

Cyanobacterial Composition of Microbial Mats, Found in Brejo do Espinho and in Artificial Saline of Araruama, RJ, Brazil

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ABSTRACT

Microbial mats are organo-sedimentary ecosystems formed by autotrophic and heterotrophic bacteria. Cyanobacteria contribute to the formation of these structures from the entrapment and engulfment of sediments contributing to the formation of carbonate rocks found in the geological record from the Precambrian to the Recent. In this research we identified the living cyanobacteria mats type 1 (gelatinous) and type 2 (cohesives) present in *Brejo do Espinho* and *Araruama* saline, east Fluminense, Rio de Janeiro state. The microbial mats type 1, were found in compartment A and B of *Brejo do Espinho* and the *Araruama* saline in the tank with a depth of 10 cm. The mats type 2 were only found in compartment A and in tank with 15 cm depth. In the microbial mats of compartments A and B predominated the spherical forms of genres *Chroococcus* Nägeli 1948 and *Aphanothecace* Nägeli 1948. In the tanks predominated filamentous forms of the *Leptolyngbya* Anagnostidis and Komárek 1988 genre. In mats type 1, the diversity indices (H') were 1.95 and 2.24 and in type 2 of 2.16 and 2.14 for the *Brejo do Espinho* and *Araruama* saline, respectively. The highest values of diversity indices found in saline tanks occurred by stable salinity conditions.

Keywords: Cyanobacteria; Microbial Mat; *Brejo do Espinho*; *Araruama* Saline

1. Introduction

The microbial mats are organo-sediment [1] formed by a microbial consortium of autotrophic and heterotrophic bacteria that acquire carbon and energy for the formation of biomass [2].

Cyanobacteria are common in microbial mats that develop in marine and hypersaline ecosystems. In general, they are distributed in the photic zone of these structures and remain abundant in the upper strata [3].

In microbial mats cyanobacteria are major primary producers [4,5] and contribute to the formation and development of these structures from the laminar growth by trapping and aggregation of sediments [6].

In Brazil, the presence of microbial mats in the east Fluminense of the state of Rio de Janeiro, in the *Araruama* Lagoon Complex, allows the research of the relationship with microbial sedimentation and the formation of carbonate rocks present in the geological record.

Despite the importance of diversity to ecological studies, few surveys have been developed with cyanobacteria

[7]. The ecological treatment of this study was a pioneer for the microbial mats of *Brejo do Espinho* and *Araruama* saline.

The microbial mats found in *Brejo do Espinho* and *Araruama* saline, state of Rio de Janeiro, Brazil, were selected in order to identify the composition and diversity of cyanobacteria in different salinity gradients.

2. Field Site

The *Araruama* Lagoon Complex consists of a dual system of sand ridges, which serve as a shield against the current ocean beach.

Small and narrow merged lagoons occur in this complex between sea and the *Araruama* lagoon [8].

This region is characterized by a semiarid microclimate in response to the flashing of a coastal upwelling at Cabo Frio, with annual rainfall around 830 mm and annual average evaporation around 1400 mm [9].

This microclimate results in the formation of hypersaline lagoons and deployment of artificial salts on salt

production in the area.

2.1. Brejo do Espinho

In *Brejo do Espinho* ($22^{\circ}56'S$ and $42^{\circ}14'W$) sampling occurred from September 2007 to October 2008, being selected in eight sites aboard two compartments marked as A and B (Figure 1) [10]. This low anthropic impact area is characterized by having a length of 1 km^2 and the water column that varies from 2 cm to 1.5 m [11-14].

The ongoing action from the frequent winds blowing in the region associated with low depth makes their water show no stratification. The large oscillations of the water surface and salinity have caused this system to be classified as a pond, lagoon [12,14], marsh [10] as well as *Sabkha* [11].

2.2. Araruama Saline

In the artificial saline of *Araruama* ($22^{\circ}51'$ to $22^{\circ}55'S$ and $42^{\circ}20'W$) samplings were conducted from August 2008 to July 2009, being selected evaporation tanks with 15 cm and 10 cm deep (Figure 1).

