

Epiphyton Colonization on Artificial Substrates in the Aburrá-Medellin River, Colombia

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Abstract- This work aims at determining the effect of wastewater input and hydrologic regimes on the epilithic algal colonization over artificial substrates in the Aburrá-Medellin River. For this purpose, at eight stations in the river were installed 14 polypropylene baskets with 2000 grams of gravel as substrates in order to know the algae response to changes in water quality (reflected in changes in biological indices and also affected by fluctuations in water levels; i.e., flow rates, and therefore changes in its composition according to the hydrological cycle).

Alterations generated in the composition and abundance of algae by wastewater discharges and change in water levels did not produce a pattern of colonization. Based on the above, it allowed to conclude that algae are sensitive organisms to particular events that affect water quality in the Aburrá-Medellín River.

Keywords- Epiphyton; Colonization; Algae; Water Quality of Aburrá-Medellín River

I. INTRODUCTION

Pollution and direct alteration of their banks, slopes and even watersheds, have been the main reason in many degraded river systems. Any alteration produces responses in biological communities and these responses can help to understand the degree of degradation of the system, thus, the biological monitoring could be used as a useful tool for assessment of river pollution [1].

Algae are aquatic microscopic organisms, sensitive to changes in their environment. These algae's behaviors are the main indicator of the ecological status of a water system. Among algae, the taxonomic groups (used in many environmental assessment studies) are diatoms. Due to its diversity, its large number of species, and especially because it's owned by colonization of any immersed substrate [2].

Periphytic algae studies in Colombia have been developed in dams, lakes and rivers [3-6]. Studies in rivers have been addressed mainly to water quality and environmental impact evaluation based on scrapings on natural substrates at different sampling stations, where the upper part of the river is used as a standard station or control point [6]. In this study, the headwaters of the river were used as a reference for evaluating the deterioration of water quality for the Aburrá-Medellín River.

Research question: The objectives of this research are to investigate the effect of wastewater input and flow on the colonization of epilithic algae assemblages in the Aburrá-Medellin River.

II. MATERIALS AND METHODS

A. Subject of Study

The Aburrá Valley is located in the south-central province of Antioquia in Colombia, amid the Central Andes. Medellín is a city ranked second in terms of population and importance in the country, where nine other municipalities are located there (more than three million inhabitants together). The valley has a large river that has become a hub for the historical development of the region [7].

The water resources of the main channel of the basin are continuously exposed to human contamination exerted on it. The discharges of domestic and industrial wastewater untreated, exploitation of materials in the channels and the slopes of the river and creeks, disposal of solid waste in the beds of streams, poor land use and deforestation around birth of streams that produce soil degradation by erosion and sedimentation processes.

In this research, eight sampling stations were located properly in order to permit to understand the behavior of the river profile, in some specific points. Fig. 1 shows the locations and the general characteristics and Table 1 presents the sampling stations.

