

Temporal Variation of the Extracellular Enzymatic Activity (EEA): Case of Study: Aburra-Medellín River, in the Valle de Aburra in Medellin, Antioquia, Colombia

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Abstract- This work aims to determine the enzymatic extracellular activity for the transformation of high molecular weight complex organic compounds and their temporal variation at the Aburrá-Medellín river “Aula Ambiental“ monitoring station. In order to evaluate the enzymatic activity of β -glucosidase and alkaline phosphatase in relation to the metabolism of carbon and phosphorus, in the water column and the biofilm attached to the rocky substrate of the river bed, seven monitoring campaigns were held between December 2010 and November 2011 at this site. By using a spectrophotometer, two specific substrates, namely 4-Nitrofenil- β -D-Glucopiranososa and 4-Nitrofenilfosfato were evaluated and analysed. From this evaluation, no significant differences were found among the monitoring campaigns, despite climatic variations presented during the study period, which may be due to the constant availability of nutrients in the river and to the continuous discharges of wastewater into the river. This research is a pioneering study in the region, which helps the understanding of the dynamics related to the microbial metabolism of nutrients in water bodies.

Key words- *β -Glucosidase; Alkaline Phosphatase; Enzymatic Activity; Aburrá-Medellín River*

I. INTRODUCTION

Part of the metabolic activity of the organic matter entering the river as allochthonous sources is performed through the biological complex that colonizes hard substrata like rocks or sediments of different granulometry in the riverbed (biofilm). The analysis of its structure and function is important to interpret the river energy balance, its self-cleansing ability, and the water quality. This biological complex layer, where bacteria and fungi form a consortium with algae, is constituted by a hydrated polysaccharide with gel-like properties, on which protozoa and small invertebrates live and move. The thickness of this layer can vary between 100 microns and a few millimeters and is called biofilm [1].

The degradation of complex large organic molecules is better carried out by extracellular enzymes. These enzymes transform outside the cell complex molecules into small monomers that can be easily absorbed by the organisms, thus helping the organism have the necessary nutrients available [2, 3].

Bacteria and fungi, as well as some algae and protozoa, have the capacity to synthesize extracellular enzymes and ectoenzymes. These enzymes are on the surface of microbial cells or in the periplasmic space of Gram negative bacteria cells and act outside of it [3].

The production of most of the extracellular enzymes is suppressed by most aquatic microorganisms, when cells grow on dissolved organic matter sources easily usable, avoiding its wastage, i.e. it is only released when the concentration of easily usable nutrients in water drops below critical levels and can be inhibited by the accumulation of final products from hydrolysis into the surrounding medium [4].

Different research studies have proven that extracellular enzymatic activity is mainly related to the availability of the substrate [3-5]. However, in rivers, this activity can be affected by environmental factors such as temperature, concentration of dissolved nutrients, light, and the presence of humic material [5]. Furthermore, biological parameters, like the presence of algae and bacteria are one of the causes for the variation of heterotrophic activities [5]. Therefore, in this research the variation of the extracellular enzymatic activity (EEA) was assessed in two substrates: Water column and the biofilm attached to the rocky substrate of the river bed.

Recent applications of some artificial substrates (reagent) hydrolyzed by natural enzymes have proved to be useful in studies of enzymatic processes in aquatic ecosystems [6]. For the aim of this research, two substrates: 4-Nitrophenyl- β -D-glucopyranose and 4-Nitrophenyl-phosphatase, which are hydrolyzed by the enzymes β -D-glucosidase (EC 3.2.1.21) and alkaline phosphatase (EC 3.1.3.1) were used. They play an essential role in nutrient recycling and transference of matter and energy through the food chain.

The β -D- glucosidase is relevant because of its influence in the last step of cellulose decomposition. That is, the transformation of cellobiose to glucose is known to be mainly associated with heterotrophic bacteria, and it is produced in waters and sediments of freshwater and marine ecosystems.

The enzyme catalyzes the hydrolysis of phosphoric acid monoesters and also catalyzes the transphosphorylation reaction in presence of high concentrations of phosphate acceptors [7]. The final product is orthophosphate, which is the form of phosphorus considered as directly available and rapidly assimilated by algae and bacteria [8].

In order to determine the enzymatic extracellular activity for the transformation of high molecular weight complex organic compounds and their temporal variation at the Aburrá-Medellín river "Estación Aula Ambiental", seven samples were collected between December 2010 and November 2011 to measure the enzymatic activity of β -glucosidase and alkaline phosphatase in relation to the metabolism of carbon and phosphorus, as both are macronutrients necessary for life. This work aims at answering the following research question: How is the temporal variation of extracellular enzymatic activity at the Aburrá-Medellín River, "Estación Aula Ambiental" in function of the climatic periods?

II. MATERIALS AND METHODS

A. Subject of Study

Aburrá Valley is located in the south-central side of the state of Antioquia-Colombia, in the middle of the Central Range of the Andes. The relevance of the Aburrá Valley lies on the fact that it has ten municipalities and the second largest city in the country (Medellín), with a population of about 3 million people. This valley is crossed by a large river that runs through the ten municipalities and has become a hub for the historical development of the region [9].

