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Effects of biological treatments on water quality in neotropical fishponds

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ABSTRACT

Effects of biological treatments in the water quality in neotropical fishponds

The aim of this study was to investigate the effects of the aquatic macrophyte (*Eichhornia crassipes*) and periphyton communities on the neotropical fishponds water quality, and to determine the ability of such communities to remove potential pollutants from the fishponds, making them interesting tools for the maintenance of desirable water quality in aquaculture under continuous water flow. In general, the biological treatments used in this study showed a positive effect in reducing BOD_5, orthophosphates, total phosphorus, conductivity and thermotolerant coliforms from the water column and also decreasing organic matter, Cu, Fe, Mn and Zn, from the sediment. The differences in physical and chemical properties of the water column and sediment between the treatments showed an improvement in the overall water quality when the treated fishponds are compared with the control fishpond. The best growth rate performance of Nile tilapia (*Oreochromis niloticus*) occurred in the fishpond with periphyton treatment.

Key words: Periphyton, macrophytes, aquaculture, Nile tilapia.

RESUMEN

Efecto de diferentes tratamientos biológicos en la calidad del agua de estanques de peces neotropicales

*El objetivo de este estudio fue investigar los efectos de la macrófita acuática (*Eichhornia crassipes*) y las comunidades de perifiton sobre la calidad del agua en estanques de peces neotropicales, y determinar la capacidad de tales comunidades para eliminar potenciales contaminantes de los estanques, mostrando su interés como herramientas para el mantenimiento de la calidad del agua para la acuicultura que utiliza flujo de agua continuo. En general, los tratamientos biológicos ensayados en este estudio mostraron un efecto positivo en la reducción de DBO_5, ortofosfatos, fósforo total, conductividad y coliformes termotolerantes en la columna de agua, y también disminuyendo la materia orgánica, Cu, Fe, Mn y Zn, en el sedimento. Las diferencias en las propiedades físicas y químicas de la columna de agua y los sedimentos entre los tratamientos mostraron una mejora en la calidad general del agua cuando los estanques tratados se comparan con el control. El mejor rendimiento de la tasa de crecimiento de la tilapia del Nilo (*Oreochromis niloticus*) se observó en el estanque con el tratamiento periphyton.*

Palabras clave: *Perifiton, macrófitas, acuicultura, tilapia del Nilo.*

INTRODUCTION

Appropriate knowledge of the management and control of the ecological and biological aspects of aquaculture are important for maintaining proper water quality and sediment balance, to yield higher fish biomass production. The need to make aquaculture systems more efficient arises, from the fact that nutrients that are not incorporated as biomass will accumulate in pond sediments, volatilize, or be discharged into the environment (Van Dam *et al.*, 2002), among other reasons. Improvements in ponds following eutrophication can be achieved by the use of floating aquatic macrophytes, such as *Eichhornia crassipes* (Sipaúba-Tavares *et al.*, 2003). The performance of such macrophytes in improving pond water quality is largely enhanced by the periphyton that forms on the submerged parts of macrophytes (Kiss *et al.*, 2003). The importance of periphyton in providing biomass and habitat for varied benthic consumers, as well as its sensitivity to phosphate levels, has been extensively described in the literature (Mei & Zhang, 2013). Periphyton communities are ubiquitous and ecologically important components of many shallow lakes; as a basis of the fluvial food web, they have been used for assessing the quality of these ecosystems (Serra *et al.*, 2010). The autotrophic organisms of the periphyton yield organic material and oxygen by using light energy and absorbing nutrients (Kosáros *et al.*, 2010).

Periphyton-based aquaculture systems offer the possibility of increasing both primary production and food availability for fish (Azim *et al.*, 2003). It has also been shown that periphyton can be inserted into and later harvested from an ecosystem with the objective of removing excess nutrients, in a process described by Drenner *et al.* (1997).

