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Spatial variation of periphyton structural attributes on *Eichhornia crassipes* (Mart.) Solms. in a tropical lotic ecosystem

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ABSTRACT. This study evaluated the influence of physical, chemical and physico-chemical variables of water on the biomass of periphyton community and verified the differences between six sampling sites over the course of São Mateus river: two upstream of the city of São Mateus, Espírito Santo State (E1, E2), two along (E3, E4), and two downstream of the city (E5, E6). The periphyton was collected from roots of *Eichhornia crassipes* (Mart.) Solms. Samplings were undertaken every week in September and October 2010. The periphyton biomass was estimated through chlorophyll 'a', biovolume, dry mass, ash-free dry mass, and ash. Higher values of chlorophyll 'a' were found at E1, while the total biovolume featured greater values in E4 and E3. Regarding the values of periphyton dry mass, the inorganic fraction was higher at sites along and downstream of the city of São Mateus. The variation of periphyton biomass was influenced by the availability of nutrients (phosphorus and nitrogen) and turbidity, as evidenced by the CCA. The results suggest that the input of allochthonous material, especially from human activities (fish farming and discharge of domestic and industrial wastewater), has changed the water quality (as pointed out by the PCA), as well as the communities present.

Keywords: chlorophyll 'a', dry mass, biovolume.

Variações espaciais dos atributos estruturais do perifíton em *Eichhornia crassipes* (Mart.) Solms em um ecossistema lótico tropical

RESUMO. Este estudo avaliou a influência das variáveis físicas, químicas e físico-químicas da água sobre a biomassa da comunidade perifítica e verificou as diferenças entre seis estações amostrais ao longo do rio São Mateus: duas a montante da cidade de São Mateus, Estado do Espírito Santo (E1, E2), duas ao longo (E3, E4) e duas a jusante da cidade (E5, E6). O perifíton foi coletado de raízes de *Eichhornia crassipes* (Mart.) Solms. As amostragens foram realizadas em intervalos semanais, em setembro e outubro/2010. A biomassa perifítica foi estimada por meio da clorofila *a*, biovolume, massa seca, massa seca livre de cinzas e cinzas. Maiores valores de clorofila foram registrados em E1, enquanto o biovolume total apresentou valores mais elevados em E4 e E3. Quanto aos valores de massa seca perifítica, a parte inorgânica foi mais elevada, nas estações ao longo e a jusante da cidade. A variação da biomassa perifítica foi influenciada pela disponibilidade de nutrientes (fósforo e nitrogênio), assim como pela turbidez, como constatado pela CCA. Os resultados sugerem que a entrada de material alóctone, proveniente principalmente das atividades antrópicas (piscicultura e lançamento de efluentes domésticos e industriais) alteram a qualidade da água (como evidenciado na PCA), assim como as comunidades presentes.

Palavras-chave: clorofila 'a', massa seca, biovolume.

Introduction

Rivers are open aquatic ecosystems, with unidirectional flow, continuous from headwaters to the mouth, strongly influenced by natural characteristics, which integrates with the adjacent terrestrial environments, forming a more balanced functional unit (MARGALEF, 1983). With increased use of water resources for multiple purposes, several stretches of the longitudinal axis of the rivers have been increasingly impacted by events located at distinct points of the basin which results in a serial discontinuity (MARQUES et al., 2003; SILVA et al., 2010), where human interference like the construction of reservoirs and discharge of wastewater of any nature, disrupts the river gradient in relation to environmental conditions, changing biotic and abiotic processes (WARD; STANFORD, 1983; WARD; STANFORD, 1995), disrupting the predicted continuum of the system (BRIGANTE; ESPINDOLA, 2003).

On biological communities several researches have been performed in Brazil to evaluate periphyton biomass (FELISBERTO; RODRIGUES, 2005; LEANDRINI; RODRIGUES, 2008; FERRAGUT et al., 2010; MARTINS; FERNANDES, 2011), through determining total dry weight, ash free dry weight (= organic matter), photosynthesizing pigments and total biovolume. These data are excellent bases for debates about the organic/inorganic characteristic of the periphyton, as well as its heterotrophic or autotrophic status, besides allowing discussions on the real importance of this community in aquatic ecosystems. The biovolume, when compared with density data, permits to evaluate the actual contribution of algae with larger or smaller size in the dynamic processes of the system (FERNANDES; ESTEVES, 2011).

