



Phytoperiphyton associated with lagoons generated by mining, San Juan river basin, Chocó-Colombia

Fitoperifíton asociado a lagunas generadas por minería, cuenca del río San Juan, Chocó-Colombia

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ABSTRACT

Mining activity generates transformations in aquatic ecosystems and adjacent areas, as well as the creation of land depressions or "lagoons" that act as water reservoirs where aquatic communities develop, including the phytoperiphyton. Considering the role of this community in these environments, the purpose of this research was to study the phytoperiphytic community present in lagoons generated by mining processes, in the San Juan basin, Chocó (Colombia). Lagoons with different formation times were selected: 1-5 years, 5-10 years and 10-15 years; three lagoons for each time period. Physical and chemical variables were measured, and phytoperiphyton

was collected, using an 8cm² quadrant, obtaining a scraping area of 120cm². The community was composed of six divisions, nine classes, 16 orders, 24 families and 33 genera, for a total density of 13108 org/cm². The lagoons of 10-15 years presented the highest density, with Bacillariophytes as the most representative in all the studied lagoons, both in density and richness. There were differences in density between lagoons, but not in specific richness and diversity. Similarity values indicate that the lagoons have little variability in their phytoperiphytic communities. Conductivity and dissolved total solids are the variables with the greatest variation throughout the study; there are significant differences between lagoons for the variables dissolved oxygen, pH and alkalinity (p<0.05). This study

constitutes a baseline for understanding the ecological dynamics of lentic environments generated by mining processes and on the development of phytoperiphytic communities in them.

Keywords: Mining activity; Bacillariophytas; Lagoons; Phytoperiphyton.

RESUMEN

La actividad minera genera transformaciones en los ecosistemas acuáticos y zonas adyacentes y, entre ellas, se crean depresiones en el terreno o “lagunas”, que actúan como reservorios de agua, en los que se desarrollan comunidades acuáticas, como el fitoperifiton. Considerando el papel de esta comunidad en estos ambientes, el propósito de esta investigación fue estudiar la comunidad fitoperifítica presente en lagunas generadas por procesos de extracción minera, en la cuenca del San Juan, Chocó (Colombia). Se seleccionaron lagunas con diferente tiempo de formación: 1-5 años, 5-10 años y 10-15 años; tres lagunas por cada periodo de tiempo. Se midieron variables físicas y químicas y se colectó fitoperifiton, utilizando un cuadrante de 8cm², obteniéndose un área de raspado de 120cm². La comunidad, se compuso de seis divisiones, nueve clases, 16 órdenes, 24 familias y 33 géneros, para una densidad total de 13108 org/cm². Las lagunas de 10-15 años presentaron la mayor densidad con las Bacillariophytas, como las más representativas en todas las lagunas estudiadas, tanto en densidad como en riqueza. Existieron diferencias en densidad entre lagunas, pero no en la riqueza específica y diversidad. Los valores de similitud indican que las lagunas presentan poca variabilidad en sus comunidades fitoperifíticas. La conductividad y los sólidos totales disueltos son las variables de mayor variación, a lo largo de estudio, existiendo diferencias significativas entre lagunas, para las variables oxígeno disuelto, pH y alcalinidad ($p < 0,05$). Las variables pH y conductividad, se relacionaron positivamente con la densidad de algunos grupos fitoperifíticos. Este estudio, se constituye en una línea base para el conocimiento de la dinámica ecológica de los ambientes lénticos generados por los procesos mineros y sobre el desarrollo de las comunidades fitoperifíticas en ellos.

Palabras clave: Actividad minera; Bacillariophytas; Lagunas; Fitoperifiton.

INTRODUCTION

Phytoperiphyton is a complex community of microbiota adhered to any submerged substrate, mainly composed of microalgae and cyanobacteria, as well as bacteria, fungi and microinvertebrates; its complex structure also includes mucilage and organic debris, along with an inorganic component, which originates from different types of particles (Irbojević *et al.* 2018).

This community is a fundamental component in aquatic biota, being the main primary producers in bodies of water, both lotic and lentic, which is why they play an important role in the metabolism of aquatic ecosystems (Pompêo & Moschini-Carlos, 2003). Equally, their ability to reveal the effects of contamination and abiotic factors has promoted their use as bioindicators (Lambert *et al.* 2015).