This saline is provided by the *Araruama* Lagoon, considered as a system suffocated by its close connection with the sea.

Its high degree of evaporation, the strong action of winds, intense insolation, low rainfall, the slow turnover of water and little fluvial inflow remains a hypersaline environment [15] with average salinity of $52\text{ g}\cdot\text{L}^{-1}$ [16].

The artificial saline environments are formed by anthropic intervention for salt exploration (NaCl), and obtaining salt based on the evaporation of brines from the sun and wind energy. Initially, the brine is stored in confined areas of the lagoon. Then, that brine travels to a tank system with continuous evaporation and concomi-

tant increase of salinity to salt precipitation [17].

3. Materials and Methods

In *Brejo do Espinho* and *Araruama* saline, during the collection period were measured pH (microprocessor—pH meter GG867) and salinity (portable refractometer) with range 0 - 100‰.

The microbial mats were taken at different stages of maturity, were separated into type 1 (gelatinous) and type 2 (cohesive). The structures type 1 were found in compartment A and B of *Brejo do Espinho* and in the salinity tank with 10 cm depth. While the structures type 2 were only in compartment A and in the tank with 15 cm depth.

These microbial mats were removed from the collection site and placed in sterile plastic containers in the dark with a solution of 4% formalin buffered with borax.

These structures were measured in thickness with the aid of calipers Mitutoyo (JIF B7507). The samples were horizontally sectioned for the initial 5 mm separation of the structure, being withdrawn subsamples from a 2 mm dermatological punch for making permanent blades. Reading of these was performed by using an optical microscope Olympus CX40, increases in the 40 to 100 times, sweeping three random columns per blade.

The identification of cyanobacterial composition followed [18,19].

The methodology for ecological quantification was modified and adapted by [20]. A total number of 1500 individuals was considered to evaluate the representativeness of species by microbial mat. The ecological interpretation occurred from the diversity index proposed by Shannon Weaver (H'), equitability and richness. The Software program used was the Paleontological Statistics (PAST) version 2.17 [21].

4. Results

The pH measured in the field in compartments A and B of the *Brejo do Espinho* and tanks of *Araruama* saline always remained basic. In compartment B and in salinity tanks obtained the minimum value of 7.0 and a maximum of 10 (Table 1).

Salinity in the compartments of *Brejo do Espinho* showed great variation, ranging from a minimum value of 16‰ and above 100‰. In the *Araruama* saline, the salinity maintained values greater than 100‰ (Table 1).

The microbial mats analyzed for this research were disposed in *Brejo do Espinho* and *Araruama* saline as flat, smooth, continuous and uniform structures.

In *Brejo do Espinho* the structures of type 1 were observed in compartment A and B with 16 mm thick, being found in the dry period and in the initial lagoon filled period in the submerged portion of the lagoon. These

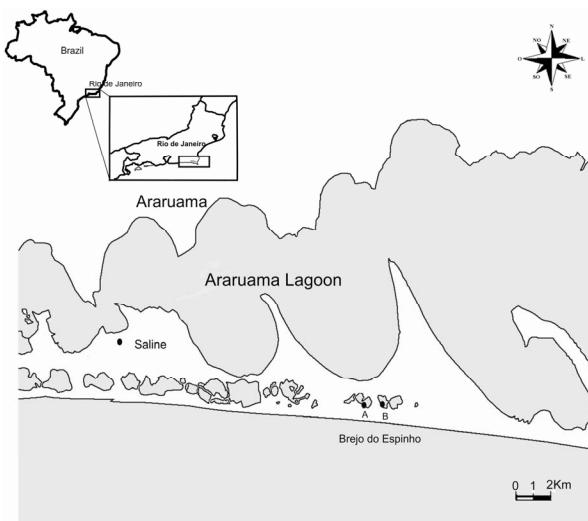


Figure 1. Map showing the location of the research area, pointing the *Brejo do Espinho* and *Araruama* saline.

