

## Structure and dynamics of the phytoplankton community within a maturation pond in a semiarid region

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### Abstract

In northeastern Brazil, stabilization ponds are very suitable for wastewater treatment because of the relative great land availability and environmental conditions (e.g., high temperature) favorable for microorganism optimal development. However, blooms of potentially toxic cyanobacteria may affect the use of these treatment ponds due to resulting effluent poor quality. The objective of this study was to evaluate the dynamics of phytoplankton communities and the occurrence of cyanobacteria in a maturation pond located immediately after a series of two ponds. Temperature, dissolved oxygen, pH, BOD, N, and P were measured during a period of four months when samples were collected from the surface and the bottom of 7 sampling points distributed inside the pond. The phytoplankton of collected samples was also identified and classified using a conventional optical microscopy. Analysis of variance and Tukey test were used to evaluate the results. The three phytoplankton divisions found (Cyanophyta, Chlorophyta, and Euglenophyta) did not change considerably through surface and bottom. However, they changed greatly over the sampled months; great dominance of Cyanophyta was found at April and October, while Chlorophyta dominated the lagoon in September. Low superficial organic loads (between 78 and 109 kg BOD.ha<sup>-1</sup>.d<sup>-1</sup>) and N:P ≤ 10 were the determinant factors that favored the predominance of Cyanophyta. The presence of two potentially toxic species of Cyanophyta, *Oscillatoria* sp. and *Microcystis aeruginosa*, indicates that caution is required when considering the final destination of treated effluent and suggests a need to assess the risks and benefits associated with the use of the treatment technology.

**Keywords:** cyanobacteria, ecology, temporal variability, domestic sewage, effluent quality.

### Estrutura e dinâmica da comunidade fitoplanctônica de uma lagoa de maturação em região semiárida

#### Resumo

No nordeste do Brasil, as lagoas de estabilização são muito adequadas para o tratamento de águas residuárias por causa da disponibilidade relativamente grande de terra e das condições ambientais (por exemplo, altas temperaturas) favoráveis ao melhor desenvolvimento dos microorganismos. Entretanto, florações de cianobactérias potencialmente tóxicas podem afetar o uso dessas lagoas de tratamento, devido à consequente qualidade inferior do efluente. O objetivo deste estudo foi avaliar a dinâmica das comunidades de fitoplâncton e a ocorrência de cianobactérias em uma lagoa de maturação situada após duas lagoas em série. Temperatura, oxigênio dissolvido, pH, DBO, N e P foram medidos durante um período de quatro meses, durante o qual amostras foram coletadas na superfície e fundo em sete pontos de amostragem da lagoa. As comunidades de fitoplâncton das amostras coletadas foram também identificadas e classificadas utilizando-se um microscópio óptico convencional. Para avaliar os resultados utilizou-se a análise de variância e o teste de Tukey. Para as três divisões de fitoplâncton encontradas (Cyanophyta, Chlorophyta e Euglenophyta), não houve diferença significativa para as amostras de superfície e de fundo de um mesmo mês. Entretanto, ocorreu grande variação para as amostras dos diferentes meses; nos meses de abril e outubro houve uma predominância de Cyanophyta, ao passo que em setembro o domínio na lagoa foi de Chlorophyta. Os fatores determinantes que favoreceram o domínio de Cyanophyta foram a baixa carga orgânica superficial aplicada (entre 78 e 109 kg DBO.ha<sup>-1</sup>.d<sup>-1</sup>) e N:P ≤ 10. A presença

de duas das espécies de Cyanophyta, *Oscillatoria* sp. e *Microcystis aeruginosa*, consideradas potencialmente tóxicas, indica que é necessária precaução quando se considera o destino final do efluente tratado e sugere a necessidade de avaliar os riscos e benefícios associados ao uso da tecnologia de tratamento.

*Palavras-chave:* cianobactéria, ecologia, variabilidade temporal, esgoto doméstico, qualidade de efluente.

## 1. Introduction

Biological processes are usually applied for the treatment of domestic sewage. Anaerobic reactor followed by aerobic reactor or stabilization pond is the main technology used in tropical countries, due to climate favorable conditions. A conventional aerobic system is usually applied to cold climate or more developed countries. However, in developing countries stabilization ponds alone are also commonly used as alternative systems. The advantages of stabilization ponds include their low cost of construction and maintenance, satisfactory pollutant removal efficiency and self-sustainability. In Brazil, for example, several wastewater treatment plants (WWTP) use this technology due to the climate and the availability of land at a relatively low cost (Kellner and Pires, 2002; Pearson et al., 2005; Von-Sperling and Mascarenhas, 2005). However, cyanobacteria blooms have occurred in water reservoirs in northeastern Brazil (Molica et al., 2005; Bittencourt-Oliveira et al., 2010, 2012). These blooms are associated with certain abiotic factors, as reported by Paerl and Paul (2012), such as vertical stratification, nutrient availability and high temperatures throughout the year. These abiotic factors are also commonly observed in stabilization ponds (Kellner and Pires, 2002).

Reports on toxic cyanobacteria are more commonly associated with reservoirs for power generation or water supply, where high temperatures and eutrophication of the water bodies are typical (Azevedo et al., 1994; Domingos et al., 1998; Lagos et al., 1999; Molica et al., 2005; Bittencourt-Oliveira et al., 2010). In 1996, the first proven human deaths caused by cyanotoxins occurred in the city of Caruaru, Pernambuco, Brazil; 52 chronic kidney disease patients died due to the presence of microcystin in water, collected at a supply reservoir, treated through filters system, and then used in hemodialysis machines (Carmichael et al., 2001; Yuan et al., 2006).