The hydrographic basin of the Aburrá-Medellín River consists of the Central and Eastern branch that belong to the Central Range of the Andes [10]. The altitude of the mountains around the valley can reach up to 2,500 to 3,000 meters above sea level and are divided by the river that runs in south-north direction. This valley extends from 1,795 m of elevation (Caldas) to 1,048 m (to the mouth of Rio Grande) [9].

In the hydrographic basin of the Aburrá-Medellín River some geological formations from different nature can be distinguished including: igneous rock, metamorphic and unconsolidated sediments. Overall, the Aburrá Valley is a high depression in the Central Range with steep slopes and partly stepped portions, consisting of broken rocks, firmly seated where the slope is softened and with terraces and recent alluvium, deep, deposited by the River [11].

The climate of the basin can fall within the regime of tropical or equatorial mountain climate, and is mainly characterized by two phenomena as follows:

a) Small variations in the average temperature during the course of the year. Colombia's geographical position within the tropical latitudinal region, determines that its temperature is defined by the altitude above sea level. In Medellín, the average annual temperature is 22°C, with average maximum and minimum of 29 and 16°C, respectively. The temperature usually ranges between 18 and 26°C.

b) Presence of two periods of high rainfall and two intermediate periods of lower rainfall [12].

The water resource of the main stream of the basin is continuously exposed to contamination caused by human activity, mainly because of misuse of the land, exploitation of materials on the beds of the river, hillsides and streams, soil degradation by erosion, sedimentation processes, disposal of solid waste in the beds of streams, settlements in high risk areas on the river bank, deforestation at the mouth of streams, and domestic and industrial wastewater discharge without a pre-treatment.

The level of the river, as it passes through Medellín, was drastically affected in the late thirties with the rectification of the river. [13]. These morphological changes led to biotic modifications, such as loss of riparian vegetation, changes in the composition of periphytic algae and macroinvertebrate; consequently, many communities disappeared. For decades, changes in the hydrodynamic characteristics of the river alongside with the dumping of sewage and solid waste in the region, have led to changes in physicochemical processes that have deteriorated the ecosystem. [14] presents an analysis of the spatial variation of the forms of nitrogen: ammonia, nitrites, and nitrates in the Medellín River profile, and phenomena associated with its transformation; likewise, a temporal analysis based on information collected since 1973 from different physicochemical studies have been carried out on the river.

For the development of this research, the "Estación Aula Ambiental" was defined as the monitoring site. It is located at 15°53.45'N 6°75'34"18"W 74". In this point, the river has received wastewater discharges from the municipality of Caldas, wastewater treated at San Fernando plant and several streams that do not yet have the full array of sanitation services (some of which present deterioration in the quality of water caused by the exploitation of material at the top of their banks), and from industrial companies wastewaters that are not connected to the collection system of Empresas Públicas de Medellín.

Fig. 1 shows a map of the study site location, made from drawings provided by the Management and Development Plan of the Aburrá river basin (POMCA).

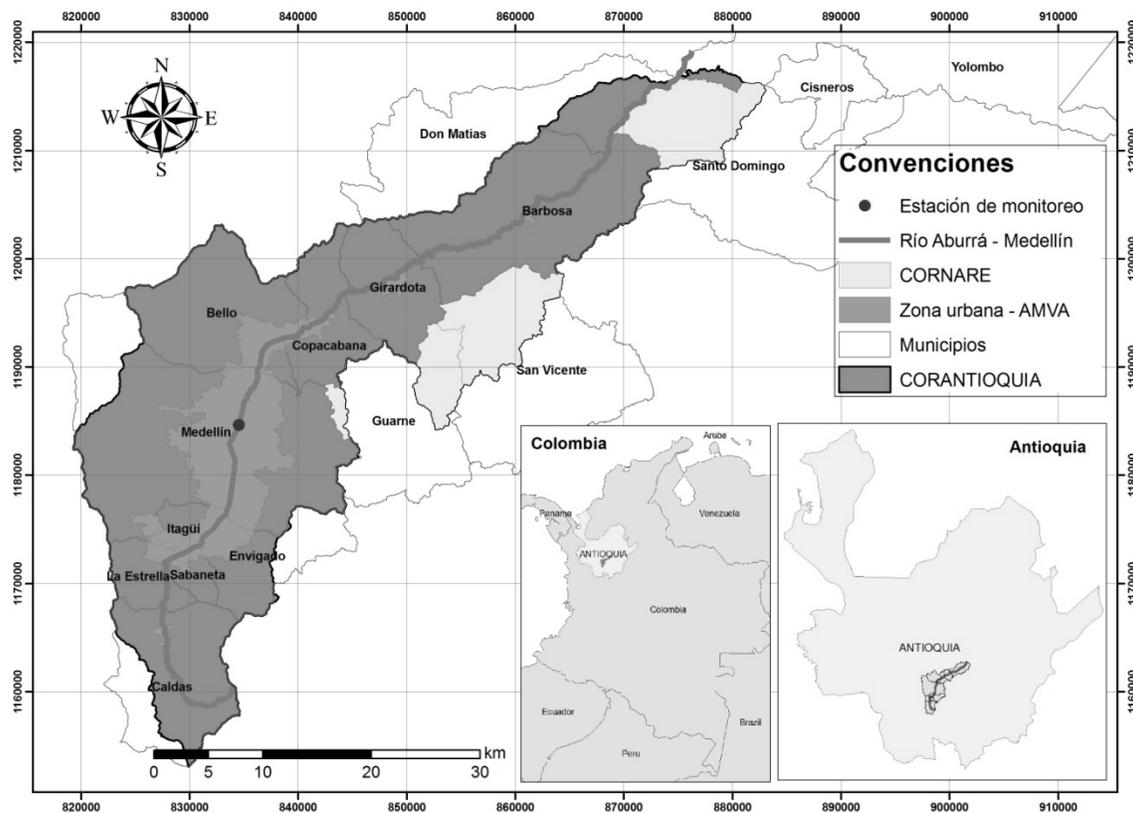


Fig.1 Geographical location of the study site (Modification [15])

