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Evaluating the periphyton as a bioreactor for removal of nutrients in a shallow hypereutrophic reservoir

Avaliando o perifíton como biorreator para remoção de nutrientes em um reservatório raso hipereutrófico

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Abstract: Aim: We evaluated the periphyton on artificial substrate in the treated sewage effluent, effluent patch, inside and after the macrophyte stand in a shallow hypereutrophic reservoir. Specifically, we investigated the relationship between N and P contents and algal biomass in the periphyton with N and P availability, focusing on nutrient retention. **Methods**: Periphyton sampling was performed at the effluent inlet, effluent path, inside, and two sites after macrophyte stand. Periphyton sampling was performed after 30 days of colonization. Abiotic variables were determined in the sewage effluent and in the reservoir water. **Results**: Biomass and N and P contents in the periphyton were significantly different among sampling sites. The highest nutrient concentrations were found in the sampling sites with effluent. The highest periphyton chlorophyll-a were found inside and after the macrophyte stand, while N and P contents were the highest in the effluent inlet and effluent. **Conclusions**: In conclusion, N and P contents in the periphyton were associated with N and P availability, evidencing the ability of nutrient retention of the community. Our findings suggest that periphyton on artificial substrate can as a potential tool for removing N and P from the effluent from the sewage treatment system, contributing to minimizing the nutrient load discharged in a shallow reservoir.

Keywords: biomass; biofilm; nutrient removal; nutrient content; secondary sewage treatment.

Resumo: Objetivos: Avaliamos o perifíton em substrato artificial no efluente de esgoto tratado, no percurso do efluente, dentro e após o banco de macrófitas em um reservatório raso hipereutrófico. Especificamente, investigamos a relação entre os conteúdos de N e P e a biomassa de algas no perifíton com a disponibilidade de N e P do ambiente, com foco na retenção de nutrientes. **Métodos**: A amostragem do perifíton foi realizada no efluente e ao longo do percurso do efluente, dentro e em dois locais após o banco de macrófitas. A amostragem do perifíton foi realizada após 30 dias da colonização. As variáveis abióticas foram determinadas no efluente do esgoto e na água do reservatório. **Resultados**: Os valores de biomassa e conteúdo N e P no perifíton foram significativamente diferentes entre os locais de amostragem. As maiores concentrações de nutrientes foram encontradas nos locais com efluente. As maiores concentrações de clorofila-*a* no perifíton foram encontradas dentro e após o banco de macrófitas, enquanto os maiores teores de N e P foram encontrados no efluente e no percurso do efluente. **Conclusões**: Em conclusão, os conteúdos de N e P no perifíton foram associados à disponibilidade de N e P, evidenciando a capacidade de retenção de nutrientes da comunidade. Nossos resultados sugerem que o perifíton em substrato artificial pode ser uma ferramenta para a remoção de N e P do efluente do sistema de tratamento de esgoto, contribuindo para minimizar a carga de nutrientes descarregada em um reservatório raso.

Palavras-chave: biomassa; biofilme; conteúdo de nutrientes; esgoto com tratamento secundário remoção de nutrientes.

1. Introduction

Eutrophication is a well-documented environmental problem worldwide (Schindler, 2012, Le Moal et al., 2019). The increase in algal biomass, changes in species composition, and structural simplification are some of the effects of eutrophication on biological communities (Wetzel, 2001). In freshwater ecosystems, phosphorus is a necessary macronutrient for primary producers, and it is considered a primary nutrient limiting algal growth. A reduction in phosphorus supply has successfully reduced eutrophication in lakes and reservoirs (Schindler, 2012). Thus, the reduction of phosphorus availability is essential for the restoration of most eutrophic ecosystems. In this context, periphyton can play an important role in the removal of nutrients due ability to assimilate, immobilize, and internally recycle N and P efficiently, reducing nutrient supply in the ecosystem (Dodds, 2003; Sutherland & Craggs, 2017). Different types of technologies using periphyton for the removal of nutrients in wastewater treatments have been successfully tested (Sutherland & Craggs, 2017; Wu et al., 2018, Gao et al., 2015). Studies reported the potential application of periphyton to minimize non-point nutrient pollution and remove phosphorus from sewage treatment systems (Lu et al., 2014, Cao et al., 2014, Sutherland & Craggs, 2017). Although the use of periphyton bioreactor to remove nutrients from enriched systems is recognized worldwide, this approach is still little investigated in Brazil (Morashashi et al., 2019).

