



# OCCURRENCE OF INTENSIVE BLOOMS OF CYANOBACTERIA

## *Microcystis aeruginosa* IN A TROPICAL ESTUARY

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**RESUMO.** As amostras foram coletadas em um perfil longitudinal do estuário do rio Jaboatão, cobrindo a área marinha (pluma estuarina) através de quatro estações no estuário e quatro na pluma durante as estações seca e chuvosa, com o objetivo de avaliar a ocorrência e a distribuição da cianobactéria *Microcystis aeruginosa*. Em ambas as regiões, foi possível observar a influência de *M. aeruginosa* na diversidade local, que foi considerada de muito baixa a baixa, onde esta espécie foi dominante. *M. aeruginosa* representou, em média, 95% do total de cianobactérias no estuário, enquanto na pluma esse percentual atingiu 65%. A espécie *M. aeruginosa* foi responsável pela predominância de cianobactérias, tanto no estuário quanto na pluma. As razões N/P no estuário e a pluma foram <16:1, indicando N como um fator limitante. Uma questão-chave na ciência da eutrofização é o potencial das cianobactérias fixadoras de N compensarem qualquer deficiência no N biologicamente disponível, principalmente durante o período seco. As águas com alta temperatura, ricas em nutrientes e com pouco oxigênio contribuíram para a abundância e formação da floração de *M. aeruginosa*. Os valores positivos de AOU (média: +2,7 e +2,1 ml/L, para os períodos úmido e seco, respectivamente) indicaram altas taxas de respiração.

**Palavras-Chave:** Cianobactéria, Floração Algal, Nutrientes, Estuário Tropical, Brasil.

**ABSTRACT.** Samples were collected in a longitudinal profile from the Jaboatão River estuary, covering the marine area (estuarine plume) through four stations in the estuary and four in the plume during the dry and rainy seasons, with the objective of evaluating the occurrence and the distribution of *Microcystis aeruginosa* cyanobacterial blooms. In both regions, it is possible to observe the influence of *M. aeruginosa* on the local diversity, which was considered to range from very low to low where this species was dominant. *M. aeruginosa* represented on average 95% of the total cyanobacteria in the estuary, while in the plume, this percentage reached 65%. The species *M. aeruginosa* was responsible for the predominance of cyanobacteria, both in the estuary and in the plume. The N/P ratios in the estuary and the plume were <16:1, indicating N as a limiting factor. A key issue in eutrophication science is the potential for N-fixing cyanobacteria to compensate for any deficiency in biologically available N, principally during the dry period. The high-temperature, nutrient-rich and polluted waters with little oxygen contributed to the abundance and bloom formation of *M. aeruginosa*. The AOU positive values (average: +2.7 and +2.1 ml/L, for the wet and dry periods, respectively) indicate high respiration rates.

**Keywords:** Cyanobacteria, Algal Blooms, Nutrients, Tropical Estuary, Brazil.

## INTRODUCTION

Eutrophication is the process of adding nutrients to recipient bodies and the effects of this addition. Therefore, it is a phenomenon associated with the nutritive enrichment of these bodies by substances, mainly nitrogenous and phosphorus compounds, that are organic and inorganic (Molica and Azevedo, 2009). The phytoplankton first responds to these changes in the water body, and one of the main consequences of this response is the formation of blooms.

Cyanobacteria can become dominant in a phytoplankton community in lakes, rivers and reservoirs affected by these conditions and may form these flowering (Carmichael, 1992; Tundisi, 2003). They are especially influenced by factors such as temperature increases, nutrient decreases and increases in water column stability, favoring the formation of blooms (Oberholster *et al.*, 2004). Algal flowering are phenomena that are likely to occur in natural aquatic systems, and they are sometimes due to the direct or indirect intervention of man (Torgan, 1989).

Global warming is one of the intensifiers of the occurrence of these blooms due to the increase in the average temperature of aquatic ecosystems, especially in temperate climates, promoting longer periods and thermal stratification, which favors cyanobacteria (Paerl and Huisman, 2008; Paerl and Paul, 2012). In Brazil, however, phytoplankton development periods vary by region. In the northern, north-eastern and central-western regions, they are affected by longer seasonal periods (rainy and dry seasons), which regulate the nutrient intake, considering the relative constancy of water temperature and light intensity (Esteves, 1998).

According to Meire *et al.* (2005), climatic factors that favor the emergence of blooms are high temperatures and environments without rain. The population of cyanobacteria may appear to be dense when dispersed in the water column; however, when atmospheric conditions are favorable, the cells concentrate on the surface of the water in a few hours. These can then be drawn to the edges of the water body by wind action and generate a higher concentration of cells. The decomposition of enormous amounts of biomass accumulated by these microorganisms leads to the deoxygenation of the water, causing damages in the whole ecosystem.

According to Lourenço and Marques-Júnior (2002), in addition to staining, blooms can cause the mass death of marine organisms, whether related to the total consumption of dissolved oxygen in the water column or to the fact that some algae are toxin producers. A striking fact in relation to cyanobacteria is that almost one-third of their approximately 150 genera are related to the production of potent toxins (Apeldoorn *et al.*, 2007).

The genus *Microcystis* (Lemmermann), class Cyanophyceae and order Chroococcales, is composed of cyanobacteria that potentially form large blooms and possess gaseous vacuoles that give them the ability to disperse rapidly in the water column. The consequences of this

state are a reduction in the dissolved oxygen of the water due to the increase in the metabolic activity of the aerobic bacteria responsible for the decomposition of the organic matter and the production of toxins by some species of cyanobacteria (Sivonen and Jones, 1999). The species *M. aeruginosa* (Kützing) Kützing 1846, is generally associated with toxicity problems in water, and its occurrence is indicated at several localities in south and southeastern Brazil (Takenaka *et al.*, 2007; Yunes *et al.*, 1998). Blooms of this species produce toxins and have been implicated in the mass mortality of aquatic animals and the destabilization of food webs (Oberholster *et al.*, 2004). The Jaboatão River estuary represents a very vulnerable area to the degradation provoked by the increase of urban pressure, real estate and pollution caused by domestic wastes and industrial effluents. The present work has the objective of evaluating the occurrence and the distribution of flowering of the *Microcystis aeruginosa* cyanobacteria in the estuary of the Jaboatão River and its plume.

## MATERIAL AND METHODS

### STUDY AREA

The Jaboatão river basin belongs to the group of small coastal rivers, located in the southern forest of Pernambuco (Brazil), draining areas of cities of the Metropolitan Region of Recife and contributing significantly to the water supply of the region (Moreira, 2007). Jaboatão River constitutes the most important hydrographic system, with the Duas Unas River as main tributary, where the same name dam is located and supplies part of the Metropolitan Region of Recife.

With an approximate extension of 72 km, the drainage of the basin of the Jaboatão River is quite dense, with ramifications, characteristic of the alluvial plain. Outstanding in this region is the Olho D'Água lagoon and the wetlands surrounding it (SECTMA, 1999).

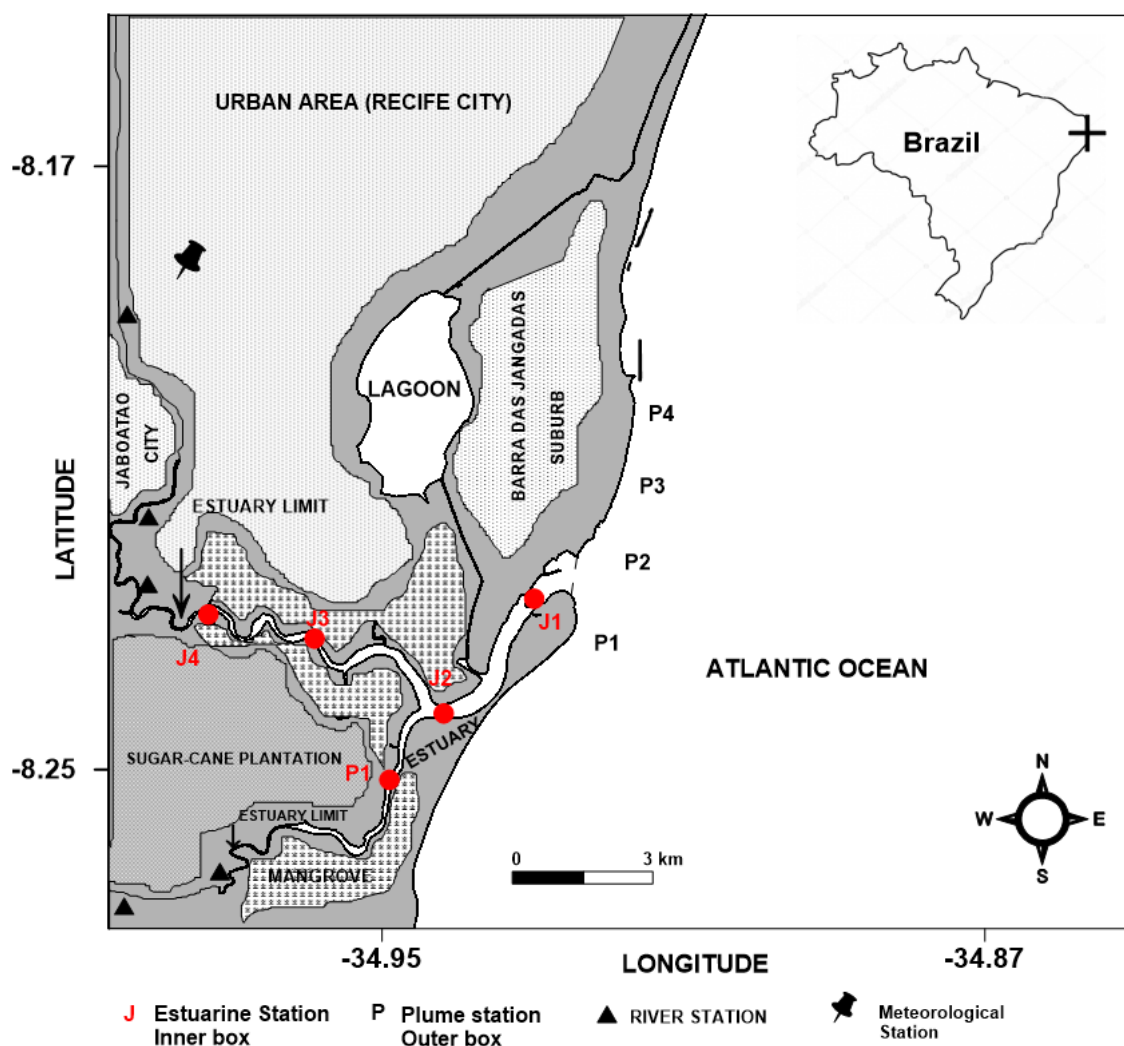
The climate is hot and humid and rainfall regime is subdivided into dry season (Sep-Feb) and rainy season (Mar-Aug). The estuary extends for approximately 14 km<sup>2</sup>, with an average depth of 2.6 m (Noriega and Araujo, 2011).

