Vol.67: e24220827, 2024
https://doi.org/10.1590/1678-4324-2024220827
ISSN 1678-4324 Online Edition

Brazilian Archives of Biology and Technology

Article - Environmental Sciences

Ecotechnologies for Aquaculture Wastewater Treatment in a Water-Scarce Region

Augusto César Cavalcanti Gomes[1*]
https://orcid.org/0000-0002-7845-3873

Alineaurea Florentino Silva[2]
https://orcid.org/0000-0003-1744-1593

Maria Carolina Tonizza-Pereira[3]
https://orcid.org/0000-0001-9284-5147

[1]Universidade Federal do Rio Grande do Norte, Centro de Biociências, Natal, Rio Grande do Norte, Brasil; [2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Semiárido, Petrolina, Pernambuco, Brasil; [3]Universidade Federal do Vale do São Francisco, Campus Ciências Agrárias, Petrolina, Pernambuco, Brasil.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Ana Cláudia Barana

Received: 20-Oct-2022; Accepted: 22-Aug-2023

*Correspondence: augustocesarpe@hotmail.com; Tel.: +55-84-99933-8149 (A.C.C.G.).

HIGHLIGHTS

- We assessed ecotechnologies with aquatic macrophytes and with periphyton.
- *Azolla filiculoides*, *Pontederia crassipes* and *Salvinia auriculata* were used.
- Nutrient removal was greater using aquatic macrophytes than using periphyton.
- Periphyton was not efficient in reducing the ammonia and nitrate contents.

Abstract: In aquaculture, biological treatments usually have an excellent benefit-cost ratio. This study evaluated the efficiency of different ecotechnologies on aquaculture wastewater treatment. Two experimental units were installed. In the first one, tanks were individually vegetated with free-floating aquatic macrophytes. In the second experimental unit, 10.72 m^2 of artificial substrate were added for periphyton colonization. The hydraulic retention time of the wastewater was of 30 days. Both physical and chemical characterizations of the effluent were carried out at the beginning and at the end of the experiment. The periphyton community attached to the substrate was catalogued. In the first unit, the pH, nitrite, and orthophosphate values were significantly different. The tanks vegetated with *Azolla filiculoides* were the only ones where the electrical conductivity values were reduced. Tanks vegetated with *Azolla filiculoides* and *Pontederia crassipes* presented decreased the nitrate concentration. On the other hand, the concentration of ammonia and total phosphorus decreased in all vegetated tanks. In the second unit, only the nitrate and ammonia values did not decrease in the last day of the experiment. 33 taxa belonging to the classes Cyanophyceae, Chlorophyceae, Bacillariophyceae, Trebouxiophyceae, Coleochaetophyceae, Coscinodiscophyceae and Zygnematophyceae were identified in the substrate. The experimental units were effective in mitigating the nutrients in aquaculture wastewater that cause eutrophication.

Keywords: aquatic community; bioremediation; eutrophication; fish farming; phytoremediation process; semi-arid region.

INTRODUCTION

Several countries have been through a massive expansion of aquaculture, especially in captive breeding, whose output exceeds capture harvest [1]. This growth results from the high capacity of this segment to meet the global food demand, which is stressed by the global human population increase [2]. Moreover, freshwater aquaculture accounts for about 64% of total aquaculture [3]. In Brazil, targeting the domestic market, aquaculture has grown faster than other sources of food production [4,5].

The Brazilian Semi-arid region, located predominantly in the Northeast region, is characterized by low rainfall levels and high evapotranspiration [6] due to the occurrence of severe dry periods over the years [7]. As a result, water resources are scarce in this region, with two great rivers standing out – São Francisco and Parnaiba – since most rivers are temporary [8]. Despite such conditions, aquaculture is growing in the region, especially because the São Francisco River presents a rich fish diversity whose potential for economic use is high [9–11]. However, this activity poses a great challenge because there must be sufficient water resources for it to be installed and reach its entire potential production volume [12], and, also in this context, rainfall in the Brazilian Semi-arid region is irregularly distributed across time and space [13–15], leading to an increase in the ratio between water demand and availability in times of drought, as well as causing an even higher water demand instead of a rationing in times of water drought [16]. These characteristics aggravate the social context of the Brazilian semiarid region, especially when the little water resource available in this region also suffers from the change in quality resulting from effluents when untreated wastewater is discarded [17].

Legislation in Brazil is inefficient to limit the expansion of anthropogenic pressures on the aquatic ecosystems, especially when water pollution from aquaculture enterprises is considered [18,19]. In the Semi-arid region, the shortage of technology and specialized labor, which contribute to the harmlessness of effluents in water systems, enhance the harmful effects of this type of livestock production. Treatment using ecotechnologies that employ metabolic properties of aquatic communities such as aquatic macrophytes and periphyton can work as a cost-effective alternative [20,21]. There are other methods to mitigate the effects of aquatic pollution caused by these enterprises. Dauda and coauthors [2] highlighted that most cultivation system ponds are absent of effluent management and one of the primary solutions is the feeding management of the cultivated species, preventing the cultivation system from having a high rate of undigested feed. Additionally, they discussed some technologies used, such as the Aquaculture Recirculation System (ARS). However, the ARS needs improvements in order to increase efficiency to mitigate the negative effects of nitrogen, phosphorus and dissolved solids accumulation, consequently, the high costs of installing the ARS and the high level of knowledge required for operationalization make the system not very desirable in enterprises.

Thus, the use of biological systems to mitigate pollution would replace chemical and physical techniques, which are disadvantageous because are expensive to implement and to operate and, above all, inefficient to purify pollutants in low concentrations, which accumulate in the long run to levels that cause adverse changes to natural environments [22]. These aquatic communities deploy several different mechanisms to extinguish pollutants. Aquatic macrophytes use rhizofiltration, phytoextraction, phytostabilization, phytovolatilization and phytotransformation as mechanisms [19,23], whereas periphyton works in the transformation and degradation of nutrients that attenuate water quality through a complex assembly of organisms [24,25].

The introduction of autotrophic aquatic communities into wastewater treatment systems has the potential to reduce nutrient concentrations in natural aquatic environments, contributing to nutrient cycling and self-depuration of aquatic ecosystems. When vegetated in tanks known as constructed wetlands, aquatic macrophytes use a technology that does not demand continuous management, especially when vegetation using free-floating macrophytes is used, since their roots cannot reach the substrates placed in the tanks [26] and do not enter effluent inlet and outlet pipes, preventing clogs in the system. Another important feature of aquatic vegetables is that they are phenotypically accumulators of substances that harm water quality and store pollutant concentrations that are thousands of times higher than the adjacent water [19]. Regarding the periphyton, the use of periphytic biofilm is advantageous because this community responds quickly to nutrient enrichment of waters despite being influenced by seasonality [27,28]. Periphyton can absorb persistent pollutants with biomagnification and bioaccumulation properties of several effluents because of its high microbial diversity, which is sensitive to a wide range of environmental stressors [29]. Moreover, the complex periphyton community has self-regulation skills, which allow these organisms to increase their resistance to changes in the characteristics of the effluent and maintain high levels of metabolic activity in wastewater treatment [30].

In this setting, this study evaluated the efficiency of different ecotechnologies on aquaculture wastewater treatment using aquatic communities of aquatic macrophytes and periphyton. For this purpose, we tested the following hypotheses: (i) the concentration of nitrogen and phosphorus in the final stage of the treatments is lower than the initial one and (ii) the ecotechnologies employed are equally efficient in minimizing the concentrations of nutrients that reduce water quality in aquaculture businesses.

