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Evaluation of extracellular enzymatic activity in the Aburrá-Medellín river as a response to variations in water quality and flow regime

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Summary

The Aburrá-Medellín river has been affected by urbanization processes in its basin, which has altered its physicochemical profile. In order to identify patterns in the enzymatic activity associated with variations in water quality and flow regime, the activity of the β-glucosidase and phosphatase enzymes, related to carbon and phosphorus metabolism, respectively, was measured in the water and biofilm. For this purpose, nine monitoring stations with different degrees of anthropic intervention were selected, which were evaluated during low, medium and high flow regimes associated with meteorological variability in the basin. The hypothesis was that the physicochemical profile, water quality and flow regime affect the activity of both enzymes, both in the water and in the biofilm, since they influence the concentration of nutrients transported by the river. According to the results obtained, no statistically significant differences were found in the enzymatic activity at low, medium and high flow rates, although a higher concentration of nutrients was observed at low and medium flow rates than at high flow rates. On the other hand, it was observed that the concentration of nutrients in the river changed according to the degree of urbanization in the basin, due to the discharge of wastewater from densely populated municipalities. The activity of both enzymes was higher in the stations with higher concentrations of nutrients, while in the monitoring stations located in the upper zone of the basin, where anthropic intervention and the concentration of nutrients was low, the lowest enzymatic activities were found both in the water and in the biofilm. In this regard, enzymatic activity can be used as an indicator of the health of the Aburrá-Medellín river and it is suggested that the measurement of this variable be included in the framework of the RedRío monitoring network.

Evaluación de la actividad enzimática extracelular en el río Aburrá-Medellín como respuesta a variaciones en la calidad del agua y el régimen de caudal

Resumen

El río Aburrá-Medellín ha sido modificado debido a los procesos de urbanización de la cuenca, lo que ha afectado el perfil fisicoquímico del agua. Con el fin de identificar patrones en la actividad enzimática asociados a la variación en las condiciones fisicoquímicas y el régimen de caudal, se midió la actividad de las enzimas β-glucosidasa and fosfatasa, relacionadas con el metabolismo del carbono y el fósforo respectivamente, en el de agua y el biofilm. Para ello, se seleccionaron en nueve sitios de monitoreo con diferente grado de intervención antrópica, durante régimen de caudal bajo, medio y alto, asociado a la variabilidad meteorológica en la cuenca. La hipótesis planteada fue que el perfil fisicoquímico y el régimen de caudal afectan la actividad de ambas enzimas, tanto en el agua como en el biofilm, puesto que influyen en la concentración de nutrientes transportada por el río. Según los resultados obtenidos, no se encontraron diferencias estadísticamente significativas en la actividad enzimática en régimen de caudales bajo, medio y alto, aunque se observó una mayor concentración de nutrientes en caudales bajos y medios respecto a caudales altos. Por otro lado, se observó que la concentración de nutrientes en el río cambió conforme el gradiente de urbanización en la cuenca, debido a las descargas de aguas residuales provenientes de los municipios densamente poblados. La actividad de ambas enzimas fue mayor en los sitios donde la concentración de nutrientes fue más alta, por el contrario, en los sitios de monitoreo localizados en la zona alta de la cuenca, donde la intervención antrópica y la concentración de nutrientes fue baja, se midieron las menores actividades enzimáticas, tanto en el agua como en el biofilm, en ese sentido, la actividad enzimática puede ser usada como un indicador de la salud del río Aburrá-Medellín y se sugiere incluir la medición de estas variables en el marco de la Red de Monitoreo RedRío.

1. Introduction

Rivers are complex socio-ecological systems with interconnected natural and human components [Dunham et al. (2018); Thoms and Sheldon (2019)]. Rivers provide numerous goods and services [Martin-Ortega et al. (2015); Assessment Millennium Ecosystem (2005)], which can generate economic benefits [The Quintessence Consortium (2016)]. However, rivers are considered among the most negatively affected ecosystems in the world due to alterations in their basins, which has had a negative impact on the goods and services that they facilitate for society [Arthington et al. (2010); Assessment Millennium Ecosystem (2005); Rossi, et al. (2019)]. In urban basins, anthropic intervention has caused ecological degradation of riparian ecosystems, which has been termed "urban stream syndrome". The symptoms of this include flashier hydrograph, altered channel morphology and stability, reduced biotic richness with presence of more tolerant species, and elevated concentrations of nutrients and pollutants [Paul and Meyer (2008)]. This syndrome is associated with the discharge of treated and untreated wastewater, legacy pollutants, and stormwater runoff [Paul and Meyer (2008)].

Microorganisms play a fundamental role in the functioning of aquatic ecosystems, since they participate in the decomposition of organic matter, the flow of matter and energy, and the structure of trophic webs [Findlay (2010); Wetzel (1991)]. In aquatic ecosystems, most organic matter (>95%) is made up of high molecular weight compounds [Wetzel (1991); Chróst and Siuda (2002); Chróst (1990)]. Bacteria, fungi, protozoa, and algae can produce extracellular enzymes [Wetzel (1991); Chróst and Siuda (2002); Chróst (1990)].; in this regard, enzymatic activity corresponds to the beginning of the degradation of organic compounds [Tiquia (2011)]. This allows them to obtain low molecular weight products that can be transported through the cell membrane and used as a source of nutrients and in the construction of biomass [Wetzel (1991); Chróst and Siuda (2002); Chróst (1990)]. The main extracellular enzymes studied in aquatic ecosystems include β-glucosidase and phosphatase [Cunha et al. (2010); Romaní et al. (2009)]. β-Glucosidase associates with heterotrophic bacteria and is involved in the hydrolysis of β-glucosidic bonds in disaccharides and oligosaccharides to release glucose [Cunha et al (2010); Romaní

(2006)]. Phosphatase is produced by bacteria, phytoplankton, and zooplankton, and catalyzes phosphate esters to release inorganic phosphate [Cunha et al. (2010)].

The activity of enzymes associated with the metabolism of carbon, nitrogen, and phosphorus in water and biofilm has been analyzed in urbanized rivers, in order to obtain information on the relationships between urbanization and the decomposition of organic matter, and between extracellular enzymatic activity and physical, chemical, and biological variables [Tiquia (2011); Harbott and Grace (2005); Williams et al. (2011); Millar et al. (2015); Lehto and Hill (2013); Hosen et al. (2014); Sabater et al. (2016)]. However, there is little information on extracellular enzymatic activity in urban rivers located in the tropics [Giraldo et al. (2014); Jaramillo et al. (2016); Cifuentes (2015)]. The Aburrá-Medellín river receives treated and untreated wastewater along its route. This causes gradual deterioration in water quality, which is also affected by the flow regime [AMVA and UdeA (2019)]. Giraldo et al. (2014) showed that, in the central and southern sections of the Aburrá-Medellín river, the activity of β-glucosidase and phosphatase enzymes in the water and biofilm were related to the concentration of nutrients; however, in that study enzymatic activity was not measured in the northern section of the river. This part of the river presents the most critical water quality conditions while, in the final stretch of the river, there is an improvement in water quality, related to the entry of tributaries that are less polluted than the river [AMVA and UdeA (2019)]. According to various authors, the enzymatic profiles of rivers are related to their physicochemical and biological conditions, as well as to the characteristics of the basin [Harbott and Grace (2005); Millar et al. (2015)]. In order to have a complete picture of the enzymatic activity along the river, it is important to consider the variation in the physicochemical conditions of the current.

The aim of our study was to evaluate the activity of β-glucosidase and phosphatase enzymes in the water and biofilm in the Aburrá-Medellín river in order to identify patterns related to the variation in physicochemical conditions along the river and changes in the flow regime. The hypotheses were that: i) the activity of the β-glucosidase and phosphatase enzymes varies with the physicochemical characteristics of the river; and ii) the flow regime affects the enzymatic profile of the river, since it influences the concentration of nutrients transported by it.

2. Materials and methods

2.1. Study area

The Aburrá-Medellín river basin (Fig. 1) is located in western Colombia, in the central-southern department of Antioquia [CORANTIOQUIA et al. (2018)]. Its area corresponds to 120,758.6 ha and it has an irregular and sloping topography [CORANTIOQUIA et al. (2018)]. There are 15 municipalities within the basin, including Medellín, the second largest city in the country, with approximately 3 million inhabitants [CORANTIOQUIA et al. (2018); DANE (2019); Giraldo et al. (2014)]. The climate in the basin is tropical, due to its geographical location [CORANTIOQUIA et al. (2018)]. The temporal variability of precipitation is bimodal, with two rainy seasons in the year (between March and June and between September and November), interspersed by two dry periods [CORANTIOQUIA et al. (2018)]. The most significant land uses are agro-sylvo-pastoral (29.98%) and urban (21.47%) [CORANTIOQUIA et al. (2018)].