B. Field Procedures

The site for regular sampling was the “Estación Aula Ambiental”. Routine sampling was at about monthly intervals. Samples were collected in December 2010 and February, March, April, May, September, and November 2011 with the purpose of evaluating the influence of different climate periods (bimodal regime: high and lower rainfall) in the extracellular enzymatic activity.

The procedure developed was as follows:

Surface water samples were collected in order to perform enzyme analysis and to measure physicochemical variables (orthophosphates, nitrates, ammonia nitrogen and dissolved organic carbon), avoiding their contamination with sediments, according to the “Guide for the monitoring of discharges, surface water, and groundwater” given by the Institute of Hydrology, Meteorology and Environmental Studies [16]. In this sense, samples were collected in two plastic containers with a capacity of 2 liters each. Other samples were collected in amber bottles with a capacity of 100 ml for determination of dissolved organic carbon (DOC).

The biofilm samples used to investigate extracellular enzyme activity were taken of rocks from the river bed under the guidelines proposed by Tümpling and Friedrich [17], corresponding to the sampling criteria for natural substrates. These were collected in two opaque plastic bottles of 300 ml per season with a chlorine freewater.

The collected samples were transported at 4°C, except those needed for the analysis of extracellular enzymatic activity that were kept at room temperature. The samples were not refrigerated because they were immediately carried to the laboratory located near the river. In the study area, the ambient temperature and water are approximately equal due the tropical conditions. The measurement of the extracellular enzyme activity was made at room temperature in the laboratory and this is similar to the temperature of the river. Table 1 presents the chemical variables, materials and methods used for their determination.

TABLE 1 CHEMICAL VARIABLES ANALYZED IN THIS STUDY

Variable	Unit	Material	Method
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Nitrates	mg/L NO ₃ ⁻	Potentiometer – Ion-Selective Electrodes	4,500-NO ₃ ⁻ -D
Ammonia Nitrogen	mg/L NH ₃	Titrimetric – Preliminary Distillation	4,500-NH ₃ -C
Orthophosphates	mg/L PO ₄ ⁻³	Colorimetric - Ascorbic Acid	4,500-P-E
Dissolved Organic Carbon (DOC)	mg C/L		SM 2,310B; Ed. 2005

C. Experimental Procedure

The determination of extracellular enzymatic activity was performed by the spectrophotometric method because the river has high levels of organic pollution and therefore high activity of microbiota.

In the determination of extracellular enzymatic activity the following equipment was used: spectrophotometer, water bath with magnetic stirrer plate and centrifuge (Memmert and Hettich respectively). NaCl (0.14 mol/L), Na₂CO₃ (1 mol/L), 4-Nitrophenyl-phosphatase (C₆H₄NNa₂O₆P₆H₂O) at 98%, 4-Nitrophenyl-β-D-glucopyranose (C₁₂H₁₅NO₈) at 99% and 4- Nitrophenol (O₂NC₆H₄OH) solutions were used.

To read the enzymatic activity in the spectrophotometer, a calibration curve was constructed from the stock solution of 4-Nitrophenol (O₂NC₆H₄OH). The dilutions of the stock solution were made from a mixture of 2 parts of sodium chloride solution and 1 part of sodium carbonate solution. The trend line fitted the linear equation: $y=16460x+0.0104$ con R²=0.9979.

Analysis of the Extracellular Enzymatic Activity

Measurement of the extracellular enzymatic activity was based on the methodology proposed by Marxsen et al., [18], which consists of a test sample coming from the Aburrá-Medellin river, in this case of water column and biofilm. To each sample a colorless substrate was added: 4-Nitrophenyl-β-D-glucopyranose and 4-Nitrophenylphosphate, to be hydrolyzed by β-glucosidase (EC 3.2.1.21) and alkaline phosphatase (EC 3.1.3.1) respectively. The procedure was developed as follows:

Blank preparation: mixing 2 ml of NaCl, 2 ml of substrate (β-glucosidase or alkaline phosphatase) and 2 ml of Na₂CO₃.

Determination of enzymatic activity: 5 ml of each steam sample (water column and biofilm) were stirred; these were dissolved in 50 ml of the sodium chloride solution, stirred again and 2 ml were taken and then mixed with 2 ml of substrate solution (β-Glucosidase alkaline phosphatase). Subsequently, the samples were incubated for 3 hours at 30°C in a water bath with stirring. After three hours, 2 ml of Na₂CO₃ were added to stop the hydrolysis reaction. Afterwards, the samples were centrifugated at 4,500 rpm during 10 minutes at room temperature. In order to measure the extracellular enzyme activity, a spectrophotometer at 405 nm against a blank was used. The liberated Nitrophenol in the hydrolysis presented a yellow coloration.