Despite the difficult in clarifying which physical, chemical and biological factors control the periphyton development (SAND-JENSEN, 1983), this can be influenced by the availability of nutrients (RODRIGUES; BICUDO, 2001; MURAKAMI; RODRIGUES, 2009; FERRAGUT; BICUDO, 2010), light (HILL, 1996, TUJI, 2000), temperature (DENICOLA, 1996; RODRIGUES; BICUDO, 2004; ALGARTE et al., 2006; MURAKAMI; RODRIGUES, 2009), quality and velocity of the water and suspended material transported by the current (STEVENSON et al., 1996; RODRIGUES et al., 2003), in addition to quality and nature of substrate (MOSCHINI-CARLOS et al., 2001).

Considering that anthropogenic activities interfere on physical, chemical, and biological natural conditions by promoting the discontinuity of the system along the river – stretches upstream and downstream of the cities, this study has hypothesized that periphyton structural attributes in *E. crassipes* respond to eutrophication gradient of the São Mateus river. Thus, the objective of this study was to evaluate the spatial variation of the periphyton structural attributes and verified the differences between the limnological factors between sampling sites.

Material and methods

Study area

The São Mateus river, located in the Espírito Santo State, formed by the confluence of the rivers Cotaxé (North arm), with around 244 km and Cricaré (South arm), with around 200 km (Figure 1), is the major supplier of water for several cities, and also supply water for several projects of irrigation, and is inevitably used as a receiver of domestic and industrial wastewater from these and other locations (ANA, 2009).