Phytoperiphytic communities maintain a constant interaction with a variety of factors, such as water temperature, light, nutrients, substrate, sediments and disturbances, which condition and modify their distribution in the environment (Allan & Castillo, 2007). In the case of disturbances, they can occur naturally or anthropogenic and communities can respond in different ways, depending on the resistance capacity of the ecosystem, the type of disturbance, its frequency and its intensity (Lake, 2000).

One of the main disturbances affecting the phytoperiphyton is mining, since it causes the suspension of sediments at the bottom of water sources and the availability of nutrients, making it difficult for light to penetrate the aquatic ecosystem (Luttenton & Baisden, 2006). According to authors, such as Lake (2000) and Castro & Donato (2008), disturbances of this type generate heterogeneous periphytic communities, which modify their structure and physiological properties in responses to these events, throughout the succession process.

Non-technical mining has been developing for decades in the department of Chocó, especially in the San Juan basin (Medina-Mosquera *et al.* 2011), with the consequent alterations in aquatic ecosystems, which have undergone great changes in its natural state. This situation has generated that in the aquatic ecosystems of the area, alterations associated with this activity are evident, such as sedimentation, the change of the channel and shape, when creating depressions in the terrain or “lagoons”, which act as reservoirs of water (Fierro-Morales, 2012), where diverse aquatic communities develop, among them, phytoperiphyton, which has not been studied in this type of environment.

In Colombia, are important contributions to the study of phytoperiphytic communities in lotic and lentic ecosystems the investigations of Osorio-Ávila *et al.* (2015), De la Hoz-Barrientos & Osorio-Ávila (2016), Lozano-Peña *et al.* (2019) and Huertas-Farías *et al.* (2019), among others. In the department of Chocó, this community has been little studied, and the investigations of Abuhatab & Asprilla (2005) and Salas *et al.* (2011) can be cited. However, there are no published works on periphytic communities in aquatic environments generated by the development of mining activities.

This research carried out a comparison of the phytoperiphytic communities (microalgae and cyanobacteria) present in lagoons generated by mining, with different formation times, in the municipality of Condoto, basin of the San Juan river, Chocó-Colombia. Equally, the physical and chemical characteristics of the lagoons and how they relate to current phytoperiphytic communities were analyzed

MATERIALS AND METHODS

Study area: The present study was carried out in lagoons generated by mining extraction processes, with different formation times, located in the municipality of Condoto, in the San Juan river basin (Chocó). The municipality of Condoto, is located at coordinates

5°06'01 "N; 76°32'44" W, at 50m of altitude. Due to its location in the equatorial calm zone, it is characterized by high temperatures of an average of 28°C, high rainfall, whose annual average varies between 7,000 and 8,000mm and marked relative humidity, of an average of 90%, which define a tropical rainy jungle climate; Due to these characteristics, Condoto is classified as tropical rain forest (bp-T) (IGAC & MMA, 2000).

Sampling design: For the data collection in the field, a specific sampling was carried out during February 2018. Mining lagoons with different formation times were selected: lagoons between 1 and 5 years, between 5 and 10 years and between 10 and 15 years. Three lagoons were selected for each period of time, with two sampling points, in each one. In general, the lagoons of 1-5 years and 5-10 years were characterized by being surrounded by herbaceous vegetation, presenting sandy or muddy substrata, as well as little or no submerged vegetation, while the lagoons of 10-15 years were surrounded by a large wooded area, bottom covered with plant material and a variety of submerged vegetation.

Physical and chemical aspects: The physical and chemical characteristics of the lagoons were analyzed at each of the two selected sampling points, through the measurement of the variables: dissolved oxygen (mg. l^{-1}), pH (pH units), water temperature ($^{\circ}\text{C}$), dissolved total solids (mg.l^{-1}) and conductivity ($\mu\text{S.cm}^{-1}$), with a HACH HQ30d multi-parameter equipment; alkalinity (mg. l^{-1}) was measured with the potentiometric method. Transparency was measured with a Secchi disk.