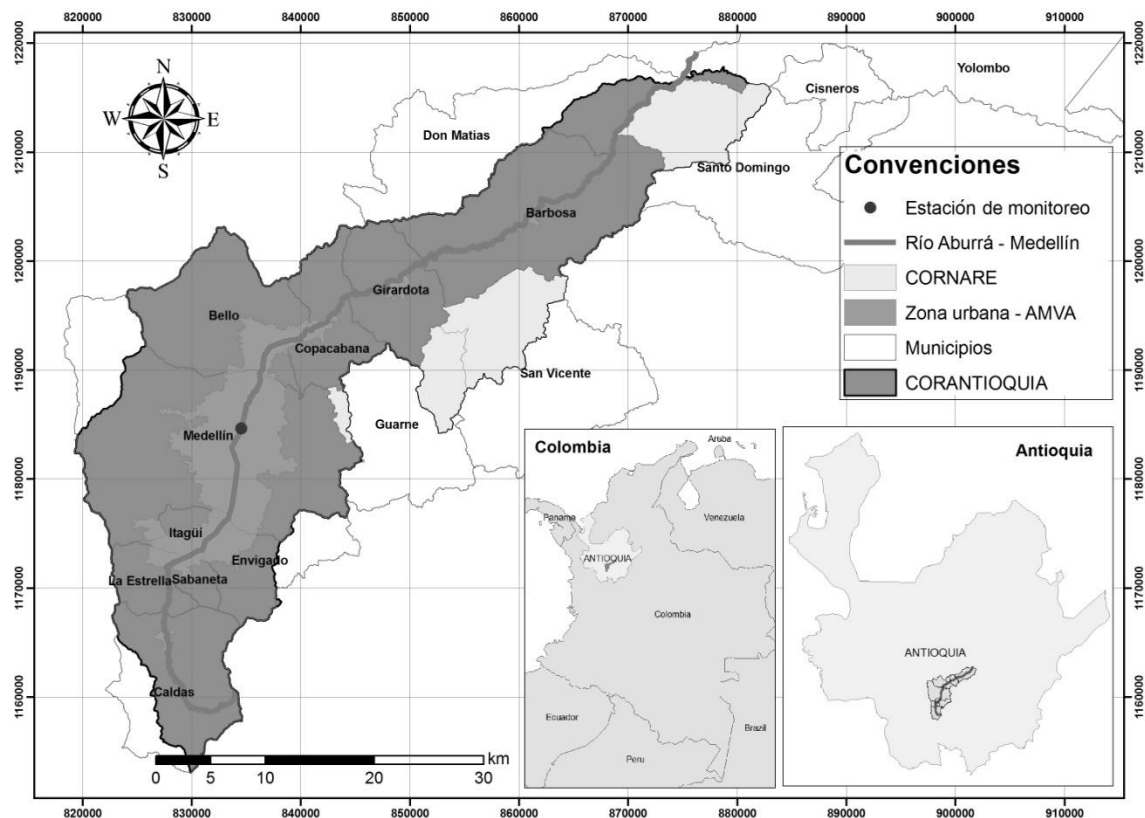


Fig. 1 Geographical location of the study area (Modified from [8])

TABLE 1 SAMPLING STATIONS

Station	Coordinates		Distance from the source	Distance from San Miguel station	Town
	Latitude	Longitude	Km	Km	
Alto San Miguel (SM)	6°02'50.4''	75°37'09.9''	5.8	0.0	Caldas
Antes quebrada La Valeria (AV)	6°5'46.6''	75°38'08.7''	14.3	8.5	Caldas
Después Quebrada La Valeria (DV)	6°5'46.6''	75°38'08.7''	14.4	8.6	Caldas
Ancón Sur without channeling (SAWC)	6°09'07.8''	75°37'54.9''	21	15.2	La Estrella
Ancón Sur Canalized (SAC)	6°09'07.8''	75°37'54.9''	21.2	15.4	La Estrella
Antes de San Fernando (ASF)	6°11'26.78''	75°34'79.15''	28.5	22.7	Medellín
Después de San Fernando (DSF)	6°11'43.5''	75°34'53.3''	28.6	22.8	Medellín
Aula Ambiental (AA)	6°15'51.8''	75°34'20.4''	37.1	31.3	Medellín

B. Sampling Design and Procedures

In the experimental work, artificial substrates containing 2000 grams of crushed-gravel were placed in polypropylene plastic baskets at the monitoreo stations. In Figs. 2 and 3, assemblage and sample designs that were used on the 14 crates games are showed. The basket was tied by cables and shackles to the vegetation of the river banks in order to ensure their stay in the station during rising waters of the river level. Table 2 shows the analyzed variables and the measurement frequency.