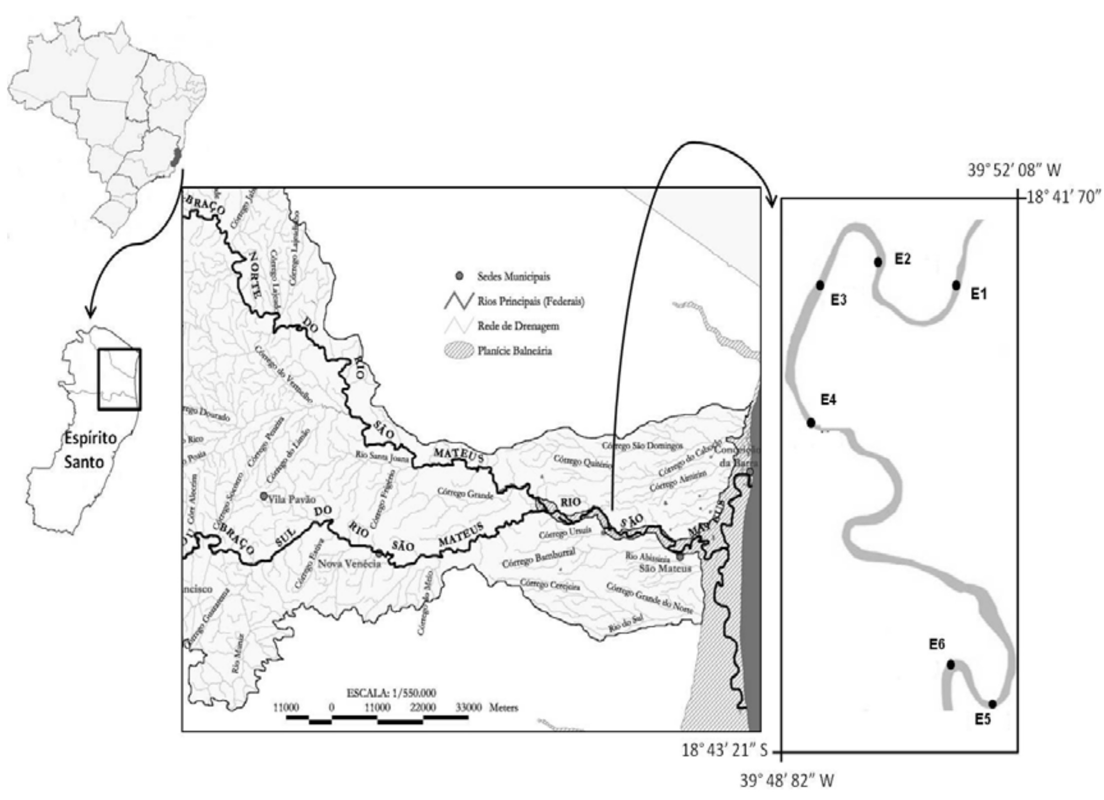

Figure 1. Map of Espírito Santo State, highlight of São Mateus river basin and sampling sites (E1, E2, E3, E4, E5, E6) (modified from the satellite image, Google Earth).

Sampling and abiotic analyses

Six sampling sites were established over a stretch of the São Mateus river, two located upstream of the city of São Mateus (E1, E2), two along the city (E3, E4), and two downstream of the city, under the influence of intensive fish farming (net cages) and discharge of domestic and industrial wastewater (E5, E6) (Table 1). Samplings were undertaken every week in September and October 2010, totaling four samplings.

Table 1. Characterization of sampling of the São Mateus river.

Sampling sites	Geographical coordinates	Localization	Features
E1	S 18° 41.703' W 39° 52.084'	M - Water uptake station CESAN	Riparian Forest preserved
E2	S 18° 42.284' W 39° 52.728'	M – Before the bridge of the BR101-North	Riparian Forest Just preserved
E3	S 18° 42.836' W 39° 51.642'	C – Station water intake - SAAE	Urban core
E4	S 18° 42.659' W 39° 51.246'	C – Historic site of the city	Discharge of domestic wastewater untreated
E5	S 18° 43.112' W 39° 48.853'	J – Fish farming (net-cages) APESAM	150 fish farming of tilápia (*Oreochromis sp*.)
E6	S 18° 43.217' W 39° 48.822'	J – APESAM	Discharge of domestic and industrial wastewater

M = upstream of the city of São Mateus, C = along the city, J = downstream of the city, CESAN = Espírito Santo's station company; SAAE = Autonomous water and sewage; APESAM = Fishermen's Association of São Mateus.

At each sampling site was determined in field electric conductivity. In laboratory it was determined: turbidity, pH, total suspended solids (APHA, 1992), and main nutrients: total nitrogen (VALDERRAMA, 1981), nitrite (GOLTERMAN et al., 1978) and ion nitrate MACKERETH et al., 1978), besides ammonium, silicate (CARMOUZE, 1994), orthophosphate (STRICKLAND; PARSONS, 1960) and total phosphorus (VALDERRAMA, 1981). Climatic data (mean monthly air temperature, rainfall) of the September were obtained from the meteorological station at Centro Universitário Norte do Espírito Santo (CEUNES).

Biotic analyses

Periphyton was collected from the roots of the floating aquatic macrophyte *Eichhornia crassipes* (Mart.) Solms and removed from the substrate with brushes and jets of distilled water. The determination of chlorophyll 'a' was performed through the filtration of 100 mL of the periphyton samples in vacuum pump on glass fiber filter GF-1 (Whatman), extraction with a volume of 10 mL of 90% acetone and subsequent centrifugation. The results were obtained using spectrophotometric method (PARSONS et al., 1984).

The periphyton biomass expressed in dry mass, ash free dry mass, and ash was determined according to Schwarzbold (1990).

The cell volume of the quantified species was estimated considering the mean dimension of the individuals, using geometrical shapes approximated to algal shapes, according to Hillebrand et al. (1999), Sun and Liu (2003) and Vadrucci et al. (2007). It was measured 20 individuals per taxon (distributed into several samplings), according to the frequency they occurred in the samples and then it was calculated the mean volume of each species. The biovolume was obtained by multiplying the mean of volumes by the densities of each species, being the results expressed in $mm^3\ mg^{-1}$.

All the results of the periphyton structural attributes were expressed as root dry weight of *E. crassipes.*

For interpretation of results, was made an average of four samples for each variable.