The Aburrá-Medellín river crosses the basin and runs through the municipalities within it, making it the historical axis of the region [Giraldo et al. (2014)]. The river is approximately 107.9 km long. Its source is at 2,980 meters above sea level. (municipality of Caldas) and it converges with the Grande river at 1,048 meters above sea level (municipality of Santo Domingo), where it forms the Porce river [CORANTIOQUIA et al. (2018)]. The Aburrá-Medellín river has been canalized and rectified in some sections due to development and urbanization processes in the basin [26]. Additionally, its physicochemical and hydrobiological profile has been affected by the dumping of treated and untreated wastewater, the inadequate disposal of solid waste, confluence with contaminated streams, and other factors [AMVA and UdeA (2019)].

Nine monitoring stations were selected, of which eight were located on the Aburrá–Medellín river and one on the Porce river. The monitoring stations were selected taking into account historical information on the water quality of the river and its main tributaries, land uses in the basin, water uses, and other factors [AMVA and UdeA (2019); AMVA (2012)], Figure 1 shows their locations.

Figure 1. Study area of the Aburrá-Medellín river basin. Monitoring stations S0, S1, S2, S3, S4, S5, S6 and S7 are located on the Aburrá-Medellín river. The S8 monitoring station is located on the Porce river. The Porce river is formed by the confluence between the Grande river and the Aburrá-Medellín river.
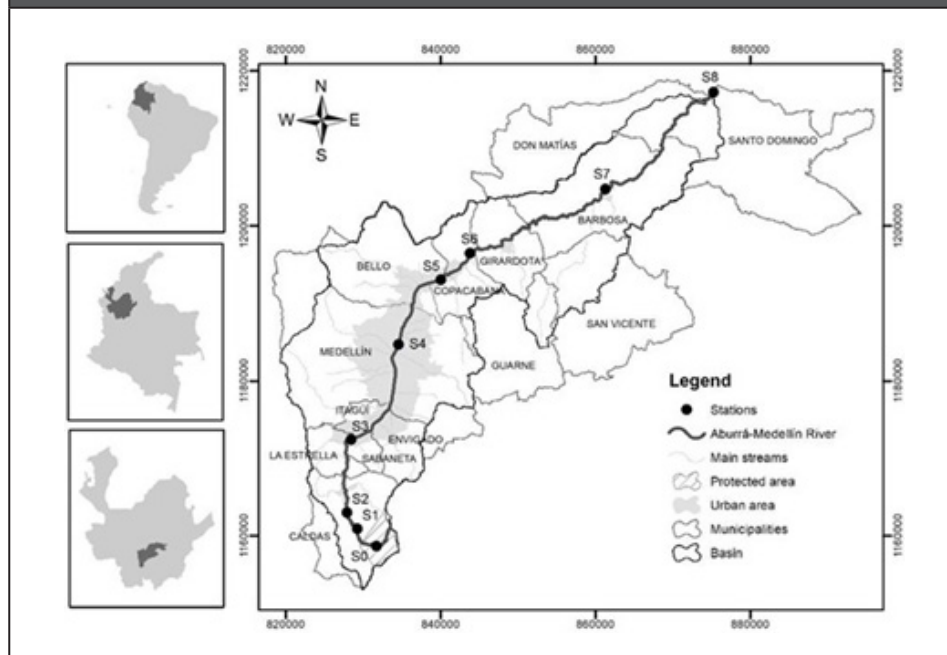
2.2 Sampling

At each monitoring station, water and biofilm samples were collected over five sampling campaigns, in periods of low, medium and high flow, according to the rainfall regime in the basin [CORANTIOQUIA et al. (2018)]. The collection of water and biofilm samples was carried out in triplicate at 12:00 hours. At this time the river carries a greater polluting load, according to the results obtained by the RedRío monitoring network, which is due to wastewater discharges [AMVA and UdeA (2019)]. The water samples were taken in the center of the section, avoiding contamination with sediments [IDEAM, (2003)]. The water samples were collected manually in a plastic container. Subsequently, the following environmental variables of the water were measured using a previously calibrated HACH multiparameter device (model HQ40D): pH, dissolved oxygen concentration, electrical conductivity, and temperature.

Regarding the biofilm samples, rocks were collected from the riverbed and adhered material was removed using a soft-bristled

toothbrush and an 8 cm^2 quadrat [Tümpling and Friedrich (1999)]. The removal was carried out in 30 randomly chosen areas in the collected rocks, and the total area removed corresponded to 240 cm^2. The material obtained from scraping was stored in opaque plastic containers containing chlorine-free water. The collected water and biofilm samples were stored at 4.00 °C until they arrived at the laboratory and were processed in less than 24 hours after collection. Fording and suspension gauging were carried out to measure the flow, and subsequently the flow was calculated using the area-velocity method. An OTT MF Pro sensor was used for flow measurement [SIATA, (2017, 2018)].

2.3 Classification of flow regime in monitoring campaigns

The flow regime classification was carried out taking into account the historical record of flows measured between 2004 and 2019 by the RedRío monitoring network. The Q1 and Q3 quartiles enabled the limits for low (Q<Q1), medium (Q1<Q<Q3) and high (Q>Q3) flows at each monitoring station to be determined [Área Metropolitana del Valle de Aburrá, Universidad de Antioquia (2019)].

2.4 Measurement of physicochemical parameters

Physicochemical variables were measured in the water samples. These variables were Total Kjeldahl Nitrogen -TKN (SM 4500-Norg-B), Total Phosphorus-PT (SM 4500-P-B-E), Orthophosphates-PO4 (SM 4500-P-E), Dissolved Organic Carbon-COD (SM 5310 B), Chemical Oxygen Demand-COD (SM5220-D), Biochemical Oxygen Demand-BOD5 (SM 5210-B, EPA360.3) and Total Suspended Solids-TSS (SM 2540-D). The methods used were based on the 22nd edition of the Standard Methods for the Examination of Water and Wastewater [APHA, AWWA, WEF (2012)].

2.5 Measurement of extracellular enzymatic activity

The activity of the enzymes β-glucosidase (EC 3.2.1.21 according to the Enzyme Commission number) and phosphatase (EC 3.1.3.1, according to the Enzyme Commission number) was determined using the spectrophotometric method [Marxsen et al. (1998)]. The colorless substrates 4-Nitrophenyl-β-D-Glucopyranose 98% (CAS No. 2492-87-7 Alfa Aesar Thermo Fisher Scientific, Haverhill, USA)

and 4-Nitrophenyl-Phosphate 99% (CAS No. 4264-83-9 Alpha Aesar Thermo Fisher Scientific, Haverhill, USA) were used. These substrates are hydrolyzed by the β-glucosidase and phosphatase enzymes, respectively, and release 4-Nitrophenol ($O_2NC_6H_4OH$), a yellow compound detected at 405 nm.

To carry out the test, 5 ml of each sample (water and biofilm) was taken and dissolved in 50 ml of NaCl (0.14 mol/l). Subsequently, 2 ml of this solution was added to test tubes and mixed with 2 ml of the 4-Nitrophenyl-β-D-Glucopyranose or 4-Nitrophenyl-Phosphate solution, according to the enzymatic activity to be determined. The test tubes were incubated at 30 °C for 3 hours. After this period, and to stop the hydrolysis reaction, 2 ml of Na_2CO_3 (1 mol/l) were added to the test tubes and centrifuged at 4500 rpm for 10 minutes. Subsequently, the absorbance was measured on a NANOCOLOR UV/VIS spectrophotometer (Macherey-Nagel GmbH & Co. KG, Düren, Germany) in test tubes with 16 mm thickness (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The absorbance was measured with respect to a blank (2 ml of the NaCl solution, 2 ml of the substrate solution and 2 ml of Na_2CO_3). The concentration of 4-Nitrophenol was contrasted with a calibration curve previously constructed from the dilution of the 4-Nitrophenol solution in a solution made up of NaCl and Na_2CO_3 in a ratio of 2:1. The extracellular enzymatic activity is calculated according to Equation 1:

Equation 1. Calculation of extracellular enzymatic activity

$$AEE_x = \frac{Abs_x * D * F}{t}$$

AEE_x : extracellular enzymatic activity of enzyme x (mol/l/h).

Abs_x: absorbance of the final incubation product measured at λ=405 nm

D: dilution factor

F: photometric factor given by the inverse of the slope of the calibration curve (mol/l).

t: time (h).