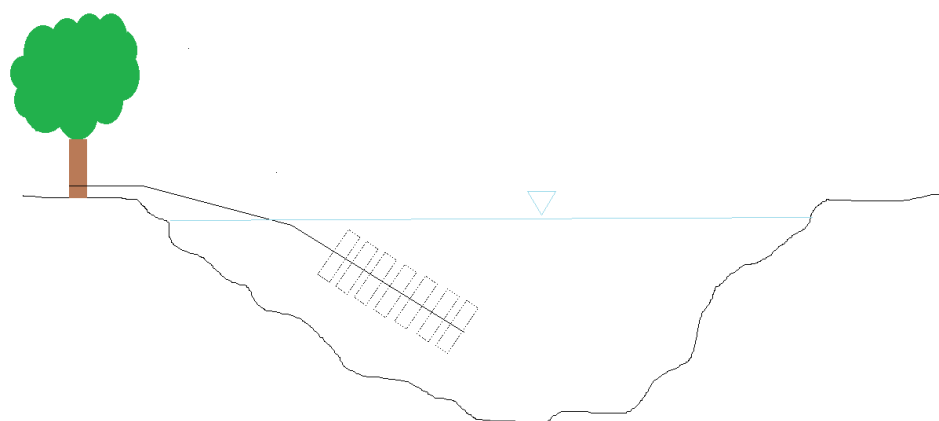


Fig. 2 Basketsin assembling on monitoring station at the river

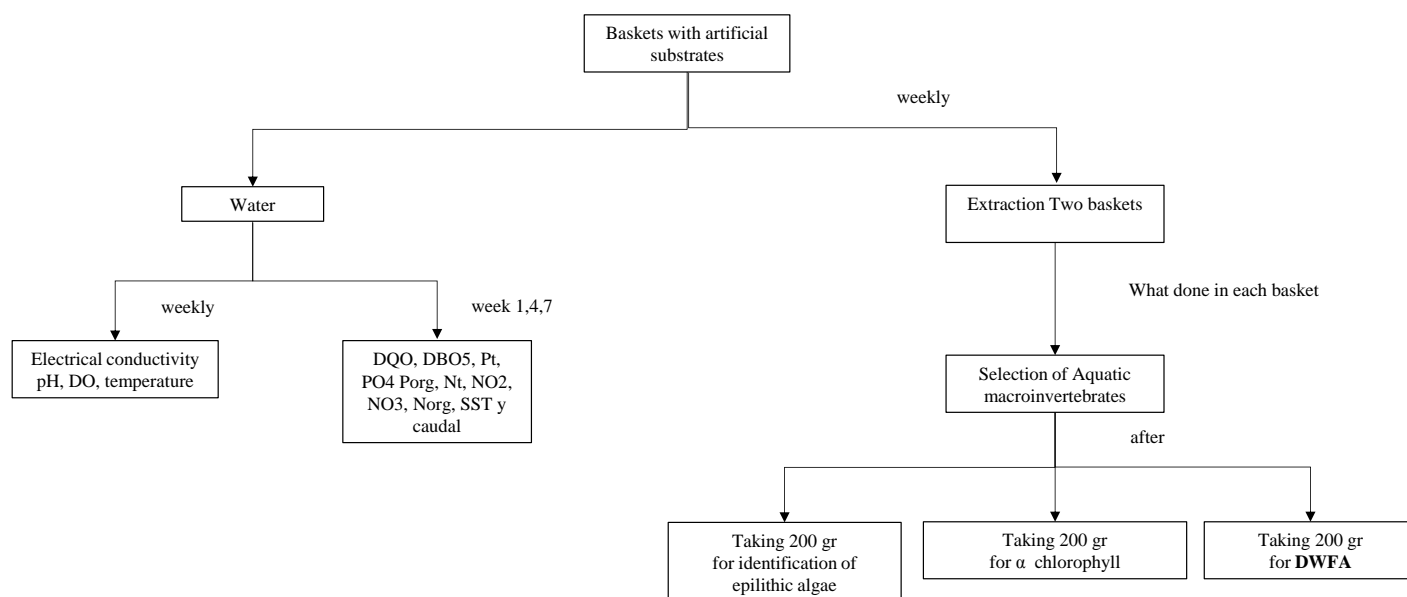


Fig. 3 Schematic of experimental design

TABLE 2 VARIABLES ANALYZED IN THIS STUDY

Frequency	Evaluated parameters	Measurement
weekly	pH, water temperature, electric conductivity, dissolved oxygen in field, dry weight free of ash and α chlorophyll.	pH, water temperature, electric conductivity, dissolved oxygen a multi-parameter equipment was used HACH model HQ40D.
weekly 1, 4 and 7	Flow, speed and a sample of water was carried to laboratory for determining the following parameters: DBO5, DQO, nitrogen total-TNK, nitrites-NO ₂ , nitrates-NO ₃ , total phosphorus -TP, organic phosphorus –org P, phosphates -PO ₄ , total suspended solids -SST (evaluated on week1, 4 and7)	The analytic methods were based on the Standard Methods for the examination of water and wastewater. [9]

1) Dry Weight Free of Ash (DWFA)

The dry weight (in mg/cm³), was used as an estimate of the biomass from the organic components (autotrophic and heterotrophic), and inorganic periphytic community [10]. Gravel sample was weighed along with adhering periphyton and dried in an oven at 105 °C. Finally, the samples were dried to 505 °C in an oven. Dry weight free of ash (DWFA) was calculated as the difference between these values and the dry weight [11].