The occurrence of cyanobacterial blooms, which can produce cyanotoxins in stabilization ponds, has also been reported in several studies (Oudra et al., 2000; Vasconcelos and Pereira, 2001; Furtado et al., 2009; Kotut et al., 2010). However, the factors and mechanisms that regulate the predominance of some phytoplankton groups are not fully understood and could represent an important tool for water resource management. In this sense, the objective of this study was primarily to investigate the dynamics of cyanobacterial blooms in a maturation pond of a WWTP; further if possible, to suggest improved management processes for treated effluent discharge into water bodies and some additional tools to help operate lagoon systems during periods with the greatest susceptibility to algal blooms.

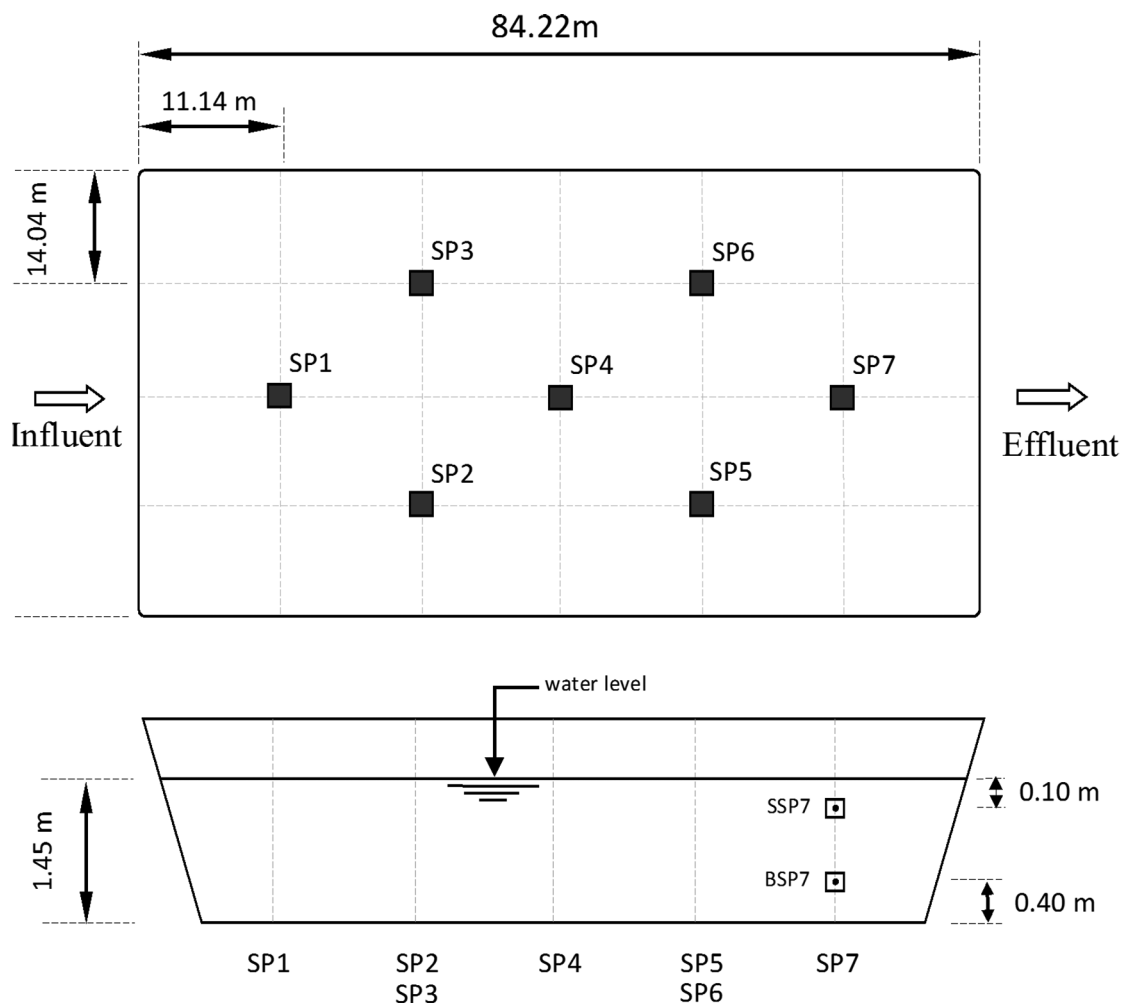
## 2. Material and Methods

The WWTP of Petrolândia city, located in northeastern Brazil (9° 4' 8" S, 38° 18' 11" W), was studied in this work. The region has semiarid climate and the average annual rainfall is less than 450 mm. The WWTP includes a series of three stabilization ponds, the first of which is a facultative pond followed by two maturation ponds with a total volume of 31,850 m<sup>3</sup>. The average inflow is 16.47 L.s<sup>-1</sup>, resulting in a total hydraulic retention time (HRT) of 22.39 days. The WWTP has been the subject of several research studies since 2005 to monitor the influent and effluent water quality, to study the algal populations and to determine the potential for effluent reuse for agricultural purposes.

This work was conducted in the second maturation pond (volume of 8,294 m<sup>3</sup>, HRT of 6.8 days) because the final treated effluent is discharged from this unit into the receiving body (River São Francisco, Itaparica reservoir) or reused in two agricultural pilot areas (2 hectares each). To assess the largest possible area and volume, 14 sampling points were established inside the pond, where seven points were near the surface, and seven points were located near the bottom. Sampling was conducted only during four months (April, August, September and October of 2008), and a total of 56 samples were collected. The spatial distribution of the sampling points is shown in Figure 1. The samples were collected at a depth of 0.10 m from the surface and 0.40 m from the bottom of the pond; for the bottom samples, a Van-Dorn collector bottle was used. The sampling was performed at 2:00 pm, corresponding to the period of highest daylight intensity at the pond site.

The temperature (T), pH, total dissolved solids (TDS), and dissolved oxygen (DO) were determined in the field using a multi-parameter instrument (HQ30D Hach, USA), and the turbidity was determined using a portable turbidimeter (DM-TU Digimed, Brazil). Standard methods were used to analyze the biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (N-NH<sub>3</sub>) and phosphorus as orthophosphate (P-PO<sub>4</sub><sup>-3</sup>) (APHA, 2012). The depth of the euphotic zone (EZ) was determined by multiplying the transparency value, obtained using a 30-cm Secchi disk, by a factor of 3 (Esteves, 1998).