2.6 Chlorophyll a

Each sample (water or biofilm) was passed through a 0.45 μm pore size glass fiber filter and stored at 4.0 °C, protected from light. The extraction was carried out with 90% ethanol (10ml), and the filter was macerated to facilitate this process. Absorbance at 665 and 750 nm, both unacidified and acidified with HCl (3M), was measured using a NANOCOLOR UV/VIS digital spectrophotometer (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The acid ratio corresponding to 1.7 was verified after adding the HCl [Hernández et al. (2011)].

2.7. Global Water Quality Index – Global WQI in the Aburrá-Medellín river

The Global WQI rates water quality according to five categories: good, acceptable, regular, bad, and very bad. This index was formulated from the historical record of physicochemical and hydrobiological variables measured in the Monitoring Network (RedRío), which has monitored the quality of water resources in the basin from 2003 to present. [AMVA and UdeA (2019)]. The quality index is calculated from the biological index BMWP-Col [Roldán (1996)], electrical conductivity, COD, total phosphorus, total Kjeldahl nitrogen and dissolved oxygen (Equation 2).

Equation 2. Global Water Quality Index – Global WQI

Global WQI=- 0.58*STANDARDIZE(LN(BMWP_Col)) + 0.96

*STANDARDIZE(LN(Electrical conductivity))+0.79

*STANDARDIZE(LN(DQO))+0.80*STANDARDIZE(LN(total P))

+0.94*STANDARDIZE(LN(NTK))-0.61*STANDARDIZE(LN(OD))

+10

Table 1 shows the classification of the index according to the numerical value and its respective color code. Low values indicate better quality classifications and, as these increase, the water quality progressively deteriorates [AMVA and UdeA, (2019)].

Table 1. Category Index of Global WQI		
CLASSFICIATION OF WATER RESOURCE QUALITY	**NUMERICAL VALUE RANGE**	**COLOR**
Good	<=3.00	Blue
Acceptable	3.10 – 6.00	Green
Regular	6.10 – 9.00	Yellow
Bad	9.10 – 12.00	Orange
Very bad	>12.00	Red

2.8 Statistical Analysis

An analysis of variance (ANOVA) was performed to detect variations in the extracellular enzymatic activity, the physicochemical variables and the Global WQI with respect to experimental factors considered (flow regime, monitoring stations and matrix) (p value < 0.05). The assumptions of normality and homoscedasticity were validated using the Kolmogorov-Smirnov and Levene tests, respectively. The samples were taken simultaneously and were considered as independent. Variables were transformed using natural logarithm. For those groups of factors in which differences were obtained, Tukey's HSD test was applied.

Pearson correlations were calculated between extracellular enzymatic activity and physicochemical and biological variables. The assumption of normality was validated using the Kolmogorov-Smirnov goodness-of-fit test. It is noteworthy that the variables were transformed using the natural logarithm, except for the activity of the β-glucosidase enzyme, for which a negative power transformation was used. A factorial analysis was carried out, taking into account the assumption of normality for each of the variables considered. The factor that summarized the variability of the data and represented the problem analyzed was selected, according to the weights assigned to each variable within the factor and the percentage of variance

explained. Subsequently, a cluster analysis and a discriminant analysis were carried out for the results obtained on the extracellular enzymatic activity from the monitoring stations. The data set was analyzed using the Statgraphics Centurion XVI statistical package.

3. Results

### 3.1 Physical chemical variables and Global WQI

In accordance with the classification, the flow regime of campaign 1 (April 26, 2017) was classified as high, in campaign 3 (August 2, 2017) it was classified as low, and in the remaining campaigns as medium. The data set evidenced contrasting behavior between the different monitoring stations. In the monitoring stations located in the upper zone of the basin (S0, S1, and S2) there were low values for the physicochemical variables, except for dissolved oxygen, and the Global WQI varied between good and acceptable. In S3 an increase in the physicochemical variables was observed, and the water quality was classified as marginal. The EC, DOC, COD, TP, BOD_5, TKN, PO4 and TSS presented the highest values between S4 and S6 and the water quality was classified as very bad. In S7 and S8 a decrease in these variables was observed and the water quality oscillated between regular and bad (Fig 2). There were no statistically significant differences in the physicochemical variables and in the Global WQI in low, medium and high flows (p value>0.05). The physicochemical variables and the Global WQI presented statistically significant differences between monitoring stations (ANOVA, value p<0.05). In general, the variables and the WQI were associated in three or four homogeneous groups: the first group was made up of S0, S1 and S2; the second corresponded to S3, S7 and S8 and the third was formed by S4, S5 and S6 (Table 2). In the first group, the lowest values were recorded for the evaluated variables and stations were classified with good and acceptable water quality. Meanwhile, in the third group, the highest values were recorded for the physicochemical variables and the water quality of the stations was classified as bad.

Table 2. Average and standard deviation of physicochemical and hydraulic variables, Global WQI and extracellular enzymatic activity of β-glucosidase and phosphatase in the water and biofilm. Letters

indicate the homogeneous groups formed among the monitoring stations according to results obtained from ANOVA and Tukey's HSD test. "<" means data lower than the quantification limit of the method.

Variable/ station	S0	S1	S2	S3	S4	S5	S6	S7	S8
pH (pH units)	6.83±0.17 [a]	7.60±0.43 [b]	7.46±0.11 [b]	7.61±0.11 [b]	7.76±0.14 [b]	7.50±0.15 [b]	7.71±0.28 [b]	7.32±0.23 [b]	7.43±0.13 [b]
Electrical conductivity (µS/cm)	24.80±1.91 [a]	33.80±3.92 [a]	58.92±8.32 [a]	141.79±32.43 [b]	518.47±163.66 [c]	476.13±124.11 [c]	430.27±128.83 [c]	220.67±77.79 [b]	198.59±51.30 [b]
Dissolved oxygen (mg/l)	7.70±0.09 [a]	7.19±0.23 [a]	7.52±0.14 [a]	5.82±1.48 [ab]	3.77±1.97 [bc]	1.43±2.40 [c]	1.76±2.30 [c]	6.47±0.49 [ab]	6.87±0.55 [a]
BOD_5 (mg/l)	<3.04 [a]	<3.04 [a]	3.23±0.43 [a]	22.76±5.63 [b]	111.48±32.48 [c]	146.14±62.00 [c]	147.32±50.86 [c]	29.44±24.51 [b]	34.26±27.12 [b]
COD (mg/l)	<10.00 [a]	10.30±0.63 [a]	11.02±1.55 [a]	39.90±5.95 [b]	215.00±33.66 [c]	293.80±76.82 [c]	243.60±32.50 [c]	81.04±63.65 [b]	79.10±62.34 [b]
DOC (mgC/l)	1.83±0.57 [a]	2.63±1.80 [ab]	1.79±0.43 [a]	4.41±1.40 [bc]	13.24±4.93 [de]	14.73±5.95 [e]	13.39±3.50 [de]	7.24±2.80 [cde]	6.20±2.38 [cd]
TKN (mgN/l)	<1.00 [a]	1.27±0.60 [a]	1.03±0.06 [a]	3.20±0.46 [b]	18.99±5.98 [d]	25.06±8.03 [d]	23.56±7.82 [d]	7.70±2.61 [c]	5.27±1.02 [bc]
TP (mgP/l)	<0.05 [a]	0.05±0.01 [a]	0.08±0.03 [a]	0.53±0.18 [b]	2.07±0.57 [c]	2.94±0.81 [c]	2.63±0.84 [c]	0.89±0.51 [b]	0.63±0.10 [b]
PO_4^{3-} (mgP/l)	<0.05 [a]	<0.05 [a]	0.08±0.03 [a]	0.21±0.08 [b]	0.95±0.35 [c]	1.30±0.48 [c]	1.30±0.49 [c]	0.23±0.10 [b]	0.22±0.15 [b]
TSS (mg/l)	<5.00 [a]	14.20±12.40 [ab]	54.40±42.24 [bc]	75.40±70.55 [cd]	143.60±93.67 [cde]	350.40±169.47 [e]	390.00±252.23	251.20±156.48 [d]	323.60±371.78 [d]
β-Glucosidase (mmol/g/h)	0.20±0.02 [a]	0.19±0.01 [a]	0.20±0.02 [a]	0.28±0.13 [a]	2.10±0.97 [b]	3.68±1.32 [b]	2.19±0.72 [b]	0.30±0.23 [a]	0.33±0.12 [a]
Phosphatase (mmol/g/h)	0.27±0.05 [a]	0.21±0.04 [a]	0.19±0.01 [a]	0.31±0.13 [a]	1.39±0.72 [b]	1.92±0.94 [b]	1.48±0.59 [b]	0.39±0.17 [a]	0.40±0.31 [a]
Q (m^3/s)	0.97±0.68	1.26±0.91	2.87±2.33	7.89±7.47	15.98±6.91	32.31±10.93	48.50±23.76	85.87±66.86	111.05±61.76
β-Glucosidase biofilm (mmol/g/h)	0.83±0.70 [ao.]	0.84±0.95 [a]	0.77±0.75 [a]	1.82±1.88 [ab]	14.17±8.16 [c]	4.30±3.94 [ab]	7.30±4.51 [abc]	4.42±4.64 [bc]	8.52±9.14 [abc]
Phosphatase biofilm (mmol/g/h)	2.46±2.41 [ab]	1.07±0.59 [ab]	0.30±0.16 [a]	1.19±0.98 [ab]	11.28±6.42 [c]	2.45±2.23 [ab]	5.13±2.80 [abc]	2.73±1.81 [ab]	5.46±5.12 [bc]