Estimate of the enzymatic extracellular activity

According Marxsen et al., 1998, the extracellular enzymatic activity as measured by the spectrophotometric method can be calculated using the following equation:

$$EEA_x = (Abs_x * D * F) / t$$

EEA_x: Extracellular enzymatic activity of the enzyme x (mol/h)

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

D: Dilution factor

F: Photometric factor given by the inverse of the slope calibration curve of 4 – nitrophenol (mol/l)

t: Time in hours

D. Statistical Analysis

In order to find relationships between physicochemical variables and enzymatic activities, Spearman product moment correlations were used (measure the strength of the linear relationship among the variables).

Additionally, in order to analyze the composition and the weight of the variables, and to determine the similarity of the samples (1, 2, 3, 4, 5, 6 and 7) according to the variable characteristics, we prepared sunray plots, each of which represents a sample. The study variables were: EEA Alkaline Phosphatase, EEA β-glucosidase, nitrates, ammonium, phosphate, DOC and chlorophyll a; it is emphasized that each edge represents a variable and the distance from this edge to the center of the polygon represents the importance within the sample as shown in Fig. 2.

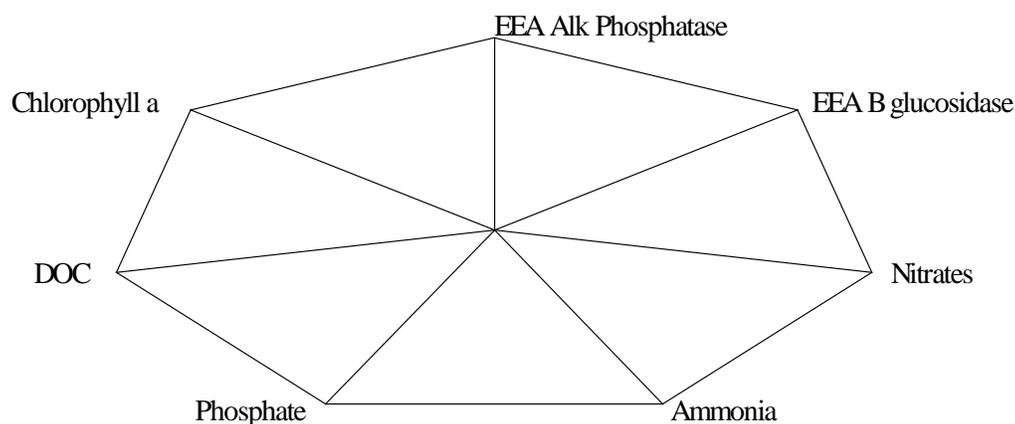


Fig. 2 Distribution of variables in a sunray plot

In order to observe graphical data box plot of the enzymes β -glucosidase and alkaline phosphatase in water and biofilm were drawn. This graph gives information about the central tendency, dispersion, and symmetry of the obtained data. In addition, it helps to clearly and individually identify observations that are far in an unusual way from the rest of the data, the atypical values.

Once the exploratory analysis was done, we carried out an analysis of the variance (ANOVA) of the components in order to establish which factor, sampling campaigns and matrix (water and biofilm) contributed most to the variability of enzymatic activities in Aburrá-Medellín River, "Estación Aula Ambiental". After determining the most influential factor, an analysis was carried out to determine whether there was a difference among the medians of the samples for each enzymatic activity of β -glucosidase and alkaline phosphatase in the matrices, evaluating the hypotheses required in the ANOVA. As the hypotheses were not accepted, a nonparametric Kruskal-Wallis test was performed. All statistical analyses were performed using Statgraphics Centurion XVI statistical program.

III. RESULTS

The prevailing weather conditions during sampling were wet, with rainfall of different intensity in every month. Table 2 shows the climatic values during the sampling months, obtained from the meteorological station 801100 (SKMD) located in the Olaya Herrera airport [19].

TABLE 2 WEATHER CONDITIONS DURING SAMPLING

SAMPLING	MONTH	AVERAGE TEMPERATURE (°C)	PRECIPITATION (MM/MONTH)	MISSING DATA
1	December 2010	21.9	184.15	2
2	February 2011	22.4	134.87	6
3	March 2011	22.4	112.24	3
4	April 2011	21.9	344.42	0
5	May 2011	23.4	50.29	14
6	September 2011	23.2	210.05	1
7	November 2011	21.9	197.61	2

NOTE: It is important to note that average temperature and monthly precipitation are presented based on available data, if any missing daily measurement, the average or total is from the days in which data was available.

The detail of temporal variation by extracellular-enzyme activity and physicochemical variables during the seven samplings campaigns are shown in Table 3.

TABLE 3 STATISTICAL RESULTS OF THE EXTRACELLULAR-ENZYME ACTIVITY AND OTHER PHYSICOCHEMICAL VARIABLES MONITORED DURING THE SEVEN SAMPLING CAMPAIGNS.