MATERIAL AND METHODS

Field of study

The ex-situ experimental units were installed in the facilities of the Bebedouro Integrated Center for Fishery Resources and Aquaculture (CIB), which belongs to Brazil's Development Company of the São Francisco and Parnaiba Valleys (Codevasf), near the municipality of Petrolina, Pernambuco, Brazil, in the Bebedouro Irrigated Perimeter (Figure 1). The climate of the region is classified, according to Köppen, as BSwh – Semi-arid climate – hot and dry [31]. Regarding hydrography, the locality is in the São Francisco Basin.

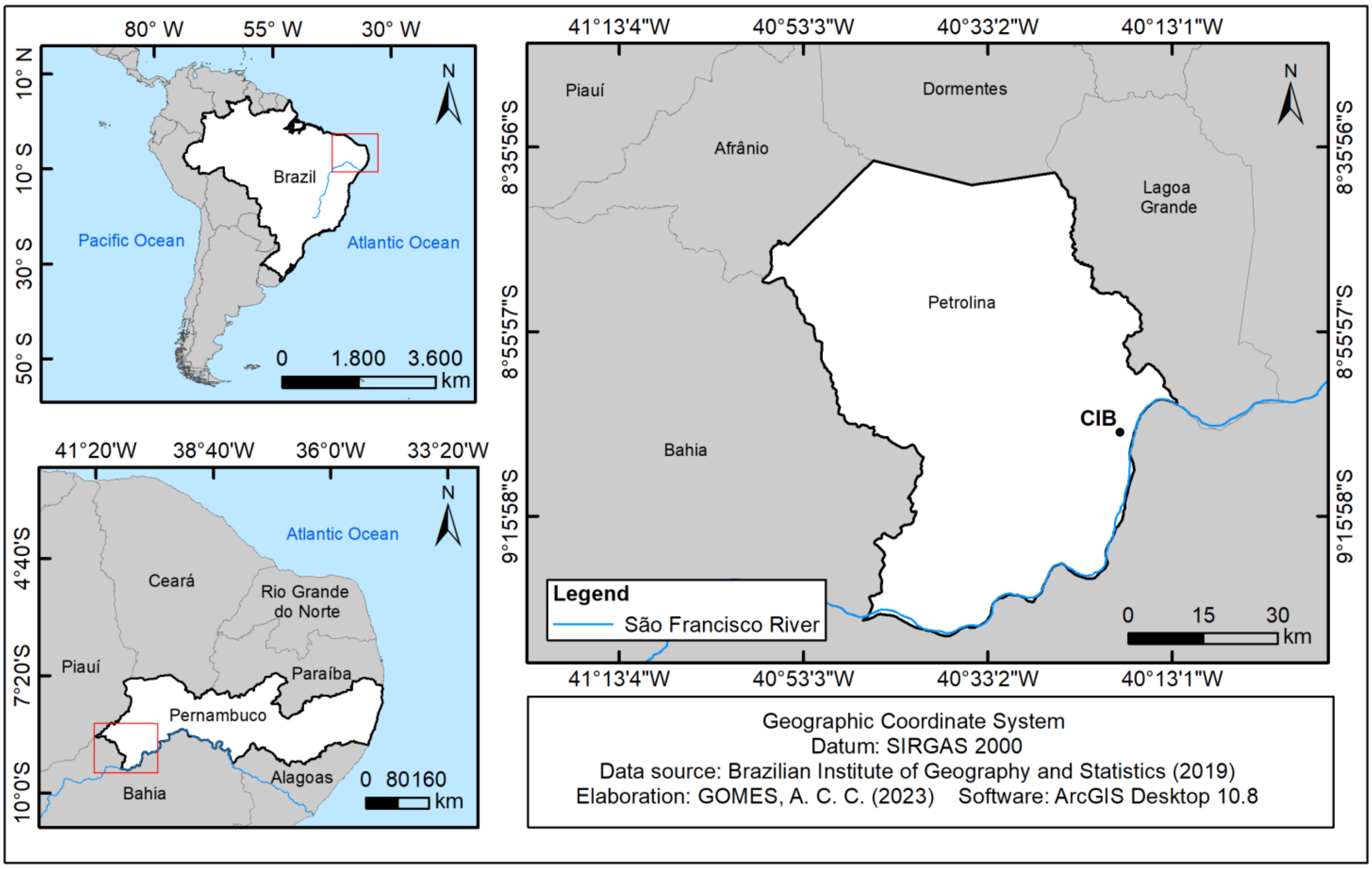

Figure 1. Geographical location map of the experimental units installed in the facilities of the Bebedouro Integrated Center for Fishery Resources and Aquaculture (CIB), in the municipality of Petrolina, Pernambuco, Brazil.

Experimental design

The wastewater generated by one of the excavated ponds was drained weekly by means of a hydraulic pump for eighteen individualized polyvinyl chloride (PVC) tanks with a volume of 1,000 L and a depth of 0.80 m. The tanks were organized in triplicates and a different ecotechnology was placed in each of them. The studies were carried out using two different experimental units, one using aquatic macrophytes and the other using periphyton, and were not performed simultaneously due to the interest of the CIB to add an experimental unit with the periphyton community after the beginning of the experiment with aquatic macrophytes.

The first experimental unit (Figure 2) used the aquatic macrophyte community with tanks in triplicates named as follows: control treatment for the ecotechnology using aquatic macrophytes (CAM), treatment with the aquatic macrophyte species *Azolla filiculoides* Lam. (TAf), treatment with the aquatic macrophyte species *Pontederia crassipes* Mart. (TPc), and treatment with the aquatic macrophyte species *Salvinia auriculata* Aubl. (TSa). The choice of these species was made because they are free-floating macrophytes with a well-developed root system, as well as due to their presence in many aquatic ecosystems of the Brazilian semiarid region, especially in those that present a change in the trophic state of the water. As for the second experimental unit, it had the periphyton community and the tanks in triplicates were organized as follows: control treatment for the ecotechnology using the periphyton community (CPC) and treatment with periphytic biofilm (TPB). The hydraulic retention time of the effluent in each tank was of 30 days (HRT-30).

The aquatic macrophyte species used in the tanks were free-floating. In addition, we selected species whose life cycle allowed the experiment to be carried out within the 30-day hydraulic retention time of the effluent in the treatment systems, with the subsequent removal of the species before decomposition. The specimens were collected in the CIB, except for *S. auriculata*, which was collected in the São Francisco River, and were separated in the laboratory from those of other species. We used young plants, in process of development, and discarded aging individuals undergoing senescence. To standardize initial biomass in the tanks, about 37 g of each species were vegetated in each tank and we used parts of whole individuals in this biomass.

The second experimental unit (Figure 2) was composed of the treatment with periphytic biofilm, where 0.10 mm transparent plastic substrates were placed, structured in 6 columns immersed in the effluent and 20 cm apart from each other. Thus, we obtained an area of 5.36 m² of substrate and of 10.72 m² in contact with the drained effluent for periphyton adhesion on both sides of the substrate. The plastics were immersed in the effluent using concrete weights wrapped in plastic.