2) Chlorophyll α

The extraction B protocol according to an international standard (ISO 10260) was used to analyze chlorophyll α ; which includes the use of glass fiber filter of 0.45 μ m diameter pore. As extraction solvent 90% ethanol (10ml) was used (to facilitate this extraction to macerate the filter while adding the solvent, heating to 75 °C for 5 minutes and immediately equilibrium at room temperature). Subsequently, it was centrifuged at 3000 rpm in order to obtain a clear supernatant. Finally, the step of

measuring absorbance at 665 and 750 nm without acidifying and after acidified with HCl (3M) (the absorbance were estimated in a spectrophotometer SECOMAM Uvi Ligh xs2 and analysis program Lab Power Junior). The final concentrations of chlorophyll α periphytic (μg of pigment/ cm^2) were calculated using the equation proposed by Marker et al. [12].

$$Chla \left(\frac{\mu\text{g}}{\text{cm}^2} \right) = \left\{ (DO_{665} - DO_{750}) - (DO_{665(a)} - DO_{750(a)}) \right\} * 2,43 * 10,48 * V_e * \frac{\text{Bucket width}}{\text{area}}$$

Where:

V_e : volume of extract (ml)

Area: Surface area (cm^2)

Bucket Width: 1cm

a: acidified

DO_{665} : absorbance sample at 665 nm

DO_{750} : absorbance sample at 750 nm

3) Determination of Epilithic Algae

200 grams of crushed material from each basket were collected to obtain the samples of epilithic algae. The adhering material to the substrate was scraped softly with a brush removing attached algae. Subsequently, the brush was washed with 100 ml of distilled water contained in a plastic amber bottle of 120 ml. The collected samples were added 10 ml of 10% lugol not resublimed, and finally stored under refrigeration at $\pm 4^\circ\text{C}$ [13].

Before the observation of each samples, it was precipitated for 72 hours in 40 ml volume. Subsequently, it was gently homogenized for one minute (using a magnetic stirrer) and a drop of sample (0,05ml) was taken to an optical microscope.

Thirty fields of observation were evaluated per sample following a sinusoidal trajectory. An asymptote behavior was observed in the accumulation curve (based on the criteria established by Uehlinger [14]). Observations were made with a total magnification of 400X and to quantify, the density per milliliter. The Ros's expression was applied [15]:

$$\text{organisms per milliliter} = \frac{(n * F)}{(s * c)}$$

Where:

n: number of organisms counted

s: surface or area of the visual field of the microscope mm^2

c: number of fields counted

F: conversion factor = 50

The number of organisms per milliliter calculated by the above expression is associated with the final volume of the sample as follows:

$$\text{number of de organisms} = \left(\frac{\text{org}}{\text{ml}} \right) * V_f(\text{ml})$$

And this in turn is related to the sampling area in question, in order to obtain the density by converting:

$$\text{Density} = \frac{\text{Organisms}}{\text{Sampled area}(\text{cm}^2)}$$

Taxonomic determination was made to genus, based on different studies [16-20]. Results were pooled using three classifications: division, physiognomic group and index.

Division: Bacillariophyta, Chlorophyta, Cyanophyta and Euglenophyta divisions were considered.

Physiognomic groups: After determining the architecture of the periphytic community, the epilithic algae taxa recorded were grouped into five categories (taking into account the appearance and growth habit, which directly affects reproductive rates and assimilation of resources of the organisms): prostrate, erect, filamentous, forming chains and free-living algae.

Indices evaluated: Abundance, Diversity, Dominance, Equity and richness.