Fig 2. Physicochemical variables and Global WQI water quality index in the Aburrá-Medellín river during low (1, red color), medium (2, green color) and high flows (3, blue color). A) Dissolved oxygen, B) electrical conductivity, C) TSS, D) DOC, E) TP, F) Flow, G) Global QWI.
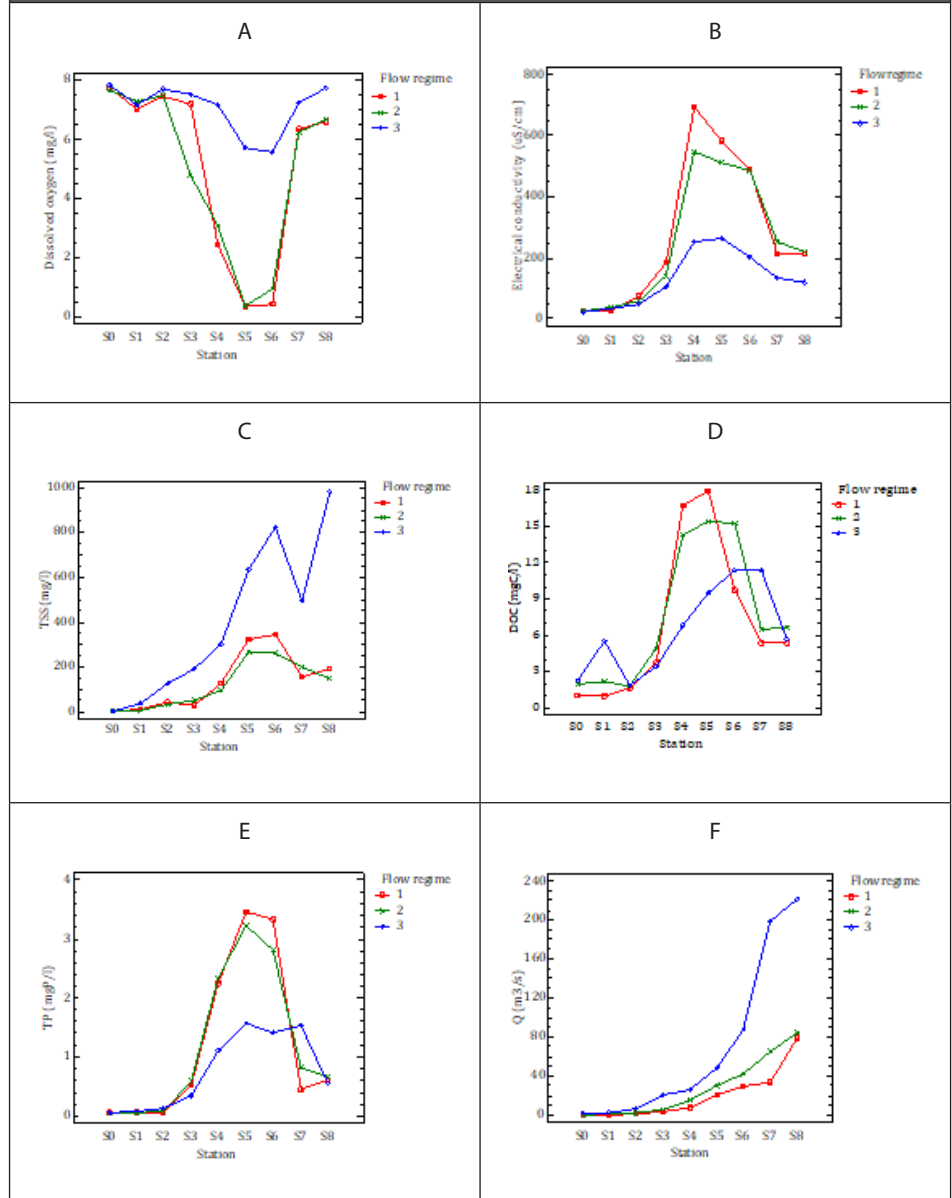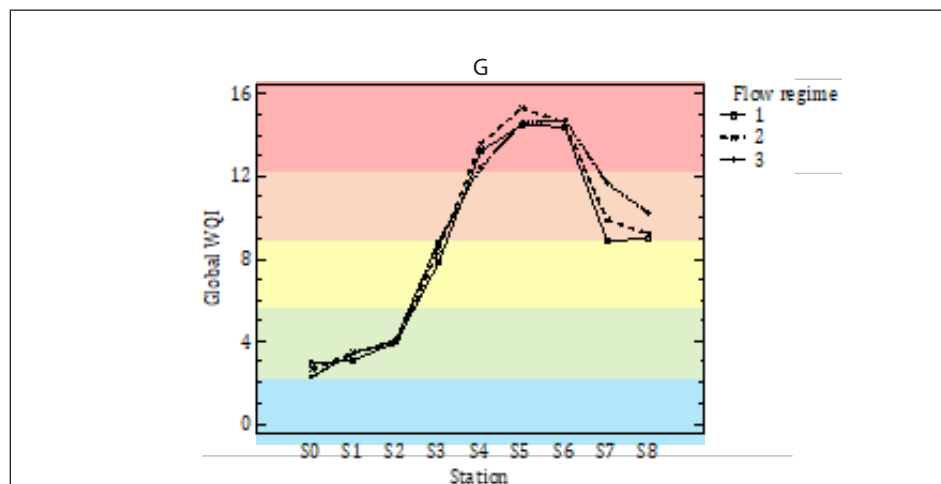
3.2. Activity comparison between matrices

The activity recorded for both enzymes in the water was lower than in the biofilm. The β-glucosidase and phosphatase activities presented statistically significant differences between the water and the biofilm (ANOVA, $p < 0.05$), (Fig. 3).