The N:P ratio used to evaluate the primary nutrients available for phytoplankton assimilation in the maturation pond was based on N-NH<sub>3</sub>:P-PO<sub>4</sub><sup>-3</sup>, and aimed to exclude the nitrogen and phosphorus that were incorporated by the phytoplankton. The applied surface organic loading rate was obtained according to Equation 1:



**Figure 1.** Location of the sampling points (SP1, SP2, SP3, SP4, SP5, SP6 and SP7) in the studied maturation pond (SP = sampling point, SSP = surface sampling point, BSP = bottom sampling point).

$$OLRs = \frac{Q \times C}{S} \quad (1)$$

where, OLRs = Surface organic loading rate ( $\text{kg BOD} \cdot \text{ha}^{-1} \cdot \text{d}^{-1}$ ); Q = Flow rate ( $\text{m}^3 \cdot \text{d}^{-1}$ ); C = Substrate concentration in the maturation pond influent ( $\text{kg BOD} \cdot \text{m}^{-3}$ ); S = Maturation pond surface area (ha).

The precipitation data was obtained from a meteorological station located in Mata Grande city ( $9^\circ 7' 53.70'' \text{ S}$ ;  $37^\circ 44' 22.56'' \text{ W}$ ), 50 km far from the WWTP, available at the Brazilian National Space Research Institute (INPE, 2014).

The samples used to assess the phytoplankton composition and enumeration were fixed with acidic Lugol's solution (Karlson et al., 2010). The identification and classification were based on previously published methods (Komárek and Anagnostidis, 1986; Anagnostidis and Komárek, 1988; Bourrelly, 1972). A Palmer-Maloney chamber was used to quantify the phytoplankton (Karlson et al., 2010), and observations were performed using a conventional optical

microscope (DM2000 Leica, USA). The cell density of each taxon was regarded as the number of cells per milliliter ( $\text{cell} \cdot \text{mL}^{-1}$ ). The relative abundance (percentage) was calculated by dividing the number of cells of each taxon by the total number of cells in the sample.

Analysis of variance (ANOVA) was applied for evaluating differences among surface and bottom samples, and also among the samples of different months. When significant difference was detected, Tukey test was used for comparisons. All tests of significance were made at  $p=0.05$ . The statistical analyses were implemented with BioEstat 5.3 Software.

### 3. Results

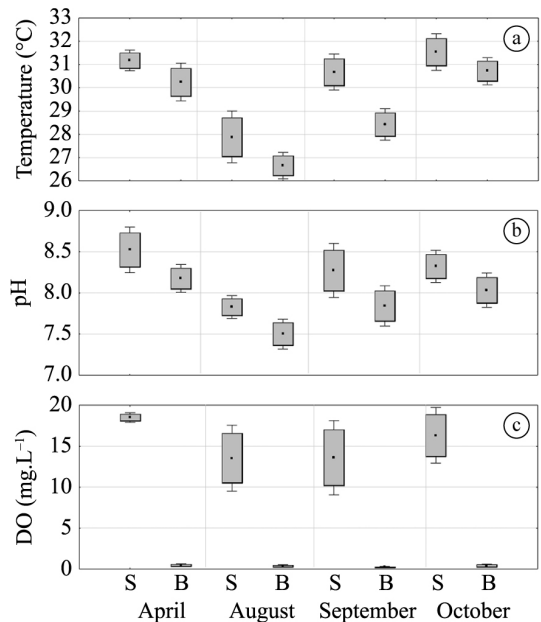
The precipitation was low during the study period, with total of 51 mm, 11 mm, 12 mm and 27 mm in the months of April, August, September and October, respectively. Thus, given these low values, rainfall was not considered a relevant parameter affecting phytoplankton dynamics.

The results found for temperature, pH, DO, TDS, TKN, ammonia, total phosphorus (TP), orthophosphate, and turbidity are shown at Table 1 for surface and bottom samples. Considering the behavior found for each month individually, no significant differences (ANOVA,  $p > 0.005$ ) were observed among the seven sampling stations located on the surface or among the seven sampling stations located at the bottom, for all parameters evaluated. Thus, for a specific month all surface samples collected at the seven points were similar, and all bottom samples collected at the seven points were also similar. However, the set of surface samples in a specific month was different of the set of bottom samples for the following parameters: T, pH, and DO. Additionally, it is important to highlight that among the months significant differences were observed for all parameters, for both surface and bottom samples. The results of these parameters are shown in Figure 2.

The results of T, total phytoplankton, EZ and turbidity for the surface samples are shown in Figure 3. October was the month with the highest water temperature (Figure 3a) and also with a high total phytoplankton cell density (Figure 3b), resulting in high turbidity (Figure 3c). These factors seemed to have led to a lower EZ value (Figure 3d). In contrast, August was the month with the lowest temperature and exhibiting lower turbidity (Figure 3c), and a greater EZ value (Figure 3d).