In the present study, we evaluated the periphyton on artificial substrate in the treated sewage effluent, inside and after the macrophyte stand, investigating the potential of the community as a bioreactor. Specifically, we investigated the relationship between N and P contents and algal biomass in the periphyton with N and P availability, focusing on nutrient retention. The present study contributes to a better understanding of the use of periphyton as a bioreactor to remove phosphorus in shallow tropical reservoirs.

2. Material and Methods

2.1. Study area

The Garças Reservoir is located in the Fontes do Ipiranga State Park (23° 38'40.6 "S, 46° 37'28.0" W), which is located São Paulo, Brazil (Figure 1). It is a shallow urban reservoir (Zmax = 4.7 m; Zmed = 2.1 m) and classified as hypereutrophic (Bicudo et al., 2020). The reservoir has a surface area of 88,156 m^2, a volume of 188,785 m^3, and a mean theoretical residence time of 71 days. The climate is classified as high altitude tropical with rainy summers and dry winters. About 20 years ago, the macrophyte coverage reached 40-70% of the water surface causing mosquitoes' proliferation (Bicudo et al., 2007). To mitigate this problem, macrophytes were removed mechanically and management actions were initiated (Bicudo et al., 2020). Currently, macrophytes are trapped by a structure made of bamboo and metal mesh. Despite the discharge of untreated domestic sewage had been interrupted, the secondary treatment effluent is still discharged into the ecosystem. The effluent from the secondary sewage treatment is discharged at a point in the riparian forest, then it percolates into the macrophyte stand in the littoral zone of the reservoir. This effluent is secondary wastewater treatment plant in the city zoo. During the experimental period (09/11/2016 to 09/12/2016), the air temperature ranged from 14.7 to 23.8ºC and daily rainfall varied from 0 to 40 mm (Instituto de Astronomia, Geofísica e Ciências Atmosféricas – IAG/USP, 2019).

2.2. Sampling design

In the littoral zone, effluent or water and periphyton sampling were performed at the effluent inlet, effluent path, inside, and after macrophyte stand (replication: n = 3), totaling 15 sampling sites. In the forest, a pipe discharges the secondary sewage treatment effluent, forming a place of the accumulation, which is called the effluent inlet. In this place, the effluent overflows and runs about 10 m until it enters the macrophyte stand (*Eichhornia crassipes* Mart. (Solms); *Eichhornia azurea* (Sw) Kunth); this path was called the effluent path. The sampling sites were designated as: EI, effluent inlet; EP, effluent path (10-15 m away from the source); M, macrophyte stand (*Eichhornia crassipes*; 20-30 m away from the source); AS, 1 m after stand (40 m away from the source); AM, 10-15m away from the macrophyte stand (50 m

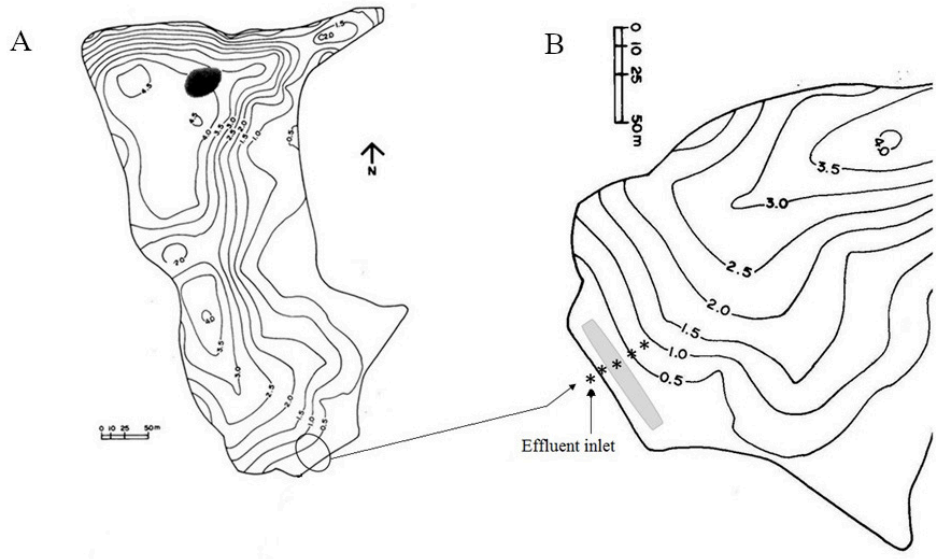

Figure 1. A. The location of the sampling area in the Garças Reservoir bathymetric map (B; Bicudo et al., 2002). B. Scheme showing the sequence of the sampling sites from the discharge of the effluent (effluent inlet; effluent path; inside macrophyte stand; after 1 m from the macrophyte stand; 10-15m away from the stand). The gray area represents the macrophyte stand.

away from the source). The water column depth varied from 20 to 50 centimeters at the first four sampling sites and was 1.5 meters at the last one.