Fig. 3. Extracellular β-glucosidase and phosphatase enzyme activity in water and biofilm. BW (β-glucosidase in water), BB (β-glucosidase in biofilm), PW (phosphatase in water), PB (phosphatase in biofilm).
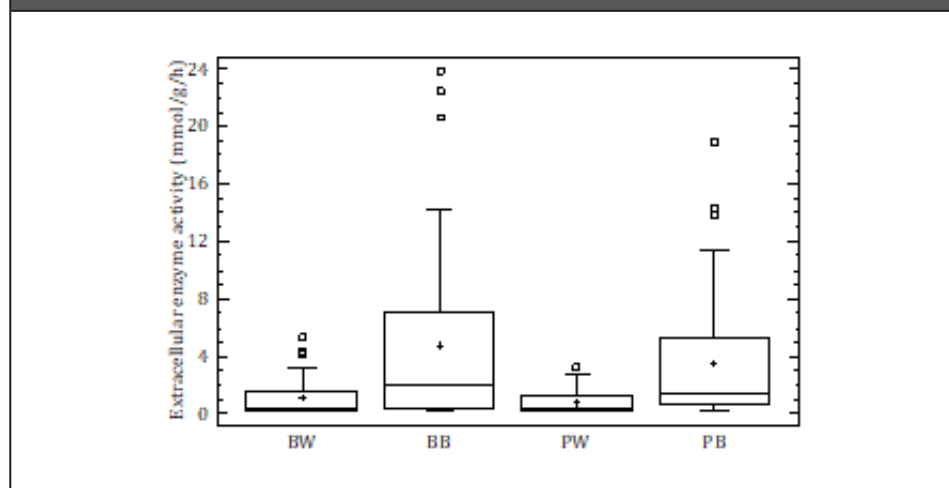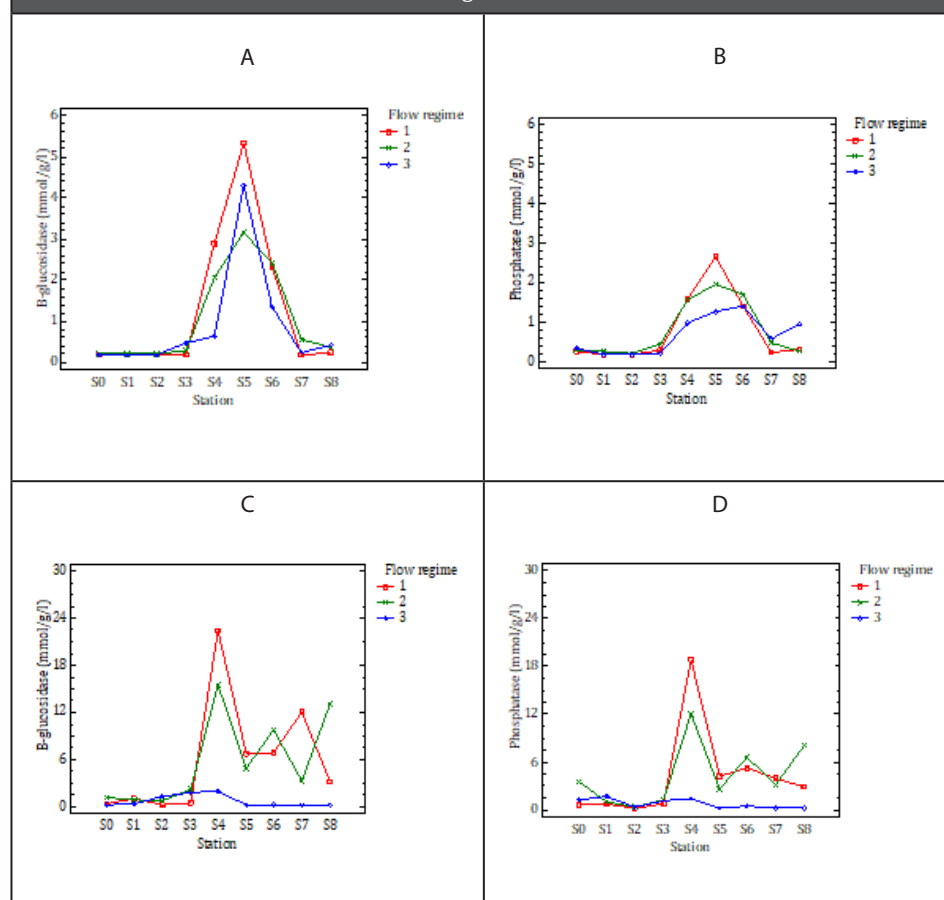
3.3. Extracellular enzymatic activity in the water

In spatial terms, β-glucosidase and phosphatase in water presented low activities in S0, S1 and S2, while in S3 a slight increase was observed. In S4 and S6 the highest activities were recorded; however, in S7 and S8 a decrease was observed compared to S6 (Figure 4). β-glucosidase and phosphatase showed statistically significant differences between monitoring stations (ANOVA, $p<0.05$). Two homogeneous groups were formed along the river (Table 2), the first of which corresponded to monitoring stations S0, S1, S2, S3, S7 and S8, and the second to stations S4, S5 and S6.

Regarding the flow regime, higher activity was recorded for both enzymes at low and medium flows than at high flows in the section between stations S4 and S6. There were no statistically significant differences between flow rates for either enzyme (ANOVA, $p>0.05$).

The β-glucosidase and phosphatase measured in the water presented a statistically significant correlation with all the variables. In the case of the phosphatase enzyme, a positive correlation was presented with all the variables, while β-glucosidase showed a negative correlation, which is associated with the negative power transformation used to comply with the assumption of normality (Table 3). However, this enzyme increased in quantity along the river, as well as the physicochemical variables measured.

A factor was selected that corresponded to 93.01% of the variance of the original data and represents the decomposition of organic matter in the water (Figure 4a). The factor loadings are presented in Table 4. A greater decomposition of organic matter in the river was observed in the S4 and S6 sections, while lower values were recorded in S0, S1, and S2. According to the cluster analysis, three homogeneous groups were obtained: the first group corresponded to the results obtained S0, S1 and S2, the second was made up of S3, S7 and S8, and the third was made up of S4, S5 and S6. According to the results obtained from the discriminant analysis, 100% of the cases presented a correct classification.

Fig. 4. Extracellular β-glucosidase and phosphatase enzymatic activity in the water (A, B) and biofilm (C, D) in the Aburrá-Medellín river under low, medium and high flow regimes.

3.4. Extracellular enzymatic activity in the biofilm

Regarding the activity of both enzymes in the biofilm, it is noteworthy that, spatially, low activity was recorded for S0, S1, and S2. In the section between S4 and S8, variable behavior was presented in the activity of both enzymes, and S4 was had notably higher values (Fig. 3). There were statistically significant differences between monitoring stations for both enzymatic activities (p<0.05), and three homogeneous groups were formed for each of them, according to the Tukey HSD test (Table 2). Regarding these homogenous groups for the β-glucosidase enzyme, the first was made up of stations S0, S1, S2, S3, S5, S6 and S8; the second of stations S3, S5, S6, S7 and S8; and the third of stations S4, S6, S7 and S8. Regarding the alkaline phosphatase enzyme, the homogeneous groups were formed by stations S0, S1, S2,

S3, S5, S6 and S7; S0, S1, S3, S5, S6, S7 and S8, and S4, S6 and S8. There were no significant differences in the enzymatic activities for low, medium and high flow rates (p>0.05). However, between S4 and S8, high activities were recorded for both enzymes in times of low and medium flows, while in times of high flows, low activities were recorded at all stations. The activity of both enzymes presented a positive correlation with the physicochemical variables measured in the water and with the chlorophyll measured in the biofilm; however, no correlation was observed with the pH of the water and the concentration of suspended solids (Table 3).

Table 3. Pearson correlation between enzymatic activity and physicochemical variables measured in the water and ICA Global. "*" Correlations significantly different from zero with a p value of 0.01. "**" Correlations significantly different from zero with a p value of 0.05. βGLU: β-glucosidase enzyme, PHO: phosphatase enzyme. The negative correlations between β-glucosidase and the variables in the water were negative because a negative power transformation was used to satisfy the assumption of normality.

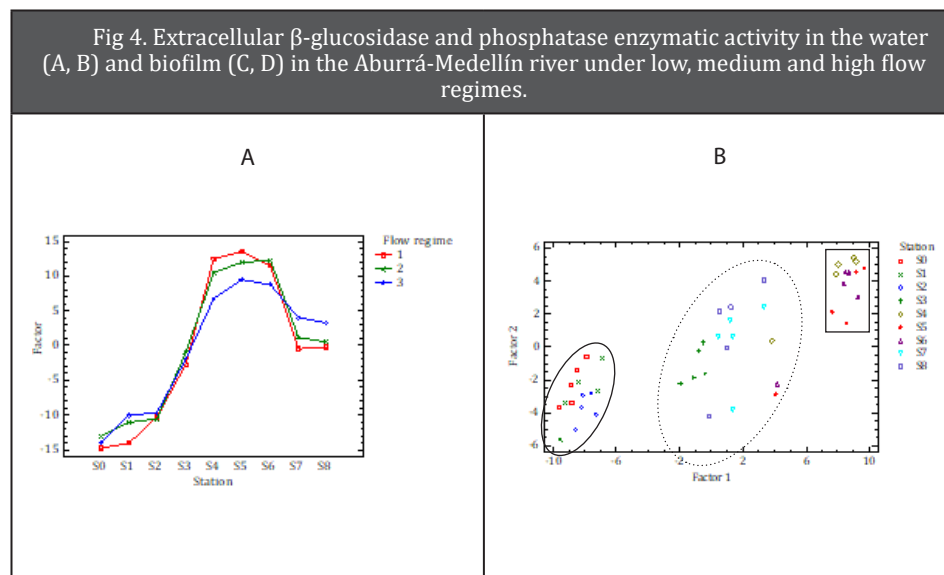| Variable	Water matrix		Biofilm matrix	
	βGLU	PHO	βGLU	PHO
pH	-0.44*	0.34**		
EC	-0.81*	0.76*	0.65*	0.52*
BOD_5	-0.86*	0.87*	0.50*	
COD	-0.88*	0.88*	0.49*	0.38*
TSS	-0.68*	0.62*		
TKN	-0.86*	0.84*	0.66*	0.56*
TP	-0.82*	0.80*	0.57*	0.45*
PO_4^{3-}	-0.88*	0.84*	0.66*	0.58*
DOC	-0.83*	0.82*	0.58*	0.53*
Chlorophyll a	-0.62*	0.60*	0.57*	0.66*
Global WQI	-0.80*	0.76*	0.53*	0.37**

From the factorial analysis carried out for the enzymatic activities and chlorophyll measured in the biofilm and the physicochemical variables measured in the water, two factors were selected that correspond to 98.51% of the variance of the original data and represent the decomposition of organic matter in the biofilm (Fig. 4b). Factor 1 represents 80.43% of the variance, and Factor 2 18.09%. Factor 1 describes the physicochemical conditions of the river, since the highest

loads observed in it corresponded to the physicochemical variables measured in the water. Meanwhile, Factor 2 represents the enzymatic activities, since their weights were higher (Table 4).