The relative contribution of the three phytoplankton divisions found in this study (Cyanophyta, Chlorophyta and Euglenophyta) is shown in Figure 4. The division Euglenophyta was the less abundant in this study, with

*Phacus* sp. as the highest occurrence among the detected taxa. Members of the Chlorophyta division were most abundant in September, when the abundance of Cyanophyta was low. The average of the WWTP influent flow rate was

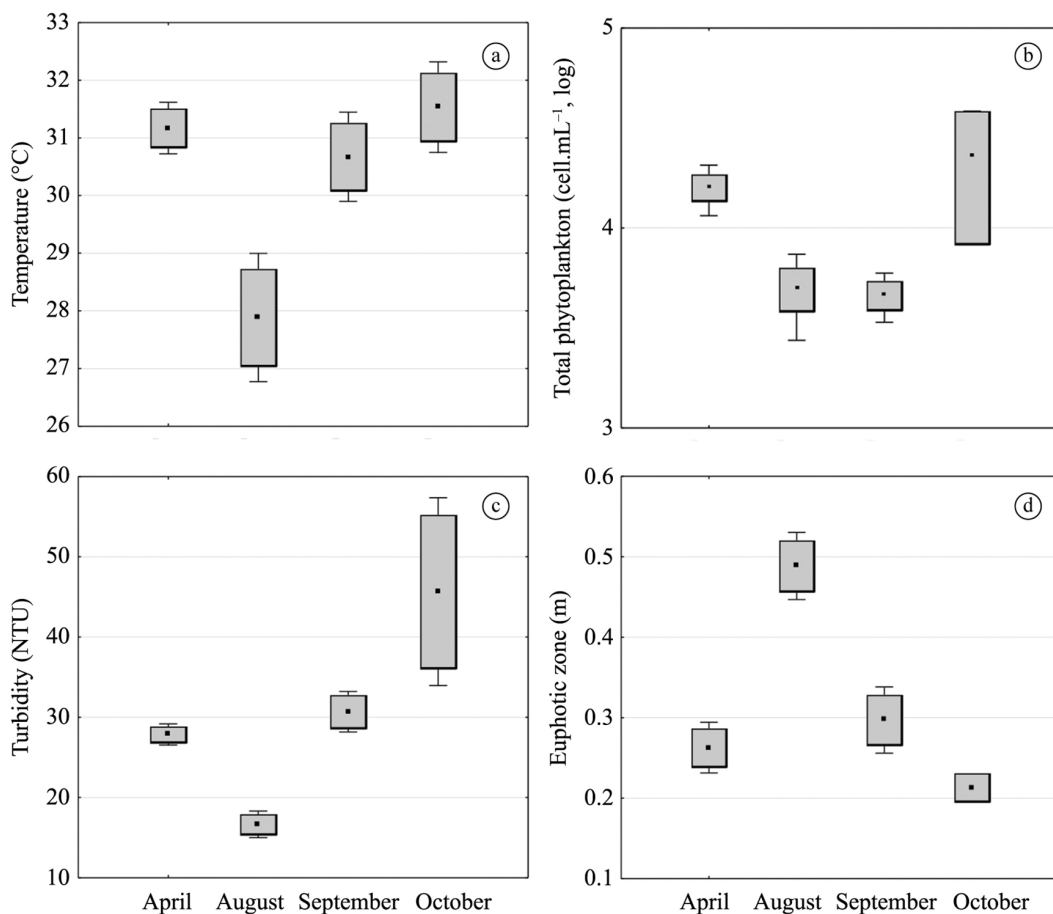


**Figure 2.** Results of the analyses of the surface (S) and bottom (B) samples: (a) temperature ( $^{\circ}\text{C}$ ); (b) pH and (c) dissolved oxygen ( $\text{mg}\cdot\text{L}^{-1}$ ). Values: maximum ( $\top$ ), minimum ( $\perp$ ), average ( $\bullet$ ) and standard deviation ( $\blacksquare$ ).

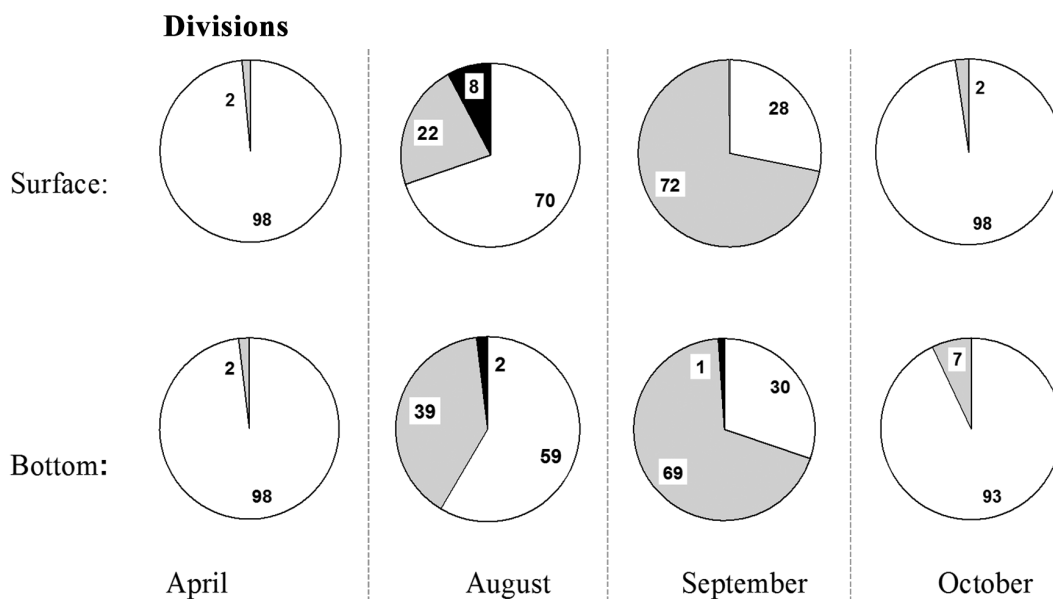
**Table 1.** Average (standard-deviation) of abiotic parameters measured at surface (S) and bottom (B) samples during the experimental time.