EEA Phosfatasa	EEA Glucosidase	Nitrates (mg/l)	Ammonia (mg/l)	Phosphate (mg/l)	DOC	Chlorophyll a
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	(mM/g)/h	(mM/g)/h	NO ₃	NH ₃	PO ₄	(mgC/l)	(µg/l)
Average	6.37	2.53	2.42	3.77	1.15	6.21	7.10
Median	5.23	0.94	2.18	3.77	1.12	6.44	6.58
Variance	21.90	16.61	3.98	0.41	0.19	1.56	4.34
Standard deviation	4.68	4.08	1.99	0.64	0.44	1.25	2.08
Coeff. of variation	0.74	1.61	0.82	0.17	0.38	0.20	0.29
Minimum	0.40	0.00	0.00	2.48	0.56	4.40	5.13
Maximum	23.79	15.24	5.44	4.48	1.72	7.70	11.46
Range	23.39	15.24	5.44	2.00	1.16	3.30	6.33
Std. kurtosis	7.28	5.14	-0.47	1.75	-0.63	-0.58	2.20

In accordance with the statistical results presented in the table shown above, only four variables present kurtosis values in a range from -2 to 2, therefore is possible to affirm that the nitrates, ammonia phosphate and DOC results, proceed from a normal distribution. Also, it is important to mention that the similarity between average and median values of physicochemical variables indicates that both are good to be used as a measure of central tendency. Based on the information provided in Table 3, there is higher Alkaline Phosphatase enzyme activity than β -glucosidase activity according to the mean and median. Additionally, based on the values found for the other variables.

Fig. 3 shows different sun ray plots generated with the weight each measured variable in water had during the seven samples. It can be noticed how the Alkaline Phosphatase activity was more significant during the first sampling, while glucosidase activity was more relevant in the third and sixth one. In the other samples, there was not a significant increase in the weight of the enzymatic activity.

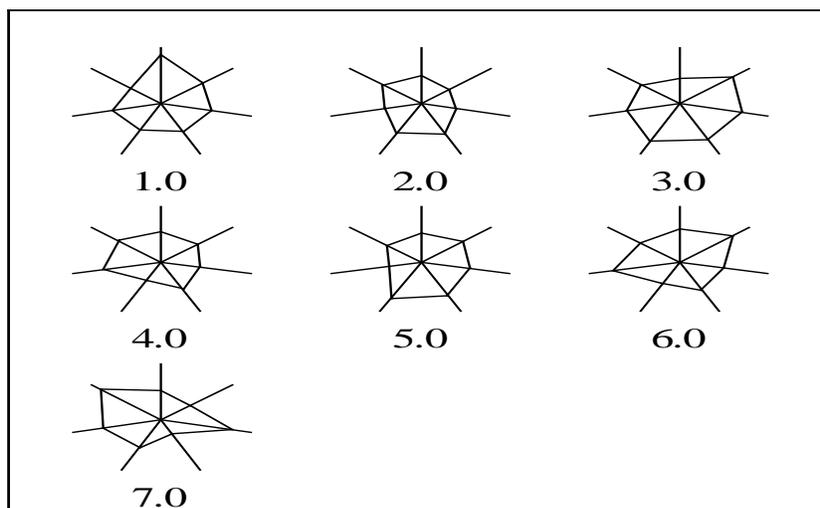


Fig. 3 Sun ray plots for the seven samplings preformed, following distribution of Fig. 2

Table 4 presents the p-values for Spearman's rank correlation between pairs of variables (physicochemical and EEA).

After reviewing the results of the Spearman correlations, between each pair of variables, it was found that there is no significant difference between correlations, with a trust level of 95.0%.

Fig. 4 presents a comparison between activities of both enzymes in box plots, which confirms a higher β -glucosidase activity in biofilm and similar activity of Alkaline Phosphatase in both matrixes. It is also noticed that Alkaline Phosphatase activity is higher in both matrixes, water and biofilm.

TABLE 4 SPEARMAN'S RANK CORRELATION *P VALUES <0,05

	EEA Alk Phosphatase $\mu\text{m/ml/h}$	EEA β glucosidase $\mu\text{m/ml/h}$	Nitrates mg/l de NO_3	Ammonia mg/l NH_4	Phosphate mg/l PO_4	DOC mg/l C	Chlorophyll a
EEA Alk Phosphatase $\mu\text{m/ml/h}$		0.840	0.825	0.182	0.052	0.453	0.566
EEA β glucosidase $\mu\text{m/ml/h}$	0.840		0.689	0.170	0.656	0.755	0.142
Nitrates mg l de NO_3	0.825	0.689		0.860	0.382	0.600	0.793
Ammonia mg l NH_4	0.182	0.170	0.860		0.058	0.133	0.122
Phosphate mg l PO_4	0.052	0.656	0.382	0.058		0.162	0.431
DOC mgC l	0.453	0.755	0.600	0.133	0.162		0.189
Chlorophyll a	0.566	0.142	0.793	0.122	0.431	0.189	