Table 4. Factor loading matrix				
Water		**Biofilm**		
Variable	**Factor**	**Variable**	**Factor 1**	**Factor 2**
β-Glucosidase	-0.89	β-Glucosidase	0.43	0.82
Phosphatase	0.86	Phosphatase	0.29	0.91
TSS	0.8	EC	0.93	0.28
Chlorophyll a	0.74	BOD_5	0.97	0.09
EC	0.96	COD	0.99	0.08
BOD_5	0.96	TKN	0.94	0.32
COD	0.99	TP	0.98	0.17
TKN	0.97	PO_4^{3-}	0.89	0.36
TP	0.98	DOC	0.89	0.26
PO_4^{3-}	0.94	Chlorophyll a	-0.02	0.77
DOC	0.94			
pH	0.48			

Based on the cluster analysis, three homogeneous groups were obtained. The first of these corresponded to the results recorded in S0, S1, and S2 in all five monitoring campaigns (ellipse). The second cluster was made up of S3, S7, and S8 in all the days monitored, as well as the data recorded for S4, S5, and S6 E12 in the first campaign (high flow) (dotted ellipse). Finally, the third cluster corresponded to S4, S5, and S6 in all monitoring campaigns, except the first (rectangle). The discriminant analysis showed that in 100% of the cases there was a correct classification. The above shows that the decomposition of organic matter in the biofilm of the river, presented variable behavior in the current, where the highest values were observed in the section S4-S6, in which the water quality presents the most critical conditions, and the lowest in S0, S1, and S2, where the water quality varies between good and acceptable.

Fig 4. Extracellular β-glucosidase and phosphatase enzymatic activity in the water (A, B) and biofilm (C, D) in the Aburrá-Medellín river under low, medium and high flow regimes.

4. Discussion

In the Aburrá-Medellín river, a high concentration of nutrients and a deterioration in water quality were observed in response to the discharge of wastewater and the pollutant load contributed by the tributary streams, which in turn result from the urbanization of the basin, mainly in the central zone [Paul and Meyer (2008); AMVA and UdeA (2019); Freixa et al. (2020)]. However, there was a low concentration of nutrients in the upper zone of the basin and a good to acceptable water quality, due to low anthropic intervention; while in the lower zone of the basin there was a decrease in the concentration of nutrients and an improvement in the quality of the water compared to section S4-S6, due to dilution processes [AMVA and UdeA (2019)]. The physicochemical variables and the water quality presented dynamic behavior over time, due to variations in the pollutant load contributed to the river and its tributaries, as well as the flow regime. At low flows, the quality of the water is critical, because the current carries a smaller volume of water (base flow), so there is little dilution of the pollutants contributed to the river; meanwhile, the opposite occurs in times of high flows [AMVA and UdeA (2019)].

The highest decomposition rates were recorded in the biofilm, which indicates that a large part of the decomposition of organic matter in the river is associated with the biofilm, which is in agreement with

what has been found by other authors [Romaní et al. (2004); Osorio et al. (2013); Ylla et al. (2014); Coundoul et al. (2014); Freixa et al. (2020)]. The rates at which carbon and phosphorus are processed along the river, both in the water column and in the biofilm, are of great importance in the basin, since the pollutant load that the river carries is high and the aquatic system does not have the capacity to process it, which influences the structure and functioning of the river and affects the uses of water and the ecosystem services that the river provides [Giraldo et al. (2014); Cifuentes (2015)]. Likewise, the process of decomposition of organic matter in the river is also very important in the Porce River hydrographic subzone, since this water source supplies the Porce II and Porce III reservoirs [Fundación EPM, Gobernación de Antioquia (2018)]. The activity of β-glucosidase and phosphatase in the water in the Porce II reservoir was higher than that found in the Aburrá-Medellín river [Giraldo et al. (2014); Grajales (2019)].

On another note, the variation observed in the activity of the β-glucosidase and phosphatase enzymes in the water is a response to changes in the physicochemical conditions of the river and in the nutrient content due to wastewater input. In the stations located in the most urbanized areas of the basin, where the highest concentrations of total phosphorus and COD were recorded, the highest activities were also found for the enzymes evaluated. On the other hand, the opposite was observed in the stations located in the upper zone of the basin, where anthropogenic activity is low. The enzymatic activity measured in the sites where a higher concentration of total phosphorus and COD was found in the river could be related to an increase in the concentration of nutrients susceptible to hydrolysis [Tiquia (2011); Harbott and Grace (2005); Williams et al. (2011); Millar et al. (2015)]. According to other authors, a high concentration of COD can induce a high processing of carbon in the current [Tiquia (2011); Harbott and Grace (2005)], which may also occur for phosphorus, since a high activity of phosphatase enzyme has been recorded in eutrophic ecosystems and wastewater, associated with the recycling of phosphate from the hydrolysis of dissolved organic phosphorus compounds [Nedoma et al. (2006)].

In contrast, the low activities recorded in the upper part of the basin, where the water quality varied between good and acceptable, may be related to a lower concentration of nutrients susceptible to

hydrolysis, and may indicate that microorganisms also use carbon sources other than those present in the most contaminated sampling sites [Harbott and Grace (2005); Millar et al. (2015)]. Harbott and Grace (2005) found that fluvial ecosystems in sparsely urbanized basins were dominated by the activities of the enzymes β-N-acetylglucosaminidase and β-xylosidase, which process plant-derived compounds and carbohydrates such as chitin and murein. It is noteworthy that in these sites the activity of β-glucosidase was similar in the different water bodies evaluated, meaning that the polysaccharides hydrolyzed by this enzyme were a source of carbon in all the streams evaluated. In this regard, chitin and murein could be considered as another carbon source used by microorganisms in S0, S1 and S2, since in this sparsely urbanized section there are contributions of plant material.

The contrasting behavior described for β-glucosidase and phosphatase activity in water has also been observed in other river ecosystems with a higher level of basin urbanization and nutrient concentration [Tiquia (2011); Harbott and Grace (2005); Williams et al. (2011); Millar et al. (2015); Jaramillo et al. (2016)]. The results of these studies were associated with the land uses in the basin, the degree of urbanization in the basins, and the concentration of organic and inorganic matter in the water bodies [Tiquia (2011); Harbott and Grace (2005); Williams et al. (2011); Millar et al. (2015); Jaramillo et al. (2016)]. Estimated enzyme activities in the Aburrá-Medellín river were lower than those reported in the Chinchiná river, where DOC and total phosphorus concentrations were higher [Jaramillo et al. (2016)], while in tributaries of the Lower Mississippi and streams in southern Ontario, Canada the opposite was observed [Williams et al. (2011); Millar et al. (2015)].

Regarding the temporal variation, no statistically significant changes were observed in the enzymatic activities of β-glucosidase and phosphatase between flow regimes. This behavior could be related to a higher concentration of complex organic carbon and phosphorus compounds, which are used by microorganisms as a source of nutrients [Chróst and Siuda (2002); Harbott and Grace (2005)]. In river systems located in other latitudes, it has been reported that the activities of various enzymes showed a dynamic behavior with respect to the season of the year, associated with variations in energy inputs, the density of microorganisms and changes in their physiology, as

well as physicochemical factors such as water temperature, light and nutrient concentration [Millar et al. (2015); Tiquia (2011); Wilczek et al. (2005); Artigas et al. (2009); Artigas et al. (2011)].

The correlation observed between the physicochemical variables in the water and the enzymatic activities recorded in the biofilm is related to the use of complex carbon and phosphorus compounds found in the water as a source of nutrients by microorganisms [Sabater et al. (2002)]. Regarding the biofilm, significant differences were found in the activity of both enzymes between seasons; however, the homogeneous groups did not show variation related to the physicochemical profile and water quality. It should be noted that during low and medium flows, a higher level of activity was measured at all monitoring stations, except those located in the upper part of the basin, which could be associated with an increase in the concentration of nutrients and chlorophyll a, this is in agreement with what was reported by Freixa et al., (2020) and Rossi et al (2019). However, authors such as Lehto and Hill (2013) and Sabater et al (2016) observed that extracellular enzymatic activity was positively correlated with forest cover and inversely with micropollutants and urbanization indicators, suggesting a deterioration in biofilm capacity to decompose organic matter in contaminated sections. Romaní and Sabater (1999) observed that the biofilm was less efficient (extracellular enzyme activity per bacterial cell) in the Ter river where nutrient concentrations were higher.