C. Processing and Analysis of Information, Redundancy Analysis (RDA)

In order to determine the length of the gradient matrix of biological and physicochemical parameters a Detrended Canonical Correspondence Analysis (DCCA) was used to choose the application of a linear or unimodal method [21]. The result found allowed apply the linear method corresponding to a Redundancy Analysis (RDA), in which by manual selection, significant physicochemical variables were chosen (Monte Carlo test with 499 permutations, $\alpha < 0.05$) [22] and [23]. Both matrix physicochemical and biological variables were standardized (x-min/max-min) and transformed to log (x+1). This analysis was performed in CANOCO4.5 for windows program.

III. RESULTS

Table 3 shows the average daily precipitation and weather conditions during the execution of each test day. It can be seen that the first test corresponds to drier conditions, while the second corresponds to wet conditions and the third test corresponds to transition (dry to wet or wet to dry). These data were obtained from the meteorological station 801100 (SKMD) located in the Olaya Herrera airport [24].

TABLE 3 WEATHER CONDITIONS

Test	Day	Month	Average temperature (°C)	Precipitation (mm/month)	Average of Precipitation
1	18	3	22.6	0	4.28
1	26	3	25.8	2.03	
1	1	4	24.8	1.02	
1	8	4	23.4	10.92	
1	15	4	25	0	
1	29	4	22.6	9.91	
1	6	5	22.6	4.06	
2	19	8	21.4	2.03	7.59
2	26	8	20.7	9.91	
2	2		24.6	0	
2	9	9	23.6	14.99	
2	16	9	22.7	0	
2	23	9	21.4	2.03	
2	30	9	22.4	7.87	
2	7	10	23.1	0	4.92
3	20	1	23.4	0	
3	27	1	21.7	35.05	
3	3	2	25.3	0	
3	10	2	21.9	-	
3	17	2	22.1	0.76	
3	24	2	20.7	27.94	
3	3	3	21.9	0	
3	10	3	21.1	14.99	

NOTE: It is important to note that average temperature and monthly precipitation are presented based on available data (the average or total is from the days in which data was available).

1) Variables Measured Weekly

The physicochemical variables: electrical conductivity, dissolved oxygen, pH, temperature, DWFA and chlorophyll α were measured weekly. Using the results, the physical chemistry matrix was structured to explain the biological matrix algae (division, physiognomic group, indices).

Redundancy Analysis results showed total variance percentages explained ranging from 9.4 % (algae indices) to 19.8 % (division algae) ($\alpha = 0.002$, Table 4) for all combinations. It suggests the existence of correlation between biological variables and physicochemical measures weekly. The pH and chlorophyll α variables were less significant in the different groups formed, while electrical conductivity, dissolved oxygen and DWFA variables generally obtained the highest proportion of the total variance.

TABLE 4 SIGNIFICANCE AND EXPLAINED VARIANCE IN THE COMBINATIONS FROM BIOLOGICAL AND PHYSICAL-CHEMICAL VARIABLES MEASURED WEEKLY

Group	Grouping	Test of significance from all canonical axis	Value F	Value p	Explained Variance (%)	Significant Variables
Algae	Division	0.198	10.07	0.002	19.8	Electrical conductivity, OD DWFA, pH
Algae	Physiognomic groups	0.148	5.367	0.002	14.8	Temperature, Electrical conductivity, OD DWFA, pH
Algae	Index	0.094	4.216	0.002	9.4	Temperature, Electrical conductivity, OD DWFA

Fig. 4 shows the graphical results of the Redundancy Analysis with groups of algae per division and the physicochemical variables for testing and sampling stations. It can be seen that the data from the low-water period (test 1) were located in quadrant II, which was associated with the presence of Bacillariophyta and Chlorophyta algae, where physicochemical variables measured had little influence on the groups per divisions.

In triplot rated stations, a clear formation of two groups is observed: San Miguel and After San Fernando. In the last station, the electrical conductivity variable is related to the presence of algae where Cyanophyta division dominates followed by Euglenophyta.