Fifteen acrylic supports with ten glass slides for periphyton colonization each were submerged at a depth of 15 cm in the sampling sites. After 30 days of exposure, the colonized substrates were collected. In the macrophyte stand, the artificial substrates were placed near *Eichhornia crassipes*, aiming at a better representation of the epiphyton. The periphyton on artificial substrates was removed by scraping and jets of distilled water. The artificial substrate was chosen to exclude the influence of macrophyte on periphyton nutrient status.

2.3. Environmental variables analyzed

In the field, underwater radiation was measured using quantometer (Li-Cor LI-250A) and temperature and pH using underwater multiparameter probe (Horiba U-50, Kyoto, Japan). The concentrations of dissolved oxygen (DO; azide-modification method), free CO_2 (calculated from alkalinity and pH), nitrate (cadmium reduction method), nitrite (diazotization method), ammonium (phenate method), orthophosphate (ascorbic acid method), total nitrogen (TN) and total phosphorus (TP) (alkaline persulfate method) were determined according to APHA (2005). The nitrite, nitrate and ammonium concentrations were summed to determine dissolved inorganic nitrogen (DIN). The water samples for the dissolved nutrients were filtered through glass-fiber filters (GF/F Whatman, Maidstone, UK).

2.4. Periphyton

We determined periphyton chlorophyll-*a* (corrected for phaeophytin) from a sample filtered on glass-fiber filters (GF/F Whatman, Maidstone, UK), following 24 h extraction with 90% ethanol in the dark (Sartory & Grobbelaar, 1984). We filtered periphyton samples on glass-fiber filters (GF/F Whatman, Maidstone, UK), which were pre-calcined and weighed to determine dry mass (DM) and ash-free dry mass (AFDM). AFDM:Chlorophyll ratio (autotrophic index) was calculated according to APHA (2005).

The periphyton TP content was determined by combusting samples at 550 °C for 1 h in a muffle and then leaching the samples in HCl 1N at 80°C for 30 min (Andersen, 1976; Pompêo & Moschini-Carlos, 2003). Subsequently, the samples were analyzed for soluble reactive phosphorus according to the ascorbic acid method (Strickland & Parsons, 1965). Periphyton TN content was determined by the micro-Kjeldahl method (Umbreit et al., 1964). N and P contents were expressed per dry mass unit (%DM).

2.5. Data analysis

Principal components analysis (PCA) was applied for the abiotic data. PCA was performed based on covariance matrix with limnological data log-transformed, except pH (PC-ORD 7.0;

McCune & Mefford, 2011). Based on eigenvalues from randomizations (Peres-Neto et al., 2005), we determined the dimension of interpretation. Pearson correlation (*r*) was used to evaluate the relationship between axis 1 scores and environmental variables, as well as the correlation between the periphyton nutrient content and dissolved nutrient concentration (α = 0.05). Permutational multivariate analysis of variance (one-way PERMANOVA) was applied to detect whether periphyton on artificial substrates were significantly different among sampling sites. This analysis was performed with logarithmic data, using the Bray-Curtis similarity measure and 9999 permutations (Past 4.02, Hammer et al., 2001).

3. Results

3.1. Environmental variables

Table 1 summarizes the abiotic variables in the water surrounding periphyton at sampling sites. The highest DO concentrations were detected inside the macrophyte stand and at the sites after the stand (1 m and 50 m). The highest TN, TP and conductivity values were found at the effluent inlet and effluent path sites. Similarly, the highest DIN and PO_4-P concentrations were found in the effluent inlet and effluent path sites (Figures 2A-B). Light availability was higher after the macrophyte stand (Figure 2c).