The shortage of sanitation in the vicinity of the Jaboatão river basin is the main reason for the existence of numerous pollution points in the area, adding to it the discharge of untreated industrial effluents, the lack of solid waste management and the use of pesticides and chemical fertilizers (Moreira, 2007).

Organic matter pollution by the sugar-cane agroindustry substantially increases during the harvest and milling season, which is from September to February. CPRH (2003) reported a high biochemical oxygen demand (BOD) of 69.6 mg/L in the harvest periods (Jaboatão River).

## SAMPLING AND ANALYSIS

For this study, the estuarine region was divided into 2 segments (estuary and plume) based on the longitudinal saline gradient classification proposed by McLusky (1993). Seven campaigns were carried out at low tide, in longitudinal profiles in the estuary and plume, with four stations in each, during the rainy (May 2010, July 2010, May 2011 and July 2011) and the dry seasons (November 2010, February 2011 and September 2011) (Fig. 1).



**Figure 1.** Sampling stations located in the estuary and plume of the Jaboatão River (northeastern Brazil). The segmented region indicates the plume's box boundary.

The samples for the phytoplankton study were collected with a Niskin oceanographic bottle, fixed with a Lugol solution 1% and analyzed according to the sedimentation method of Utermöhl (Edler, 1979; Ferrario *et al.*, 1995). The abiotic and Apparent Oxygen Utilization (AOU) data used was those described by Silva *et al.* (2017) to the same location.

## METEOROLOGICAL DATA

The rainfall data were obtained through the website of the Pernambuco State Agency for Water and Climate (APAC) and the National Institute of Meteorology (INMET), for the months studied, as well the historical average.

## STATISTICAL ANALYSES

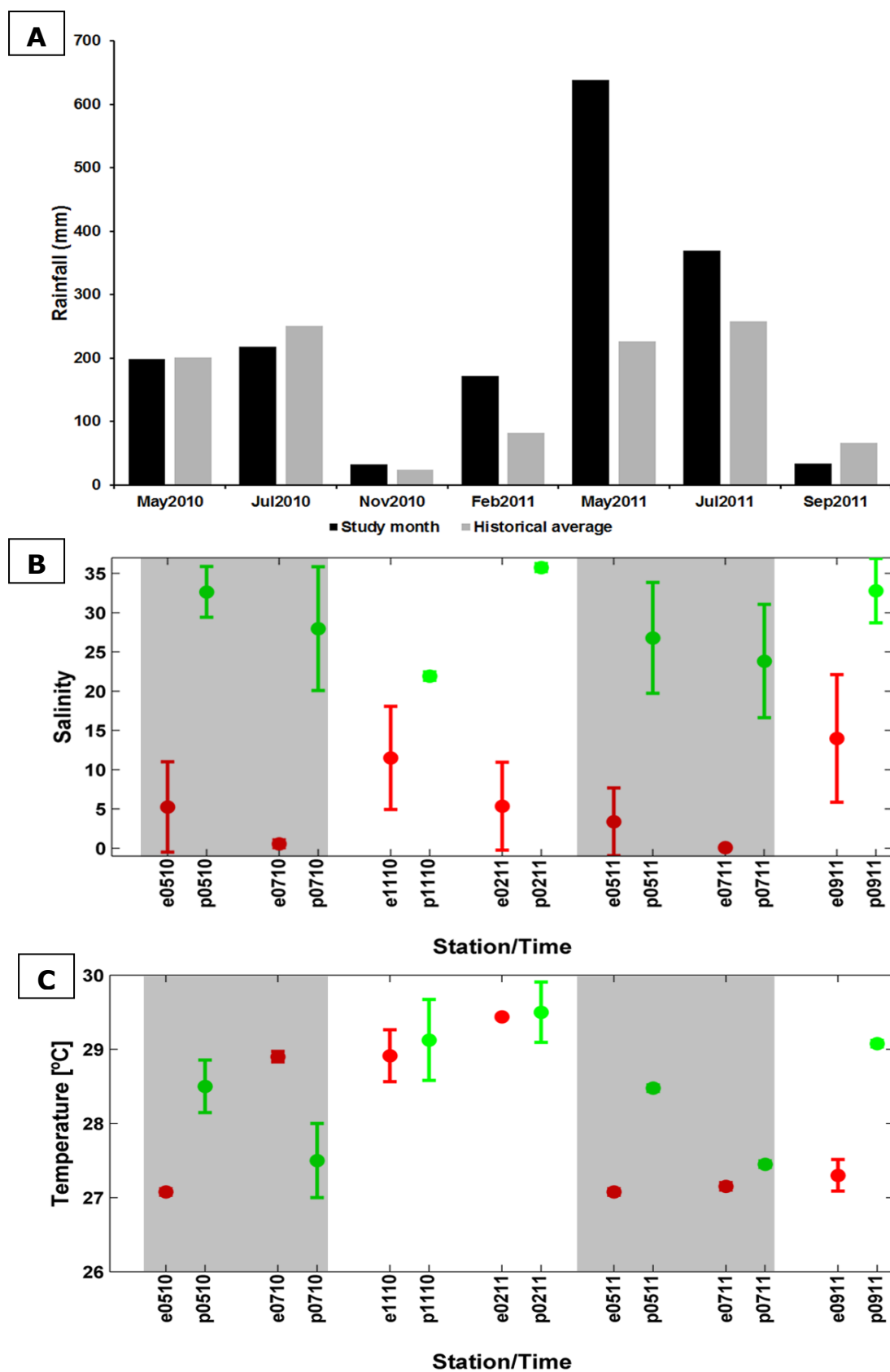
The Principal Components analysis (PCA) was based on the hydrological parameters and the *M. aeruginosa* cell density, applying the Pearson's moment-to-product correlation coefficient, with the self-value of the main components and the auto-vector being extracted. For the tests, the program PRIMER 6® (Plymouth Routines in Multivariate Ecological Research) was used.

## RESULTS

### RAINFALL, PHYSICAL AND CHEMICAL FACTORS

Total monthly rainfall ranged from 32 mm (Nov 2010) to 639 mm (May 2011), following the historical trend for the region (Fig. 2A). The study period did not show significant differences with the historical values recorded for the same months ( $t$ -test;  $P = 0.38$ ;  $\alpha = 0.05$ ). The salinity showed no significant difference between the rainy and dry periods, but it varied significantly between the two study regions (estuary and plume) (Fig. 2B, Table 1).

Temperature showed significant differences between wet and dry periods, however, no significant differences between the estuarine and plume regions (Fig. 2C, Table 1). Table 1 presents the climatic, physical and chemical parameters evaluated by Silva *et al.* (2017) for the same place and period of study.



**Figure 2.** Rainfall (A) - study period and historical average; salinity (B); surface temperature (C) - in the estuary and plume of the Jaboatão River. The gray region indicates the wet period. e= estuary, p=plume. The 4 digits in the axis of the abscissa indicate: month and year, respectively.

**Table 1.** Statistical *t*-test for the categories. The means with significant differences are in bold ( $\alpha = 0.05$ ). The average and standard deviation values are between brackets.