Statistical analyses

To test significant differences ($p < 0.05$) between the mean values of the biotic and abiotic variables along sampling sites, we used the Kruskal-Wallis non-parametric test. When significant differences were detected, the Dunn's test was applied to check between which pairs of sites those differences were observed. These analyses were performed in the software BioStat. Thus, the continuity of the system was tested considering the following pairs of sites: 1 and 5; 1 and 6; 2 and 5; 2 and 6; 3 and 5; 3 and 6; 4 and 5; 4 and 6.

The Principal Component Analysis (PCA), applied to verify the longitudinal variation of abiotic variables, was performed with eight variables (turbidity, silicate, nitrate, nitrite, ammonium, total nitrogen, orthophosphate and total phosphorus). To interpret the PCA results, it was retained the axes with eigenvalues higher than generated by Broken-Stick model and used Pearson' correlation. The influence of the five abiotic variables (orthophosphate, total phosphorus, turbidity, ammonium, and total nitrogen) on the periphyton biomass (chlorophyll 'a', ash-free dry mass, classes biovolume: Cyanophyceae, Bacillariophyceae, Chlorophyceae, Euglenophyceae, Chrysophyceae, Oedogoniophyceae) was evaluated by the Canonical Correspondence Analysis (CCA), with significance by the Monte Carlo test ($p < 0.05$), with 999 randomizations. For the PCA and CCA, the variables were transformed into log and the analyses were run using the software PC-ORD 5.15.

Results

The São Mateus river presented higher values of turbidity, total suspended solids, nitrite, ammonium, and total nitrogen in the sites downstream of the city São Mateus (E5 and E6) (Table 2).

Table 2. Longitudinal variation of abiotic variables, mean ± standard deviation, registered in the sampling sites in September and October 2010 (E1, E2 = upstream of the city of São Mateus; E3, E4 = along the city; E5 e E6 = downstream of the city).

Variable	E1	E2	E3	E4	E5	E6
pH	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.2	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
Turb (NTU)	14.5 ± 7.4	10.6 ± 4.0	9.5 ± 3.4	9.6 ± 4.2	36.3 ± 9.8	38.0 ± 9.4
EC (μS cm^{-1})	209.8 ± 6.3	213.7 ± 11.7	208.9 ± 5.9	214.0 ± 9.9	309.6 ± 75.6	275.0 ± 32.2
Si-SiO_4 (mg L^{-1})	7740 ± 130	7615 ± 310	76180 ± 430	7242 ± 800	75770 ± 220	7202 ± 1150
TP (μg L^{-1})	33 ± 4	30 ± 10	35 ± 10	28 ± 9	67 ± 14	114 ± 47
P-ortho (μg L^{-1})	17 ± 4	11 ± 2	14 ± 3	15 ± 4	25 ± 40	67 ± 70
TN (μg L^{-1})	452 ± 20	519 ± 80	538 ± 70	607 ± 120	894 ±1 10	866 ± 150
NO_3^- (μg L^{-1})	96 ± 40	108 ± 40	126 ± 50	151± 60	159 ± 30	127 ± 20
NO_2^- (μg L^{-1})	2 ± 2	3 ± 4	2 ± 1	1 ± 2	11 ± 3	13 ± 2
NH_4^+ (μg L^{-1})	10 ± 4	14 ± 9	9 ± 5	16 ± 7	288 ± 42	329 ± 80

*Turb = turbidity; EC = electric conductivity; TSS = total suspended solids; Si-SiO_4 = silicate; NO_3^- = nitrate; NO_2^- = nitrite; NH_4^+ = ammonium; TN = total nitrogen; P-ortho = orthophosphate; TP = total phosphorus.

The average monthly air temperature was high, ranging from 19.8 to 25.4°C with an average of 22°C. The average monthly precipitation ranged from 0.0 to 0.42 mm, averaging 0.05 mm, characterizing the sample period as the dry season (Figure 2). The annual average flow of 2010 at the São Mateus river was 80 m^3 s^{-1} (ATLAS DIGITAL DAS ÁGUAS DE MINAS, 2012).