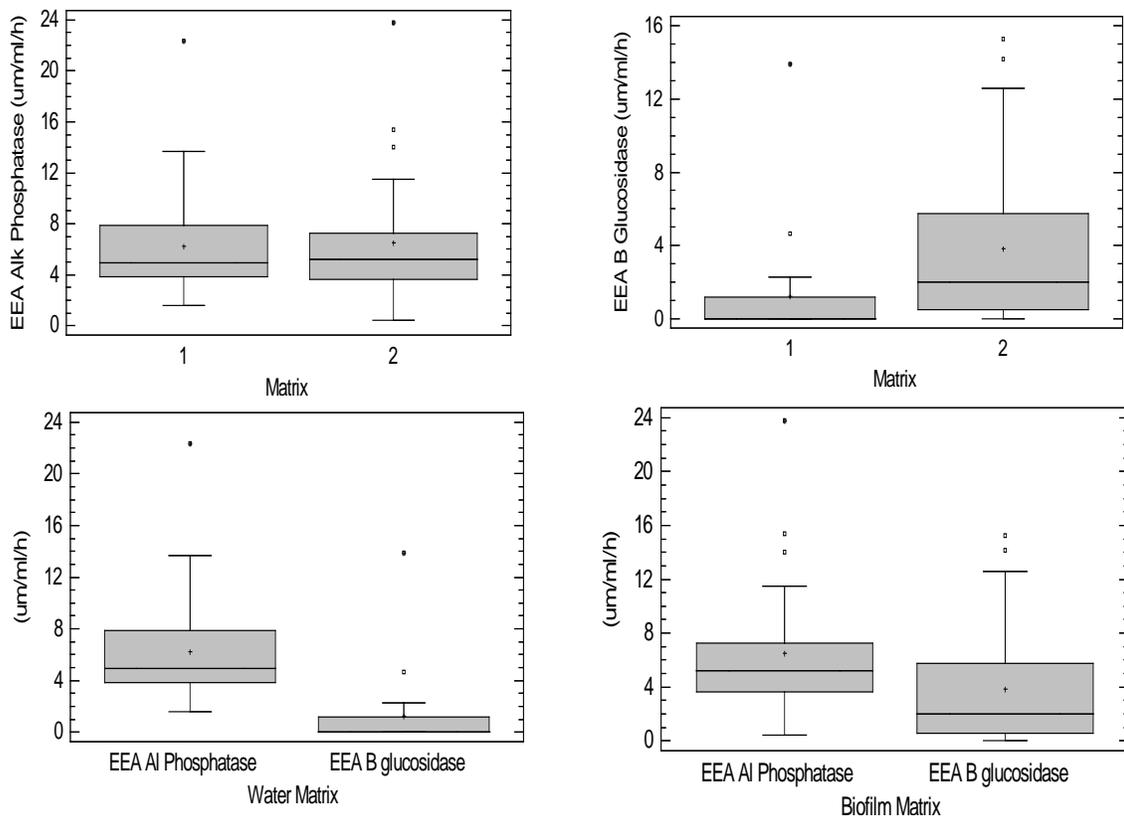


Fig. 4 Comparison between the activities of β -glucosidase and alkaline phosphatase in the matrices

Fig. 5 shows a significant Alkaline Phosphatase activity in biofilm from the sampling corresponding to: December (2010), february, march and april (2011), while in the other months it is highest in the water than in biofilm. On the other side, although the mean and median β glucosidase activity was slightly higher in the biofilm, this figure does not show a clear trend and it is only possible to state that it was highest in four samples (2, 5, 6 and 7).

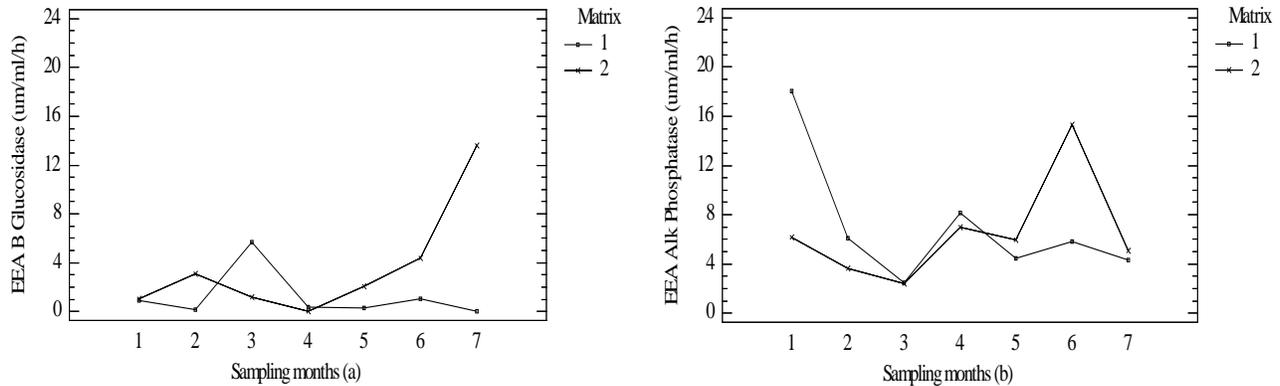


Fig. 5 Comparison of the activities of β -glucosidase (a) and alkaline phosphatase (b) among samples

In order to identify which of the factors: time (sampling month) or matrix (biofilm or water) has more influence on the variability of the data, a variance component analysis was performed for the variables associated to the extracellular enzymatic activity in relation with alkaline phosphatase and β -glucosidase enzymes.

TABLE 5 VARIANCE COMPONENTS ANALYSIS

Source	EEA alkaline phosphatase	EEA β -Glucosidase
Matrix	0	7.85
Sampling months	61.18	68.57
Repetition	0	0.97
Sample	38.82	22.61

The components variance analysis shows that the “Sampling months” factor generated the highest contribution to the variance of both activities. Hence, the Kruskal-Wallis test was applied considering each of the extracellular enzyme activities as dependent variables. This test established that the medians of the data belonging to each matrix, do not present a statistically significant difference regarding the enzyme activity associated with the β -glucosidase (p -value = 0.189), this means both activities are similar in water and in biofilm while for Alkaline Phosphatase enzyme activity, this difference is significant (p -value = 0.00074) and when the Fisher's least significant difference (LSD) method was applied, two homogeneous groups were identified: (February, March, April, May, September) and (November, December 2010).