Category Parameters	Wet period	Dry period	P valor	Estuary	Plume	P valor
Secchi disk (m)	(1.1±1.2)	(1.4±1.1)	0.37	<b>(0.5±0.2)</b>	<b>(2.2±1.1)</b>	<b>0.0001</b>
Temperature (°C)	<b>(27.7±0.7)</b>	<b>(28.8±0.8)</b>	<b>0.0001</b>	(28.0±1.0)	(28.4±0.8)	0.046
Salinity (psu)	(14.5±14)	(19.0±12.2)	0.24	<b>(5.7±7.2)</b>	<b>(27.7±7.7)</b>	<b>0.0001</b>
DO (ml/L)	(2.8±1.6)	(3.2±1.7)	0.45	<b>(1.6±1.1)</b>	<b>(4.4±0.3)</b>	<b>0.0001</b>
pH	(7.7±0.7)	(7.8±0.6)	0.83	<b>(7.2±0.4)</b>	<b>(8.2±0.3)</b>	<b>0.0001</b>
Ammonia (µmol/L)	(1.9±2.8)	(1.0±1.3)	0.12	<b>(2.7±2.8)</b>	<b>(0.2±0.3)</b>	<b>0.0001</b>
Nitrite (µmol/L)	(0.6±0.6)	(0.8±1.1)	0.36	<b>(1.0±1.0)</b>	<b>(0.2±0.3)</b>	<b>0.0001</b>
Nitrate (µmol/L)	(5.4±4.1)	(6.0±8.5)	0.73	<b>(8.8±7.2)</b>	<b>(2.4±2.0)</b>	<b>0.0001</b>
Phosphate (µmol/L)	(1.8±2.0)	(1.5±2.0)	0.57	<b>(2.9±2.1)</b>	<b>(0.4±0.3)</b>	<b>0.0001</b>
Silicate (µmol/L)	(63.0±53.0)	(42.1±45.0)	0.14	<b>(81.1±52.0)</b>	<b>(26.0±30.0)</b>	<b>0.0001</b>
Cyanobacteria (cell/L)	<b>(786.0±800.0)</b>	<b>(270.0±380.0)</b>	<b>0.006</b>	(626.0±617.0)	(514.0±780.0)	0.56
<i>M. aeruginosa</i> (cell/L)	<b>(764.0±810.0)</b>	<b>(259.0±360.0)</b>	<b>0.008</b>	(602.0±609.0)	(502.0±790.0)	0.60
Bacillariophyta (cell/L)	<b>(15.0±16.0)</b>	<b>(65.0±70.0)</b>	<b>0.0002</b>	(44.0±66.0)	(27.0±33.0)	0.24
Chlorophyta (cell/L)	(0.2±0.9)	(1.7±7.3)	0.25	(1.6±6.6)	(0.1±0.3)	0.24
Rainfall (mm)	<b>(356.0±179.0)</b>	<b>(75.0±65.0)</b>	<b>0.0001</b>	-	-	-

### BIOLOGICAL FACTORS: PHYTOPLANKTON

Considering the contribution percentage of the phytoplankton community recorded, the phylum Bacillariophyta represented the majority (55%), with Cyanobacteria representing 11%. There was no significant differences between the estuarine (*t*-test;  $P = 0.24$ ;  $\alpha = 0.05$ ) and plume regions (*t*-test;  $P = 0.56$ ;  $\alpha = 0.05$ ) (Figs. 3A and 3B). However, considering total cell count cyanophyte group was higher in both studied regions, making 93% of the total cells in the estuary and 97% in the plume. Bacillariophyta did not reach 10% of the total in both regions (Figs. 3C and 3D).

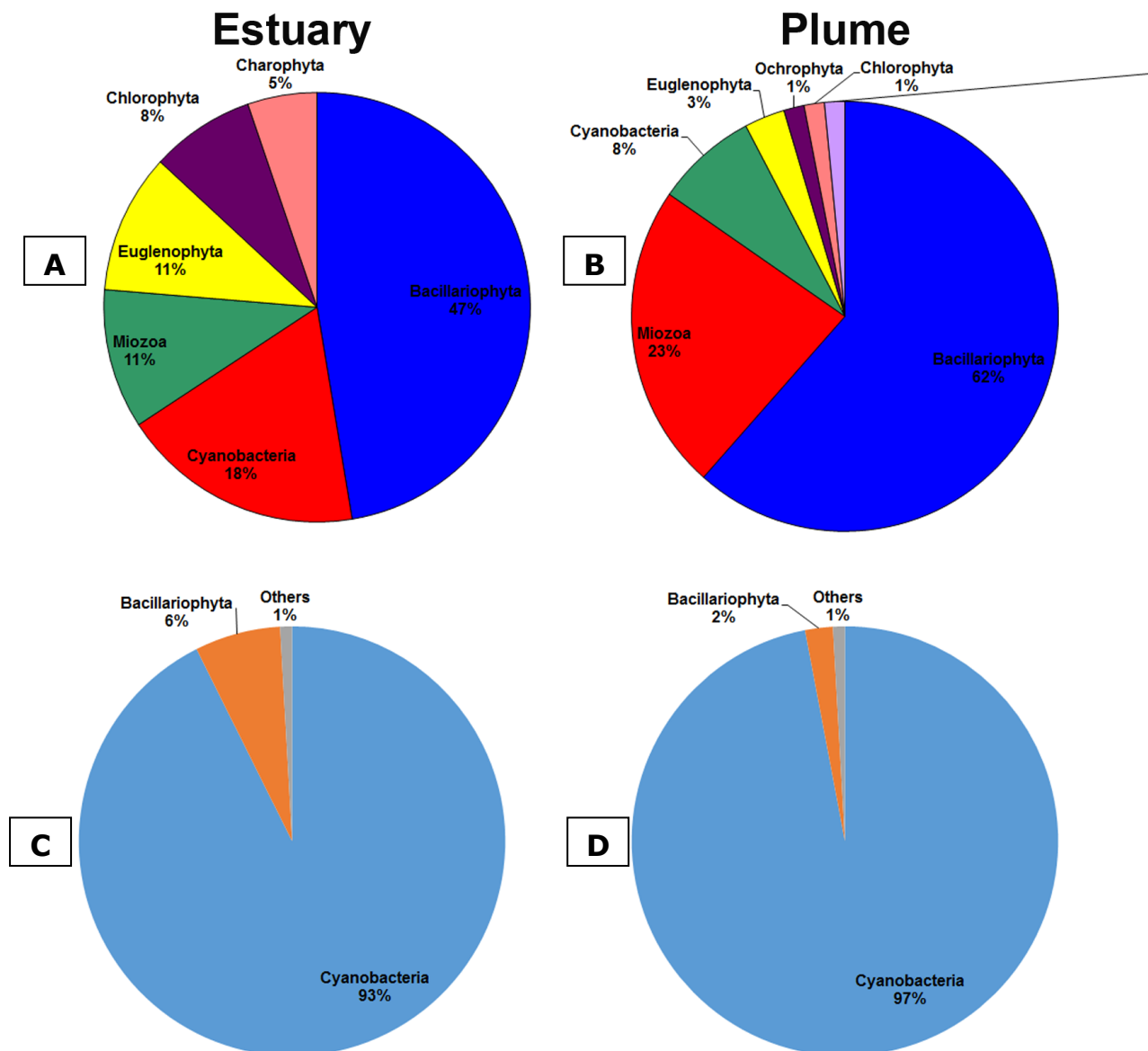
In the estuary, only two species were dominant: the cyanobacteria *M. aeruginosa* Kützing and the bacillariophyta *Cyclotella meneghiniana* Kützing, with the first occurring in all stations and months in the estuary (Fig. 4A). In Figures 4A and 4B, we observed these two species alternating dominance in the system, especially in the plume region during the dry period.

*M. aeruginosa*, *Planktothrix agardhii* (Gomont) Anagnostidis & Komarek, *Lepocylindrus danicus* (O. F. Muller) Marin & Melkonian, *Protoperdinium bispinum* (Schiller) Balech, *Coscinodiscus centralis* Ehrenberg, *Cyclotella meneghiniana* and *Paralia sulcata* (Ehrenberg) Cleve dominated the plume (Fig. 4B). It is possible to observe *M. aeruginosa* and *C. meneghiniana* alternating dominance in the system, with this more evident in the plume (Figs. 4A and 4B).

Also, *M. aeruginosa* represented on average 95% of the total cyanobacteria in the estuary, while in the plume, this percentage reached 65%. This species was responsible for the predominance of cyanobacteria, both in the estuary and in the plume (Fig. 5).