Another point to consider is that when water is drained directly from one pond to the next, the composition of the first environment may affect

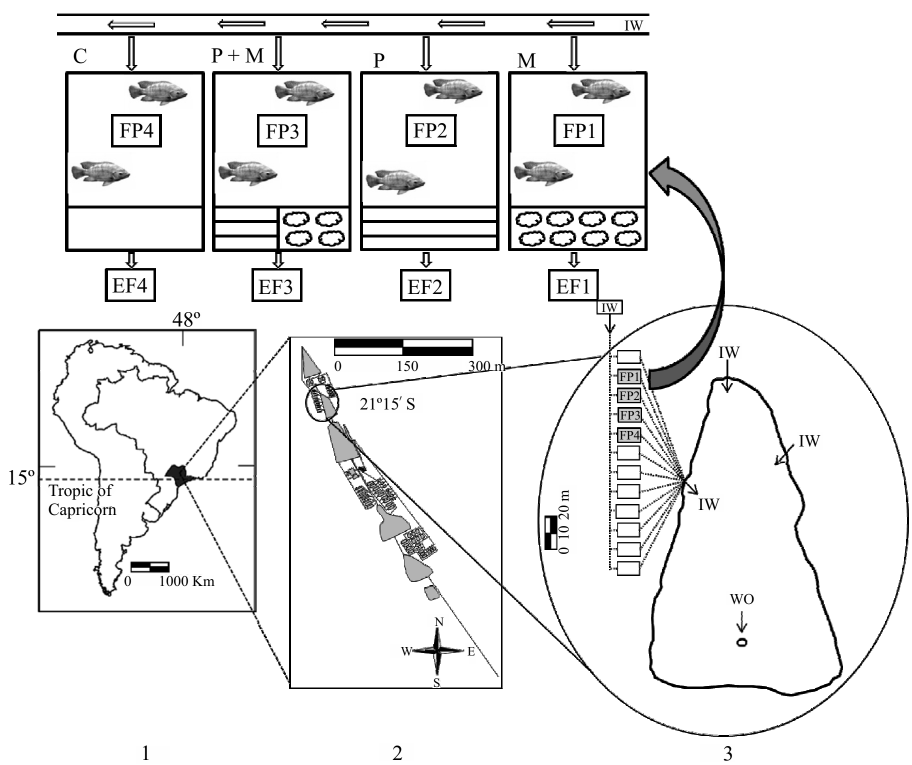

Figure 1. Outline of the flow-through fishponds used. FP1-FP4 = study fishponds; EF1-EF4 = effluent of fishponds; M, P, P + M = treated fishponds, C = control (without treatment); IW = inlet water; WO = Water outlet; ▤ = periphyton substrates; ⬭ = macrophytes. Inset 1: shade area indicates São Paulo state, located in southeastern Brazil. Inset 2: UNESP-Jaboticabal pond system. Inset 3: indication of the fishponds used in this study. *Esquema de los estanques de flujo continuo utilizados. FP1-FP4 = Estanques estudiados; EF1-EF4 = Efluentes de los estanques; M, P, P +M = Estanques tratados, C = Control (sin tratamiento); IW = Entrada de agua; WO = Salida de agua; ▤ = Sustratos perifiton; ⬭ = Macrófitas. Inset 1: Área sombreada indica el estado de Sao Paulo, localizado en el sureste de Brasil. Inset 2: Sistema de estanques de la UNESP-Jaboticabal. Inset 3: Indicación de los estanques utilizados en este estudio.*

the characteristics of the second. Macrophytes and periphyton thus become a good alternative treatment to remove excess nutrients from fishponds in this type of continuous water flow systems.

Detailed knowledge on the concomitant use of periphyton and floating macrophytes in aquaculture may increase the number of tools available for improving water quality in fishponds. The specific aims of this study were to investigate the contribution of the macrophyte *Eichhornia crassipes* and the periphyton community in maintaining water quality by removing incoming pollutants from the fishponds, and consequently determine if they are a good tool for aquaculture farms employing continuous water flow systems. The following hypotheses were proposed: *i*) the water and sediment parameters would improve with macrophyte and/or periphyton treatments, and *ii*) the presence of macrophyte and/or periphyton would have a positive effect in reducing nutrient discharge from fishponds.