Table 1. Physico-chemical parameters of pH and salinity and the types of microbial mats found in Brejo do Espinho (compartment A and B) and Araruama saline (evaporation tanks of 10 and 15 cm depth).

Site of samples	pH			Salinity (%)			Type mats
	Min.	Max.	Standard deviation	Min.	Max.	Standard deviation	
Compartment A	7.3	9.7	1.2	16	>100	34.26	Type 1
							Type 2
Compartment B	7.0	10.0	1.2	9.3	75	23.6	Type 1
Tank of evaporation 1	7.0	10.0	0.8	>100	—	—	Type 2
Tank of evaporation 2	7.7	10.0	1.0	>100	—	—	Type 1

mats were observed multicolored strata with yellow, green, red and brown colors.

In saline, these structures showed little cohesive, thin and brittle with a thickness of 5 mm, green color and evaporate minerals on their surface. They were found throughout the collection period in evaporation tanks of 10 cm.

The structures of type 2 of *Brejo do Espinho* proved to be cohesive with 20 mm thick, made of multicolored layers, with yellowish, green, red and brown that are repeated throughout the structure. They were found only in the dry lagoon period.

In *Araruama* saline this type of structure was found in the initial evaporation tanks with water layer of 15 cm, it had a thickness of 15 mm. These structures were found throughout the collection period, with upper stratum green, intermediate red and brown bottom.

In mat type 1 the composition cyanobacterial presented a richness of 30 species in *Brejo do Espinho* and 17 species in *Araruama* saline. In *Brejo do Espinho* the genres more representatives in number of species were *Chroococcus* Nägeli 1849 with 24% *Aphanothece* Nägeli 1849 and *Leptolyngbya* Anagnostidis and Komárek 1988 with 11%. In *Araruama* saline the genres *Aphanothece* Nägeli 1849 and *Chroococcus* Nägeli 1849 were the ones who gathered the largest number of species, both with 29% (**Figure 2**).

In mats type 2 showed a richness of 27 species in *Brejo do Espinho* and 16 in *Araruama* saline. In both structures the genres most representative were *Aphanothece* Nägeli 1849 with 29% and 32% and *Chroococcus* Nägeli 1849 14% and 20% (**Figure 2**), respectively.

In relation to gelatinous mat type 1 were common to *Brejo do Espinho* and the saline species *Aphanothece clathrata* West and West 1906, *Chroococcus minor* (Kützing) Nägeli 1849, *Chroococcus turgidus* (Kützing) Nägeli 1849 (**Table 2**).

In mat type 2 were common *Aphanothece castagnei* (Brébisson) Rabenhorst 1865, *Aphanothece microscopica* Nägeli 1849, *Aphanothece stagnina* (Sprengel) A. Braun 1863, *Microcoleus cthonoplastes* (Thuret) Gomont 1892 and *Schizothrix friesii* (Agardh) Gomont 1892 (**Table 2**).

In gelatinous mat of *Brejo do Espinho* was possible to see from the values of the relative abundance the predominance of species *Chroococcus* sp. (0.42) *Leptolyngbya* sp. (0.24) (**Figure 2**).

In the saline the same structure type showed from the relative abundance of predominant species *Leptolyngbya hypolimnetica* (0.24), *Leptolyngbya tenuis* (0.19) and *Chroococcus minimus* (0.13) (**Figure 3**).

In *Brejo do Espinho* and saline, gelatinous mats showed diversity index H'1.95 and 2.24 and equitability of 0.57 and 0.79 (**Table 3**), respectively.

In structure type 2 of *Brejo do Espinho*, from the values of relative abundance the species that show dominant, were species *Aphanothece stagnina* (Sprengel) A. Braun 1863 (0.43), *Aphanothece clathrata* (0.16) (**Figure 3**).

In mat type 2 from saline occurred predominantly three species based on relative abundance, the *Leptolyngbya hypolimnetica* (0.31), *Chroococcus minutus* (0.20) and *Chroococcus minimus* (0.11) (**Figure 3**).