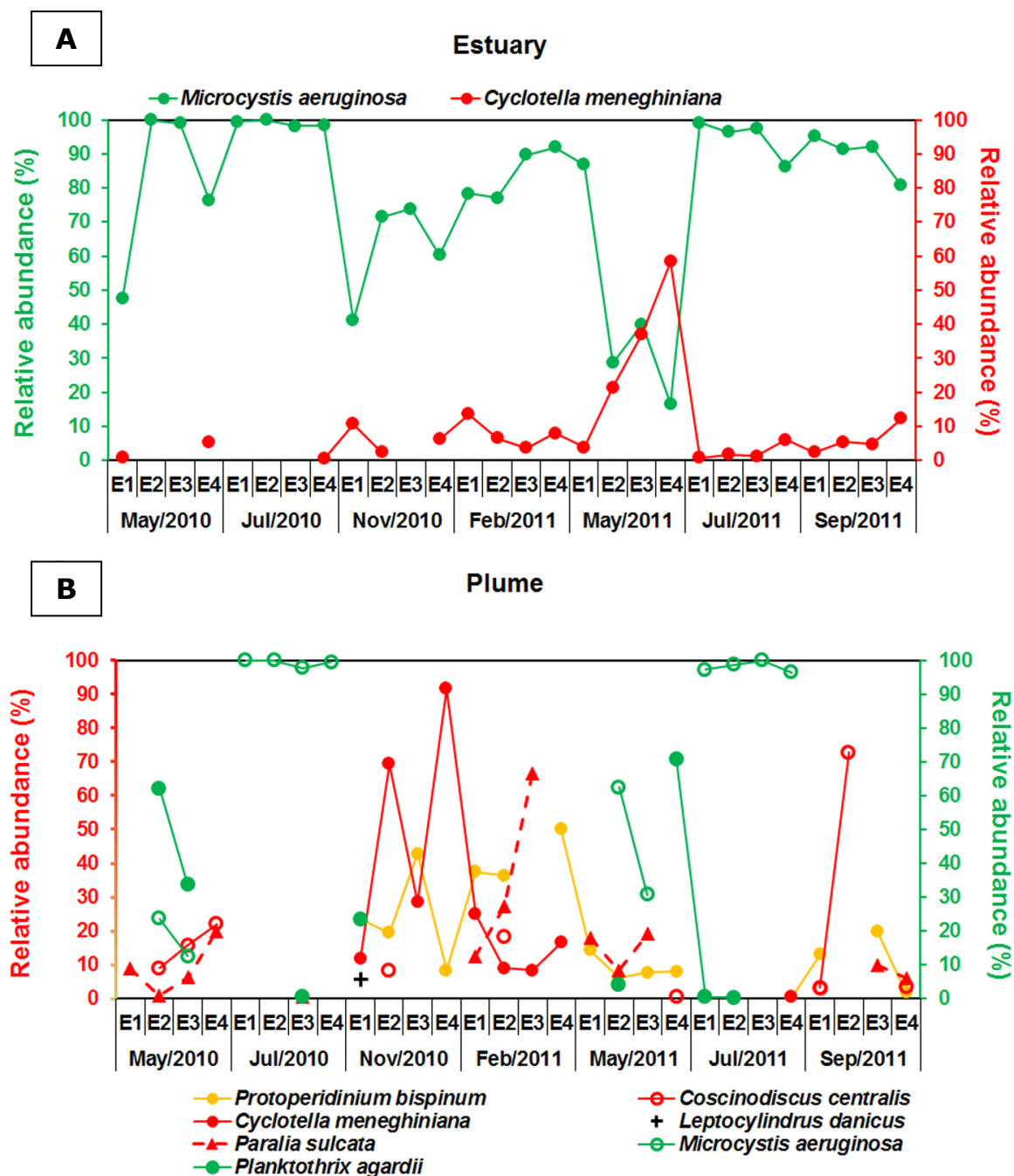


In both regions, it is possible to observe the influence of *M. aeruginosa* on the local diversity. Where this species dominated, the specific diversity varied from very low to low (Figs. 6A and 6B).

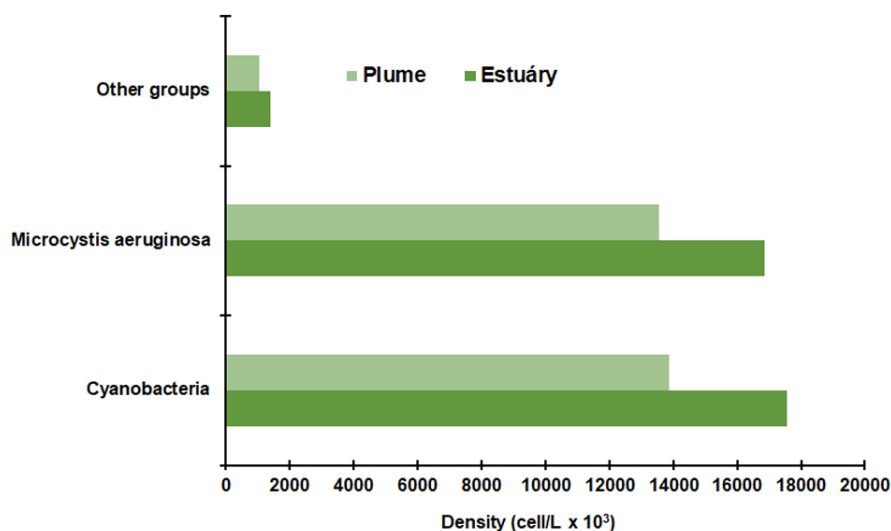


**Figure 3.** Percentage of occurrence of the phyla (A-B) and total cells (C-D) in each studied area.

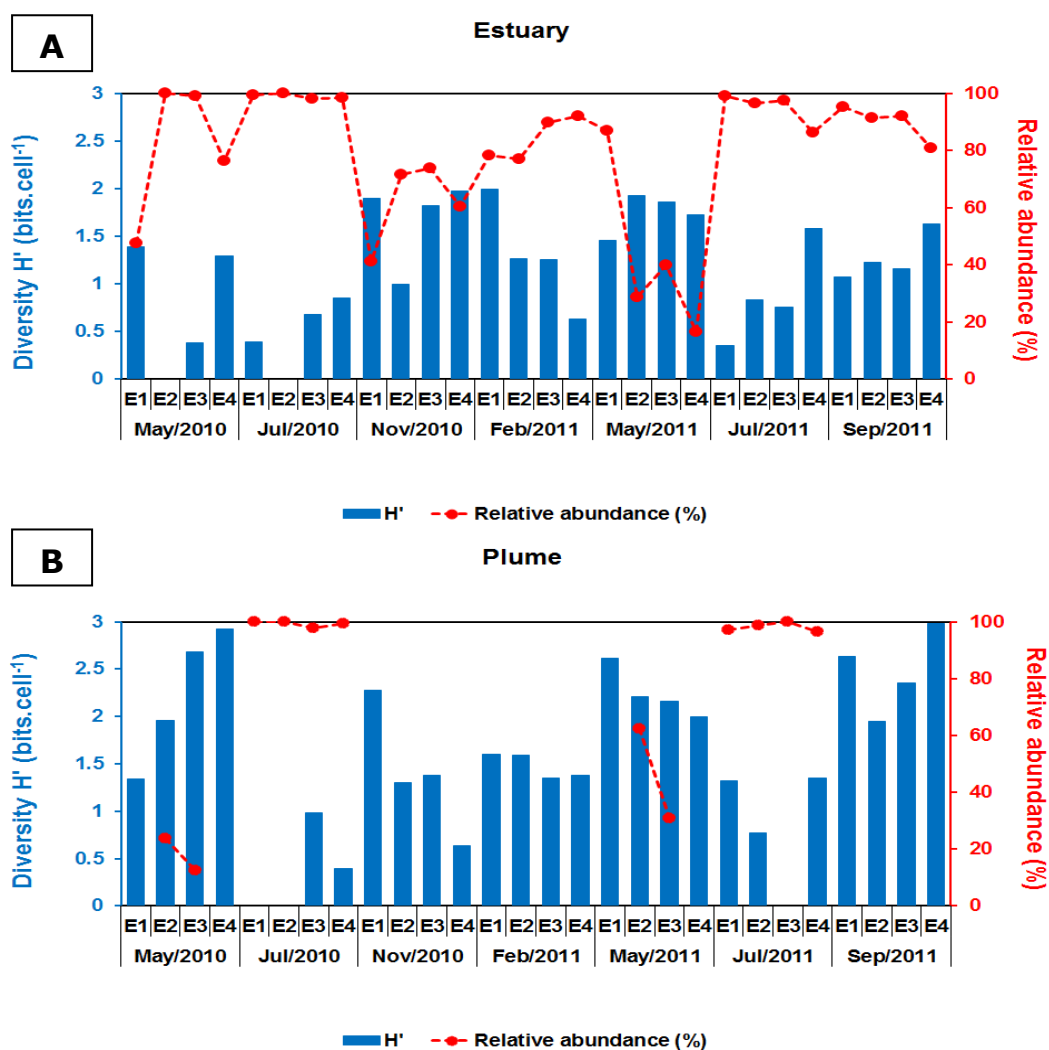




**Figure 4.** Relative abundance (%) of the most representative species in the studied areas (Silva et al., 2017).



**Figure 5.** Total density of *Microcystis aeruginosa* in the studied areas, in comparison with total density of cyanobacteria and the other groups.