Phytoperiphyton sampling: To collect the phytoperiphyton at each of the sampling points, the modified methodology of Jiménez-Pérez *et al.* (2014) was used, which consisted of the selection of three random stones, from which the attached phytoperiphytic community (microalgae and cyanobacteria) was removed, using an 8cm^2 quadrant, scraping five times with a plastic brush. The total scraping area was 120cm^2 per site, taking one sample and one repetition of each, for a total of two samples at each point. The samples were preserved with Lugol (10%), in amber plastic containers.

Qualitative and quantitative analysis of phytoperiphyton: To observe the phytoperiphyton samples, an inverted microscope and a Sedgwick-Rafter (SR) counting chamber (1mL) were used. 30 observation fields were defined through a species accumulation curve, which were taken at random, following the criteria established by Uehlinger (1964). Counting under the microscope, made a total magnification of 400X and applied the Ross equation (1979), to quantify the organisms/ml, which is related to the sampling area at each point (120cm^2), obtaining the density of organisms/ cm^2 ; unicellular organisms, colonies and filaments were specific as individuals. The taxonomic identification was carried out up to the gender category, following the keys of Prescott *et al.* (1983), Krammer & Lange-Bertalot (1991), Komárek & Anagnostidis (2005), Bicudo and Menezes (2006). In addition, digital databases will be used (Guiry & Guiry, 2019).

Data analysis: Descriptive statistics were applied to analyze the physical and chemical data, estimating the average and the coefficient of variation, as well as an analysis of variance (ANOVA), to analyze the differences between lagoons, according to the time of formation. To evaluate the phytoperiphytic community, the density, specific richness and the diversity index of Shannon-Weaver were estimated and to analyze their differences between lagoons, an analysis of variance (one way ANOVA) was used; this same analysis was used to evaluate the changes in the proportion of the divisions of microalgae and cyanobacteria, among the types of lagoons evaluated. The assumptions inherent in the ANOVA test were verified, in all cases, being acceptable ($p > 0.05$). In order to analyze the similarity of the phytoperiphytic community between the lagoons, the Jaccard index was determined, with which they performed qualitative grouping analyzes, using the presence and absence criteria of the taxa registered in the study. Pearson's multiple linear correlation test was used to evaluate the effect of physical and chemical variables on the components of the phytoperiphytic community, after reviewing the condition of normality ($p > 0.05$).

The statistical packages used for the different analyzes were Minitab version 17.1.0, Statgraphics Centurion XV, Past 1.57, and BioDiversity Pro.

RESULTS AND DISCUSSION

Physical and chemical aspects: Table 1 shows the average values of the physical and chemical variables registered in the studied lagoons. The lowest dissolved oxygen and pH averages were found in the lagoons of 1-5 years and the highest, in the lagoons of 5-10 years; equally, the latter report the highest average water temperature values, while the lowest, in the lagoons of 10-15 years.

In general, in all the lagoons studied, the dissolved oxygen values are recorded within the normal ranges found, both in natural and lentic environments, greater than 4mg.l^{-1} (Lewis, 2000). In the case of pH, the values correspond in all cases to acidic waters, especially in the most recently formed lagoons (1-5 years), which is associated, in all cases, with the origin of the same, as a product of mining, which tends to acidify the waters, as a result of the oxidation and hydrolysis of sulfides, with formation of sulfates and acid sulfates (acid mine drainage), which constitutes one of the greatest problems generated by mining. However, the values recorded are within the range required for the survival of aquatic organisms, between 4.5 and 8.5 (Roldán & Ramírez, 2008). On the other hand, the water temperature presents values above 28°C , which is characteristic of aquatic environments located in tropical regions, where there are no significant variations in the amount of solar radiation received throughout the year, with values between 25 and 30°C (Roldán & Ramírez, 2008).

In the case of dissolved total solids, the conductivity and the transparency present their lowest averages in the lagoons of 10-15 years and the highest, in the lagoons of 5-10 and 1-5 years. Finally, alkalinity exhibits its highest averages in the lagoons of 1-5 years and the lowest in the lagoons of 5-10 years. Conductivity and dissolved

Table 1. Average values, coefficients of variation (CV) and significant difference for the physical and chemical variables.