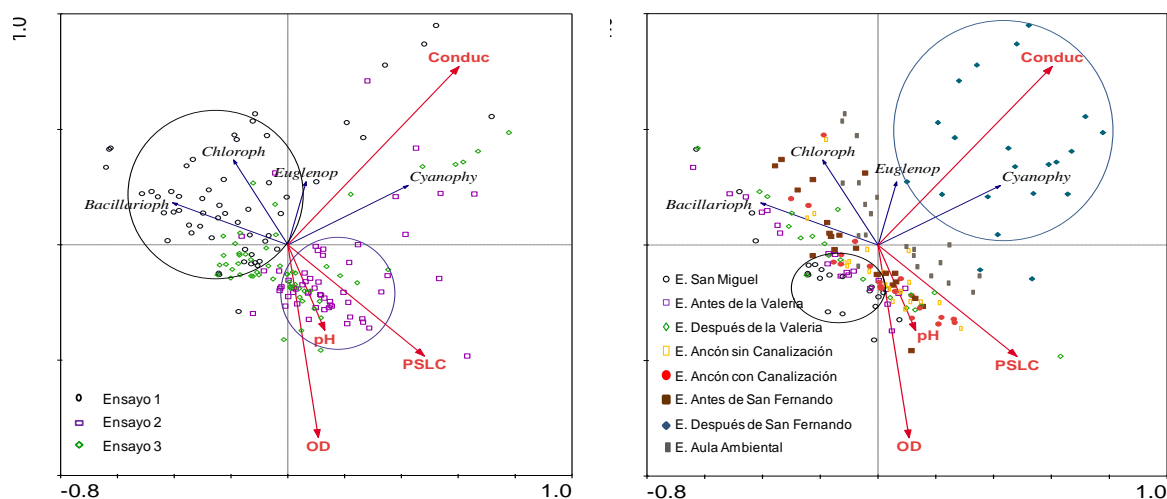


Fig. 4 triplot Redundancy Analysis between algae (division) - physicochemical variables measured weekly

2) Variables Measured Every Twenty Days

Redundancy Analysis results showed percentages of total variance explained, ranging from 14.1% (algae indices) to 25.1% (algae division and physicochemical parameters) ($\alpha=0.002$ Table 5). It suggests, for all combinations, the existence of correlation between the biological and physicochemical variables measured at a frequency of twenty days. Only ten of the eighteen variables measured were considered significant in the different groups formed, from which the most important, in its order, were: Electrical conductivity, DO, DWFA, org P and flow, they had the highest proportion of the total variance explained.

TABLE 5 SIGNIFICANCE AND VARIANCE EXPLAINED IN THE COMBINATIONS OF BIOLOGICAL AND PHYSICOCHEMICAL VARIABLES MEASURES EVERY TWENTY DAYS

Group	Grouping	Test of significance of all canonic axis	Valor F	Valor p	Variance explained (%)	Significant Variables
Algae	Division	0.251	7.605	0.002	25.1	Electrical conductivity, flow, DO
Algae	physiognomic group	0.194	3.959	0.002	19.4	DQO, DO, electrical conductivity, PO4
Algae	Index	0.141	11.515	0.002	14.1	Flow

Fig. 5 shows the graphical results of the Redundancy Analysis with groups of algae per division, and the physicochemical variables for testing and monitoring stations. It can be seen that for the low-water period (test 1), data were located in quadrant II, which was associated with the presence of Chlorophyta and Bacillariophyta algae, where physical-chemical variables measured had little weight. A direct relationship is also observed between the divisions Cyanophyta and Euglenophyta with the electrical conductivity in the first quadrant and in the fourth quadrant absence of divisions and a high weight of the flow vector.

In triplot rated stations, a parallel group conformation is seen to the flow vector and stands out at the After San Fernando station, the electrical conductivity variable determines the presence of algae where prevails divisions Cyanophyta followed by Euglenophyta.