Type 2 structures found in *Brejo do Espinho* and saline showed diversity index (H') of 2.16 and 2.14 and equitability of 0.65 and 0.79 (**Table 3**), respectively.

5. Discussion

Brejo do Espinho is a dynamic system governed by seasonal cycles of drought and flood lagoons, which are determined by regional climatic conditions [10] and cause large fluctuations in salinity as observed during the research period.

The mats type 1 found in *Brejo do Espinho* during drought and flood lagoon proved well adapted to conditions of low and high salinity. While that type 2 structures, present only in the dry lagoon, highlighted the need for a stable conditions and high salinity for its formation.

Araruama saline despite of having tanks with low depth had found some stability in salinity always above 100‰.

According to [22] the location of saline in dry climates with low rainfall justifies the high salinities found in these salines and the continuous exploitation of salt.

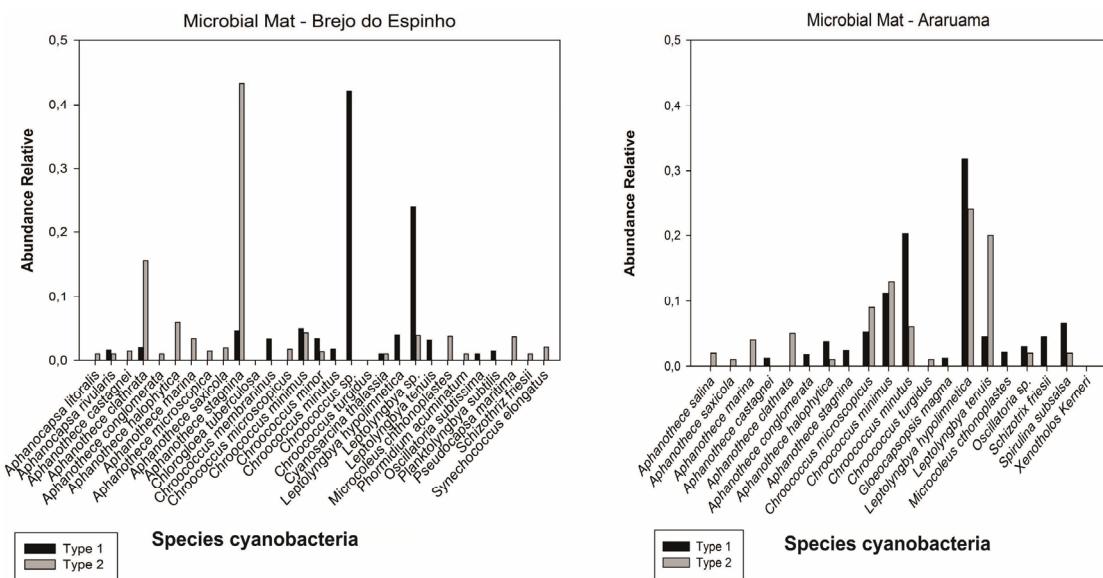


Figure 2. Graphs of the qualitative distribution of genres in mats type 1 and type 2 of *Brejo do Espinho* and *Araruama* saline.