Variables	Units	1-5 years	5-10 years	10-15 years	CV %	p-value
		Averages				
Dissolved oxygen	mg. l ⁻¹	5.81	7.26	6.70	15.14	0.028
pH	units	4.60	5.38	5.32	9.42	0.002
Water temperature	°C	30.45	30.92	29.73	5.86	0.539
Alkalinity	mg. l ⁻¹ CaCO ₃	12.71	6.17	9.50	35.19	0.000
Conductivity	μS.cm ⁻¹	126.65	126.97	77.32	59.15	0.336
Dissolved total solids	mg. l ⁻¹	81.68	80.53	78.43	45.99	0.989
Transparency	m	0.47	0.46	0.39	36.17	0.677

total solids are the variables with the greatest variation throughout the study. There were significant differences in dissolved oxygen, pH and alkalinity between lagoons with different formation times ($p < 0.05$) (Table 1).

The values recorded for alkalinity, dissolved total solids, and conductivity were found within the ranges reported for neotropical ecosystems, with values less than 100mg.l⁻¹, 1500μS.cm⁻¹ and 200mg.l⁻¹, respectively (Roldán & Ramírez, 2008), which shows a great recovery of these environments, taking into account that these variables are affected, to a large extent, by the development of mining-type activities (Fierro-Morales, 2012). In the case of transparency, the values recorded are low, which may be associated with the shallow depth of the lagoons, given their origin. However, they are within the range established for Colombian natural lentic systems, by Arias (1985), of between 0.17 and 1.13m.

Phytoperiphytic community: The phytoperiphytic community of the studied lagoons registers a total density of 13108 org/cm², grouped into 33 genera, 24 families, 16 orders, 9 classes and 6 divisions (Table 2). The average density ranges from 936.11 org/cm² (CV=59.67%), in the lagoons of 1-5 years, to reach a significant increase in the lagoons of 10-15 years, with 2319.37 org/cm² (CV=7.94), there being statistically significant differences between the lagoons ($F=9.20$; $p=0.015$) (Figure 1). On the contrary, the specific richness does not present significant differences between lagoons, with values of between 23 and 24 genera ($F=1.16$; $p=0.374$) (Figure 1). The diversity presented averages of 2.16 bits/ind in the lagoons of 1-5 years (CV=18.80%); 2.12 bits/ind, in lagoons of 5-10 years (CV=16.08%) and 2.02 bits/ind, in lagoons of 10-15 years (CV= 9.58%), but not statistically significant differences were found between them ($F=0.14$; $p=0.873$).

According to Wetzel (2001), the density of phytoperiphytic communities tends to increase, as the time of exposure to the environment increases, until reaching stable values or until its decline begins, as a result of grazing. In this sense, the variation in the density of the phytoperiphytic community between lagoons can be attributed to the time of their formation, including colonization processes, spatial changes, as well as the prevailing environmental

conditions, which influence, among others, in reproduction processes, which can cause a significant increase in density (Zhang *et al.* 2013).

Regarding diversity and richness, the values recorded can be attributable to the mining processes, which gave rise to the lagoons, since the disturbances, in general, contribute to making phytoperiphytic communities heterogeneous and modifying their structure and physiological properties during the succession process (Castro & Donato, 2008), which prevents the existence of a homogeneous development of the species and, therefore, the values of diversity and richness, are reduced. According to Pedrozo *et al.* (2001), acidic environments, both lotic and lentic, are characterized by low diversity and richness, which coincides with the characteristics of the studied lagoons.

In general terms, the Bacillariophyta division registers the greatest contribution in terms of density, a situation that remains in all the lagoons with different formation times, followed by the Charophyta division. In this sense, the ANOVA evidenced the existence of statistically significant differences in the density of the Bacillariophyta and Euglenophyta divisions between lagoons ($p < 0.05$) (Figure 2). Regarding species richness, once again Bacillariophytas registered the highest contribution, with 39.39% of the genera, followed by Charophytas, with 33.33%, while Ochrophytas only contributed 3.03% (Table 2); equally, within the Bacillariophyta division, the genera with the highest density are found in the three types of lagoons, according to their formation time.

Bacillariophytas are the dominant group in the abundance and richness of the phytoperiphyton, both in lotic and lentic ecosystems (Bustamante-Toro *et al.* 2009; Osorio-Ávila & Manjarres-García, 2015). These organisms are considered as fast and efficient colonizers, capable of colonizing a substrate, in a matter of days to weeks, starting from the initial stages of succession and remaining throughout the process (Fonseca & Rodríguez, 2005).