Parameter	Layer	Month				ANOVA* Months
		April	August	September	October	
Temperature ( $^{\circ}\text{C}$ )	S	31.2 <sup>a</sup> (0.4)	27.9 <sup>b</sup> (1.1)	30.7 <sup>a</sup> (0.8)	31.5 <sup>a</sup> (0.8)	$p < 0.01$
	B	30.2 <sup>a</sup> (0.8)	26.7 <sup>b</sup> (0.6)	28.4 <sup>c</sup> (0.7)	30.7 <sup>a</sup> (0.6)	$p < 0.01$
pH	S	8.5 <sup>a</sup> (0.3)	7.8 <sup>b</sup> (0.1)	8.3 <sup>a</sup> (0.3)	8.3 <sup>a</sup> (0.2)	$p < 0.01$
	B	8.2 <sup>a</sup> (0.2)	7.5 <sup>b</sup> (0.2)	7.8 <sup>b</sup> (0.2)	8.0 <sup>a</sup> (0.2)	$p < 0.01$
DO ( $\text{mg}\cdot\text{L}^{-1}$ )	S	18.5 <sup>a</sup> (0.6)	13.5 <sup>a</sup> (4.0)	13.6 <sup>a</sup> (4.5)	16.3 <sup>a</sup> (3.4)	$p < 0.05$
	B	0.5 <sup>a</sup> (0.2)	0.4 <sup>a</sup> (0.1)	0.2 <sup>a</sup> (0.1)	0.4 <sup>a</sup> (0.2)	$p > 0.05$
TDS ( $\text{mg}\cdot\text{L}^{-1}$ )	S	992.4 <sup>a</sup> (2.4)	1065.9 <sup>b</sup> (20.7)	921.3 <sup>c</sup> (21.4)	1012.0 <sup>a</sup> (7.8)	$p < 0.01$
	B	991.7 <sup>a</sup> (1.8)	1085.4 <sup>b</sup> (18.2)	982.4 <sup>a</sup> (19.7)	1040.3 <sup>b</sup> (1.2)	$p < 0.01$
TKN ( $\text{mg}\cdot\text{L}^{-1}$ )	S	23.1 <sup>a</sup> (2.0)	33.9 <sup>b</sup> (5.5)	34.6 <sup>b</sup> (3.6)	25.1 <sup>a</sup> (2.5)	$p < 0.01$
	B	24.3 <sup>a</sup> (1.3)	34.0 <sup>b</sup> (5.3)	34.4 <sup>b</sup> (2.8)	26.6 <sup>a</sup> (6.2)	$p < 0.01$
Ammonia ( $\text{mg}\cdot\text{L}^{-1}$ )	S	18.0 <sup>a</sup> (1.4)	18.4 <sup>a</sup> (1.5)	23.1 <sup>b</sup> (2.3)	14.4 <sup>c</sup> (2.4)	$p < 0.01$
	B	17.4 <sup>a</sup> (1.0)	17.9 <sup>a</sup> (2.3)	22.9 <sup>c</sup> (2.2)	14.4 <sup>d</sup> (1.6)	$p < 0.01$
TP ( $\text{mg}\cdot\text{L}^{-1}$ )	S	3.3 <sup>a</sup> (0.2)	3.2 <sup>a</sup> (0.2)	3.9 <sup>c</sup> (0.2)	3.9 <sup>c</sup> (0.3)	$p < 0.01$
	B	2.9 <sup>a</sup> (1.2)	3.6 <sup>a</sup> (0.3)	4.0 <sup>a</sup> (0.1)	4.0 <sup>a</sup> (0.6)	$p > 0.05$
Orthophosphate ( $\text{mg}\cdot\text{L}^{-1}$ )	S	2.2 <sup>a</sup> (0.2)	2.2 <sup>a</sup> (0.2)	1.4 <sup>a</sup> (0.1)	4.0 <sup>a</sup> (0.0)	$p < 0.01$
	B	2.2 <sup>a</sup> (0.2)	2.3 <sup>a</sup> (0.2)	1.2 <sup>a</sup> (0.3)	4.4 <sup>a</sup> (0.1)	$p < 0.01$
Turbidity (NTU)	S	21.9 <sup>a</sup> (3.4)	16.6 <sup>a</sup> (1.7)	32.5 <sup>a</sup> (3.5)	63.6 <sup>c</sup> (22.5)	$p > 0.05$
	B	24.3 <sup>a</sup> (3.3)	31.6 <sup>a</sup> (8.2)	34.7 <sup>a</sup> (4.4)	55.6 <sup>b</sup> (16.8)	$p < 0.01$

\* ANOVA test applied to the set surface and to the set bottom samples did not indicated differences among them ( $p > 0.05$ ). S - surface sample; B - bottom sample. Superscript letters a, b, and c indicates results from Tukey test applied to compare monthly results: same letter indicates similarity while different letter implies significant differences between the results.



**Figure 3.** Results of analyses in the surface samples: (a) temperature; (b) total phytoplankton; (c) turbidity and (d) euphotic zone. Values: maximum ( $\Upsilon$ ), minimum ( $\perp$ ), average ( $\bullet$ ) and standard deviation ( $\text{I}$ ).



**Figure 4.** Relative contribution (%) of the three phytoplankton divisions Cyanophyta ( $\square$ ), Chlorophyta ( $\square$ ) and Euglenophyta ( $\blacksquare$ ).

16.47 L/s, and the BOD (mg O<sub>2</sub>/L) at the maturation pond influent was 30.9, 25.9, 36.2 and 32.4 for April, August, September and October, respectively; the resulting applied OLRs were (average values, in kg DBO<sub>5</sub>.ha<sup>-1</sup>.d<sup>-1</sup>): 93, 78.1, 108.9 and 97.5, respectively. These values are significantly different between the months (ANOVA, p<0.01).