**Figure 6.** Relative abundance and diversity index of *M. aeruginosa* in the studied areas.

## DISCUSSION

### PHYSICAL AND CHEMICAL FACTORS

Changes in the composition and structure of the phytoplankton community can cause profound changes in all trophic levels, considering the dynamic of these organisms, their high reproduction rates and rapid response to changes in environmental conditions. This importance is even more relevant when there are processes of eutrophication of aquatic ecosystems, through massive blooms and quantitative changes in their populations (Esteves, 1998; Ricklefs, 2003; Valiela, 1995).

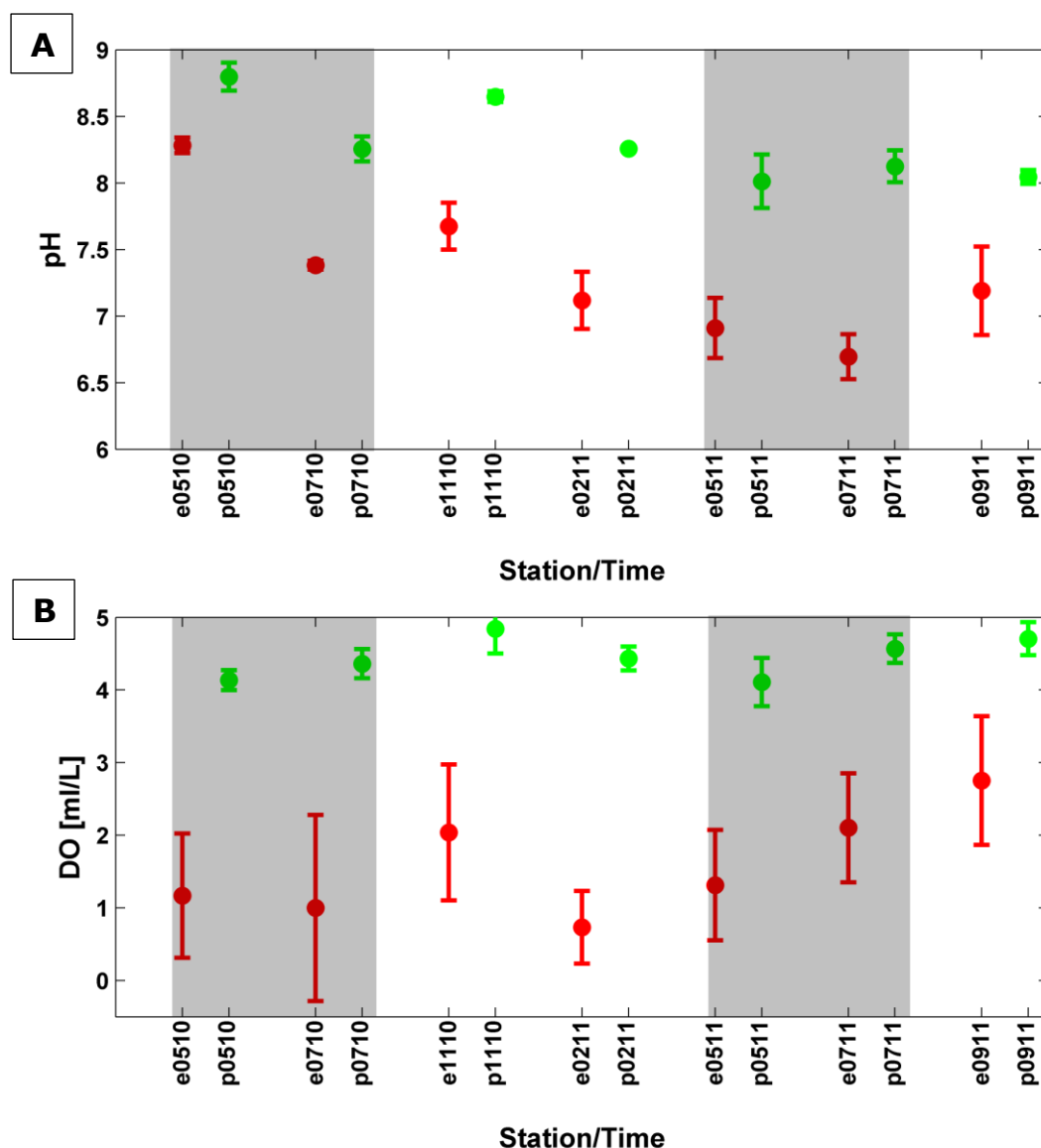
The lack of planning in the use and occupation of the soil in the Jaboatão river basin has caused a degradation in the environmental quality, with one of the main consequences the degradation of the quality of the superficial water resources, characterized by the discharge of urban and industrial wastewaters and solid residues in their water bodies, and runoff water from agro-industrial areas (Gomes *et al.*, 2003).

In addition, another important contribution of organic load to the estuarine system is the presence of Lagoon Olho d'Água, with an area of 3.7 km<sup>2</sup> (see Fig. 1), a population of 35,000 and a low environmental sanitation coverage. According to CPRH (2010), the remaining organic load for the Jaboatão River was 20.58 t BOD / day, while the organic load (BOD) calculated for the urban area around the lagoon was 756 kg / BOD / day. This value represents approximately 4% of the total generated by the urban area surrounding the estuary system of the Jaboatão River.

DO values obtained for the Jaboatão River estuary showed lower values than those recommended by Brazilian legislation (3.5 ml/L). During the wet period observed, no sample in the estuarine region reached the required minimum value (3.5 ml/L). Throughout the year, 7% of the samples reached this limit, which shows low water quality and a change in the processes of oxidation, decomposition and cycling of organic matter in the aquatic system (Fig. 7).

Analyzing data from nitrogen compounds from 2000 to 2011 obtained from the monitoring station of the CPRH near the estuary shows that during this period there was an increase in the concentrations of nitrogen compounds (mainly NH<sub>4</sub><sup>+</sup>) entering the estuary system of the Jaboatão River during the dry period. This positive trend for the dry season can be observed in Figure 9A. The PO<sub>4</sub><sup>-</sup> did not show a trend according to the data obtained for the dry and rainy periods between 2001 and 2011.

In addition, we use demographic data (population density) obtained from IBGE (2011) for the main city (Jaboatão dos Guararapes) adjacent to the aquatic system studied. We observed that the population growth rate of this municipality was 11%, and the population density grew from 2,249 inhab/km<sup>2</sup> in 2000 to 2,513 inhab/km<sup>2</sup> in 2011. Population growth is the main direct input of nitrogenous and phosphate compounds to the adjacent aquatic system.



**Figure 7.** Surface pH (A) and DO (B) in the estuary (red) and plume (green) of the Jaboatão River. The gray region indicates the wet period. e= estuary, p=plume. The 4 digits in the axis of the abscissa indicate: month and year, respectively.

During the dry season, the river discharge also decreases. According to Noriega and Araujo (2011), 75% of the annual rainfall occurs during the wet season in this region, and the residence time in the dry period can reach 13 days in the estuarine system. According to Smith (2003) and Dodds and Smith (2016), in some rivers and streams with reduced water replacement times, phytoplankton blooms can become problematic, with cyanobacterial blooms more likely in excess-nutrient conditions.

These factors directly affect the water quality of this system, increasing the nutrient load and causing changes in the biota of the site. These observations showed that the anthropic factor added to climatic factors can generate important changes in the phytoplankton biomass

of the estuarine system and may be mainly responsible for the change in the dominant species of this aquatic system.

*M. aeruginosa* predominated at most estuarine stations, with blooms in July 2010 and September 2011. In the plume region its occurrence was punctual, with blooms in July 2010 and 2011.

It was observed an alternating dominance between *M. aeruginosa* and *Cyclotella meneghiniana* in the plume region during the dry period. During the month of greatest density for *Cyclotella meneghiniana* (November of 2010),  $\text{NO}_3^-$  was the element available in the water column. Other elements such as  $\text{NH}_4^+$  and  $\text{PO}_4^-$  had low concentrations (Fig. 8).

This alternation entails changes in the relative abundance of species in a community, in a typical sequence. These organisms respond to a rapid increase in the availability of limiting nutrients by increasing their growth rate and changing their composition favoring the fastest growing species (r-strategists). With the depletion of nutrients, the relative abundance of species changes again, favoring species with adaptations for nutrient scarcity (k-strategists) (Lewis Jr., 1978).

In this case, if it is possible to identify the direction of planktonic succession, then qualitative changes through increase or decrease of nutrients concentration can be predicted. However, environmental disturbances can lead to a complete or partial initial return, with high levels of nutrients, causing abrupt change in community composition. This constitutes a reverse direction, a fact common to estuarine areas.

As in highly dynamic systems species adaptability is an important factor, different specific sets of the community reach high rates of growth at specific moments. Thus, the temporal distribution patterns would not reflect the phytoplankton community as a whole, but of certain populations that respond to fluctuations of certain environmental factors (Scott and Marcarelli, 2012), that is the case of *M. aeruginosa*.

There is no consensus on an operational definition of flowering, and it is agreed that blooms cannot be characterized by a single universal criterion of cell density or biomass. The biomass value that characterizes flowering differs from species to species; it is only possible to determine if the population of a species is flowering or not when its relative contribution is evaluated within the totality of the community (CETESB, 2007), which in the case of the present study, can be understood as the direct influence on local diversity and species richness.