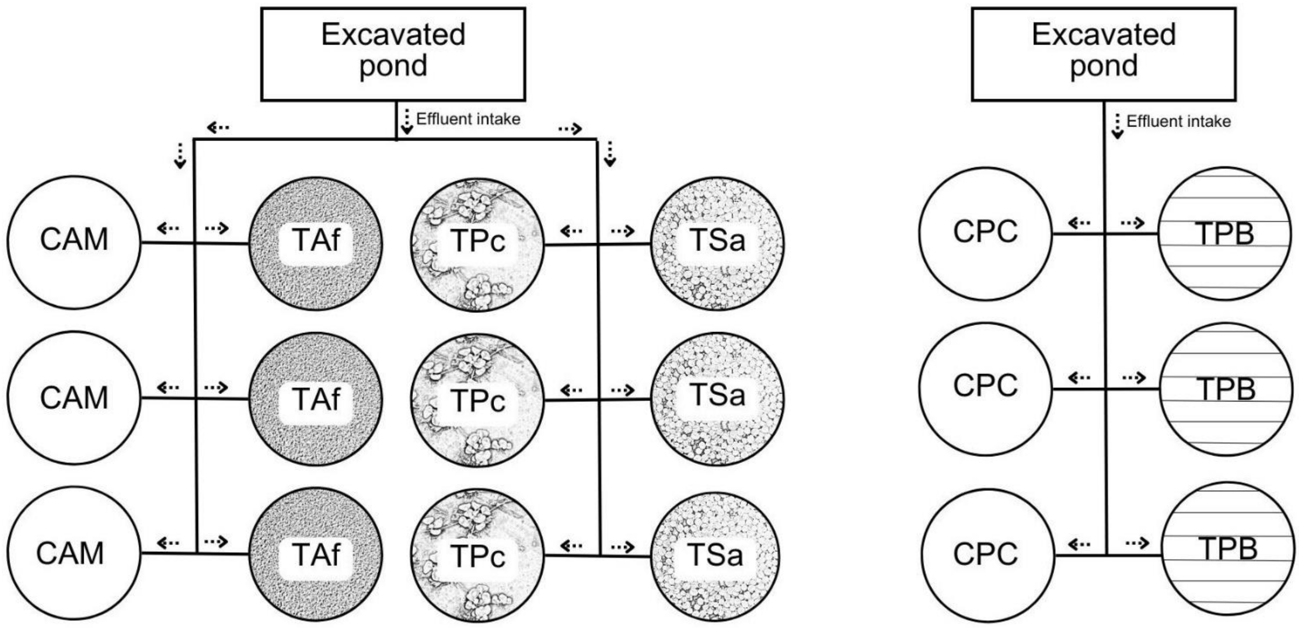

Figure 2. Schematic design of the experimental units, one using aquatic macrophytes and the other using periphyton. CAM: control treatment for the ecotechnology using aquatic macrophytes; TAf: treatment with the aquatic macrophyte species *Azolla filiculoides* Lam.; TPc: treatment with the aquatic macrophyte species *Pontederia crassipes* Mart.; TSa: treatment with the aquatic macrophyte species *Salvinia auriculata* Aubl.; CPC: control treatment for the ecotechnology using the periphyton community; TPB: treatment with periphytic biofilm.

Effluent analysis

In this stage, we collected 1-liter aliquots in the horizontal subsurface in polyethylene vessels. The effluent was collected at the beginning of the hydraulic retention time (HRT-0), that is, prior to the adding of the ecotechnologies, and at the end of the 30-day hydraulic retention time (HRT-30). Prior to collection, effluent homogenization was performed. Also, the amount of effluent released in the tanks weekly was important for the recovery of the effective volume and for the periodic homogenization of the water column.

The vessels were stored in a thermal box and sent to the Agro-Environmental Laboratory of the Brazilian Agricultural Research Corporation (Embrapa) unit in the Semi-arid region (Embrapa Semiárido). These samples were preserved following the recommendations for each analysis, according to the procedures of the American Public Health Association [32]. For the analysis of abiotic variables, we determined the pH by the electrometric method; electrical conductivity ($\mu S \cdot cm^{-1}$) by the conductivimetric method; and turbidity

(NTU) by the nephelometric method. In addition, we evaluated the concentrations of the following nutrients (in $mg \cdot L^{-1}$): nitrate, by the second-derivative UV spectrophotometric method; nitrite, by the colorimetric method; ammonia, by the sodium salicylate method – the procedures of these analyses were described by the American Public Health Association [32]; orthophosphate (OP), by the ascorbic acid method [32,33]; and total phosphorus (TP) by acid hydrolysis and phosphorus digestion using ascorbic acid [32,34].

Analysis of the periphyton community attached to the substrate

After HRT-30, the plastic substrates of each tank were cut into squares of about 10 x 10 cm below the water-air interface. The cuts were made parallel to the diameter of the tank in each curtain of the plastic substrate immersed in the effluent. Using a flat brush with soft synthetic bristles, the plastic substrate was tenderly brushed to remove the periphyton community attached to it. As the substrate was brushed, jets of distilled water poured out with the help of a wash bottle. The water that flowed to a 1-liter plastic beaker was transferred to 60 mL ember glasses and three drops of 5% Lugol's iodine solution were added for community conservation. The use of distilled water is important because using the water from the effluent itself may insert components of the phytoplankton community that are present in the effluent water column.

The samples were placed in a thermal box for sunlight protection and taken to the Laboratory of Microscopy and Magnifying Glasses of the Federal University of the São Francisco Valley (UNIVASF). In the laboratory, we prepared semi-permanent slides for microscopic analysis in the NOVA 180IT trinocular infinity-corrected biological microscope. In the taxonomic identification of the organisms that form the periphyton community, we followed the specialized literature [35–43].

Statistical analysis

We performed the Shapiro-Wilk test for normality and the Levene test for the homogeneity of variance of the raw data of each variable, using the Car package (version 3.0.11) for R (version 3.6.2). The data showed a heteroskedastic non-normal distribution. Kruskal-Wallis analyses with Bonferroni post-hoc tests were performed to assess statistical differences in data ordination between the experimental units (control, aquatic macrophyte species and periphyton) and treatments (HRT-0 and HRT-30), using the DescTools package (version 0.99.42) for R (version 3.6.2).

RESULTS

Table 1 displays the limnological and statistical data of the effluent at HRT-0 and HRT-30 obtained in the experimental units. In general, it was observed that, at the beginning of the experiment, the tanks presented similar values for all physical and chemical variables of the effluent, only changing at HRT-30. After 30 days of effluent retention in the ecotechnologies, we observed an increase in the phytomass of the aquatic macrophytes and the attachment of the periphyton community to the artificial substrates. Likewise, significant differences were observed, mainly, in pH, nitrite, orthophosphate and total organic phosphorus.

Experimental unit using the aquatic macrophyte community

In the experimental unit with aquatic macrophytes, the species *P. crassipes* (Kruskal-Wallis, H=2.3333, df=1, p=0.1266) and *S. auriculata* (Kruskal-Wallis, H=1.1905, df=1, p=0.2752) did not present significant differences in electrical conductivity. Similarly, the nitrate concentrations in the control tanks for aquatic macrophytes (Kruskal-Wallis, H=0.42857, df=1, p=0.5127) and vegetated with *S. auriculata* (Kruskal-Wallis, H=1.1905, df=1, p=0.2752) were not significant after the HRT-30. The same occurred for total organic phosphorus in the control tank for aquatic macrophytes (Kruskal-Wallis, H=0.42857, df=1, p=0.04953).

Comparing the tanks with aquatic macrophytes in the last day of treatment, the Bonferroni post-hoc test indicated significant differences between the control tank and the tank vegetated with *A. filiculoides* regarding electrical conductivity, turbidity, nitrate and total organic phosphorus; the same occurred for orthophosphate between the tank vegetated with *S. auriculata* and the control tank.

The species *A. filiculoides* was the one that stood out at the end of the experiment (HRT-30). In these tanks, the pH, the electrical conductivity, and the turbidity presented significant attenuation. Among the results obtained for the nitrogen series, the best performance in nitrate attenuation was provided by the species *A. filiculoides*. With this species and with *S. auriculata*, the ammonia concentration was obliterated. All the free-floating aquatic macrophyte species were successful in decreasing the nitrite content of the aquaculture effluent. In addition, for the phosphorus series, dissolved inorganic phosphate – orthophosphate – concentrations were more attenuated than total organic phosphorus, demonstrating a preferential chemical configuration of assimilation by these plants.

Table 1. Limnological and statistical data of aquaculture wastewater in the experimental units with free-floating aquatic macrophyte community and periphyton community.