The representatives of the Bacillariophyta division present different morpho-physiological characteristics (silicate cell wall, fine drops of oil, as nutritive reserves, high durability of the membrane), as well

Table 2. Taxonomic composition and average density (org/cm³) of the phytoplankton in lagoons, with different formation times, San Juan - Chocó.

DIVISION / CLASS	ORDER	GENERA	1-5 years	5-10 years	10-15 years
BACILLARIOPHYTA					
Bacillariophyceae	Naviculales	<i>Frustulia</i>	204.10	102.05	463.61
		<i>Navicula</i>	32.07	34.99	32.07
		<i>Neidium</i>	8.75	8.75	0.00
		<i>Stauroneis</i>	0.00	20.41	2.92
		<i>Pinnularia</i>	87.47	215.77	288.66
	Fragilariales	<i>Synedra</i>	32.07	14.58	0.00
		<i>Fragilaria</i>	0.00	14.58	0.00
	Eunotiales	<i>Actinella</i>	17.49	35.02	52.48
		<i>Eunotia</i>	2.92	10.85	670.63
	Cymbellales	<i>Cymbella</i>	72.89	294.49	271.17
<i>Gomphonema</i>		20.41	0.00	0.00	
Coscinodiscophyceae	Aulacoseirales	<i>Aulacoseira</i>	23.33	5.83	0.06
Mediophyceae	Stephanodiscales	<i>Cyclostephanos</i>	0.00	0.09	0.00
EUGLENOPHYTA					
Euglenophyceae	Euglenales	<i>Trachelomonas</i>	151.62	29.86	14.87
		<i>Euglena</i>	0.00	0.00	5.90
CHAROPHYTA					
Conjugatophyceae	Desmidiiales	<i>Closterium</i>	87.47	119.55	96.22
		<i>Cosmarium</i>	26.24	40.82	172.03
		<i>Actinotaenium</i>	29.16	14.58	23.33
		<i>Desmidium</i>	0.09	2.92	32.07
		<i>Micrasterias</i>	0.00	0.00	2.92
		<i>Pleurotaenium</i>	0.00	40.82	61.23
		<i>Stauroidesmus</i>	0.00	0.00	2.92
		<i>Gonatozygon</i>	0.00	11.66	0.26
	Zygnematales	<i>Netrium</i>	5.83	3.05	14.93
		<i>Spirogyra</i>	0.06	0.00	0.00
		<i>Mougeotia</i>	23.33	0.00	0.03
CHLOROPHYTA					
Ulvophyceae	Ulotrichales	<i>Ulothrix</i>	23.33	0.00	0.00
Chlorophyceae	Oedogoniales	<i>Oedogonium</i>	17.49	29.16	38.14
	Chlamydomonadales	<i>Chlamydocapsa</i>	0.00	0.09	0.00
OCHROPHYTA					
Xanthophyceae	Tribonematales	<i>Tribonema</i>	2.92	0.00	0.00
CYANOBACTERIA					
Cyanophyceae	Oscillatoriales	<i>Oscillatoria</i>	2.92	14.61	0.03
	Synechococcales	<i>Pseudanabaena</i>	64.15	49.57	69.98
	Spirulinales	<i>Spirulina</i>	0.00	0.00	2.92