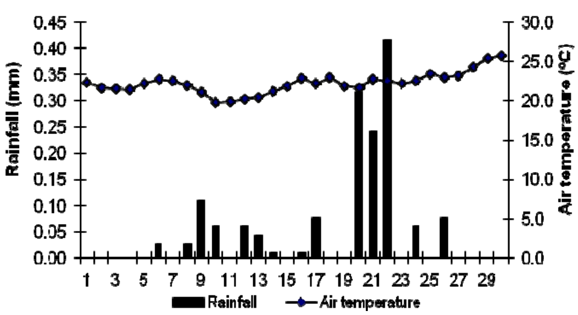

Figure 2. Temporal variation of daily mean values (n = 30) of air temperature and rainfall during the study period.

Turbidity, electric conductivity, total phosphorus, nitrite and ammonium of the sites E5 and E6 were significantly different ($p < 0.05$) from the other sites. Total suspended solids at E6 differed from the other sites; orthophosphate at E5 differed from E2 and E3, while E6 significantly differed from the sites E1, E2, E3 and E4. Regarding nitrogen compounds, the total nitrogen at E5 and E6 differed from E1, E2 and E3; the nitrate at E1 differed from E5 (Table 3).

The PCA results explained 91.1% of total data variability along the first two axes (Table 4). On the axis 1 (72.9%), there was a distinction between the sampling sites, with E5 and E6 located on the left, and mainly associated with turbidity, total phosphorus, orthophosphate, and nitrogen compounds (nitrite, ammonium, and total nitrogen). Although with a low explanation (18.2%), the axis 2 evidenced a clear differentiation between the sites upstream of the city (E1) and along the city (E4); being positively influenced by higher values of silicate, and negatively influenced by nitrate values, respectively (Figure 3).

Table 3. Comparison between the sampling sites along the longitudinal axis of a stretch of São Mateus river, through Kruskal-Wallis/Dunn test, using physical, chemical and biological parameters.

Pairs of sites	EC	Turb	TSS	NH_4	NO_2	NO_3	TN	P-ortho	TP	A
1x5	*	*	*	*	*	*	*	ns	*	ns
1x6	*	*	ns	*	*	ns	*	*	*	ns
2x5	*	*	*	*	*	ns	*	*	*	ns
2x6	*	*	ns	*	*	ns	*	*	*	*
3x5	*	*	*	*	*	ns	*	*	*	ns
3x6	*	*	ns	*	*	ns	ns	*	*	ns
4x5	*	*	*	*	*	ns	ns	ns	*	ns
4x6	*	*	ns	*	*	ns	ns	*	*	ns

*Significant differences ($p < 0.05$); ns = non significant; EC = electric conductivity; Turb = turbidity; TSS = total suspended solids; NH_4^+ = ammonium; NO_2^- = nitrite; NO_3^- = nitrate; TN = total nitrogen; P-ortho = orthophosphate; TP = total phosphorus; A = ash.

Table 4. Pearson' correlation of abiotic variables with the principal components of PCA.

Results	Axis 1	Axis 2
Eigenvalues	5.838	1.457
% explained	72.9	18.2
Broken-stick	2.718	1.718
Variables	Eigen values	
Turbidity (Turb)	-0.3919	0.1942
Silicate (Si- SiO_4)	0.1430	0.6815
Orthophosphate (P-Ortho)	-0.3593	0.1797
Total phosphorus (TP)	-0.3913	0.1879
Nitrite (NO_2^-)	-0.4075	0.1138
Nitrate (NO_3^-)	-0.2292	-0.6218
Ammonium (NH^+)	-0.4976	0.0940
Total nitrogen (TN)	-0.3988	-0.1478

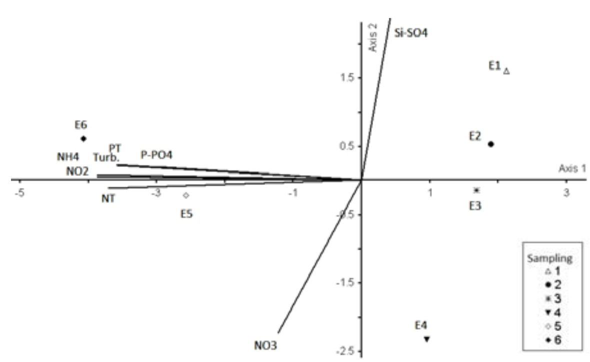

Figure 3. Principal Component Analysis (PCA) with abiotic variables (eight) and ordination of sampling sites in São Mateus river, in September and October 2010. *Turb = turbidity; Si-SiO_4 = silicate; NO_3^- = nitrate; NO_2^- = nitrite; NH_4^+ = ammonium; TN = total nitrogen; P-ortho = orthophosphate; TP = total phosphorus.