Physical and chemical variables	HRT (days)	Experiment unit with aquatic macrophytes							Experiment unit with periphyton				
		Tanks (median values of the triplicates)				Bonferroni Correction (horizontal comparison)			Tanks (median values of the triplicates)		Kruskal-Wallis Test (horizontal comparison)		
		CAM	**TAf**	**TPc**	**TSa**	***df***	***H* value**	**SD**	**CPC**	**TPB**	***df***	***H* value**	**SD**
pH	HRT-0	7.40(a)	7.54(a)	7.70(a)	7.56(a)	3	2.89	NS (p>0.05)	6.98(a)	6.90(a)	1	0.20	NS (p>0.05)
	HRT-30	6.69(b)	6.56(b)	6.93(b)	7.04(b)	3	9.05	NS (p>0.05)	7.08(b)	7.48(b)	1	3.86	CPC ≠ TPB
EC ($\mu S \cdot cm^{-1}$)	HRT-0	65.80(a)	67.90(a)	66.30(a)	67.90(a)	3	5.21	NS (p>0.05)	78.30(a)	77.10(a)	1	1.19	NS (p>0.05)
	HRT-30	111.00(b)	54.40(b)	70.10(a)	70.10(a)	3	9.38	CAM ≠ TAf only	90.00(b)	155.00(b)	1	3.86	CPC ≠ TPB
Turbidity (NTU)	HRT-0	19.90(a)	12.50(a)	17.30(a)	14.20(a)	3	8.44	CAM ≠ TAf only	17.80(a)	16.00(a)	1	3.86	CPC ≠ TPB
	HRT-30	7.62(b)	0.28(b)	0.53(b)	0.61(b)	3	7.82	CAM ≠ TAf only	17.00(a)	2.05(b)	1	3.86	CPC ≠ TPB
Nitrate ($mg \cdot L^{-1}$)	HRT-0	1.970(a)	1.130(a)	1.160(a)	1.180(a)	3	5.44	NS (p>0.05)	1.320(a)	1.290(a)	1	0.43	NS (p>0.05)
	HRT-30	2.320(a)	0.684(b)	1.050(b)	1.050(a)	3	7.52	CAM ≠ TAf only	0.630(b)	1.260(a)	1	3.86	CPC ≠ TPB
Nitrite ($mg \cdot L^{-1}$)	HRT-0	0.030(a)	0.019(a)	0.019(a)	0.019(a)	3	5.02	NS (p>0.05)	0.098(a)	0.049(a)	1	3.86	CPC ≠ TPB
	HRT-30	0.005(b)	0.001(b)	0.000(b)	0.000(b)	3	3.86	NS (p>0.05)	0.072(b)	0.002(b)	1	3.86	CPC ≠ TPB
Ammonia ($mg \cdot L^{-1}$)	HRT-0	0.054(a)	0.028(a)	0.044(a)	0.048(a)	3	6.48	NS (p>0.05)	0.069(a)	0.007(a)	1	0.05	NS (p>0.05)
	HRT-30	0.000(b)	0.000(b)	0.014(b)	0.000(b)	3	2.78	NS (p>0.05)	0.174(b)	0.041(a)	1	3.86	CPC ≠ TPB
OP ($mg \cdot L^{-1}$)	HRT-0	1.360(a)	1.060(a)	1.140(a)	1.080(a)	3	4.66	NS (p>0.05)	0.533(a)	0.526(a)	1	0.20	NS (p>0.05)
	HRT-30	0.720(b)	0.001(b)	0.000(b)	0.000(b)	3	8.28	CAM ≠ TSa only	0.421(b)	0.016(b)	1	3.97	CPC ≠ TPB
TOP ($mg \cdot L^{-1}$)	HRT-0	0.388(a)	0.367(a)	0.374(a)	0.355(a)	3	7.67	CAM ≠ TSa only	0.539(a)	0.539(a)	1	0.00	NS (p>0.05)
	HRT-30	0.406(a)	0.106(b)	0.107(b)	0.121(b)	3	7.84	CAM ≠ TAf only	0.293(b)	0.127(b)	1	3.86	CPC ≠ TPB

CAM: control treatment for the ecotechnology using aquatic macrophytes; TAf: treatment with the aquatic macrophyte species *Azolla filiculoides* Lam.; TPc: treatment with the aquatic macrophyte species *Pontederia crassipes* Mart.; TSa: treatment with the aquatic macrophyte species *Salvinia auriculata* Aubl.; CPC: control treatment for the ecotechnology using the periphyton community; TPB: treatment with periphytic biofilm; df: degrees of freedom; EC: electrical conductivity; HRT: hydraulic retention time; HRT-0: hydraulic retention time at the beginning of the experiment (day 0); HRT-30: hydraulic retention time 30 days after the beginning of the experiment; OP: orthophosphate; TOP: total organic phosphorus; SD: significant difference; NS: not significant; (a) and (b) indicate whether the values changed in relation to the beginning of the hydraulic retention time, according to the Kruskal-Wallis test.

Experimental unit using the periphyton community

As for the experimental unit with periphyton, all variables were significant in the control tank for periphyton except for turbidity (Kruskal-Wallis, H=0.42857, df=1, p=0.5127). On the other hand, at HRT-30, the tanks to which plastic substrates were added had their pH and electrical conductivity values increased, whereas turbidity presented lower values at the end of the experiment.

Regarding the nitrogen series, tanks with the substrate were not significant for nitrate (Kruskal-Wallis, H=0.4285, df=1, p=0.5127) and ammonia (Kruskal-Wallis, H=1.2255, df=1, p=0.2683). For the phosphorus series, the same characteristic of the experimental unit with aquatic macrophytes was observed, that is, total organic phosphorus concentration was less attenuated than orthophosphate. Moreover, the tanks with periphytic biofilm were significantly different from their respective control tanks through the correlation of each physical and chemical variable at HRT-30.

In the TPB triplicate, we identified 33 taxa belonging to 7 classes and 22 families. Table 2 brings the catalog of species of the periphyton community present in the artificial substrate installed and the specific richness of the taxonomic classes. Of these taxa, 16 were identified at the species level, 14 at the genus level, 1 at the family level and 2 were not identifiable.

Table 2. Algae taxa of the periphyton community present in the plastic substrate installed in the experimental treatment unit with periphyton after a 30-day hydraulic retention time of the effluent in the tanks.

Class	Family	Species
Bacillariophyceae (Specific richness: 18.75%)	Fragilariaceae	*Fragilaria crotonensis*
	Gomphonemataceae	*Gomphonema* sp.
	Naviculaceae	*Navicula* sp.
	Rhopalodiaceae	*Epithemia adnata*
	Rhopalodiaceae	*Rhopalodia gibba*
	Tabellariaceae	*Meridion circulare*
Chlorophyceae (Specific richness: 24.24%)	Hydrodictyaceae	*Pediastrum simplex*
	Hydrodictyaceae	*Stauridium tetras*
	Radiococcaceae	*Radiococcus* sp.
	Radiococcaceae	Radiococcaceae sp. 1
	Scenedesmaceae	*Scenedesmus* sp.
	Selenastraceae	*Ankistrodesmus fusiformis*
	Selenastraceae	*Monoraphidium contortum*
	Sphaerocystidaceae	*Sphaerocystis schroeteri*
Coleochaetophyceae (Specific richness: 3.03%)	Chaetosphaeridiaceae	*Chaetosphaeridium* sp.
Coscinodiscophyceae (Specific richness: 6.06%)	Aulacoseiraceae	*Aulacoseira granulata*
	Aulacoseiraceae	*A. granulata* var. *angustissima*
Cyanophyceae (Specific richness: 30.30%)	Aphanizomenonaceae	*Anabaenopsis* sp.
	Calothricaceae	*Calothrix fusca*
	Chroococcaceae	*Chroococcus* sp.
	Merismopediaceae	*Merismopedia tenuissima*
	Microcystaceae	*Gloeocapsa* sp.
	Microcystaceae	*Microcystis aeruginosa*
	Nostocaceae	*Anabaena* sp.
	Nostocaceae	*Cylindrospermum* sp.
	Nostocaceae	*Nostoc* sp.
	Oscillatoriaceae	*Phormidium* sp.
Trebouxiophyceae (Specific richness: 9.09%)	Chlorellaceae	*Dictyosphaerium* sp.
	Oocystaceae	*Eremosphaera viridis*
	Oocystaceae	*Oocystis lacustris*
Zygnematophyceae (Specific richness: 3.03%)	Desmidiaceae	*Cosmarium* sp.
—	—	sp.1
—	—	sp.2

DISCUSSION

Under the experimental conditions evaluated, the results confirmed the hypothesis that the nitrogen and phosphorus concentrations at the end of the treatments would be lower than they were at the beginning, even though the tanks vegetated with free-floating aquatic macrophytes presented more favorable results for the mitigation of the effluent when compared to the inferior performance of the phytoremediation process, performed only by the periphyton community.