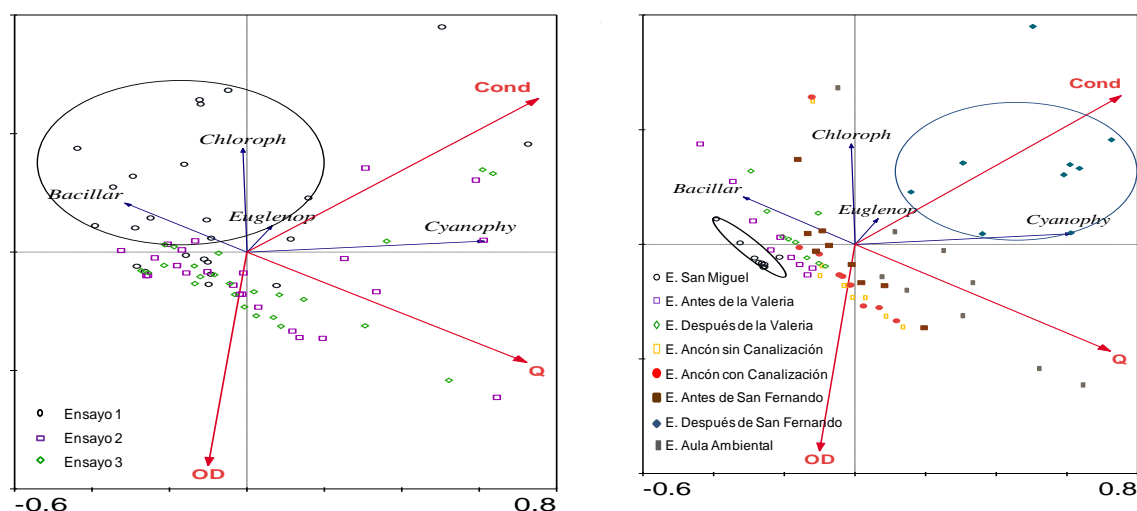


Fig. 5 Redundancy Analysis triplot between algae (division) - physicochemical variables measured every twenty days

IV. DISCUSSION

1) Variables Measured Weekly

According to the results presented in Table 4, the obtained percentages of explained variance were relatively low (19.8% algae division). However, the results were significant since only six physicochemical variables to explain the behavior of the epilithic in the river were considered, i.e. the limited number of variables that were considered were not able to explained (with high resolution) such a rich and complex biological matrix [25]. This suggests that there are probably other unmeasured weekly variables that affect algae. However, in all combinations a $p = 0.002$ value was obtained, indicating statistically significant associations between physicochemical and biological variables.

A separation between sampling periods was observed in all triplot figures. The test one contained the more grouped data, and the other two formed groups share their areas corresponding to high and middle water level. During times of low levels, the division Bacillariophyta had the most important influence, that is, their presence was common in this water level, followed by Chlorophyta algae. Opposed results in regard to the prevailing division into low water levels were found in the River Plate (Argentina), where the Bacillariophyta was the dominant division [26], during spring, winter and autumn.

The physiognomic groups and index of algae vector also played an important role during this period in the different classifications. It can be highlighted that only in few opportunities physicochemical variables showed representative in this water level, only electrical conductivity and temperature were outstanding.

As the water level rises, the division Cyanophyta began to gain importance without becoming the most representative of a group, and in high water levels, dissolved oxygen and DWFA variables influenced more strongly, while less important the algae expressed as divisions, physiognomic group and index.

The classification per sampling stations showed that the data from the San Miguel station were more concentrated with no apparent predominance of some division, and according to the results of the indices, it was the station with more richness and equity algae. Station data After San Fernando were the most dispersed and distanced from the other stations, with importance of electrical conductivity variable and dominance of Cyanophyta algae followed by Euglenophyta (similar results regarding the increase of these divisions after a wastewater discharge are found in [27]). This station was correlated with the physiognomic group of erect algae alike, which is attributed to the entry of treated wastewater from the water treatment plant to triple the electrical conductivity in the water body.

2) Variables Measured Every Twenty Days

Higher percentages of explained variance (maximum 25.1%) were obtained since it was considered a greater number of physicochemical variables, which stand out as the most significant: electrical conductivity, dissolved oxygen, DWFA, org P and flow. Among them, the variable that had the greatest influence was electrical conductivity, which to perform grouping per stations, it was characterized to be mainly associated with the After San Fernando station that has the highest electrical conductivity values (average 673 $\mu\text{S}/\text{cm}^2$) due to entries from treated sewage plant.

In all triplot graphics, a spacing between sampling periods was observed, where the test one contains the data more clustered (although test two was clustered, data were more dispersed). During periods of low water level, Bacillariophyta division was most importantly influenced, that is, its presence and variation was common at this low water level, followed by Chlorophyta algae.