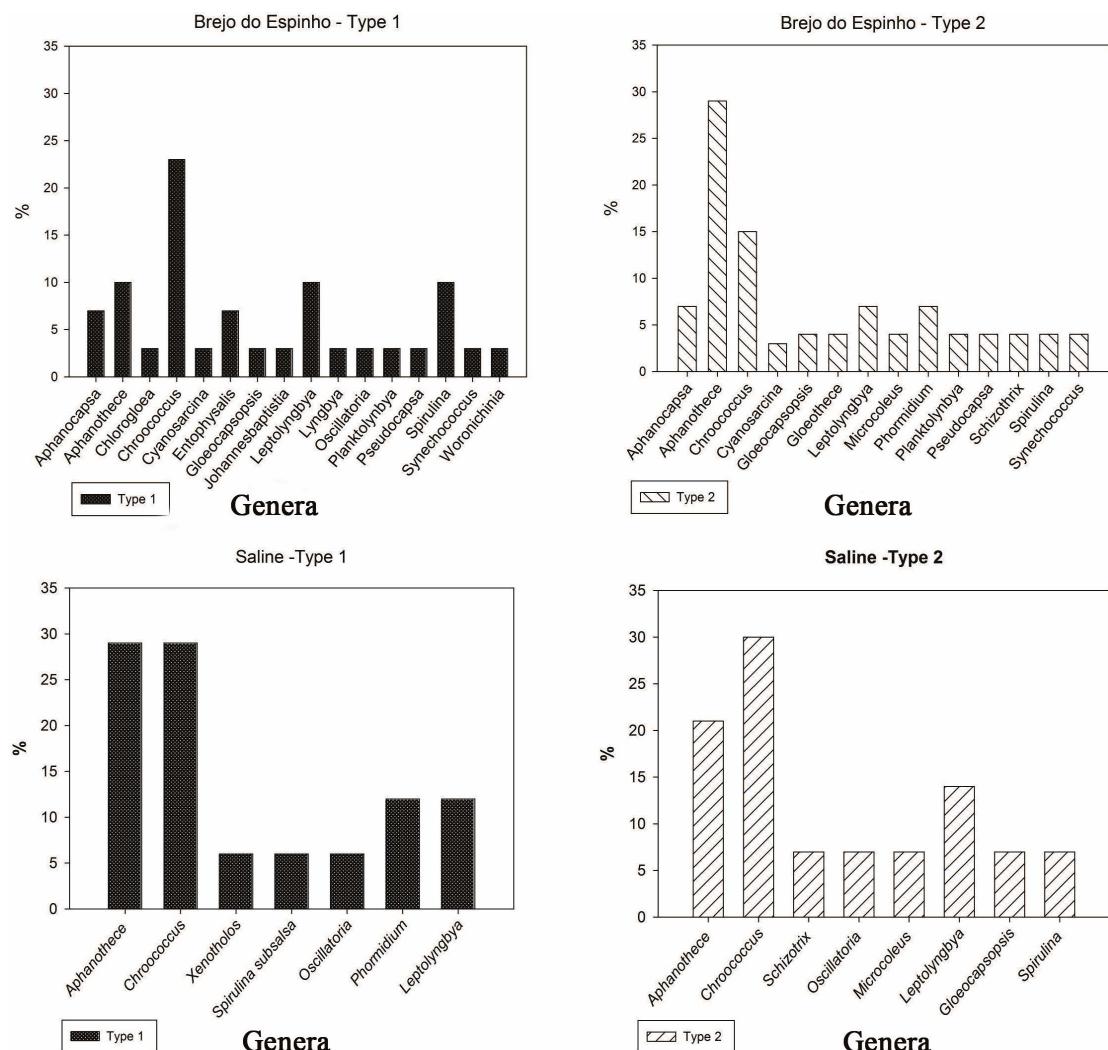


Figure 3. Graphs representing the relative abundance of species in mats type 1 and type 2 of *Brejo do Espinho* and Araruama saline.

Table 2. Composition of cyanobacterial plain microbial mats type 1 and type 2 found in Brejo do Espinho and Araruama saline.