Omori and Ikeda (1984) report that specific diversity indicates the degree of complexity of community structure, decreasing with the dominance of one or a few species, with individuals of rare species replaced by individuals of more common species, or when some species reproduce more quickly.

High density of phytoplanktonic organisms against low species richness suggests that the habitat received a polluting load, favoring organisms tolerant of this condition (Black *et al.*, 2011; Esteves, 1998). This was evidenced both in plume and estuary, where the occurrence of other species was limited by *M. aeruginosa*.

Other researches in the same region (Branco 2002; 2007; Lacerda *et al.*, 2004) point the diatoms *Belleriochea malleus*, *Coscinodiscus centralis* and *Cyclotella meneghiniana* as the key species for that ecosystem. In the present study, *M. aeruginosa* is the key species, given its dominance, frequency and high cell density.

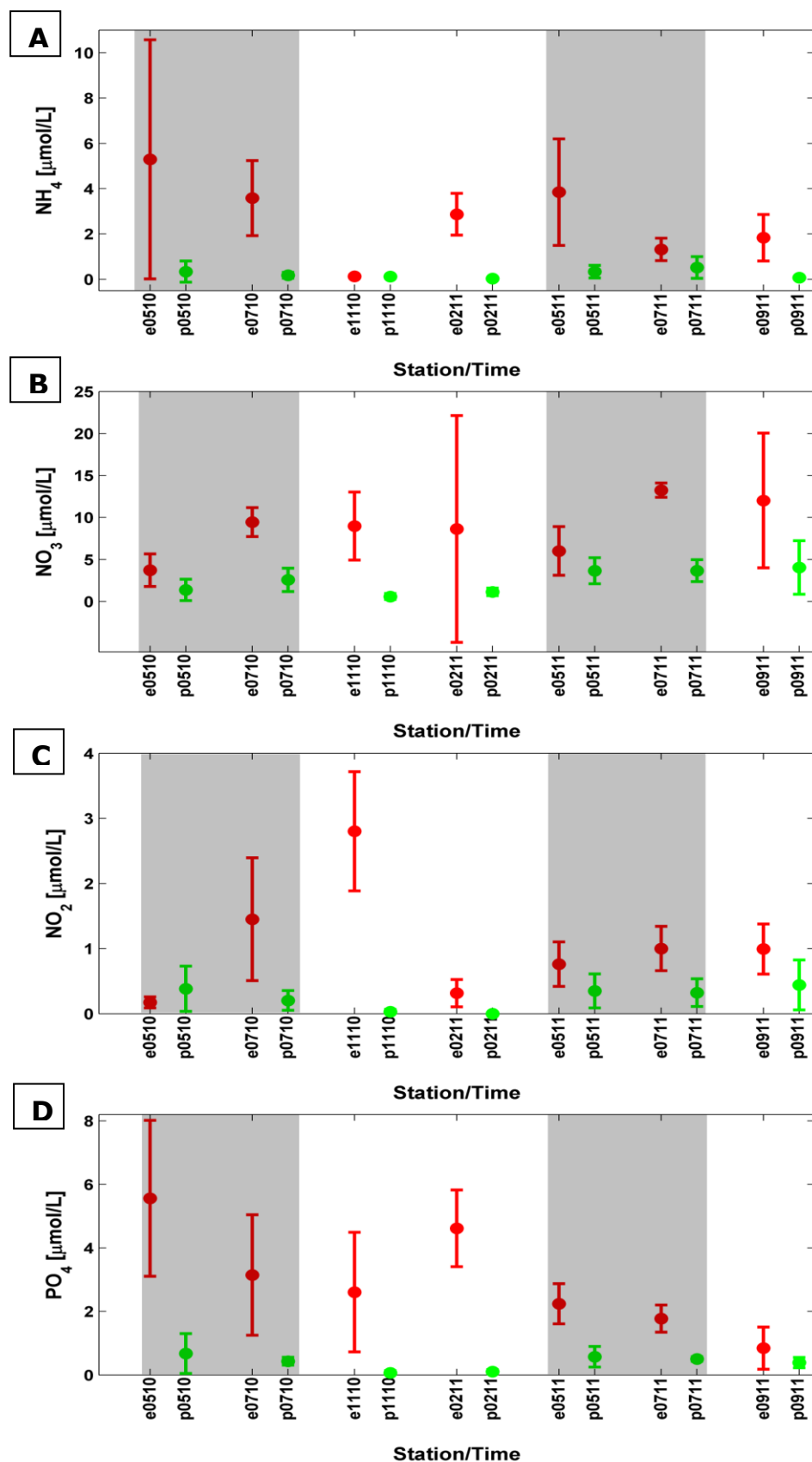
### **AOU (APPARENT OXYGEN UTILIZATION)**

AOU was used to obtain the apparent respiration rate in the estuarine system, where DO is consumed and CO<sub>2</sub> is released into the water column. The increase in algal blooms can produce a reduction in the DO of the water due to the increase in the metabolic activity of the aerobic bacteria responsible for the decomposition of the organic matter and the production of toxins by some species of cyanobacteria. AOU indicates the apparent variations between production and respiration. Positive values were observed and were indicative of high respiration rates (average: +2.7 and +2.1 ml/L, for both climatic periods, respectively) (Fig. 9B).

The *Microcystis* species also requires high light irradiation (Havens *et al.*, 1998). In temperate reservoirs, the *M. aeruginosa* species appears in the water column at the end of spring and forms blooms during the summer. After this excessive growth, it sinks and reaches the sediment in the autumn (Latour and Giraudet, 2004). On the other hand, *M. aeruginosa* can be considered a species adapted to store phosphorus, with a high capacity to absorb inorganic phosphorus (Olsen, 1989).

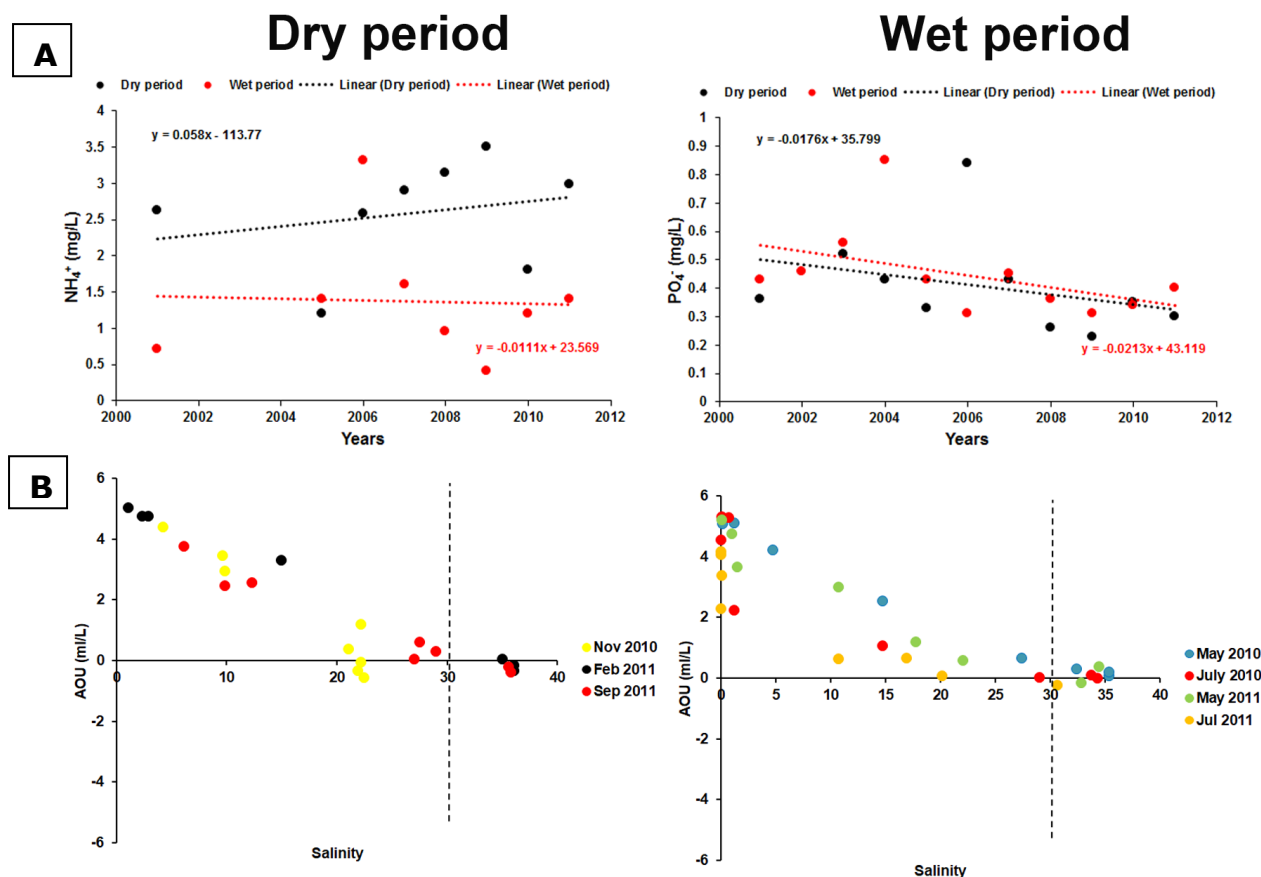
The concentrations of PO<sub>4</sub><sup>-</sup> observed in this study were considered high and outside the limit of the environmental resolution (0.1 mg/L~1.6 mol/L) in both climatic periods.