PCA summarized 97.2% of limnological data variability in the two first axes (Figure 3). The sampling sites with sewage effluent were associated with high nutrient and free CO_2 concentrations (r > -0.7) on the negative side of axis 1. In contrast, the sampling sites inside and after the macrophyte stand were correlated with high DO concentration, pH and temperature (r> 0.8). Axis 1 represented a gradient of nutrient availability from the effluent input.

3.2. Periphyton

The highest periphyton chlorophyll-*a* were found inside macrophyte stand and after the stand, while the highest dry mass was found in the effluent inlet (Figures 4A-B). The lowest values of the periphyton AFDM:Chlorophyll ratios were found inside and after the macrophyte stand (Figure 4C). Environmental conditions of the sampling sites had significant influence on periphyton nutrient contents and biomass (PERMANOVA: F= 3.85; p = 0.002).

The highest N and P contents in the periphyton were found in the effluent inlet and effluent path (Figures 5A-B). At 50 meters from the effluent inlet, periphyton P and N contents increased again. Periphyton P content was positively correlated with water PO_4-P concentration (Pearson: r = 0.8; p = 0.0003) and N content with DIN concentration (Pearson: r = 0.6; p = 0.04). Regarding the PCA scores, a negative and significant correlation was found between the P content and the scores for axis 1 (Pearson: r = -0.81; p = 0.0002). Negative and significant correlation was also found between N content and the axis 1 scores (Pearson: r = -0.54; p = 0.05).

4. Discussion

Our findings showed a difference in the dissolved and total nutrient concentrations between sampling

Table 1. Mean and standard deviation of environmental variables in the sampling sites.

	Effluent Inlet	Effluent path	Macrophyte stand	After stand	Away from the stand
Conductivity (µS cm^{-1})	279.0±53.0	428.0±33.9	296.7±38.4	280.3±1.2	286.3±4.8
Depth (m)	0.20±0.0	0.20±0.0	0.20±0.0	0.29±0.6	0.56±0.2
DO (mg L^{-1})	3.3±0.8	2.3±0.3	12.1±2.5	10.7±1.6	11.6±2.8
NO_2-N (µg L^{-1})	179.1±2.6	205.7±7.1	5.5±0.6	13.6±8.7	5.0±0.0
NO_3-N (µg L^{-1})	30.2±19.0	18.5±1.1	6.1±0.8	14.8±6.2	5.0±0.0
NH_4-N (µg L^{-1})	400.3±9.7	322.3±10.7	<5.0	7.8±3.7	<5.0
TN (µg L^{-1})	7764.0±14.3	7001.4±166.1	6003.0±707.4	2677.4±60.5	2830.8±496.6
TDP (µg L^{-1})	79.3±46.6	188.0±70.2	37.9±7.9	41.3±9.4	24.9±1.7
TP (µg L^{-1})	938.5±170.4	789.5±70.2	775.9±299.4	215.0±28.6	165.1±60.7
PO_4-P (µg L^{-1})	599.4±29.5	312.3±66.0	7.4±2.0	18.7±8.0	<4.0
Light (µmol m^{-2} s^{-1})	30.9±9.4	140.4±87.8	216.2±57.7	451.8±48.2	720.0±137.9
%Light	56.7±13.6	56.9±11.7	33.0±3.9	76.7±10.2	79.4±11.7
Temperature (°C)	23.1±0.5	22.9±0.1	24.3±0.3	24.8±0.1	25.2±0.3
pH	5.7±1.6	5.4±1.0	8.6±0.8	9.5±0.1	9.8±0.1
TDS (g L^{-1})	0.6±0.5	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0