Species	Brejo do Espinho		Araruama Saline	
	Type 1	Type 2	Type 1	Type 2
<i>Aphanocapsa litoralis</i>	x	x		
<i>Aphanocapsa marina</i>				
<i>Aphanocapsa rivularis</i>	x	x		
<i>Aphanothece castagnei</i>		x		x
<i>Aphanothece clathrata</i>	x	x	x	
<i>Aphanothece conglomerata</i>		x		x
<i>Aphanothece halophytica</i>	x	x	x	x
<i>Aphanothece marina</i>		x	x	
<i>Aphanothece microscopica</i>		x		x
<i>Aphanothece salina</i>			x	
<i>Aphanothece saxicola</i>		x	x	
<i>Aphanothece stagnina</i>	x	x		x
<i>Chlorogloea tuberculosa</i>	x			
<i>Chroococcus membraninus</i>	x			
<i>Chroococcus microscopicus</i>	x	x	x	x
<i>Chroococcus minimus</i>	x	x	x	x
<i>Chroococcus minor</i>	x	x	x	
<i>Chroococcus minutus</i>	x	x	x	x
<i>Chroococcus obliteratus</i>				
<i>Chroococcus</i> sp.	x			
<i>Chroococcus turgidus</i>	x		x	x
<i>Cyanosarcina thalassia</i>	x		x	
<i>Entophysalis conferta</i>	x			
<i>Entophysalis granulosa</i>	x			
<i>Gloeocapsopsis crepidinum</i>	x	x		
<i>Gloeocapsopsis magma</i>				x
<i>Gloeothece linearis</i>		x		
<i>Gloeothece vibrio</i>				x
<i>Johannesbaptistia pellucida</i>	x			
<i>Pseudocapsa maritima</i>	x	x		
<i>Pseudocapsa sphaerica</i>			x	
<i>Synechococcus</i> sp.	x	x		
<i>Synechococcus elongatus</i>	x			
<i>Xenotholos kernerii</i>			x	
<i>Leptolyngbya hypolimnetica</i>	x	x	x	x
<i>Leptolyngbya</i> sp.	x	x		
<i>Leptolyngbya tenuis</i>	x		x	x
<i>Lyngbya submonilifera</i>	x			
<i>Microcoleus cthonoplastes</i>		x		x
<i>Oscillatoria</i> sp.			x	x
<i>Oscillatoria subtilissima</i>	x			
<i>Phormidium acuminatum</i>		x		
<i>Phormidium okenii</i>		x	x	
<i>Planktolyngbya subtilis</i>	x	x		
<i>Schizothrix friesii</i>		x		x
<i>Spirulina meneghiniana</i>	x			
<i>Spirulina subsalsa</i>	x	x	x	
<i>Spirulina subtilissima</i>	x			x

Table 3. Diversity index, equitability and richness to the mats type 1 and type 2 of Brejo do Espinho and Araruama saline.

Field site	microbial mat	Shannon H'	Equitability	Richness
<i>Brejo do Espinho</i>	Type 1	1.95	0.57	30
	Type 2	2.16	0.65	27
<i>Araruama Saline</i>	Type 1	2.24	0.79	17
	Type 2	2.08	0.75	16

Locations where samples were collected are considered as intermediate salinity tanks (above 100‰ and below 150‰), having structures in development, as noted by [21] in saline for most authors, by [23,24]. In saline of Cabo Frio, Rio de Janeiro.

Microbial mats type 1, probably are found in evaporation reservoirs with higher salinity for presenting on its surface evaporate precipitation.

Microbial mats analyzed showed distinct cyanobacterial composition in different salinity regimes which explains the low number of species found in common in *Brejo do Espinho* and *Araruama* saline corroborating [25] in microbial mats of Arabian Gulf.

Individual filamentous *Microcoleus chthonoplastes* common in microbial mat type 2 are considered as prominent phototrophic organisms composing these structures. This species was not considered abundant as observed in saline of Guerrero Negro, Mexico [26], the saline of Giraud in southern France [27] and Puerto Rico [28].

The apparent abundance of this species is cited by several authors for their biovolume occupying an area greater microbial mat when compared with other species of cyanobacteria [29].

The spherical forms of cyanobacteria were the major constituents in microbial mats of the *Brejo do Espinho*, being also abundant in the structures of *Araruama* saline.

The predominance of spherical forms were also observed by [30] from microbial mats Salar Llámara (Atacama Desert, Chile) and [31] for microbial mats of Saline Cabo de Gata that corroborate by mentioning spherical species that are adapted to hypersaline environments.