Experimental unit using the aquatic macrophyte community

The tanks vegetated with the species *A. filiculoides* had the lowest pH value among the macrophytes – pH values below 6.6 result from lower nitrification rates [44]. Thus, at the end of the experiment, the lower nitrate concentration on the tanks vegetated with *A. filiculoides* evidenced the low nitrification. The pH values of the tanks vegetated with *P. crassipes* and *S. auriculata* were closer to neutrality, favoring water nitrification [45].

The increase in electrical conductivity in the tanks was due to the carrying of ions from the water of the excavated ponds, since all the tanks were constantly reloaded to compensate for evaporation. Despite the increase in electrical conductivity, the TPc and TSa tanks did not show significant differences in this variable. On the other hand, TAf reduced electrical conductivity. The reduction of this variable in the effluent results from either the accumulation, the adsorption or the absorption of several nutrients that contribute for electrical conductivity such as calcium, sodium, potassium, phosphate, and others [46]. In parallel, Deval and coauthors [47] verified that *A. filiculoides* reduces the concentration of ions, mainly sodium and potassium, contributing to the decrease in electrical conductivity. Amare and coauthors [48] noted that effluents treated with *A. filiculoides* presented lower values of this variable across long hydraulic retention times.

Aquatic macrophytes have a root zone capable of absorbing particles suspended in water and store debris in lignin cells for further degradation by endophytic bacteria and by the plant enzyme system [49,50]. Such features are the main reasons for the reduction in the turbidity values promoted by the aquatic macrophytes. The use of free-floating aquatic macrophytes with a dense root zone in shallow tanks favored adsorption and precipitation of suspended particles [51]. The increase in the hydraulic retention time of the effluent in the treatment system also helped attenuate turbidity [44].

In effluents with free-floating aquatic macrophytes, nitrate concentration depends on nitrification and denitrification processes, water absorption, and root-associated microbes [52]. As previously demonstrated about nitrate, the slight attenuation of its content in tanks with *S. auriculata* was not significantly different when we compare the beginning and the end of the treatment, what implies a predominance of nitrification reactions by this species, since the nitrate concentration values remained close to the initial concentration. On the other hand, lower nitrate concentrations evidence the predominance of denitrification and absorption by aquatic organisms [52]. Thus, among the plant species used in the study, tanks with *A. filiculoides* and *P. crassipes* benefited the most from denitrification.

The attenuation of nitrite concentration at HRT-30 is mediated by the ammonia mineralization performed by the aquatic macrophytes, as well as by the oxygen produced in the root zone of the plants and by the symbiosis of the periphyton microorganisms, contributing to the removal of this intermediate compound [53]. It is possible that the free-floating aquatic macrophytes used in this study presented some tolerance for ammonia assimilation. Nizam and coauthors [54] demonstrated that different macrophytes presented differences in ammonia concentration at the end of the treatment and, after 12 days, the effluent displayed a constant concentration of this compound, especially when vegetated with *P. crassipes*. Therefore, *P. crassipes* may have reached its threshold in ammoniacal nitrogen absorption. *S. auriculata* absorbs the ammoniacal fraction of nitrogen because this chemical configuration demands less energy than nitrate [55]. This characteristic may be the reason why the increase in the biomass of *S. auriculata* in the tanks with aquaculture wastewater was four times greater than that of the other species.

Among the phosphorus fractions, the inorganic chemical configuration is the one preferred by the aquatic macrophytes. This fact justified the low concentrations of orthophosphate in the effluent vegetated with the macrophyte species. The other phosphorus fractions, mainly organic phosphorus, were degraded into the inorganic form by the bacteria in symbiosis with the root zone, which, for their turn, were assimilated by the symbionts and the species vegetated in the effluent. Among the species used, *P. crassipes* was more efficient in absorbing phosphorus than *S. auriculata*. In the latter, the concomitant action of the periphyton was essential in phosphorus absorption [56]. Moreover, *P. crassipes* absorbs excess phosphorus compounds to meet its metabolic requirements and stores them in its tissue [57]. *A. filiculoides* was also less capable of absorbing phosphorus than *S. auriculata*, mainly due to its biomass mortality because of the overcrowding in the tanks [55].

Experimental unit using the periphyton community

In the experimental unit with the periphyton, we observed that the effects of the treatment using this community resembled those of the treatments with aquatic macrophytes for most of the physical and chemical variables evaluated. The presence of periphytic algae colonized in the substrate is determined by the rate of availability and loss of nutrients, especially nitrogen and phosphorus [58,59]. Thus, the periphyton community attached to the substrate can be used as an indicator of pollution in aquaculture tanks. Species of the Bacillariophyceae class are indicators of pollution from organic compounds [60,61]. The Chlorophyceae class becomes dominant as the phosphorus concentration decreases in the effluent [62]. The Cyanophyceae can take a high diluted organic load and tolerate large physical and chemical variations in the water [63]. These characteristics corroborated the results of the present study since the dominance of the three aforementioned taxonomic classes – mainly of the Cyanophyceae class – were observed.

The presence of some organisms from this community was a result of the characteristics of the effluent because aquaculture wastewater is rich in compounds that favor periphyton colonization and the study area was developed under propitious climatic conditions – frequent sunlight and high temperatures. In addition, carrying out the experiment in a location of low precipitation avoids events of hydrological turbulence that may hinder the succession of the periphyton community and, consequently, the diversity of these organisms. Discussing some properties of such organisms, we notice that the presence of *Aulacoseira granulata* and *A. granulata* var. *angustissima* are associated with shallow water bodies with high loads of suspended organic matter with high turbidity values [64]. *Microcystis aeruginosa* is a frequent cyanobacterium in effluents rich in nitrogen and phosphorus due to its potential for absorption of these elements dissolved in water [65] since it has akinetes and heterocysts that store nitrogen. *Monoraphidium contortum* and *Gomphonema* sp. are present in effluents with high concentration of nutrients [66]. *Epithemia adnata* responds proportionally to the high nitrogenous nutrient supply and the increase in temperature [67].

On the other hand, the presence of some organisms indicates that the quality of the effluent is better than prior to phytoremediation. *Oocystis lacustris, Sphaerocystis schroeteri* and some *Chroococcus* species are associated with shallow environments and with lower turbidity, phosphorus, and nitrogen values after the treatment [64]. *Meridion circulare* is frequent in waters with good physical and chemical parameters for aquaculture [68]. *Pediastrum simplex* is an indicator of low turbidity and occurs at pH values close to neutral [69]. These species, after HRT-30, witnessed the minimization of the concentration of nutrients in water caused by the periphytic biofilm.