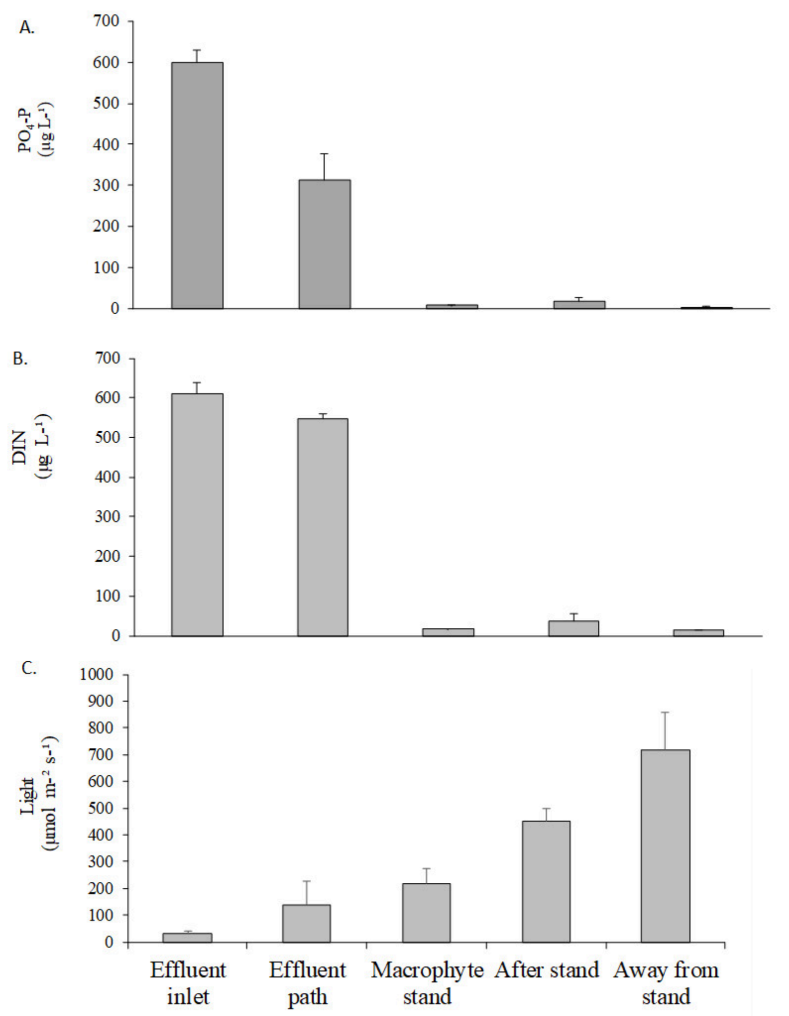

Figure 2. Dissolved nutrient concentrations and light availability (mean; SD; n = 3) in the sampling sites.

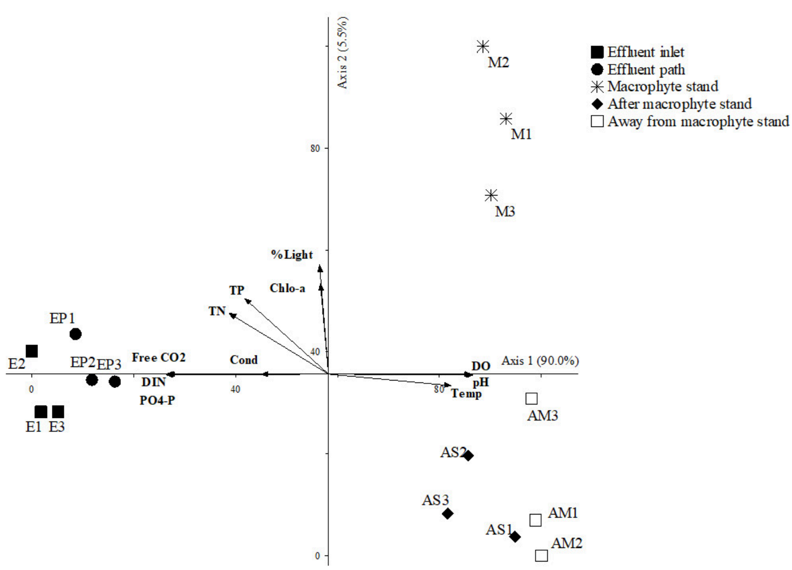

Figure 3. PCA of environmental variables in the sampling sites. Abbreviations: the letters indicate the sampling sites (E, effluent inlet; EP, effluent path; M, inside macrophyte stand; AS, after 1 m from the macrophyte stand; AM, 10-15m away from the macrophyte stand), and the last number indicates the three replicates (1,2,3) from each sampling site.