The greater abundance of *Aphanothecete stagnina* in microbial mats of *Brejo do Espinho* in mats type 2 indicates that this species is hardy and well adapted to conditions of high salinity [32]. In tanks, this species showed no significant relative abundance, being found only in the microbial mat type 2.

[23] considered this species as resistant to high salinity, being found in Salines of Cabo Frio, Rio de Janeiro, Brazil.

The genre *Aphanothecete* is reported as a major photosynthetic groups in hypersaline mats of Guerrero Negro (Mexico), Solar Lake (Sinai) and Salines of Giraud (France) by [33]. This genre was also present in microbial mats of brackish environments in the Caribbean ac-

cording [34].

The high values of relative abundance of *Chroococcus* sp. in mats type 1 of *Brejo do Espinho* is corroborated by the presence of the genre *Chroococcus* in microbial mats of several lagoons in Belize (Caribbean) [34], in Camargue (Iberia) [35] and Ebro Delta (Iberia) [36].

The high relative abundance of filamentous cyanobacteria of the genre *Leptolyngbya* found on the surface of microbial mats in *Araruama* saline contributes to the growth of the structure, as well as colony formation by spherical shapes genre *Aphanothecete* and *Chroococcus* found abundantly in mats of *Brejo do Espinho*, that showed thicker than those found in saline. In distinct environments, different cyanobacteria can occupy similar ecological niches contributing to the formation of microbial mats [37].

Spherical and filamentous species of cyanobacteria promote the formation of upper layers from the trapping of sediment particles that are deposited on the structure.

The spherical species that dominate the surface are organized in colonies, producing in conjunction with the filamentous species relevant quantities of EPS which assist in capturing and trapping of sediment according to [38].

In addition, [39] noted that the arrangement of the spherical cells in colonies serves to provide protection in environments of intense solar radiation.

The predominant species in the spherical surface of microbial mats of the *Brejo do Espinho* possibly resulted in yellowing of its upper stratum evidenced by high carotenoid production for protection of hydrous and luminous stress [30,33,40].

[41] mentions that microbial mats show high microbial diversity, agreeing with the high values of diversity and equitability found in microbial mats type 1 and 2 of *Brejo do Espinho* and *Araruama* saline.

Biodiversity depends on the salinity gradient, having a reduction of microbial diversity with its increase. However, in microbial mats of hypersaline environments the diversity for phototrophic oxygenic organisms is high with the occurrence of *Microcoleus chthonoplastes* and the genera *Phormidium* according to [27].

In this research, mats type 2 is an example of diversity and the presence of these groups.

Cyanobacterial diversity and equitability were higher in saline than in the *Brejo do Espinho*, which according

to [25] may be related to environmental conditions more stable in saline.

In *Brejo do Espinho* was found in mats type 1 the lowest values of diversity and equitability. These structures were observed under conditions of high salinity variation with predominance of species *Chroococcus* sp. and *Leptolyngbya* sp., which are probably the most favored and adapted to these environmental conditions.

Brejo do Espinho got diversity values similar to [26] in natural saline of Guerrero Negro, Mexico.

The ecological study of the composition of cyanobacteria based on classical taxonomy presented limitations. Groups of prokaryotes with similar morphologies may represent distinct phylogenetic entities [26]. However, cyanobacteria are a special case, as its botanical name is based on morphological preceding bacterial identification. In fact, the diversity and richness of cyanobacterial populations are estimated by morphology in ecological studies of phytoplankton [28].

6. Conclusions

In microbial mats type 1 and 2 of the *Brejo do Espinho* and *Araruama* saline, few species of cyanobacteria were common indicating relationship between the distribution of species and different salinity gradients.

The floatability environmental of *Brejo do Espinho* favored spherical forms with a predominance of genres *Chroococcus* Nageli 1948 and *Aphanothecace* 1948.

The stability in salinity of *Araruama* saline set higher values of diversity and dominance of filamentous species of the genre *Leptolyngbya* Anagnostidis and Komárek 1988 in the formation of these structures.

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