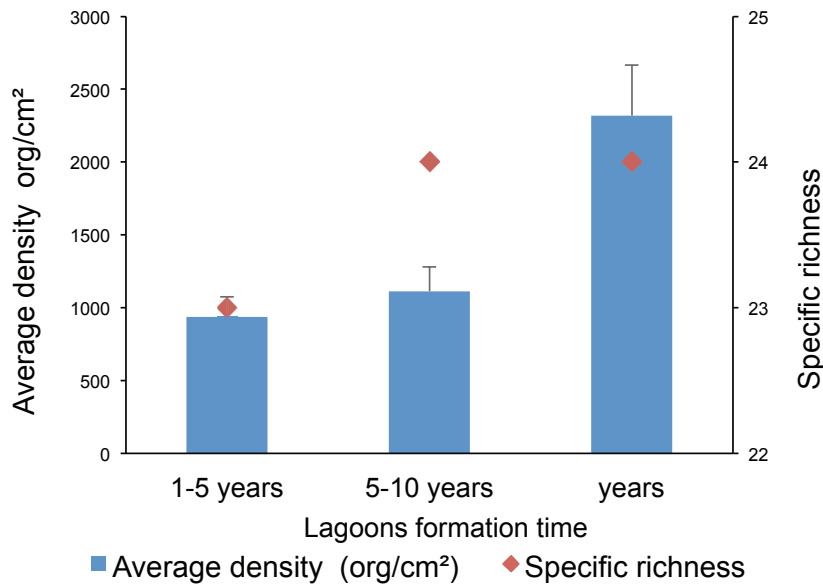


Figure 1. Average density (org/cm²) and specific richness of the phytoplanktonic community in lagoons, with different formation times. Vertical bars indicate \pm SE (n = 6).

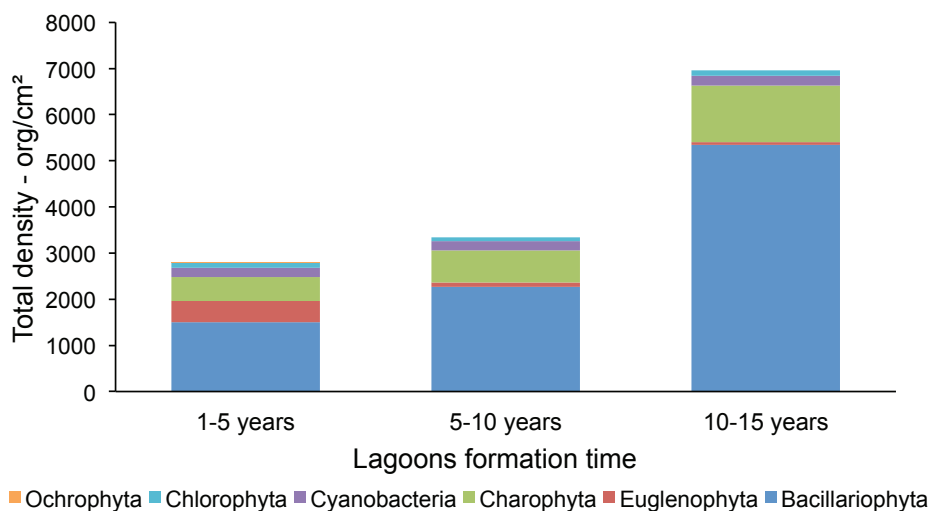


Figure 2. Total density and contribution by divisions of microalgae and cyanobacteria in lagoons, with different formation times.

as specialized structures, such as peduncles and mucilaginous matrices and formation of colonies in form of stars, which offer competitive advantages in stressful conditions, in addition to providing protection and giving them a wide range of tolerance, to a large number of vulnerability factors. This means that the majority of the representatives of this group are able to survive, in a wide variety of aquatic ecosystems and environmental conditions (Carapunarla *et al.* 2014).

Regarding the density presented by other phytoplanktonic groups, such as Chlorophytas and Cyanobacteria, it is worth mentioning that their low representativeness is attributable to their inability to

tolerate acidic pH, presenting their greatest development in alkaline media (Martín *et al.* 2004), which contrasts with the acidic conditions of the studied lagoons.

The genera *Eunotia*, *Cymbella*, *Frustulia* and *Pinnularia* are among the most abundant in all the lagoons; together, they occupy 61.44% of the total abundance (Table 2). The genus *Eunotia* has the facility to colonize environments, due to its rapid growth, developing in patches and progressively increasing its density, which makes it one of the most common and with the greatest species richness in the neotropics (Metzeltin & Lange -Bertalot, 2007).

Some investigations, such as those of Hill *et al.* (2000), report the highest abundance of the *Ennotia* genus, in environments in which the pH is below 5.5, which shows its ability to tolerate acidic waters and that classify it as acidobiontic (Siver *et al.* 2006; Fore & Grafe 2002); situation that coincides with the physicochemical characteristics of the lagoons studied in this investigation.