After comparing both enzymatic activities in the water matrix, a statistical significant difference between the medians with a trust level of 95.0% was found, because the p -value was 6.74674E-8. Similar results were found after comparing the biofilm matrix ($P = 0.005$).

IV. DISCUSSION

The maximum value in the Alkaline Phosphatase enzyme activity was presented in the water matrix during the first sampling campaign (December 2010), while the highest β -glucosidase occurred in the biofilm matrix during the seventh sampling campaign (November 2011). Both samples were characterized by monthly rainfalls that exceeded 184 mm. On the other hand, the Alkaline Phosphatase activity in the biofilm matrix, showed a similar behavior in the rainiest months as shown in Fig. 3. This apparent relationship may be due to rock washed generated by the increase in water levels in the river, and hence there was no longer much phosphorus available and there was a slight increase in the extracellular activity in order to obtain this nutrient. There was also a relocation of substrates, which provided new epilithic surfaces for the colonization of bacteria, algae and biofilm formation [20].

This result is consistent with the one found by Romani and Sabater [5]; they stated that the extracellular-enzyme activities are related to the availability of readily useful organic matter. Increased availability lowered the activity cited by Cunha et al. 2010, who claimed that enzymes are only released once the concentration of readily useful organic matter in the water falls below a critical level.

According to the results of enzymatic activity and the information reported of the weather conditions, a correlation between them was observed, similar to the data obtained by Caruso et al. [21] and Sinsabaugh and Follstad's [22].

In the sun ray plots, similar diagrams were observed in the fourth and sixth sampling campaigns (with more weight in the variable DOC) corresponding to the months of higher rainfall, indicating that variables together presented a similar behavior in the

river, when climatic conditions are alike. Comparable results were found in three phases of the RedRío project at the same site and climatic condition variation [9, 11, 12].

In this same graph, it was observed that the Alkaline Phosphatase activity was more significant in the first sampling campaign, while the β -glucosidase was more relevant in the third one, dates on which water quality - according to the measured parameters - was similar but with extreme values of these activities in the water, and hence the samples taken do not show a relationship of the enzymatic activity with the other measured parameters. Pohlen et al. [23] found that enzymatic activities depend on the ratio of biomass bacteria/algae and this is also related to the water temperature, giving little weight to the DOC. It should be noted that in the case of Aburrá-Medellin River "Aula Ambiental," average water temperature is 21.5°C with little variation of temperature above measured in the study of reference [9]; likewise, the DOC in the river was higher than those obtained in the other study.

It is also important to note that from the present study, no difference between the enzymatic activity of biofilm and water at the "Estación Aula Ambiental," was found. This is probably because nutrient limitations in the biofilm lead the organisms to use nutrients from the water column [24], thus decreasing their downstream availability. This can result in maintaining a balance of activities between both matrices.

Alkaline Phosphatase activity in both matrices was higher than the β -glucosidase, as observed from the box plots. The Kruskal-Wallis test shows that there are significant differences between enzymatic activities, due to the availability of both nutrients in the river, for example, river water is more enriched with carbon sources than with phosphorus and this is evidenced by the results of DOC and phosphates, due to the continuous water deposition into the river (discharge or tributaries streams to the water). Higher amount of carbon in relation to phosphorus is typical of the rivers of Colombia [25]. According to Hill B. et al. [26], the enzymatic activity is influenced by the anthropogenic disturbances, because it generates an imbalance in the nutrients being transported from the river.

Therefore, the available group of organisms such as bacteria, some algae, fungi and protozoa, should produce the necessary enzymes to obtain the required phosphorus, i.e. to breakdown complex organic matter into simple compounds and to use them as a source of nutrients.

According to the result of the chemical parameters measured and compared with those obtained in the samples conducted in September, when the weather conditions were rainy (210.05 mm/month), there was a dilution of nutrients and the biofilm adhered to the rocks was washed by the increased flow, caused by precipitation. This fact suggests that the enzymatic activity increases when the available organic matter decreases [4]. In the case of the month of April, there were maximum precipitations, and hence the substrata were washed, this condition, together with a decrease of organisms (detachment and/or scraping biofilm generated by the increase of suspended solids during rainfall), led to low extracellular enzyme activity.