In relation to periphyton biomass, the lower mean values of chlorophyll ‘a’ were registered at the site E2, and the higher mean values, at E1 (both sites upstream of the city of São Mateus) (Figure 4). The site E2 was significantly different ($p < 0.05$) from E1, E3 and E4 (Table 3).

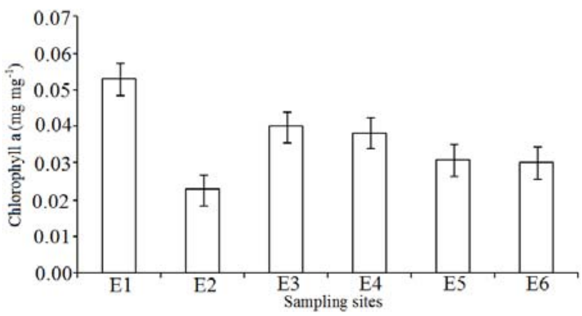

Figure 4. Longitudinal variation of chlorophyll ‘a’ of periphyton community in the sampling sites, followed by standard deviation.

The values of total biovolume of periphytic algae were not significantly different ($p < 0.05$) between the sampling sites. The total biovolume ranged from 61.348 to 156.476 $mm^3\ mg^{-1}$, with higher mean values verified at E4 and E3 (Figure 5), sites along the of city of São Mateus.

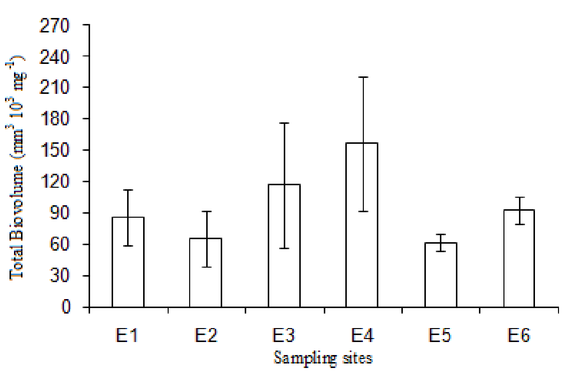

Figure 5. Longitudinal variation of mean total biovolume of periphyton community in the sampling sites, followed by standard deviation.

Bacillariophyceae was the class that contributed most to total biovolume in all sites. Cyanophyceae and Oedogoniophyceae had high contribution to total biovolume at site E2 (Figure 6).

In relation to the values of periphyton dry mass, the inorganic fraction (ash) was higher especially in the sites along and downstream of the city of São Mateus (Figure 6). The values of ash of E2 were significantly different from E6 (Table 2). The organic fraction (ash-free dry mass) ranged from 0.02 mg mg^{-1} (E4) to 0.04 mg mg-^{1} (E6) (Figure 7).

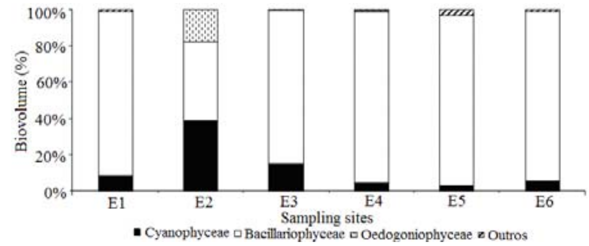

Figure 6. Relative contribution of the classes total biovolume of periphytic algae in the sampling sites.

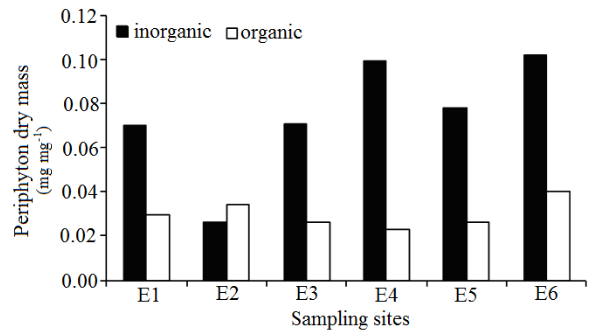

Figure 7. Longitudinal variation of periphyton dry mass in the sampling sites.