The activities measured in the biofilm can also be associated with the influence of hydraulic conditions on the colonization of substrates and on the structure and function of the biofilm [Giraldo (2013); Freixa et al. (2020); Coundoul et al. (2014)]. In accordance with previous studies carried out in the Aburrá-Medellín river on the colonization of periphytic algae on artificial substrates [Giraldo (2013)], it was found that the highest density was recorded during periods of low flow, while the lowest density was recorded during periods of high flow. This was associated with an increase in flow and the abrasive effect generated on the epilithic community by an increase in TSS along the river, in response to diffuse contributions (runoff) and resuspension of sediments [Giraldo (2013); AMVA and UdeA (2019)]. In this regard, the low activity measured in both enzymes during the high flow season can be attributed to the detachment of biofilms, due to flooding shear stress in the biofilm that generates a decrease in the density of

microorganisms [Osorio et al. (2013); Jiménez et al. (2014); Giraldo (2013); AMVA and UdeA (2019)]. In the high flow season, substrate relocation occurs, making new surfaces available for microorganism colonization and biofilm formation [Pohlon et al. (2010)]. Additionally, it is noteworthy that a section of the river is channeled and rectified (between S3 and before S5), which also has an incidence on the colonization of substrates due to changes in flow velocity [Pohlon et al. (2010)]. In this regard, the activity of both enzymes in the biofilm responds to the colonization of substrates and, therefore, to the quality and quantity of water and the morphology of the channel.

No statistically significant changes in the enzyme activities of β-glucosidase and phosphatase were observed between the flow regime in the water and the biofilm. At low and medium flow rates, higher activities were recorded for the enzymes evaluated, contrary to what was observed at high flow rates. According to the results obtained, the concentrations of phosphorus and DOC were higher during the periods of low and medium flow, due to a decrease in the base flow of the river, while in the periods of high flow, lower concentrations were recorded, due to dilution processes [AMVA and UdeA (2019)]. The changes in the flow affected the amount and composition of organic matter and nutrients in the aquatic ecosystem, which in turn influences metabolism [Sabater et al. (2016); Wilczek et al. (2005); Artigas et al. (2009); Artigas et al. (2011)]. In this regard, enzymatic activities tend to be higher at low and medium flow times due to an increase in nutrients and organic matter [Harbott and Grace (2005)].

Hypothesis i) was that enzymatic activity responds to changes in the availability of nutrients and is higher in the places where the concentrations of nutrients are higher, as a result of the discharges of treated and untreated wastewater into the river, coming mainly from of the most urbanized municipalities in the basin. According to the results obtained in this study, the physicochemical profile of the river changes as it receives specific and diffuse wastewater discharges throughout its course, which affects the concentration of nutrients and organic matter, as well as the concentration of chlorophyll a. The enzymatic activity in the biofilm was higher than in the water column, showing its importance in the decomposition of organic matter in the river. It was observed that extracellular enzymatic activity was sensitive to changes in the concentration of nutrients in the river,

which responded to the degree of urbanization in the basin. In this respect, the hypothesis is accepted, and the enzymatic activity can be used as an indicator of the health of the Aburrá-Medellín river. Hypothesis ii) was that the flow regime affects the enzymatic profile in the river, since it influences the concentration of nutrients transported by the river. Nutrients presented higher concentration in periods of low and medium flow, while their concentration was lower in periods of high flow, and so the enzymatic activities presented the same trend. In the biofilm, the enzymatic activities showed a decrease in the season of highest flow due to a detachment of the biofilm and, therefore, a decrease in the density of microorganisms. Hypothesis ii) was that the flow regime affects the enzymatic profile in the river, since it influences the concentration of nutrients transported by the river. Nutrients presented higher concentration in periods of low and medium flow, while their concentration was lower in periods of high flow, so the enzymatic activities presented the same trend. In the biofilm, enzymatic activities decreased in the highest flow season due to a detachment of the biofilm and, therefore, a decrease in the density of microorganisms.

However, there were no statistically significant changes in the activity of the enzymes β-glucosidase and alkaline phosphatase during different flow regimes. It is important to mention that, during the study period, only one sampling campaign was carried out during low and high flows and three campaigns were carried out during medium flows, so there is not enough evidence to affirm that the flow regime is not an important factor associated with the variability of the activities of the two enzymes in the river. In general, our study suggests that the physicochemical profile of the river and its water quality influence the activity of the β-glucosidase and phosphatase enzymes in the water and biofilm, and therefore, in turn influence the metabolism of carbon and phosphorus in the water body and the decomposition of organic matter. Our study also highlights the role of biofilm in the decomposition process of organic matter in urban rivers, where channeling and rectification of the river affects the colonization of substrates. This has implications for the management of river ecosystems in urbanized basins, since the enzymatic profile can be a potential indicator of the state of the river.

5. Acknowledgements

6. References

APHA, AWWA, WEF (2012) Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

Área Metropolitana del Valle de Aburrá-AMVA (2012) Resolución Metropolitana 2016 de 2012 por medio de la cual se adoptan nuevos objetivos de calidad del Río Medellín-Aburrá, para el periodo 2012-2022.

Área Metropolitana del Valle de Aburrá-AMVA; Universidad de Antioquia-UdeA (2019) Aunar Esfuerzos para la Gestión Integral del Recurso Hídrico Superficial y Subterráneo, en el marco de la operación de la Red de Monitoreo Ambiental en la cuenca hidrográfica del río Aburrá-Medellín C643/2019: Informe Final de Calidad de Agua Superficial.

Artigas J, Romaní AM, Gaudes A, et al (2009) Organic matter availability structures microbial biomass and activity in a Mediterranean stream. Freshwater Biology 54:2025–2036. https://doi.org/10.1111/j.1365-2427.2008.02140.x.

Artigas J, Gaudes A, Muñoz I, et al (2011) Fungal and Bacterial Colonization of Submerged Leaf Litter in a Mediterranean Stream. International Review of Hydrobiology 96:221–234. https://doi.org/10.1002/iroh.201111355.

Arthington AH, Naiman RJ, McClain ME, Nilsson C (2010) Preserving the biodiversity and ecological services of rivers: new challenges and research opportunities. Freshwater Biology 55:1–16. https://doi.org/10.1111/j.1365-2427.2009.02340.x

Assessment Millennium Ecosystem (2005) Ecosystems and human well-being. Washington, DC

Berrio-Restrepo JM, Saldarriaga JC, Correa MA, Aguirre NJ. Extracellular enzymatic activity of two hydrolases in wastewater treatment for biological nutrient removal. Appl Microbiol Biotechnol. 2017 Oct;101(19):7385-7396. doi: 10.1007/s00253-017-8423-1. Epub 2017 Aug 7. PMID: 28782075

Chróst RJ (1990) Microbial Ectoenzymes in Aquatic Environments. In: Overbeck J, Chróst RJ (eds) Aquatic Microbial Ecology: Biochemical and Molecular Approaches. Springer New York, New York, NY, pp 47–78.

Chróst RJ, Siuda W (2002) Ecology of microbial enzymes in lake ecosystems. In: Burns RG, Dick RP (eds) Enzymes in the Environment: Activity, Ecology, and Applicationsctivity, ecology, and applications. Marcel Dekker, Inc, New York, NY, pp 35–72.

Cifuentes A (2015) Determinación de la actividad enzimática extracelular en el tramo km 5.8-km 37.1 del río Aburrá-Medellín en el agua y el biofilm (Antioquia-Colombia). Universidad de Antioquia, Medellín, Colombia. Trabajo de Investigación de Pregrado en Ingeniería Ambiental, Facultad de Ingeniería.

CORANTIOQUIA, Área Metropolitana del Valle de Aburrá, Cornare, et al (2018) Actualización Plan de Ordenación y Manejo de la Cuenca Hidrográfica Río Aburrá.

Coundoul F, Bonometti T, Graba M, et al (2014) Role of Local Flow Conditions in River Biofilm Colonization and Early Growth. River Research and Applications 31:350–367. https://doi.org/10.1002/rra.2746.

Cunha A, Almeida A, Coelho F, et al (2010) Bacterial Extracellular Enzymatic Activity in Globally Changing Aquatic Ecosystems. Current Reseach, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 124–135

DANE (2019) Resultados Censo Nacional de Población y Vivienda 2018.