Finally, we suggest that the system varies between autotrophy (production) and heterotrophy (respiration) during the year due to the rainfall regime, human activities in the basin (density population and sugarcane plantations), and associated DIP and DIN riverine loads.



**Figure 8.** Concentrations of the dissolved nutrients in the two studied areas: ammonia (A); nitrate (B); nitrite (C) and phosphate (D). The gray region indicates the wet period. e= estuary (red), p=plume (green). The 4 digits in the axis of the abscissa indicate: month and year, respectively.





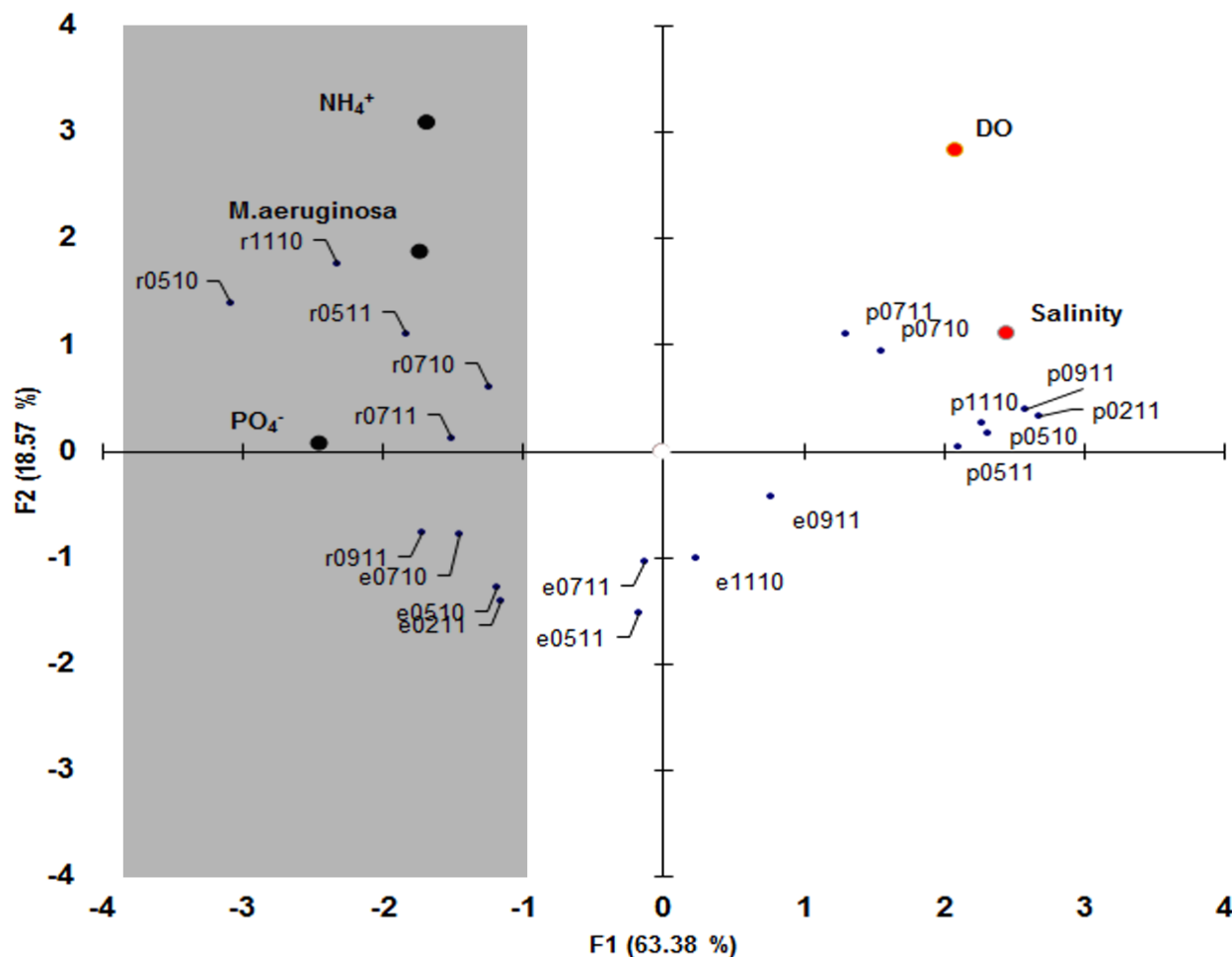
**Figure 9.** Tendency of nitrogenous and phosphate compounds in the Jaboatão River between 2001 and 2011 (A); AOU (B) (the segmented line indicates the division between the estuary and plume).

### PRINCIPAL COMPONENT ANALYSIS (PCA)

We used the estuarine, plume and river observations of the main nitrogenous ( $\text{NH}_4^+$ ) and  $\text{PO}_4^-$ . The river data were provided by the State Environmental Agency (CPRH) for the studied period. Additionally, we included observations of the salinity, DO and density of *M. aeruginosa*.

There was observed a direct association between the nutrient ( $\text{NH}_4^+$  and  $\text{PO}_4^-$ ) from the river and estuary and *M. aeruginosa*. For the plume region the observations obtained showed an association with DO values and salinity. This explains the dominance of other species in the plume, which presented waters well oxygenated, saline and with low concentration of nutrients, evidencing the influence of the marine flow in the area.

The fluvial influence of  $\text{NH}_4^+$  and  $\text{PO}_4^-$  correlates directly with this species of Cyanobacteria. According to this statistical analysis and trend analysis (Figs. 9 and 10), we can conclude that the increasing nutrient load (mainly  $\text{NH}_4^+$ ) for the estuarine region offers a favorable substrate for these cyanobacteria, affecting the spatial and temporal biodiversity of the aquatic system.



**Figure 10.** PCA of the chemical, physical and biological parameters. The black circles indicate the nutrients and *M. aeruginosa*; the red circles indicate the salinity and DO. The gray region indicates the positive correlation in Factor 1 between *M. aeruginosa* and the nutrients ( $\text{NH}_4^+$  and  $\text{PO}_4^-$ ). The codes of the observations indicate (r) = river; (e) = estuary and (p) = plume. The number labels for the observations indicate the month and year of the sampling data.

In conclusion, it is possible to observe both in the estuarine and plume regions, the influence of *M. aeruginosa* on the local diversity. *M. aeruginosa* represented on average 95% of the total cyanobacteria in the estuary, while in the plume, this percentage reached 65%. The species *M. aeruginosa* was responsible for the predominance of cyanobacteria, both in the estuary and in the plume. The DO values obtained, which are lower than recommended by Brazilian legislation (3.5 ml/L), show low water quality and a change in the processes of oxidation, decomposition and cycling of organic matter in the aquatic system.

The high values of the nitrogen compounds and phosphate evidenced the high degree and the strong influence of the anthropic action in that environment. The concentrations of  $\text{PO}_4^-$  observed in this study were considered high and outside the limit of the environmental resolution, suggesting that the system varies between autotrophy and heterotrophy during the

year due to the rainfall regime, human activities in the basin and the associated DIP and DIN riverine loads. The N/P ratio is a factor that plays an important role in the occurrence of *M. aeruginosa* in the aquatic system. The N/P ratios in the estuary and the plume were <16:1, indicating N as a limiting factor.

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## REFERENCES

- Agência Estadual de Meio Ambiente (CPRH) (2003), Report on the monitoring of water quality in the watersheds of the State of Pernambuco in 2003, in: <http://www.cprh.pe.gov.br/> (access in 01 of September of 2018).
- Agência Estadual de Meio Ambiente (CPRH) (2010), Report on the monitoring of water quality in the watersheds of the State of Pernambuco in 2010, 15p., in: <http://www.cprh.pe.gov.br/> (access in 10 of September of 2018).
- Apeldoorn, M.E.V., Egmond, H.P.V., Speijers, G.J.A. and Bakker, G.J.I. (2007), Toxins of cyanobacteria, *Molecular Nutrition and Food Research*, Vol. 51, pp.7-60, doi: 10.1002/mnfr.200600185.
- Black, K., Yilmaz, M. and Philips, E. (2011), Growth and toxin production by *Microcystis aeruginosa* PCC 7806 (Kutzing) Lemmerman at elevated salt concentrations, *Journal of Environmental Protection*, Vol. 2, pp. 669-674, doi: 10.4236/jep.2011.26077.
- Branco, E. (2002), Variação sazonal e espacial da biomassa fitoplanctônica relacionada com parâmetros hidrológicos no estuário de Barra das Jangadas (Jaboatão dos Guararapes - Pernambuco - Brasil), *Tropical Oceanography*, Vol. 39, pp. 79-96, doi: 10.5914/tropocean.v30i2.3896.
- Branco, E. (2007), *Influência das variáveis ambientais na estrutura da comunidade fitoplanctônica do sistema estuarino de Barra das Jangadas (Pernambuco- Brasil)*, Tese de Doutorado, Universidade Federal de Pernambuco, Recife, 210 p.
- Carmichael, W. (1992), A review: cyanobacteria secondary metabolites - the cyanotoxins, *Journal of Applied Bacteriology*, Vol. 72, pp. 445-459, doi: 10.1111/j.1365-2672.1992.tb01858.x.
- Companhia Ambiental do Estado de São Paulo (CETESB) (2007), Relatório de Qualidade das Águas Litorâneas no Estado de São Paulo - 2007, 50 p., Companhia de Tecnologia de