The relative abundance of Cyanophyta and Chlorophyta relative to the primary nutrients available for phytoplankton assimilation in the stabilization ponds (N-NH<sub>3</sub>:P-PO<sub>4</sub><sup>-3</sup>) is shown in Figure 5. For an N-NH<sub>3</sub>:P-PO<sub>4</sub><sup>-3</sup> ratio less than 10, Cyanophyta were predominant with a relative abundance greater than 50% (Figure 5a), whereas there was a predominance of Chlorophyta (relative abundance greater than 50%) at a N-NH<sub>3</sub>:P-PO<sub>4</sub><sup>-3</sup> ratio ≥10 (Figure 5b). Although the ability of some cyanobacteria in fixing

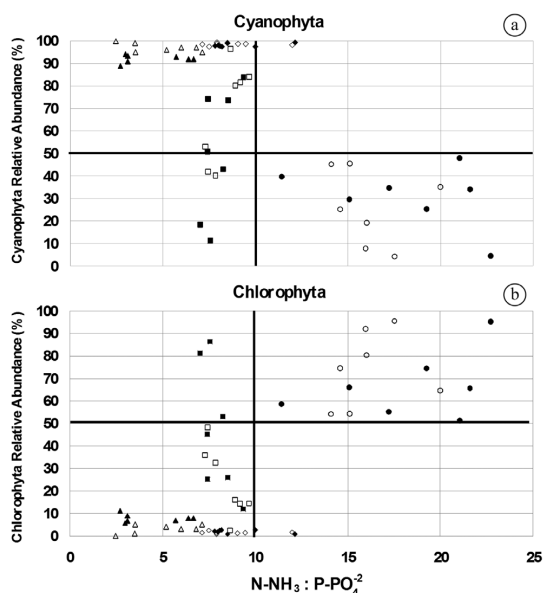
N<sub>2</sub>, none Cyanophyta detected in this work presented heterocyst cells.

The taxonomic survey of the phytoplankton community in the studied maturation pond identified 16 taxa belonging to the Cyanophyta, Chlorophyta and Euglenophyta divisions (Table 2).

The taxa distribution within the Cyanophyta division is shown in Figure 6. The turbidity was high and the depth of the euphotic zone was shallow during the months of April and October; therefore, the Cyanophyta were predominant, with *Oscillatoria* sp. being the most abundant taxon (Figure 6a). August was the month with the most distinct abiotic conditions (the lowest temperature, pH, and turbidity, Figure 2 and Figure 3c), and also with the most dissimilar taxa distribution for both Cyanophyta and

**Table 2.** Cell density of phytoplankton identified in the maturation pond during the study period.

Division	Taxa	Layer	Average (standard-deviation) of cell density (cell.mL <sup>-1</sup> ) by month of monitoring			
			April	August	September	October
Cyanophyta	<i>Aphanocapsa</i> sp.	S	88 (158)	0 (0)	0 (0)	0 (0)
		B	17 (34)	112 (161)	9 (24)	0 (0)
	<i>Chroococcus turgidus</i>	S	2075 (2214)	8813 (4366)	1292 (742)	8477 (15208)
		B	1124 (1020)	9732 (7940)	1232 (378)	1667 (2045)
	<i>Merismopedia tenuissima</i>	S	1 (2)	5 (8)	3 (7)	0 (0)
		B	2 (6)	317 (404)	8 (16)	7 (12)
	<i>Microcystis aeruginosa</i>	S	0 (0)	36 (71)	0 (0)	16 (31)
		B	0 (0)	272 (278)	0 (0)	21 (36)
	<i>Oscillatoria</i> sp.	S	85714 (23400)	4571 (5855)	6000 (6573)	125000 (151345)
		B	68929 (16534)	8000 (8000)	5143 (4451)	133333 (58287)
<i>Chlorococcum</i>	S	0 (0)	3384 (3294)	990 (1767)	0 (0)	
	B	0 (0)	10652 (6390)	1393 (2379)	0 (0)	
Chlorophyta	<i>Dictyosphaerium</i> sp.	S	243 (308)	781 (485)	42 (102)	0 (0)
		B	85 (224)	875 (625)	45 (118)	0 (0)
	<i>Sphaerocystis</i> sp.	S	202 (317)	85 (166)	16167 (7658)	5406 (10117)
		B	202 (252)	0 (0)	12942 (6296)	63 (108)
	<i>Closteriopsis acicularis</i>	S	691 (276)	27 (46)	94(52)	2844 (4083)
		B	576 (402)	54 (56)	170 (107)	3188 (2544)
	<i>Monoraphidium circinale</i>	S	69 (124)	9 (24)	0 (0)	78 (156)
		B	445 (464)	848 (693)	9 (24)	7042 (1697)
	<i>Radiococcus</i> sp.	S	9 (24)	0 (0)	0 (0)	0 (0)
		B	6 (15)	0 (0)	0 (0)	0 (0)
<i>Volvox</i> sp.	S	118 (144)	0 (0)	0 (0)	0 (0)	
	B	50 (68)	0 (0)	0 (0)	0 (0)	
<i>Euglena acus</i>	S	0 (0)	36 (46)	0 (0)	0 (0)	
	B	0 (0)	54 (56)	9 (24)	0 (0)	
Euglenophyta	<i>Euglena</i> sp.	S	15 (18)	112 (156)	0(0)	0 (0)
		B	4 (6)	80 (100)	27 (49)	0 (0)
	<i>Phacus</i> sp.	S	0 (0)	1263 (1578)	10(26)	31(36)
		B	0 (0)	469 (619)	143 (156)	0 (0)
<i>Lepocinclis</i>	S	0(0)	103(59)	0 (0)	0 (0)	
	B	0 (0)	18 (2)	54 (12)	0 (0)	



**Figure 5.** Relative abundance (%) of phytoplankton groups based on N:P ratio: (a) Cyanophyta and (b) Chlorophyta. Month-sampling point: April-surface ( $\diamond$ ), April-bottom ( $\blacklozenge$ ), August-surface ( $\square$ ), August-bottom ( $\blacksquare$ ), September-surface ( $\circ$ ), September-bottom ( $\bullet$ ), October-surface ( $\triangle$ ) and October-bottom ( $\blacktriangle$ ).