Regarding the variables analyzed during the experimental procedure carried out with the periphyton, the greater cumulative absorption of carbon dioxide and the photosynthetic activities of the periphytic algae contributed to the increase in the pH and to phosphorus precipitation [70]. It is possible that the prolonged hydraulic retention time of the effluent helped increase pH at HRT-30. The use of the periphyton community alone was not enough to ensure the assimilation of the ions present in the effluent in order to reduce electrical conductivity. In this variable, the increase can be explained by the carrying of ions that results from the dissolution of salts, since tanks were constantly refilled with the effluent. As for turbidity, the same process that the periphyton performs in symbiosis with the root zone of aquatic macrophytes occurred, that is, the mineralization of dissolved organic and inorganic compounds.

The use of periphyton as an ecotechnology, alone, was not capable of reducing the ammonia and nitrate concentrations. However, despite having a poor phytoremediation performance, it is noteworthy that this community stimulates the processes of nitrification and denitrification of the effluent [70,71]. As aforementioned, for the aquatic communities, the orthophosphate was the recommended assimilable form, causing a higher concentration of total phosphorus at HRT-30. Therefore, Yu and coauthors [72] pointed out that the presence of an aeration system in phytoremediation treatments contributes to phosphorus assimilation in shorter hydraulic retention times. Thus, it would be interesting to assess these ecotechnologies integrated with aeration mechanisms.

Caveats, difficulties experienced, and conclusions

Such considerations indicate that the use of free-floating aquatic macrophytes and the formation of periphytic biofilm in artificial substrates was effective in attenuating physical and chemical characteristics of the effluent that could be harmful both for aquaculture, making the aquatic environment unviable for production, and for the adjacent receiving water body – in this case, the São Francisco River. However, reliance on electricity for pumping large volumes of effluent proved to be disadvantageous, as some rural areas lack the electricity to run small-scale treatments, preventing these areas from being self-sufficient in managing their own effluent. Performing the study within the fishponds would not be effective because there is nutrient feedback caused by bioturbation of the sediment when the sediment is disturbed by the fish species being cultured. Also, the management of aquatic macrophytes within the mesocosms of the study proved to be important for continuous treatment requiring maintenance of the biomass constantly. However, the fact creates difficulties for the appropriate management of the green biomass of exceeding aquatic macrophytes, since the disposal in terrestrial environments may give rise to the release of nutrients stored in the plant tissues as the plant goes into senescence, especially when the plants remediate heavy metal ions that are harmful to the environment.

In the experimental units, the aquatic macrophyte community performed better than the periphyton community as an ecotechnology. Thus, it would be interesting to evaluate the integration of these two experimental units in a single system to assess the purification of nutrients that cause water eutrophication in aquaculture systems. It is also worth noting that, at the end of the experiment, the aquatic macrophytes can be used for composting and organic fertilization, since the effluent comes from aquaculture, that is, is both rich in nutrients and not contaminated by heavy metals. In addition, the use of free-floating macrophyte species was of paramount importance because plants not rooted in the sediment are in immediate contact with the effluent to work out the treatment [73–75]. At the same time, the use of a technology based on the periphyton community also helps develop an economic activity that is less impactful on ecosystems and works as a food alternative for omnivorous species, given that the periphyton community is an important food source for several aquatic organisms. Regarding the use of artificial plastic substrates for the colonization of this community, it is important to note that the substrate can be re-established and reused in new colonization processes, mitigating the environmental impacts of the disposal of plastic.

Aquatic species provide subsidies to other environments and ecosystems and are fundamental in the cycling of several elements of the biogeochemical cycles. It is also noteworthy that these ecotechnologies are effective in the treatment of other types of effluents such as those from livestock [76,77]; petroleum products [78]; tannery [79]; textile industry [80]; and agriculture [81]. Thus, exploring the potential of these organisms is important for low-cost wastewater management and contributes to the universalization of wastewater treatment in places that do not have a centralized treatment structure.

Funding: This research was supported by the Participa Project, led by Embrapa Semi-arid Region (SEG 26.16.04.004.00.00) for part of its execution in the field and laboratories.
Acknowledgments: The authors would like to thank the Federal University of the São Francisco Valley, the Brazilian Agricultural Research Corporation unit in the Semi-arid region (Embrapa Semiárido), and the Development Company of the São Francisco and Parnaiba Valleys for all the support in the conduction of the present study. We would like to thank Rozzanno Antonio Cavalcanti Reis de Figueiredo, Head of the Bebedouro Aquaculture Station, for allowing the installation of the experiment in their facilities; the researcher Paula Tereza de Souza e Silva, Coordinator of the Agro-Environmental Laboratory of Embrapa Semiárido, for allowing the effluent analysis procedures to be carried out in their facilities; the Professor Diego César Nunes da Silva, Coordinator of the Laboratory of Microscopy and Magnifying Glasses of the Federal University of the São Francisco Valley, for arranging the material necessary for the microscopic analysis of the periphyton; and the Professor Edson Gomes de Moura Júnior for the initial instruction and for reviewing the statistical data.
Conflicts of Interest: The authors have no competing interests to declare that are relevant to the content of this article.

REFERENCES

1. Li D, Liu S. Water quality monitoring in aquaculture. In: Li D, Liu S, editors. Water quality monitoring and management. Academic Press; 2019. p. 303–28.
2. Dauda AB, Ajadi A, Tola-Fabunmi AS, Akinwole AO. Waste production in aquaculture: sources, components and managements in different culture systems. Aquac Fish. 2019 May 1;4(3):81–8.
3. Ahmed N, Thompson S. The blue dimensions of aquaculture: a global synthesis. Sci Total Environ. 2019;652:851–61.
4. Associação Brasileira da Piscicultura. [2020 Peixe BR Fish Farming Yearbook]. São Paulo, SP: Associação Brasileira da Piscicultura; 2020. 135 p.
5. Calixto ES, Ferreira D, Santos B, Lange D, Galdiano MS, Rahman IU. Aquaculture in Brazil and worldwide: overview and perspectives. J Environ Anal Prog. 2020;5(1):98.
6. Moro MF, Lughadha EN, Araújo FS, Martins FR. A phytogeographical metaanalysis of the Semiarid Caatinga Domain in Brazil. Bot Ver. 2016 822. 2016 May;82(2):91–148.
7. Silva JLB, Moura GBA, Silva MV, Lopes PMO, Guedes RVS, Silva ÊFF, et al. Changes in the water resources, soil use and spatial dynamics of Caatinga vegetation cover over semiarid region of the Brazilian Northeast. Remote Sens Appl Soc Environ. 2020 Nov 1;20:100372.
8. Soares MO, Campos CC, Carneiro PBM, Barroso HS, Marins RV, Teixeira CEP, et al. Challenges and perspectives for the Brazilian semi-arid coast under global environmental changes. Perspect Ecol Conserv. 2021 Jul 1;19(3):267–78.