The canonical correspondence analysis summarized 85.3 % of total data variability along the first two axes (Table 5), with high significant relationship ($p < 0.05$) between abiotic and biotic variables used in the ordination (Figure 8). On the axis 1 (55.7 %), the site E2 (upstream of the city of São Mateus) was set apart from the other sites, due to the higher biovolume values of Oedogoniophyceae, which, possibly, have been less influenced by turbidity, whereas the sites E5 and E6 (downstream of the city) were related to the biovolume values of Ulothricophyceae, Euglenophyceae, Chrysophyceae and the inorganic fraction (ash) of periphyton community, with greater influence of high values of turbidity, ammonium, orthophosphate, and total phosphorus (Figure 8).

Table 5. Summary statistics and correlation coefficients between the periphyton and abiotic and biotic variables along the first two CCA axes.

Abiotic and biotic variable	Code	Pearson’s correlation (axis 1)	Pearson’s correlation (axis 2)
Orthophosphate	P-Ortho	0.561	-0.478
Total phosphorus	PT	0.559	-0.626
Turbidity	Turb	0.560	-0.799
Ammonium	$NH4^{+}$	0.506	-0.815
Total nitrogen	TN	0.338	-0.831
Chlorophyll *a*	Cl a	0.568	0.556
Ash		0.735	0.000
Ash free dry matter	MSLC	0.059	-0.359
Cyanophyceae	Cy	-0.740	0.362
Bacillariophyceae	Bacil	0.856	0.171
Euglenophyceae	Eugl	0.792	-0.103
Oedogoniophyceae	Oedo	-0.921	-0.320
Chlorophyceae	Chloro	-0.668	-0.679
Chrysophyceae	Chryso	0.806	0.209
Ulothricophyceae	Uloth	0.477	-0.847

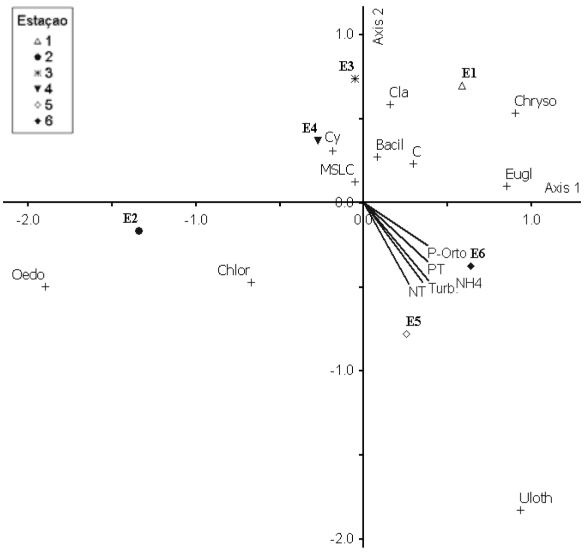

Figure 8. Ordination by the Canonical Correspondence Analysis (CCA) of abiotic and biotic variables for the six sampling sites in São Mateus river. *Cl 'a' = chlorophyll 'a'; C = ash, MSLC = ash-free dry mass; Cy = Cyanophyceae biovolume; Bacil = Bacillariophyceae biovolume; Eugl = Euglenophyceae biovolume; Chlor = Chlorophyceae biovolume; Chryso = Chrysophyceae biovolume; Oedo = Oedogoniophyceae biovolume; Uloth = Ulothricophyceae biovolume.

Discussion

The most common application in biological monitoring using periphytic algae, and non-biological, using physical and chemical parameters, involves the survey of impacts in lotic systems, especially when they are important to diverse uses of the water. In this way, evaluating these impacts requires investigation up- and downstream of the interest point (LOWE; PAN, 1996) and in this case, the periphyton organisms are ideal.