Chlorophyta divisions (Figure 6). *Chroococcus turgidus* and *Chlorococcum* dominated the Cyanophyta and the Chlorophyta division, respectively. It is also important to highlight the great dominance of the taxa *Sphaerocystis* sp. for division Chlorophyta during September (Figure 6b).

#### 4. Discussion

Considering the significant difference for the correspondent values of T, pH and DO of surface and bottom samples during the 4-month sampling period (Table 1), the maturation pond can be considered stratified for only these three parameters. The primary cause of thermal stratification in stabilization ponds can be attributed to the heating of the surface layers by solar radiation (Kellner and Pires, 2002). The high surface temperatures and the presence of a thermocline prevent mixing between the hypolimnion and epilimnion, leading to differences in parameters such as phytoplankton, pH and DO (Gu and Stefan, 1995). The primary effect of stratification on sewage treatment processes is the reduction of the pond storage volume, which results in two zones: a more active, lower-density surface layer and a deeper, denser and less-active layer. Consequently, the reduction of the total volume for treatment decreases the HRT, which may compromise the efficiency of the treatment system. A temperature gradient of  $0.6\text{ }^{\circ}\text{C}\cdot\text{m}^{-1}$  is a suitable threshold for identifying the presence of thermal stratification in ponds (Kellner and Pires, 2002). In the present work, an average gradient around of  $1\text{ }^{\circ}\text{C}\cdot\text{m}^{-1}$  was observed, thus confirming a thermal stratification.

Regarding the phytoplankton community, increased stratification in aquatic bodies favors the predominance of Cyanophyta (Paerl and Paul, 2012), thus, resulting an environment that is more favorable for the development of this potential toxin producer.

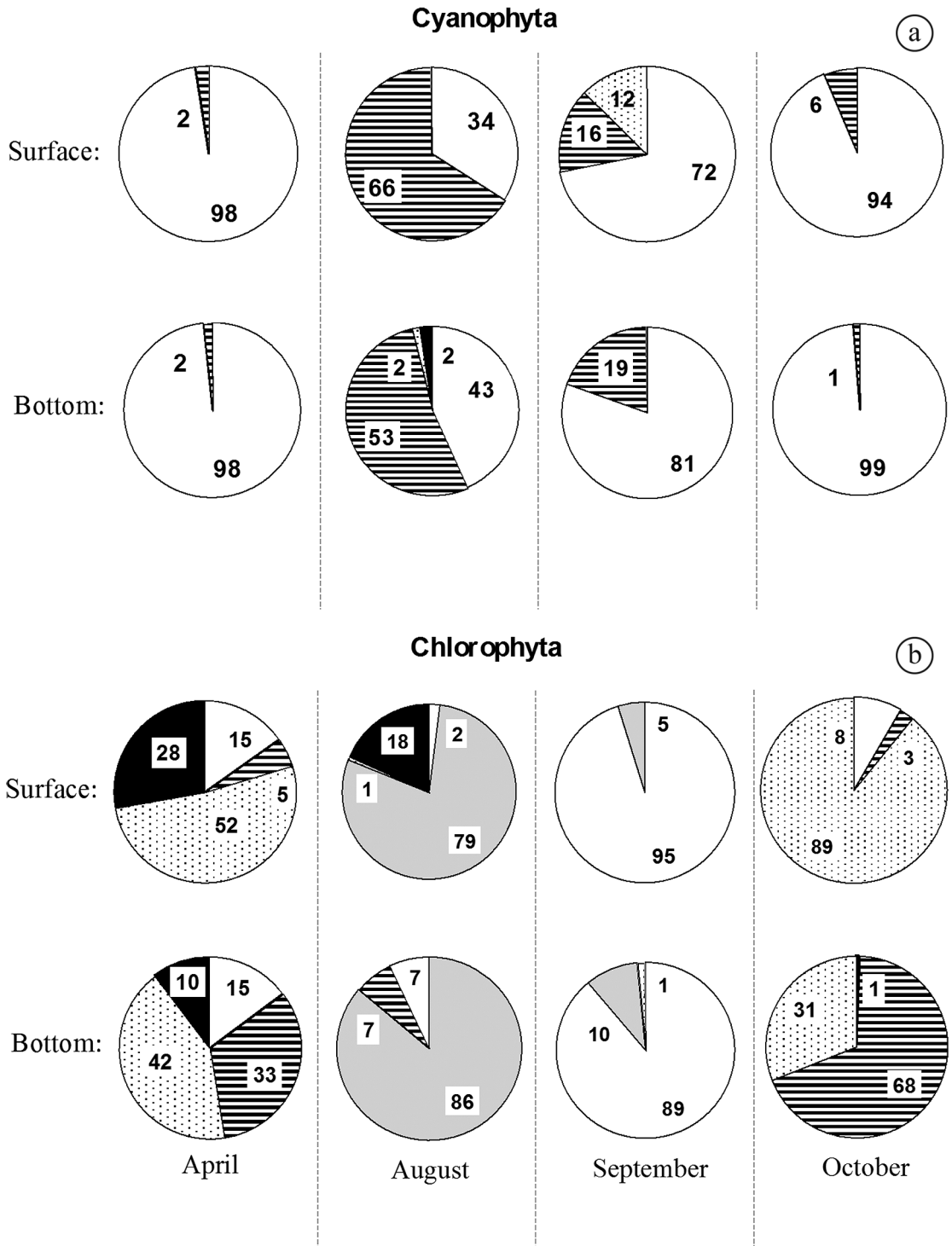
The pattern observed for T, total phytoplankton, EZ and turbidity (see Figure 3) was expected because these parameters are generally correlated, i.e., a higher temperature increases the microbial metabolism, and the growth of phytoplankton populations and increased algal biomass initiate a self-shading phenomenon (Park et al., 2011).