9. Arantes FP, Sato Y, Sampaio EV, Rizzo E, Bazzoli N, Arantes FP, et al. Spawning induction and fecundity of commercial native fish species from the São Francisco River basin, Brazil, under hatchery conditions. Agric Sci. 2013;4(8):382–8.
10. Saint-Paul U. Native fish species boosting Brazilian's aquaculture development. Acta Fish Aquat Resour. 2017;5(1):1–9.
11. Soares E, Almeida EO, Araújo K, Lima M, Gusmão-Júnior L, Oliveira WD, et al. Polyculture of curimatã-pacu (*Prochilodus argenteus*) and canela shrimp (*Macrobrachium acanthurus*) feed with dehydrated cassava leaf meal. Lat Am J Aquat Res. 2019;47(1):27–33.
12. Oliveira EG, José F, Santos S. [Fish farming and the challenges of producing in water-scarce regions]. Ciência Anim. 2015;25(1):133–54.
13. Francisco PRM, Medeiros RM, Tavares AL, Santos D. Variability space-temporal of annual precipitation of wet and dry period in the Paraíba State. J Hyperspectral Remote Sens. 2016 Apr 27;6(1):1–9.
14. Farias TRL, Medeiros PHA, Navarro-Hevia J, Araújo JC. Unpaved rural roads as source areas of sediment in a watershed of the Brazilian semi-arid region. Int J Sediment Res. 2019 Oct 1;34(5):475–85.
15. Santos CAG, Brasil Neto RM, Silva RM, Costa SGF. Cluster analysis applied to spatiotemporal variability of monthly precipitation over Paraíba State using Tropical Rainfall Measuring Mission (TRMM) data. Remote Sens. 2019 Mar 15;11(6):637.
16. Gomes MG, Maia AG, Medeiros JDF. Reservoir operation rule in semiarid areas: the quantity-quality approach. J Hydrol. 2022 Jul 1;610:127944.
17. Camargo AFM, Amorim RV. Fish farming in cages: a practice to be restricted in Brazil. Acta Limnol Bras. 2020;32:e101.
18. Ottinger M, Clauss K, Kuenzer C. Aquaculture: relevance, distribution, impacts and spatial assessments – a review. Ocean Coast Manag. 2016 Jan 1;119:244–66.
19. Hargreaves AJ, Constantino C, Dotro G, Cartmell E, Campo P. Fate and removal of metals in municipal wastewater treatment: a review. Environ Technol Rev. 2018 Jan;7(1):1–18.
20. Gangadhar B, Ramakrishna NG. Green technology vs environmental sustainability in india– an overview. Int J Curr Adv Res. 2017 Mar;6(3):2465–8.
21. Jiménez S, Micó MM, Arnaldos M, Medina F, Contreras S. State of the art of produced water treatment. Chemosphere. 2018 Feb;192:186–208.
22. DalCorso G, Fasani E, Manara A, Visioli G, Furini A. Heavy metal pollutions: state of the art and innovation in phytoremediation. Int J Mol Sci. 2019;20(14):3412.
23. Anand S, Bharti SK, Kumar S, Barman SC, Kumar N. Phytoremediation of heavy metals and pesticides present in water using aquatic macrophytes. In: Arora NK, Kumar N, editors. Phyto and Rhizo Remediation. 1st ed. Singapore: Springer; 2019. p. 89–119.
24. Afzal M, Arslan M, Müller JA, Shabir G, Islam E, Tahseen R, et al. Floating treatment wetlands as a suitable option for large-scale wastewater treatment. Nat Sustain. 2019 Aug 12;2(9):863–71.
25. Guttman L. Periphyton for biofiltration and fish feeding in an integrated multi-trophic aquaculture system: a case study in the Gulf of Aqaba. J Environ Soil Sci. 2019 Sep 18;3(5):413–8.
26. Teoh TP, Ong SA, Ho LN, Wong YS, Oon YL, Oon YS, et al. Up-flow constructed wetland-microbial fuel cell: Influence of floating plant, aeration and circuit connection on wastewater treatment performance and bioelectricity generation. J Water Process Eng. 2020;36:101371.
27. Lambrecht RW, Tavares DA, Santos TR, Ferragut C. Responses of periphyton biomass and nutrient status to experimental enrichment and its relationships with changes in seston nutrient content and chlorophyll-a. Hydrobiologia. 2019 Apr;836(1):141–53.
28. Hao B, Wu H, Zhen W, Jo H, Cai Y, Jeppesen E, et al. Warming effects on periphyton community and abundance in different seasons are influenced by nutrient state and plant type: a shallow lake mesocosm study. Front Plant Sci. 2020;11:404.
29. Carles L, Wullschleger S, Joss A, Eggen RIL, Schirmer K, Schuwirth N, et al. Impact of wastewater on the microbial diversity of periphyton and its tolerance to micropollutants in an engineered flow-through channel system. Water Res. 2021 Sep 15;203:117486.
30. Liu J, Wang F, Wu W, Wan J, Yang J, Xiang S, et al. Biosorption of high-concentration Cu (II) by periphytic biofilms and the development of a fiber periphyton bioreactor (FPBR). Bioresour Technol. 2018;248:127–34.
31. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Zeitschrift. 2013 Dec 1;22(6):711–28.
32. American Public Health Association, American Water Works Association, Water Environment Federation. Standard methods for the examination of water and wastewater. 23rd ed. Baird RB, Eaton AD, Rice EW, editors. Washington, DC: American Public Health Association, American Water Works Association and Water Environment Federation; 2017.
33. Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962 Jan 1;27(C):31–6.
34. Valderrama JC. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar Chem. 1981 Jan 1;10(2):109–22.
35. Komárek J. Phenotype diversity of the heterocytous cyanoprokaryotic genus *Anabaenopsis*. Fottea. 2005;1–35.