The various uses of river water, such as intensive fish farming and discharge of domestic and industrial wastewater on the sites downstream of the city of São Mateus, probably had influenced the periphyton biomass and contributed to the separation of the sites E5 and E6 from the other sites, in the longitudinal profile of São Mateus river (evidenced by the PCA). The input of allochthonous material contributed to increased turbidity and nutrient levels, which have essential role in controlling the composition and biomass of primary producers, besides promoting the reduction in water transparency.

Regarding the chlorophyll 'a' has higher value in studying productivity when species with high biomass have strong influence on the transference of energy and matter (LOWE; PAN, 1996). However, factors like the light incidence and nutrients deficit has changed the ratio between pigment and algal organic matter (STEVENSON et al., 1996). In the present study, the low values of chlorophyll 'a' in the periphyton community may be related, in part, with the morphology, physiology and especially with the size of the stand of macrophyte *Eichhornia crassipes* at each sampling sites. The lowest values of chlorophyll *a* recorded in the sampling site downstream of the city of São Mateus may be related to larger sizes of the macrophyte with possibility of greater assimilation of nutrients from the water and consequent lower availability of these for the algal community. Martins and Fernandes (2011) reported that the low photosynthetic biomass in Santa Maria da Vitória river (0.07 μg cm^{-2} to 0.67 μg cm^{-2} of chlorophyll 'a'), even with nutrient availability, was related to physical factors (turbulence and water current), which could remove the forms loosely attached to the substrate. Moreover, the input of allochthonous inorganic material might have had abrasive effect on the community (FERNANDES; ESTEVES, 1998; OLIVEIRA; RODRIGUES, 2002) which may also explain the low values of chlorophyll *a* of the periphytic community of São Mateus river.

In the present study, the inorganic material of the total dry mass had greater contribution when the river crossed the city of São Mateus, and after this, possibly due to the influence of fish farming and discharge of domestic sewage, downstream of the city. The great contribution of inorganic fraction can also explain the low values of chlorophyll 'a', since the variation in the content of ash and chlorophyll 'a' indicate changes of auto- and heterotrophic organisms (FERNANDES; ESTEVES, 2003).

The algal biomass can also be evaluated through biovolume that enables a more accurate analysis of the actual contribution of species to community (BIGGS; KILROY, 2000). The high biovolume values verified especially in the sites along and downstream of the city of São Mateus can be related to the higher concentration of nutrients from input of allochthonous material into the environment. This allochthonous material raises the level of the main nutrients, that it is essential for metabolism, development and cell composition (biomass) of autotrophic organisms (ESTEVES; AMADO, 2011). This condition (higher nutrients) together with higher turbidity may have favored an increase in algal biovolume, especially Bacillariophyceae at sampling stations along and downstream of the city of São Mateus.

In general, the class Bacillariophyceae was the most representative in the relative contribution to total periphyton biovolume, and its high values are related to the high density and the larger-sized cells of the individuals of this class. The second most representative class was Cyanophyceae, especially represented by high density of *Synechocystis aquatilis* Sauvaegau (small-sized, but abundant), and by the presence of filamentous Cyanobacteria, with high biomass due to their larger size. Environments with high concentration of nitrogen have favored the development of filamentous algae with high biovolumes (LAMPERT; SOMMER, 2007), once the filaments are excellent adaptive forms that grow rapidly in length, and may remain constant the ratio area:volume (MARGALEF, 1983).

Nutrients have a key role on composition and biomass of the aquatic primary producers, especially the periphytic algae (HUSZAR et al., 2005). In this way, the high levels of nutrients, mainly at sites along and downstream of the city of São Mateus (as evidenced by the PCA) possibly have favored Bacillariophyceae and Cyanophyceae individuals.

Conclusion

Our results for abiotic data corroborated the initial hypothesis (serial discontinuity concept), that is, the São Mateus river presents, along its course, pulses of allochthonous material primarily from human activities, especially along and downstream of the city. The same was not observed for periphyton biomass, due to the sites being submitted to different environmental conditions. Nevertheless, the periphyton biomass had been influenced by nutrients (phosphorus and nitrogen) and turbidity, as indicated by the CCA, suggesting that the input of allochthonous material altered the water quality (evidenced by the PCA) after the city of São Mateus and downstream of it.

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