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REFERENCES

- [1] M. Licursi and N. Gómez, "Application of biotic indices in the assessment of water quality in river systems of the Pampas Argentina from the use of diatoms," *Aquatic Biology*, vol. 21, pp. 31-49, 2003. (In Spanish).
- [2] S. Alvarez, "The decomposition of organic matter in wetlands: the importance of the microbial component," *Ecosystems: Scientific and Technical Review of Ecology and Environment*, vol. 14 (2), p. 17-29, 2005. (In Spanish).
- [3] A. Elozegi and S. Sabater, "Concepts and Techniques in Ecology Fluvial," Rubes Editorial, Spain, 2009. (In Spanish).
- [4] A. Cunha, A. Almeida, F. J. R. C. Coelho, N. C. M. Gomes, V. Oliveira and L. Santos, *Bacterial Extracellular Enzymatic Activity in Globally Changing Aquatic Ecosystems, Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, A Mendez-Vilas, 2010.
- [5] A. M. Romani and S. Sabater, "Variability of heterotrophic activity in mediterranean stream biofilms: a multivariate analysis of physical-chemical and biological factors," *Aquat. Sci.*, vol. 62, pp. 205-215, 2000.
- [6] R. Chrost, "Characterization and significance of β -Glucosidase activity in lake water," *Limnol. Oceanogr.*, vol. 34(4), pp. 660-672, 1989.
- [7] J. Millan, "Alkaline phosphatases structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes," *Purinergic Signalling*, vol. 2, pp. 335-341, 2006.
- [8] J. Nedoma, J. García, M. Comerma, K. Simek and J. Armengol, "Extracellular phosphatases in a Mediterranean reservoir: seasonal, spatial and kinetic heterogeneity," *Freshwater Biology*, vol. 51, pp. 1264-1276. 2006.
- [9] Metropolitan Area of the Valley of Aburrá, Universidad de Antioquia, National University of Colombia (Medellín Campus), Universidad Pontificia Bolivariana and Universidad de Medellín, Environmental Monitoring Network in the watershed of the Aburrá-Medellín river in the jurisdiction of the Metropolitan Area Phase-III, Medellín, 2011. (In Spanish).

- [10] Empresas Públicas de Medellín, *Revista Empresas Públicas de Medellín*, Vol. 03. Nos. 3 – 4 Jul./Dic. Medellín, Colombia, 1981.
- [11] Metropolitan Area of the Valley of Aburrá, Universidad de Antioquia, National University of Colombia (Medellín Campus), Universidad Pontificia Bolivariana and Universidad de Medellín. Environmental Monitoring Network in the watershed of the Aburrá-Medellín river in the jurisdiction of the Metropolitan Area Phase-I, Medellín, 2005. (In Spanish).
- [12] Metropolitan Area of the Valley of Aburrá, Universidad de Antioquia, National University of Colombia (Medellín Campus), Universidad Pontificia Bolivariana and Universidad de Medellín. Environmental Monitoring Network in the Watershed of the Aburrá-Medellín River in the Jurisdiction of the Metropolitan Area Phase-II, Medellín, 2007-a. (In Spanish).
- [13] Mi Rio Institute, *The Medellín River, graphic story*, Editorial Colina, Medellín, 1997. (In Spanish).
- [14] L. Giraldo, R. Agudelo and C. Palacio, Spatial and temporal variation of nitrogen in the Medellín river, *Revista DYNA Universidad Nacional de Colombia*, Medellín, Dyna, Año 76, Nro. 163, pp. 124-131, 2010.
- [15] Metropolitan Area of the Valley of Aburrá, CORANTIOQUIA, CORNARE and National University of Colombia (Medellín Campus), *Management plan and Management of Aburrá River Watershed*, Medellín, 2007-b. (In Spanish).
- [16] IDEAM, *Guide for Monitoring Discharges, Surface Water and Groundwater*, 2005. (In Spanish).
- [17] Tümppling Wolf von and Günther Friedrich, *Biological Water Analysis*, 1999. (In German)
- [18] Marxsen –Jürgen, *Microbiological Characterization of Aquatic Sediment, Enzyme Activity*, pp. 87-111, 1998. (In German)
- [19] Weather Station 801100 (SKMD) located in the Olaya Herrera Airport (Online). http://www.tutiempo.net/clima/Medellin_Olaya_Herrera/801100.htm- Accessed: 10/01/2012.
- [20] L. Giraldo, “Dynamic modeling of the fluvial system of Aburra river in k5-k44 section, through the study of the chemical quality of the water, epilithic algae, macroinvertebrates and enzyme activity,” PhD. Thesis (unpublished work), Universidad de Antioquia, Medellín, Colombia, 2013. (In Spanish)
- [21] G. Caruso, F. Azzaro, M. Azzaro, F. Decembrini, R. La Ferla, G. Maimone, F. De Pasquale, L. S. Monticelli, R. Zaccone, G. Zappalà and M. Leonardi, “Environmental variability in a transitional Mediterranean system (Oliveri-Tindari, Italy): focusing on the response of microbial activities and prokaryotic abundance,” *Estuarine, Coastal and Shelf Science*, vol. 135, pp. 158-170, 2013.
- [22] R. L. Sinsabaugh and J. J. Follstad Shah, “Integrating resource utilization and temperature in metabolic scaling of riverine bacterial production,” *Ecology*, vol. 91(5), pp. 1455-1465, 2010.
- [23] E. Pohlen, J. Marxsen and K. Kirsten Küsel, *Pioneering Bacterial and Algal Communities and Potential Extracellular Enzymeactivities of Stream Biofilms*, 2009.
- [24] A. M. Romani and S. Sabater, “Influence of algal biomass on extracellular enzyme activity,” *Aquatic Sciences*, vol. 62, pp. 205-215, 2000.
- [25] G. Roldan and J. Ramírez, *Fundamentals of neotropical limnology*, 2nd Ed., 2010. (In Spanish).
- [26] B. H. Hill, C. M. Elonen, L. R. Seifert, A. A. May and E. Tarquinio, “Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers,” *Ecological Indicators*, vol. 18, pp. 540-551, 2012.