36. Bicudo CEM, Menezes M. [Genera of algae from continental waters in Brazil: key to identification and descriptions]. 2nd ed. Bicudo CEM, Menezes M, editors. São Carlos: RiMa; 2006. 489 p.
37. Sant'anna CL, Azevedo MTP, Henrique L, Branco Z, Komárek J. New aerophytic morphospecies of *Nostoc* (Cyanobacteria) from São Paulo State, Brazil. Hoehnea. 2007;34(1):95–101.
38. Rodrigues LL, Sant'Anna CL, Tucci A. [Chlorophyceae from the Billings (Taquacetuba stream) and Guarapiranga reservoirs, SP, Brazil]. Rev Bras Botânica. 2010 Jun;33(2):247–64.
39. Domingues CD, Torgan LC. [Chlorophyta of a hypereutrophic artificial lake in southern Brazil]. Iheringia, Série Botânica. 2012 Jun;67(1):75–91.
40. Franceschini IM. [Identification key to the genera of algae (except Bacillariophyceae) most commonly found in the periphyton and metaphyton of continental aquatic environments]. In: Schwarzbold A, Burliga AL, Torgan LC, editors. Ecologia do Perifíton. 1st ed. São Carlos: RiMa; 2013. p. 245–65.
41. Ludwig TAV, Tremarin PI. [Identification key for the genera of diatoms (Diatomeae - Ochrophyta) most commonly found in the periphyton and metaphyton of continental aquatic environments]. In: Schwarzbold A, Burliga AL, Torgan LC, editors. Ecologia do Perifíton. 1st ed. São Carlos: RiMa; 2013. p. 267–315.
42. Alves FRR, Gama WA, Nogueira IS. Planktonic Radiococcaceae Fott ex Komárek of the Tigres Lake system, Britânia, Goiás State, Brazil. Braz J Bot. 2014;37(4):519–30.
43. Thakar MK, Luthra D, Khattar JS. Forensic studies of phytoplankton ecology of two water bodies of Kurukshetra area of Haryana, State in India. Egypt J Forensic Sci. 2018;8(1):1–15.
44. Akinbile CO, Yusoff MS. Assessing water hyacinth (*Eichhornia crassipes*) and lettuce (*Pistia stratiotes*) effectiveness in aquaculture wastewater treatment. Int J Phytoremediation. 2012 Mar;14(3):201–11.
45. Rezania S, Din MFM, Taib SM, Dahalan FA, Songip AR, Singh L, et al. The efficient role of aquatic plant (water hyacinth) in treating domestic wastewater in continuous system. Int J Phytoremediation. 2016 Jul;18(7):679–85.
46. Verma R, Suthar S. Synchronized urban wastewater treatment and biomass production using duckweed *Lemna gibba* L. Ecol Eng. 2014 Mar 1;64:337–43.
47. Deval CG, Mane AV, Joshi NP, Saratale GD. Phytoremediation potential of aquatic macrophyte *Azolla caroliniana* with references to zinc plating effluent. Emirates J Food Agric. 2012;24(3):208–23.
48. Amare E, Kebede F, Mulat W. Wastewater treatment by *Lemna minor* and *Azolla filiculoides* in tropical semi-arid regions of Ethiopia. Ecol Eng. 2018 Sep 1;120:464–73.
49. Spangler JT, Sample DJ, Fox LJ, Owen JS, White SA. Floating treatment wetland aided nutrient removal from agricultural runoff using two wetland species. Ecol Eng. 2019 Feb;127:468–79.
50. Sarkheil M, Safari O. Phytoremediation of nutrients from water by aquatic floating duckweed (*Lemna minor*) in rearing of African cichlid (*Labidochromis lividus*) fingerlings. Environ Technol Innov. 2020 May 1;18:100747.
51. Henry-Silva GG, Camargo AFM. Efficiency of aquatic macrophytes to treat Nile tilapia pond effluents. Sci Agric. 2006;63(5):433–8.
52. Ng YS, Chan DJC. Wastewater phytoremediation by *Salvinia molesta*. J Water Process Eng. 2017;15:107–15.
53. Muvea FM, Ogendi GM, Omondi SO. Nutrient removal efficiency by floating macrophytes; *Lemna minor* and *Azolla pinnata* in a constructed wetland. Glob J Environ Sci Manag. 2019;5(4):415–30.
54. Nizam NUM, Hanafiah MM, Noor IM, Karim HIA. Efficiency of five selected aquatic plants in phytoremediation of aquaculture wastewater. Appl Sci. 2020 Apr 14;10(8):2712.
55. Toledo JJ, Penha J. Performance of *Azolla caroliniana* Willd. and *Salvinia auriculata* Aubl. on fish farming effluent. Braz J Biol. 2011 Feb;71(1):37–45.
56. Kumar S, Deswal S. Phytoremediation capabilities of *Salvinia molesta*, water hyacinth, water lettuce, and duckweed to reduce phosphorus in rice mill wastewater. Int J Phytoremediation. 2020 Sep;22(11):1097–109.
57. Zhang Y, Liu H, Yan S, Wen X, Qin H, Wang Z, et al. Phosphorus removal from the hyper-eutrophic Lake Caohai (China) with large-scale water hyacinth cultivation. Environ Sci Pollut Res. 2019 Mar;26(13):12975–84.
58. Huh JH, Ahn JW. A perspective of chemical treatment for cyanobacteria control toward sustainable freshwater development. Environ Eng Res. 2017 Mar;22(1):1–11.
59. Cao J, Hou Z, Li Z, Chu Z, Yang P, Zheng B. Succession of phytoplankton functional groups and their driving factors in a subtropical plateau lake. Sci Total Environ. 2018 Aug 1;631–632:1127–37.
60. Tiwari A, Marella TK. Potential and application of diatoms for industry-specific wastewater treatment. In: Gupta SK, Bux F, editors. Application of microalgae in wastewater treatment. 1st ed. Springer, Cham; 2019. p. 321–39.
61. Al-Hassany JS, Alrubai GH, Jasim IM. The potential use of the diatom *Nitzschia palea* (Kützing) W. Smith for the removal of certain pollutants from Al-Rustumeyah wastewater treatment plant in Baghdad-Iraq. IOP Conf Ser Earth Environ Sci. 2021 Jun 1;779(1):012114.
62. Amano Y, Machida M. Mechanisms of algal species dominance among cyanobacteria, diatoms and green algae as a consequence of phosphorus reduction caused by river water dilution in eutrophic lake. J Water Environ Technol. 2013;11(5):391–401.
63. Bernal CB, Vázquez G, Quintal IB, Bussy AL. Microalgal dynamics in batch reactors for municipal wastewater treatment containing dairy sewage water. Water Air Soil Pollut. 2008 May 29;190(1–4):259–70.
64. Izaguirre I, Allende L, Escaray R, Bustingorry J, Pérez G, Tell G. Comparison of morpho-functional phytoplankton classifications in human-impacted shallow lakes with different stable states. Hydrobiol 2012 6981. 2012 Mar;698(1):203–16.

65. Du Y, Wang J, Wang Z, Torres OL, Guo R, Chen J. Exogenous organic carbon as an artificial enhancement method to assist the algal antibiotic treatment system. J Clean Prod. 2018 Sep 1;194:624–34.
66. Felisberto SA, Leandrini JA, Rodrigues L. Effects of nutrients enrichment on algal communities: an experimental in mesocosms approach. Acta Limnol Bras. 2011;23(2):128–37.
67. Adams JK, Peng Y, Rose NL, Shchetnikov AA, Mackay AW. Diatom community responses to long-term multiple stressors at Lake Gusinoye, Siberia. Geogr Environ. 2019;6(1):e00072.
68. Varol M, Balcı M. Characteristics of effluents from trout farms and their impact on water quality and benthic algal assemblages of the receiving stream. Environ Pollut. 2020 Nov 1;266:115101.
69. Degefu F, Mengistu S, Schagerl M. Influence of fish cage farming on water quality and plankton in fish ponds: a case study in the Rift Valley and North Shoa reservoirs, Ethiopia. Aquaculture. 2011 Jun 15;316(1–4):129–35.
70. Sandefur HN, Matlock MD, Costello TA. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. Ecol Eng. 2011 Oct;37(10):1476–80.
71. Lopardo CR, Zhang L, Mitsch WJ, Urakawa H. Comparison of nutrient retention efficiency between vertical-flow and floating treatment wetland mesocosms with and without biodegradable plastic. Ecol Eng. 2019 Jun;131:120–30.
72. Yu L, Zhang Y, Liu C, Xue Y, Shimizu H, Wang C, et al. Ecological responses of three emergent aquatic plants to eutrophic water in Shanghai, P. R. China. Ecol Eng. 2019 Oct 1;136:134–40.
73. Sipaúba-Tavares LH, Dias SG. Water quality and communities associated with macrophytes in a shallow water-supply reservoir on an aquaculture farm. Braz. J. Biol. 2014;74(2):420–8.
74. Geng Y, Han W, Yu C, Jiang Q, Wu J, Chang J, et al. Effect of plant diversity on phosphorus removal in hydroponic microcosms simulating floating constructed wetlands. Ecol Eng. 2017 Oct;107:110–9.
75. Tang Y, Harpenslager SF, Van Kempen MML, Verbaarschot EJV, Loeffen LMJM, Roelofs JGM, et al. Aquatic macrophytes can be used for wastewater polishing but not for purification in constructed wetlands. Biogeosciences. 2017;14(4):755–66.
76. Adhikari U, Harrigan T, Reinhold DM. Use of duckweed-based constructed wetlands for nutrient recovery and pollutant reduction from dairy wastewater. Ecol Eng. 2015 May 1;78:6–14.
77. Sudiarto SIA, Renggaman A, Choi HL. Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics. J Environ Manage. 2019 Feb 1;231:763–9.
78. Zeiger C, Silva ICR, Mail M, Kavalenka MN, Barthlott W, Hölscher H. Microstructures of superhydrophobic plant leaves - inspiration for efficient oil spill cleanup materials. Bioinspir Biomim. 2016;11(5):056003.
79. Alemu A, Gabbiye N, Lemma B. Application of integrated local plant species and vesicular basalt rock for the treatment of chromium in tannery wastewater in a horizontal subsurface flow wetland system. J Environ Chem Eng. 2020;8(4):103940.
80. Shabbir S, Faheem M, Ali N, Kerr PG, Wu Y. Periphyton biofilms: a novel and natural biological system for the effective removal of sulphonated azo dye methyl orange by synergistic mechanism. Chemosphere. 2017;167:236–46.
81. Yang J, Liu J, Wu C, Kerr PG, Wong PK, Wu Y. Bioremediation of agricultural solid waste leachates with diverse species of Cu (II) and Cd (II) by periphyton. Bioresour Technol. 2016 Dec 1;221:214–21.