- Saneamento Ambiental, São Paulo, em: <https://cetesb.sp.gov.br/> (acesso em 10 de Setembro de 2018).
- Dodds, W. and Smith, V. (2016), Nitrogen, phosphorus, and eutrophication in streams, *Inland Waters*, Vol. 6, pp. 155–164, doi: 10.5268/IW-6.2.909.
- Edler, L. (1979), Recommendations of methods of marine biological studies in the Baltic Sea: phytoplankton and chlorophyll, *The Baltic Marine Biologists*, Vol. 5, pp. 1-38.
- Esteves, F.A. (1998), *Fundamentos de Limnologia*, Interciência / FINEP, Rio de Janeiro, 602 p.
- Ferrario, M., Sar, E. and Sala, S. (1995), Metodologia basica para el estudio del fitoplancton con especial referencia a las diatomáceas, in Alvear, K., Ferrario, M., Oliveira-Filho, E.C. and Sar, E. (eds), *Manual de métodos ficológicos*, Ed. Anibal Pinto, Concepción, pp. 1-25.
- Gomes, S.C., Lima, E.S. and Alheiros, M.M. (2003), *Diagnóstico ambiental do meio físico para a gestão da bacia hidrográfica do rio Jaboatão*, in II Congresso sobre planejamento e gestão das zonas costeiras dos países de expressão portuguesa, Recife, Fecha... em: [http://www.abequa.org.br/mostra\\_ano.php?pageNum\\_editoria=16&ano=2003/](http://www.abequa.org.br/mostra_ano.php?pageNum_editoria=16&ano=2003/) (acesso em 01 de Setembro de 2018).
- Havens, K.E., Philips, E.J., Cichra, M.F. and Li, B. (1998), Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake, *Freshwater Biology*, Vol.39, pp. 547-566, doi: 10.1046/j.1365-2427.1998.00308.x.
- Instituto Brasileiro de Geografia Estatística (IBGE) (2011), Census 2010. <https://www.ibge.gov.br/> (acesso em 10 de Setembro de 2018).
- Lacerda, S.R., Koenig, M.L., Neumann-Leitão, S. and Flores-Montes, M.J. (2004), Phytoplankton nyctemeral variation at a tropical river estuary (Itamaracá - Pernambuco - Brazil), *Brazilian Journal of Biology*, Vol. 64, pp. 81-94, doi: 10.1590/S1519-69842004000100010.
- Latour, D. and Giraudet, H. (2004), Méthode d'échantillonnage adaptée aux cyanobactéries coloniales em milieu lacustre. Cas applique à *Microcystis aeruginosa* sur la retenue de Grangent (Loire, France), *Comptes Rendus Biologies*, Vol. 327, pp. 105-113, doi : 10.1016/j.crv.2003.12.005.
- Lewis Jr., W.M. (1978), Analysis of succession in a tropical phytoplankton community and a new measure of succession rate, *The American Naturalist*, Vol. 112, issue 984, pp. 401-414, doi: 10.1086/283282.
- Lourenço, S.O. and Marques-Júnior, N.A. (2002), Produção primária marinha, in Pereira, R.C. and Soares-Gomes, A. (eds), *Biologia marinha*, Interciências, Rio de Janeiro, pp. 195-227.
- McLusky, D.S. (1993), Marine and estuarine gradients – An overview, *Netherlands Journal of Aquatic Ecology*, Vol. 27, issue 2-4, pp. 489-493, doi: 10.1007/BF02334809.
- Meire P., Ysebaert, T., Van Damme, S., Van Den Bergh, E., Maris, T. and Struyf, E. (2005), The Scheldt Estuary: A Description of a Changing Ecosystem, *Hydrobiologia*, Vol. 540, pp. 1-11, doi: 10.1007/s10750-005-0896-8.

- Molica, R. and Azevedo, S. (2009), *Ecofisiologia de cianobactérias produtoras de cianotoxinas, Oecologia Brasiliensis*, Vol. 13, issue 2, pp. 229-246.
- Moreira, H.A. (2007), *Diagnóstico da qualidade ambiental da Bacia do Rio Jaboatão: Sugestão de enquadramento preliminar*, Dissertação de Mestrado, Universidade Federal de Pernambuco, Recife, 121 p.
- Noriega, C. and Araujo, M. (2011), Nutrient budgets (C, N and P) and trophic dynamics of a Brazilian tropical estuary: Barra das Jangadas, *Anais da Academia Brasileira Ciências*, Vol. 83, issue 2, pp. 441-456, doi: 10.1590/S0001-37652011000200007.
- Oberholster, P.J., Botha, A.M. and Grobbelaar, J.U. (2004), *Microcystis aeruginosa*: source of toxic microcystins in drinking water, *African Journal of Biotechnology*, Vol. 3, pp. 159-168, doi: 10.5897/AJB2004.000-2029.
- Olsen, Y. (1989), Evaluation of competitive ability of *Staurostrum luetkemullerii* (chlorophyceae) and *Microcystis aeruginosa* (cyanophyceae) under P limitation, *Journal of Phycology*, Vol. 25, pp. 486-499, doi: 10.1111/j.1529-8817.1989.tb00254.x.
- Omori, M. and Ikeda, T. (1984), *Methods in marine zooplankton ecology*, John Wiley & Sons, New York, 331 p.
- Paerl, H.W. and Huisman, J. (2008), Blooms like it hot, *Science*, Vol. 320, issue 5872, pp. 57-58, doi: 10.1126/science.1155398.
- Paerl, H.W. and Paul, V.J. (2012), Climate change: Links to global expansion of harmful cyanobacteria, *Water Research*, Vol. 46, issue 5, pp. 1349-1363, doi: 10.1016/j.watres.2011.08.002.
- Ricklefs, R.E. (2003), *A economia da natureza*, Ed. Guanabara Koogan, Rio de Janeiro, 503 p.
- Scott, J.T. and Marcarelli, A.M. (2012), Cyanobacteria in freshwater benthic environments, in Whitton, B. (eds) *Ecology of Cyanobacteria II*, Springer, Dordrecht, pp. 271-289.
- Secretary of Science, Technology and Environment (SECTMA) (1999), Plano Estadual de Recursos Hídricos, PERHPE, in: <http://www.inmet.gov.pe.br/> (access in 10 of September of 2018).
- Silva, A.X. da, Noriega, C., Koenig, M.L., Flores-Montes, M.J. and Araujo, M. (2017), Distribution of nutrients and changes in Phytoplankton composition in a tropical Mesotidal estuary, northeastern Brazil, *Open Journal of Ecology*, Vol. 7, pp. 460-494, doi: 10.4236/oje.2017.77032.
- Sivonen, K. and Jones, G. (1999), Cyanobacterial toxins, in Chorus, I. and Bartram, J. (eds), *Toxic Cyanobacteria in water - A guide to their public health consequences, monitoring and management*, E&FN Spon, London, pp. 55-124.
- Smith, V.H. (2003), Eutrophication of freshwater and coastal marine ecosystems - a global problem, *Environmental Science and Pollution Research*, Vol. 10, pp. 126-139, doi: 10.1065/espr2002.12.142.
- Takenaka, R.A., Dellamano-Oliveira, M.J. and Rocha, O. (2007), Toxicidade de extratos de florações de cianobactérias de reservatórios do rio Tietê, SP, aos dafinídeos *Ceriodaphnia*

*dubia* e *Ceriodaphnia silvestrii* (Cladocera, Crustacea), *Journal of the Brazilian Society of Ecotoxicology*, Vol. 2, pp. 147-156, doi: 10.5132/jbse.2007.02.007.

Torgan, L.C. (1989), Floração de algas: composição causa e consequências, *Ínsula*, Vol. 19, pp. 15-34, in: <https://periodicos.ufsc.br/index.php/insula/article/viewFile/22299/20259/>

Tundisi, J.G. (2003), *Água no século 21: enfrentando a escassez*, RIMA/IEE, Rio de Janeiro, 247p.

Valiela, I. (1995), *Marine ecological processes*, Springer-Verlag, New York, 696 p.

Yunes, J.S., Mathiensen, A., Parise, M., Salomon, P.S., Ragget, S.L., Beattie, K.A. and Codd, G.A. (1998), *Microcystis aeruginosa* growth stages and the occurrence of microcystins in the Patos Lagoon, Southern Brazil, in Reguera, B., Blanco, J., Fernandez, M.L. and Wyatt, T. (eds), *Harmful algae*, Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo, pp. 18-21.