The behavior observed in relation to the phytoplankton divisions (Figure 4) is, in general, coherent with the applied low OLRs ( $<109\text{ kg BOD}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$ ). Similar behavior was reported in a study by Amengual-Morro et al. (2012) for the Mediterranean climate of Spain, where Cyanophyta occurred at a high abundance under lower OLRs ( $64\text{ kg DBO}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$ ), whereas Chlorophyta and Euglenophyta occurred at high loads ( $192\text{ kg DBO}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$ ).

Regarding the role of the nutrients, the phytoplankton community is often based on competition between Chlorophyta and Cyanophyta in eutrophic and hypereutrophic systems. In these environments, Cyanophyta predominate at low mineral nitrogen concentrations, which in turn, results in a low N:P ratio. Arauzo et al. (2000) attributed the absence of Cyanophyta in stabilization ponds to a high ratio of dissolved inorganic nitrogen and soluble reactive phosphorus ( $>15$ ). Similarly, Smith (1983) stated that low nitrogen to phosphorus ratios favor the predominance of Cyanophyta in lakes. Therefore, the N:P ratio may be a good indicator of the dominance of Cyanophyta in stabilization ponds, as observed in our study (see Figure 5). Members of the Euglenophyta division were not so abundant during the study period, having an average cell density of only  $397\text{ cell}\cdot\text{mL}^{-1}$ , and a relative abundance of less than 8%. This division is usually reported at more polluted environments with *Phacus* sp. preference for environments rich in organic nutrients (Alves-da-Silva et al., 2011) that were highly available in this study.

The genera that were identified (Table 2) have also been recorded in other stabilization ponds and polluted environments (Amengual-Morro et al., 2012; Furtado et al., 2009; Kotut et al., 2010; Vasconcelos and Pereira, 2001). Within the Cyanophyta division, *Oscillatoria* sp. and *Microcystis aeruginosa* are noteworthy because they are considered potentially toxic species (Kaebnick and Neilan, 2001), with an average cell density reaching up to  $1.5\times 10^5$  and  $154\text{ cell}\cdot\text{mL}^{-1}$  (Table 2), respectively.

The depth of the euphotic zone was strongly negatively associated with the taxon *Oscillatoria* sp. and, according to Scheffer et al. (1997), many shaded lakes are dominated by this group. High abundance of *Oscillatoria* sp. might have outcompeted other high light requiring species due to a shading effect. Several members of the *Oscillatoria* are capable of capturing long-wavelength light, which frequently occurs in the hypolimnion of lakes (Cohen et al., 1975). Confirming the authors observation, in this work *Oscillatoria* sp. dominated both surface and bottom layers



**Figure 6.** Relative contribution (%) of taxa within the two principal divisions: (a) Cyanophyta: *Oscillatoria* sp. (□), *C. turgidus* (▨), *M. aeruginosa* (▩) and other (■); (b) Chlorophyta: *Sphaerocystis* sp. (□), *M. circinale* (▨), *C. acicularis* (▩), *Chlorococcum* sp. (▨) and other (■).

(Table 2) when the euphotic zone was low (April, September, and October – Figure 3) and, despite not dominating the Cyanophyta division in August, its cell density in bottom samples was even higher than in September. In addition,

coccoid forms present better competition than filamentous forms when the euphotic zone increases (low turbidity) (Whitton and Potts, 2000), as observed for *Chroococcum turgidus* dominance in August.



In this study (See Figures 4 and 6), during the months of April and October, the water temperature was high, which also favored the growth of the potential toxin producer *Oscillatoria* (Kaebernick and Neilan, 2001).

Regarding to results found for division Chlorophyta, *Chlorococcum*, as nonmotile green algae, competed better for light in August, dominating when the lowest turbidity (<20 NTU) and the highest euphotic zone was found. On the other hand, when the turbidity doubled and the EZ reduced (Figure 3), from August (16.6 NTU) to September (32.5 NTU), *Chlorococcum* was replaced with *Sphaerocystis* sp., which represent the highest relative dominance found for N:P ≥ 10 (Figure 5). According with Whitton and Potts (2000) chlorophytes *Sphaerocystis* showed pulses of growth in Crooked Lake-Indiana when nutrients were less available and the light conditions remained favorable for its growth. In this work, similar results were found, as September was the month with the lowest phosphorus availability (Table 1) and a great dominance of *Sphaerocystis*.

The predominance of Cyanophyta or Chlorophyta in stabilization ponds and the factors that affect their abundance can help to define improved strategies to manage the treated effluent of stabilization ponds. Recent studies have reported the successful removal of cyanobacteria or cyanotoxins using various technologies. In one study, hydrogen peroxide was used as an algicide to remove cyanobacteria and cyanotoxins in a stabilization pond, resulting in a significant reduction in their abundance (Barrington et al., 2013). In another study, ultrafiltration and nanofiltration have been used to remove intracellular and dissolved cyanotoxins with 98% and 96% removal efficiency, respectively, in a water reservoir (Gijssbertsen-Abrahamse et al., 2006). Finally, it has been reported that the biodegradation of the secondary metabolites of cyanobacteria can be achieved using a biological filter (Ho et al., 2012). When the removal of cyanobacteria and cyanotoxins is required, a broad knowledge regarding the fluctuations of the phytoplankton community in stabilization ponds will be important in determining the timing and type of treatment that is most appropriate.

## 5. Conclusions

Results indicating stratification from surface to bottom with respect to the temperature, pH and DO, as well as parameters such as the applied surface organic load and N:P ratio, influenced the predominance of Cyanophyta or Chlorophyta in a maturation pond used to treat domestic sewage. Cyanophyta were dominant at N:P ≤ 10 and OLRs ≤ 109 kg BOD.ha<sup>-1</sup>.d<sup>-1</sup>. *Oscillatoria* sp., which is potentially toxic, was the most abundant member of the Cyanophyta. Euglenophyta division was not significant during the study period. For improved management of the discharge or reuse of the effluent from stabilization ponds, an appropriate type of treatment for cyanobacteria and cyanotoxin removal will depend on knowledge of the variation in the phytoplankton community for a given season.

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