The Jaccard similarity index indicated an approximate affinity range of 60% in the composition of the phytoplankton, between the lagoons with different formation times (Figure 3), with the lagoons being 5-10 years and 10-15 years, those with the highest affinity, with 65.51%, which share some taxa of Bacillariophyceae and Conjugatophyceae, with 48.4% of the genera, being shared between the lagoons (Table 2). These results may be associated with the fact that they are cosmopolitan or generalist taxa, which present a great ecological amplitude, without following a spatial structure or pattern (Lee, 2008); equally, they show the few changes in the

phytoplanktonic community present in the studied lagoons and that the differences they present in terms of time of formation are not sufficient to generate significant changes in the composition of the community, although they do in its density.

Physical and chemical parameters and phytoplanktonic community: The multiple linear correlation analysis (Table 3) showed that there are few statistically significant relationships found: positively, the pH was related to the abundance of Bacillariophytas, Charophytas and the total density of phytoplanktonic organisms; the conductivity and abundance of Euglenophytas and Ochrophytas; the richness with the abundance of Bacillariophytas, Charophytas and Cyanobacteria.

Phytoplanktonic communities are highly dependent on environmental conditions, especially water chemistry, which has led to their frequent use as bioindicators (Hill *et al.* 2000). Some studies have

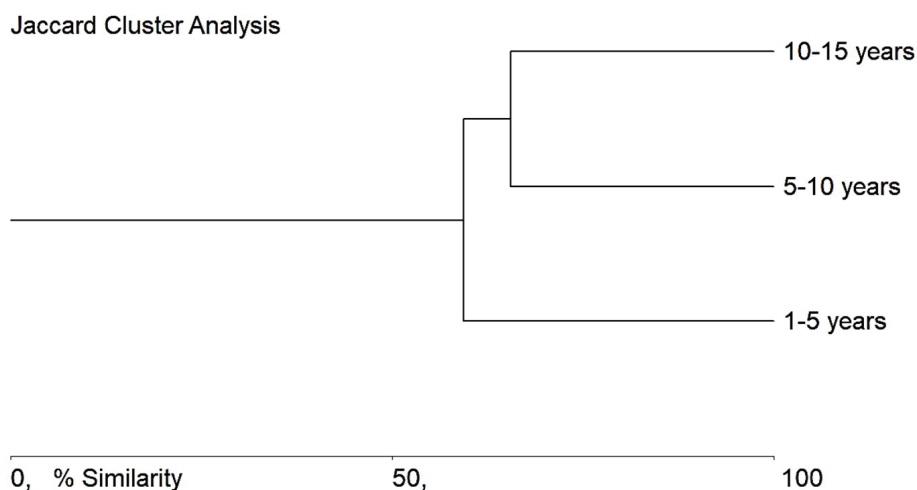


Figure 3. Similarity of the phytoplanktonic community between lagoons, with different formation times: 1-5 years, 5-10 years, 10-15 years, using the Jaccard index.

Table 3. Pearson’s correlation matrix of the physical and chemical variables, richness, density and composition of the phytoplanktonic community, in the studied lagoons. Significant correlation ($p < 0.05$ *) and very significant ($p < 0.01$ **).

	pH	Conductivity	Richness
Bacillariophyta	0.89**	0.09	0.67*
Charophyta	0.70*	0.02	0.81**
Euglenophyta	-0.64	0.66*	-0.43
Ochrophyta	-0.40	0.80**	-0.45
Cianobacteria	0.75	-0.26	0.73*
Total density (org/cm ²)	0.75**	0.28	0.15

shown the importance of variables, such as pH (Verb & Vis, 2005) and conductivity (Rusanov *et al.* 2012) in the composition of phytoperiphyton in aquatic ecosystems. In the case of pH, it has been observed that its decrease produces changes in the structure of the phytoperiphyton, because acid-tolerant taxa become dominant (Genter, 1996). For its part, conductivity is a strong integrating variable on multiple scales and is commonly reported as the most important explanatory variable in studies with phytoperiphytic organisms (Rusanov *et al.* 2012).

The present study constitutes a baseline for understanding the ecological dynamics of lentic environments, generated by mining processes and on the development of phytoperiphytic communities in them. In conclusion, changes were evidenced in the density of the phytoperiphytic community between the lagoons, with different time of formation, with an increase in it, as the colonization time advances. However, the richness and diversity presented little significant changes, attributable to the little variation in the physical and chemical conditions of the lagoons, therefore, future studies in these environments should include lagoons, with a longer formation time.

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