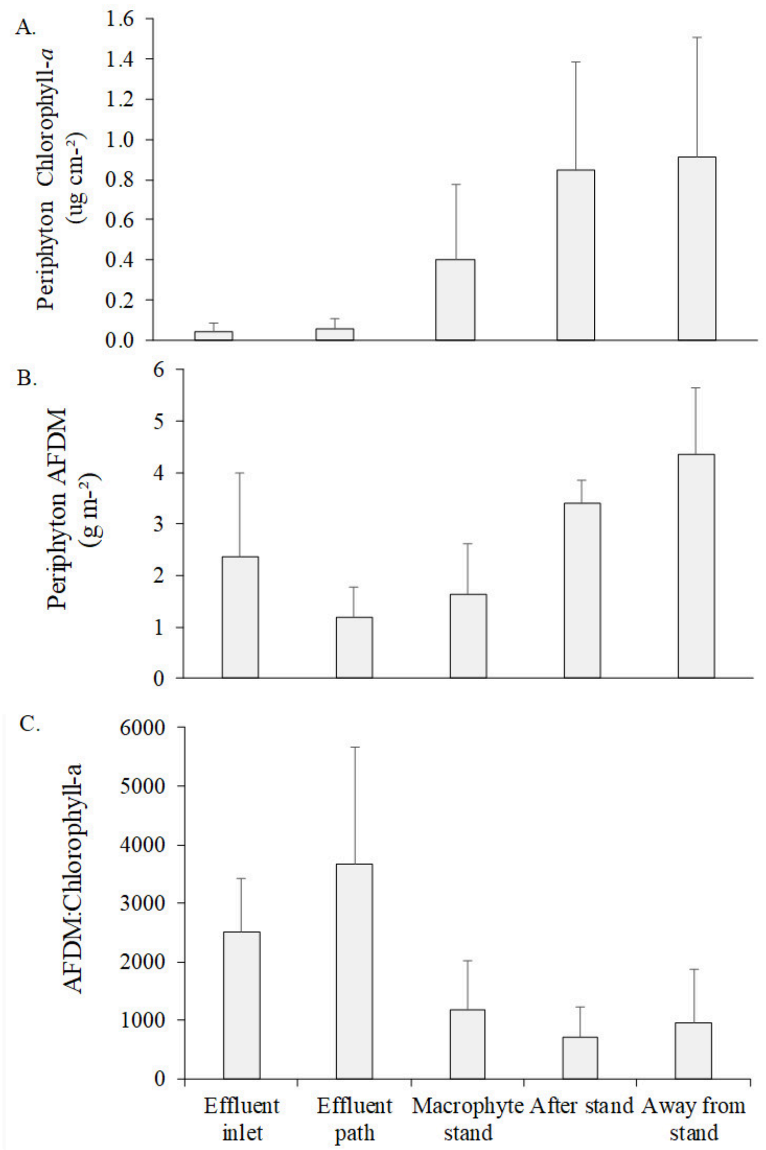

Figure 4. Periphyton chlorophyll-*a*, ash free dry mass (AFDM) and AFDM:Chlorophyll ratio (mean; SD; n = 3) in the sampling sites.

sites, showing an N and P availability gradient from the discharge of effluent from the secondary sewage treatment system. Comparing to long-term studies in the current reservoir (Crossetti et al., 2019; Bicudo et al., 2020), dissolved nutrient concentrations were extremely high in the inlet effluent, demonstrating the high nutrient load that discharged into the reservoir. We observed that total and dissolved nutrient concentrations were substantially reduced inside and after the macrophyte stand (55-76%) when compared to sampling sites with treated sewage. Several factors may have acted on reducing the nutrient concentration inside and after macrophyte stand, such as dilution and chemical processes (Vymazal, 2007). However, the macrophyte stand that is restricted to an area of the reservoir can function as an artificial wetland, in which *Eichhornia crassipes* is the dominant species. According to a study in Brazilian tropical constructed wetlands, the removal of TP and TN from effluents averaged 50%-80% efficiency depending on the climatic period (Lautenschlager, 2001; Travaini-Lima & Sipaúba-Tavares, 2012). The efficiency of *E. crassipes* in removing nutrients from different systems has been demonstrated (Patel, 2012; Zhang et al., 2019), including sewage treatment systems (Dellarossa et al., 2001; Mishra & Maiti, 2017). In constructed wetlands, macrophytes and periphyton are considered efficient in removing nutrients from