Physiognomic groups of algae and algae indices vectors also played an important role during this period in the different classifications. It is noteworthy that only in few opportunities physical-chemical variables showed representative of this water level and chlorophyll a was the most prominent. As the level rises, the Cyanophyta division begins to gain importance and in high levels of water, dissolved oxygen, DWFA, speed and flow variables are more representative.

The classification by sampling stations indicated that the data from the San Miguel station are more concentrated with no apparent predominance of some division and according to the results of the indices, was the station with more richness and equity of algae, while station data After San Fernando are more scattered and distant from other stations, with a great importance of the electrical conductivity and dominance of the division Cyanophyta algae followed by Euglenophyta. Similar results in terms of increasing divisions after a wastewater discharge were found in the San Carlos Creek tributary of the river La Plata in Argentina [27].

Contrary to what was found in high mountain river systems in Colombia [28], in the Aburrá-Medellín River, the algae assembly recorded variation, related with water level, in addition to differences between sampling stations. The stations San Miguel and After San Fernando were the most dissimilar stations, they were characterized in the study to be the better water quality and less water quality, respectively.

In the Redundancy Analysis, no direct relationship was found between the algae and the speed of the current, contrary to what was reported in the Tota river [5], in which high current speeds, promoted low algal densities. However, it could be observed a relationship of this group with the flow, and therefore the Aburrá-Medellín River, like other mountain rivers, it is mainly influenced by precipitation (Payne, 1986 in [5, 27]). The physical, chemical and biological variables match this pattern influenced by microclimate of the basin.

V. CONCLUSIONS

In the Aburrá-Medellín river epilithic algae change due to turbulence generated by both the input of effluents and flood events in the main channel, which are the product of rising in micro-watersheds and its magnitude is a result of deforestation, channeling, channel straightening, among others, so it was not possible to observe a general pattern colonization.

The algae in the river under research were highly affected by the flow, and therefore vary according to the hydrological cycle. That is why despite their relationship with nutrients and physicochemical variables, Redundancy Analysis only appears as significant in the flow, because it was the variable that most affected the structure and abundance of these organisms.

The electrical conductivity was a significant physical-chemical variable in the river, highlighting its importance in periods of low water, when pollutants are concentrated due to the decrease of the base flow of the current and permanent entries of the raw wastewater and treated waters generated in the Aburrá Valley. Its highest values were associated with lower quality water; e.g. of that, was the significance of this variable in the After San Fernando station and the emergence of algae Cyanophyta of the genus *Anabaena*.

By analyzing the clusters in the figures, it was observed that in the data set corresponding to high water (test 2), the physical-chemical variables are located, showing the changes in water quality during this period, which is related to a process of dilution and flow variation due to additional entries from drainage of the basin. Besides it is outstanding the presence of Cyanophyta algae, a result that is inconsistent with that reported in the Tota River, where some diatoms were the predominant period of higher speed [4, 5]. Additionally, biological indices are in the data set low waters (test 1), showing the importance of these indices in the river at its most critical water quality scenario, corresponding to low flow. According to Decree 3930 of 2010, biological analyzes allow us to propose potential uses of a water source and from them formulate the plan of management of water resources and the quality objectives for the Aburrá-Medellín River.

The variability of the data matrix is mainly attributed to the changes between stations, so it is understood that there are higher differences in water quality sampling stations. These results are due to the section researches considered as a low anthropic intervention (San Miguel) plus other stations where degradation product of the anthropic activities takes place in this part of basin; within uses of water identified in this section are: Agriculture, livestock, recreational primary (direct contact with the water), secondary recreation, aesthetic (harmonization and embellishment of landscape), industry (including mining use in accordance with Decree 3930 of 2010) receptor discharges, and finally receiving and transportation of household and special waste [7].

These results, alike those presented by Gómez [26] hint the stress generated by human activity on the biological integrity of a stream is complex and cumulative, and is the product of the alteration of one or more of the following main factors: physical habitat, water flow, food, interactions within the biota of the river, and water chemistry quality [28, 29].

ACKNOWLEDGMENTS

We thank the University of Antioquia, the GIGA, GAIA and GEOLIMNA groups, the Metropolitan Area of the Aburrá Valley, engineers Carlos Jaramillo and Solange Arias and biologists Orlando Caicedo and Adrian Escobar for their support, advice and contributions in the experimental work.

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