Dunham JB, Angermeier PL, Crausbay SD, et al (2018) Rivers are social – ecological systems : Time to integrate human dimensions into riverscape ecology and management. WIREs Water 1–10. https://doi.org/10.1002/wat2.1291

Hernández E., N. J. Aguirre, and J. A. Palacio, "Relación entre la determinación del pigmento Clorofila a y el Biovolumen geométrico algal en un lago de planicie de inundación (Ciénaga de Ayapel, Córdoba-Colombia)," *Revista Facultad de Ingeniería Universidad de Antioquia*, vol. 60, pp. 159–169, 2011.

Findlay S (2010) Stream microbial ecology. Journal of the North American Benthological Society 29:170–181. https://doi.org/10.1899/09-023.1

Freixa A, Perujo N, Langenheder S, Romaní AM (2020) River biofilms adapted to anthropogenic disturbances are more resistant to WWTP inputs. 96:1–12. https://doi.org/10.1093/femsec/fiaa152

Fundación EPM, Gobernación de Antioquia (2018) Antioquia un territorio para proteger. Actualización y monitoreo del estado del recurso hídrico. Medellín

Giraldo LC (2013) Dinámica de la calidad química del agua, algas epilíticas, macroinvertebrados acuáticos y actividad enzimática del sistema fluvial río Aburrá-Medellín en el tramo k5-k48. Universidad de Antioquia

Giraldo LC, Palacio CA, Aguirre NJ (2015) Epiphyton Colonization on Artificial Substrates in the Aburrá-Medellin River, Colombia. The International Journal of Environmental Protection 5(1):16-24. DOI:10.5963/IJEP0501003.

Giraldo LC, Palacio CA, Aguirre NJ (2014) Temporal Variation of the Extracellular Enzymatic Activity (EEA): Case of Study Aburra-Medellín River, in the Valle de Aburra in Medellin, Antioquia, Colombia. International Journal of Environmental Protection 4:58–67

Grajales H (2019) Dinámica ambiental de los nutrientes nitrógeno, fósforo y sílice y su relación con la calidad del agua en el embalse Porce II, municipio de Amalfi, Antioquia, Colombia. Universidad de Antioquia, Medellín, Colombia.

Harbott EL, Grace MR (2005) Extracellular enzyme response to bioavailability of dissolved organic C in streams of varying catchment urbanization. Journal of the North American Benthological Society 24:588–601. https://doi.org/10.1899/04-023.1

Hosen JD, McDonough OT, Febria CM, Palmer MA (2014) Dissolved oganic matter quality and bioavailability changes across an urbanization gradient in headwater streams. Environmental Science & Technology 48:7817–7824. https://doi.org/10.1021/es501422z

Instituto de Hidrología Meteorología y Estudios Ambientales (IDEAM), "Guia para el monitorieo de vertimientos, aguas superficiales y subterráneas," 2003.

Jaramillo MT, Aguirre NJ, Galvis JH (2016) Using Extracellular Enzyme Activity as a Pollutant Indicator: a Field Study in Chinchiná River, Caldas-Colombia. International Journal of Environmental Protection 6:47–59. https://doi.org/10.5963/IJEP0601004

Jiménez Pérez P, Toro Restrepo B, Hernández Atilano E (2014) Relación entre la comunidad de fitoperifiton y diferentes fuentes de contaminación en una quebrada de los Andes colombianos. Boletín Científico Centro de Museos Museo de Historia Natural Universidad de Caldas 18:49–66

Lehto LLP, Hill BH (2013) The effect of catchment urbanization on nutrient uptake and biofilm enzyme activity in Lake Superior (USA) tributary streams. Hydrobiologia 713:35–51. https://doi.org/10.1007/s10750-013-1491-z

Martin-Ortega J, Ferrier RC, Gordon IJ, Khan S (2015) Water ecosystem services: A global perspective. UNESCO Publishing

Marxsen J, Tippmann P, Heininger H, et al (1998) Enzymaktivität. In: Remde A, Tippmann P (eds) Mikrobiologische Charakterisierung aquatischer Sedimente: Methodensammlung. vol (Hrsg.) Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM). Oldenbourg Verlag GmbH, München. pp 87–114

Millar JJ, Payne JT, Ochs CA, Jackson CR (2015) Particle-associated and cell-free extracellular enzyme activity in relation to nutrient status of large tributaries of the Lower Mississippi River. Biogeochemistry 124:255–271. https://doi.org/10.1007/s10533-015-0096-1

Nedoma J, García J, Comerma M, et al (2006) Extracellular phosphatases in a Mediterranean reservoir: Seasonal, spatial and kinetic heterogeneity. Freshwater Biology 51:1264–1276. https://doi.org/10.1111/j.1365-2427.2006.01566.x

Osorio V, Proia L, Ricart M, et al (2013) Hydrological variation modulates pharmaceutical levels and biofilm responses in a Mediterranean river. Science of The Total Environment 472:1052–1061. https://doi.org/10.1016/j.scitotenv.2013.11.069

Paul M, Meyer J (2008). Streams in the Urban Landscape.

Pohlon E, Marxsen J, Küsel K (2010) Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms. FEMS Microbiology Ecology 71:364–373. https://doi.org/10.1111/j.1574-6941.2009.00817.x

Roldán G., *Guía para el estudio de los macroinvertebrados acuáticos del Departamento de Antioquia*. Bogotá D.C., Colombia, 1996.

Romaní A, Sabater S. (1999). Epilithic ectoenzyme activity in a nutrient-rich Mediterranean river. Aquatic Sciences 61:122-132. https://doi.org/1015-1621/99/020122-11.

Romaní AM, Guasch H, Muñoz I, et al (2004) Biofilm structure and function and possible implications for riverine DOC dynamics. Microbial ecology 47:316–328. https://doi.org/10.1007/s00248-003-2019-2

Romaní A, Fischer H, Lindblom C, Tranvik L (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569

Romaní A, Artigas J, Camacho A, et al (2009) La biota de los ríos: los microorganismos heterotróficos. In: Elosegi A, Sabater S (eds) Conceptos y Técnicas en ecología fluvial. Fundacion BBVA, p 448

Rossi F, Mallet C, Portelli C, et al (2019) Stimulation or inhibition : Leaf microbial decomposition in streams subjected to complex chemical contamination. Science of the Total Environment 648:1371–1383. https://doi.org/10.1016/j.scitotenv.2018.08.197

Sabater S, Guasch H, Romaní A, Muñoz I (2002) The effect of biological factors on the efficiency of river biofilms in improving water quality. Hydrobiologia 469: 149-156. https://doi.org/10.1023/A:1015549404082.

Sabater S, Barceló D, De Castro-Català N, et al (2016) Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. Environmental Pollution 210:303–314. https://doi.org/10.1016/j.envpol.2016.01.037

Sabater S, Timoner X, Borrego C, Acuña V (2016) Stream Biofilm Responses to Flow Intermittency: From Cells to Ecosystems. Frontiers in Environmental Science 4:1–10. https://doi.org/10.3389/fenvs.2016.00014

Sistema de Alerta Temprana (SIATA), "Informe del Componente Hidráulico," 2017.

Sistema de Alerta Temprana (SIATA), "Informe del Componente Hidráulico," 2018.

The Quintessence Consortium (2016) Networking Our Way to Better Ecosystem Service Provision. Trends in Ecology & Evloution 31:105–115. https://doi.org/10.1016/j.tree.2015.12.003

Thoms M, Sheldon F (2019) Large rivers as complex adaptive ecosystems. River Research and Applications 35:451–458. https://doi.org/10.1002/rra.3448

Tiquia SM (2011) Extracellular Hydrolytic Enzyme Activities of the Heterotrophic Microbial Communities of the Rouge River: An Approach to Evaluate Ecosystem Response to Urbanization. Microbial Ecology 62:679–689. https://doi.org/10.1007/s00248-011-9871-2

Tümpling W V., Friedrich G (1999) Methoden der Biologischen Wasseruntersuchung 2. Biologische Gewässerunchung

Wetzel RG (1991) Extracellular Enzymatic Interactions: Storage, Redistribution, and Interspecific Communication. In: Chróst RJ (ed) Microbial Enzymes in Aquatic Environments. Springer New York, New York, NY, pp 6–28

Wilczek S, Fischer H, Pusch MT (2005) Regulation and seasonal dynamics of extracellular enzyme activities in the sediments of a large Lowland River. Microbial Ecology 50:253–267. https://doi.org/10.1007/s00248-004-0119-2

Williams CJ, Scott AB, Wilson HF, Xenopoulos MA (2011) Effects of land use on water column bacterial activity and enzyme stoichiometry in stream ecosystems. Aquatic Sciences 74:483–494. https://doi.org/10.1007/s00027-011-0242-3

Ylla I, Canhoto C, Romaní AM (2014) Effects of Warming on Stream Biofilm Organic Matter Use Capabilities. Microbial Ecology 68:132–145. https://doi.org/10.1007/s00248-014-0406-5