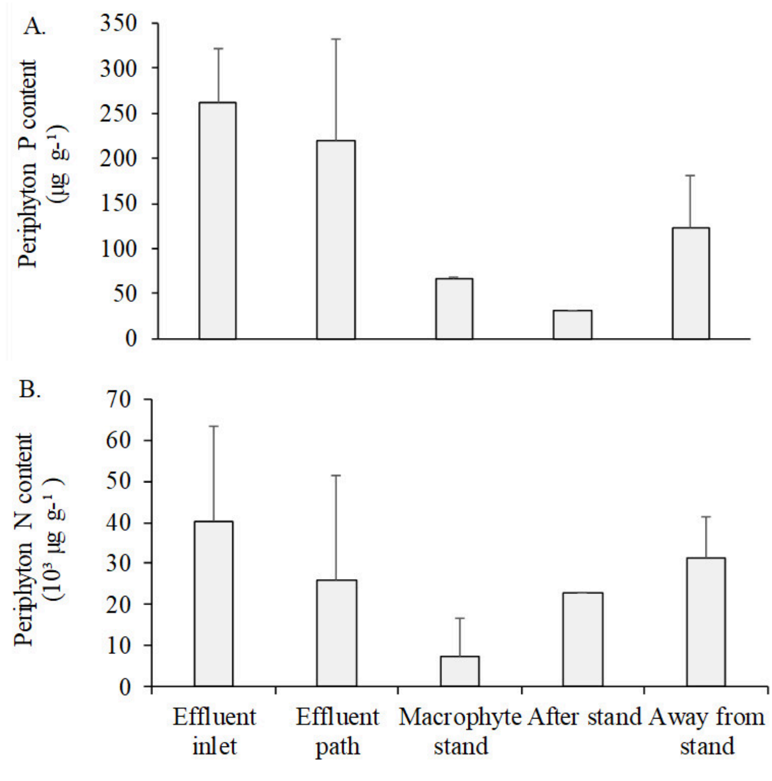

Figure 5. Periphyton N and P contents (mean; SD; n = 3) in the sampling sites.

effluents (Vymazal, 2007; Gu & Dreschel, 2008; Sutherland & Craggs, 2017). In the present study, our findings suggested that macrophyte-periphyton complex may have acted in reducing the N and P concentration from treated sewage effluent.

We found that the N and P contents in the periphyton on artificial substrate were coupled with the nutrient availability of the environment. Thus, the highest periphyton N and P contents were found in the treated sewage effluent. In freshwater ecosystems, the relationship between periphyton P content and water P concentration has been widely reported in the literature (e.g., Gaiser et al., 2004), including the study area reservoir (Santos et al., 2018). Studies demonstrated the ability of periphyton to remove and retain phosphorus in wastewater with high-load phosphorus (Cao et al., 2014; Lu et al., 2014, Gao et al., 2015). On artificial substrates of different complexities, Wu et al. (2010) demonstrated that the periphyton bioreactor was a promising tool for controlling cyanobacterial growth in Chinese lakes. According to the review study, several technologies have been developed based on the potential of the periphytic algae community to remove wastewater nutrients (Sutherland & Craggs, 2017). As described in the literature, our results also suggested that periphyton on the artificial substrate can be used to remove nutrients from sewage effluent in the hypereutrophic reservoir studied here.

We found the highest periphyton chlorophyll-*a* inside and after the macrophyte stand. In these sites, AFDM:Chlorophyll ratio also evidenced greater participation of the autotrophic component in the periphyton. The low light availability may have been a determining factor for the low algal biomass in the periphyton in the inlet and path effluent, where the riparian forest causes strong shading. Despite the low algal biomass in the periphyton, we highlight that algae have functional characteristics that can contribute to the nutrient storage under low light availability, such as mixotrophy. The low light availability can favor the mixotrophic algae growth, which ingests bacteria or particulate matter (Rothhaupt, 1996). A previous study reported a high abundance of mixotrophic algae in the periphyton and phytoplankton (Borduqui et al., 2012; Crossetti et al., 2019; Amaral et al., 2020). For example, *Trachelomonas, Ceratium*, and *Cryptomonas* species are common in the plankton and periphyton in the reservoir current. According to the literature, these genera are potentially mixotrophic (Chen et al., 2016; Hansson et al., 2019). Thus, periphyton can present algae with adaptive strategies to grow in low light availability, as observed in the treated sewage effluent.

Our findings showed that periphyton can as a tool for removing N and P from the effluent from the secondary sewage treatment system. Besides, periphytic algae contributed more to the removal of nutrients inside and after the macrophyte stand, where there was higher light availability. The results suggest that the algal community's contribution to nutrient retention should be greater in non-shaded sites. In conclusion, N and P contents in the periphyton were associated with N and P availability, evidencing the ability of nutrient retention of community. Our findings suggested that periphyton on artificial substrate can as a potential tool for removing N and P from the effluent from the sewage treatment system, contributing to minimizing the nutrient load discharged in a shallow reservoir.

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