pp. 263–277, Jul. 2021, doi: 10.22395/rium.v20n39a15.
- [47] M. Carmen *et al.*, “Diversity of aquatic macroinvertebrates and water quality of the High Andean wetlands of Chalhuanca, Arequipa-Peru,” *Biodivers J*, vol. 12, no. 2, pp. 517–528, 2021, doi: 10.31396/Biodiv.Jour.2021.12.2.517.528.
- [48] M. Springer, “Capítulo 7: Trichoptera,” *Rev. Biol. Trop*, vol. 58, pp. 151–198, 2010.
- [49] S. C. Stuijzand, S. Engels, E. Van Ammelrooy, and M. Jonker, “Caddisflies (Trichoptera: Hydropsychidae) Used for Evaluating Water Quality of Large European Rivers,” *Archives of environmental Contamination Toxicology*, vol. 36, pp. 186–192, 1999.
- [50] T. N. Docile, R. Figueiró, L. H. Gil-Azevedo, and J. L. Nessimian, “Water pollution and distribution of the black fly (Diptera: Simuliidae) in the Atlantic forest, Brazil,” *Rev Biol Trop*, vol. 63, no. 3, pp. 683–693, 2015, doi: 10.15517/rbt.v63i3.16195.
- [51] K. Lock, T. Adriaens, and P. Goethals, “Effect of water quality on blackflies (Diptera: Simuliidae) in Flanders (Belgium),” *Limnologica*, vol. 44, pp. 58–65, 2014, doi: 10.1016/j.limno.2013.08.001.
- [52] B. Rabha, S. Dhiman, K. Yadav, S. Hazarika, R. K. Bhola, and V. Veer, “Influence of water physicochemical characteristics on Simuliidae (Diptera) prevalence in some streams of Meghalaya, India,” *J Vector Borne Dis*, vol. 50, no. 1, pp. 18–23, 2013.
- [53] E. Z. Rocha Gil, L. Á. Cuellar Rodríguez, L. J. Rodríguez Vargas, and X. Díaz Soler, “Bioindicadores de la calidad del agua en áreas con restauración ecológica de la quebrada la colorada, Villa de Leyva, Boyacá.,” 2015.

- [54] D. F. Buss and F. F. Salles, "Using Baetidae species as biological indicators of environmental degradation in a Brazilian river basin," *Environ Monit Assess*, vol. 130, no. 1–3, pp. 365–372, Jul. 2007, doi: 10.1007/s10661-006-9403-6.
- [55] J. D. Gutierrez, W. Riss, and R. Ospina, "Bioindicación de la calidad del agua con logica difusa sugeno y macroinvertebrados acuaticos," *Acta Biológica Cofombiana*, vol. 7, no. 2, p. 37, 2002.
- [56] R. Manenti and B. Bianchi, "Distribution of the triclad *Polycelis felina* (Planariidae) in aezkoa mountains: Effect of stream biotic features," 2014. [Online]. Available: <https://www.researchgate.net/publication/288299581>
- [57] V. González Meléndez, O. Caicedo Quintero, and N. Aguirre Ramirez, "Aplicación de los índices NSF, DINIUS y BMWP para el análisis de la calidad de agua de la Quebrada La Ayurá, Antioquia Colombia.," *Revista Gestión y Ambiente*, vol. 16, pp. 97–108, 2013.
- [58] R. W. Flowers and C. De la rosa, "Capítulo 4: Ephemeroptera," *Rev Biol Trop*, vol. 58, pp. 63–93, 2010.
- [59] H. Nasirian, "Evaluation of water quality and organic pollution of Shadegan and Hawr Al Azim wetlands by biological indices using insects," ~ 193 ~ *Journal of Entomology and Zoology Studies*, vol. 2, no. 5, pp. 193–200, 2014.
- [60] J. Forero Duarte, "Macroinvertebrados bentónicos y su relación con la calidad del agua en la cuenca alta de del Río Frío (Tabio, Cundinamarca)," Pontificia Universidad Javeriana, 2017.
- [61] R. Espinosa, P. Andino, S. Cauvy-Fraunié, O. Dangles, D. Jacobsen, and V. Crespo-Pérez, "Diversity patterns of aquatic macroinvertebrates in a tropical high-Andean catchment," *Rev. Biol. Trop. (Int. J. Trop. Biol)*, vol. 68, pp. 29–53, 2020.