MATERIALS AND METHODS

Study area

This study was performed during a six month period (from September 2011 to February 2012) in four parallel fishponds at the Aquaculture Center (21°15′S and 48°17′W) of the University of São Paulo State (UNESP) at Jaboticabal (SP, Brazil) (Fig. 1). The fishponds (named FP1 to FP4) were rectangular in shape and, 1.5 m deep with an aerial surface of 40 m^2 and a volume of 38 m^3 (Fig. 1). The water supply was obtained from an upstream pond channelled to the experimental ponds by an underground grid of tubes. Water renovation (calculated from the discharged volume) was equivalent to 5 % of the total pond volume per day. The physical and chemical characteristics of inlet water, such as pH (5.4 ± 0.4), dissolved oxygen (DO, 5.2 ± 1.4 mg/L), temperature (Temp, 23 ± 0.9 °C), total ammonia nitrogen (TAN, 18.1 ± 6.0 µg/L), total phosphorus (TP, 19.6 ± 4.3 µg/L), and total suspended solids (TSS, 5.8 ± 3.1 mg/L), were regularly monitored during the experimental period.

Biological treatments

Before the start of this study, ponds were dried and the existing vegetation was removed with constant maintenance of the fishponds so that any type of aquatic macrophyte (either submerged or floating) would develop during the experimental period. Quicklime (CaO, 150 g/m^2) was then applied to all ponds. Subsequently, one of three different biological treatments was applied close to the effluent in each fishpond: the addition of macrophytes (M); the addition of plastic pipes as substrates for periphyton (P); or the concomitant addition of macrophytes and plastic pipes as substrates for periphyton (P+M). A control fishpond (C) that did not receive any biological treatment was also used (Fig. 1). Plastic pipes (diameter of 5 cm and, length of 10 cm or 1 m) were placed vertically in the experimental ponds to be used as an artificial substrate for periphyton. These pipes were held in place by nylon strands and hang inside the water column. They extended from approximately 5 cm below the water surface to near the bottom of the fishponds, except for the 10 cm pipes, which were used for samples collection. Pipes were located towards the water outlet so that the water would pass by them before being drained from the fishponds. Pipe substrates, submersion time and depth were the same in the P and P+M treatments. Each fishpond that received a periphyton treatment (P and P+M) was provided with 64 pipes. This setup resulted in an effective submerged substrate surface area of 10 % of total pond water surface. Three days after quicklime application, all fishponds were fertilized with NPK (10-10-10, 1.5 g/m^2), and after this initial fertilization, the fishponds were left for 12 days while filling up to allow the development of natural organisms in the water column and on the plastic pipe substrates. Nile tilapia (*Oreochromis niloticus*), weighing 4.7 ± 2.9 g was stocked at a density of 150 fish per fishpond (approximately 4 fish/m^2) and a stocking biomass of 0.7 kg/pond. Fish were fed three times a day by floating pelleted feed containing 30 % crude protein for a total of 3 % weight/day. The aquatic macrophyte *Eichhornia crassipes* was introduced into the fishponds after they were filled with water, occupying a surface area of 10 % of the total fishpond water surface with a renovation of plants occurring at the end of each collection. No aeration or water-mixing devices were used.

Water quality monitoring

Measurements of water quality parameters started on day one of the experiment and were recorded between 08:00 and 09:00 h. Samples were collected from a depth of 10 cm. Two samples were collected monthly (during a total of six months) using a 1 L Van Dorn bottle at two different sites: directly from each fishpond (FP1 to FP4) and from the output of each fishpond (EF1 to EF4) as shown in figure 1. Temperature (Temp), turbidity (Turb), pH, dissolved oxygen (DO) and conductivity (Cond) were measured using a multi-probe Horiba U-10. Total phosphorus (TP), orthophosphates (OP), nitrite (NO_2) and nitrate (NO_3) were quantified with a spectrophotometer according to Golterman *et al*. (1978), and total ammonia nitrogen (TAN) was quantified as described by Koroleff (1976). Chlorophyll-a (Chl-a) concentration was determined by colorimetric analysis using a spectrophotometer after extraction with 90 % ethanol (Nusch, 1980). Total dissolved solids (TDS), total suspended solids (TSS) and 5-day biochemical oxygen demand (BOD_5) were determined according to Boyd & Tucker (1992). Water samples for microbiological analysis, which was performed using the Most Probable Number (MPN) method, were collected in sterilized 500 mL flasks and taken to the laboratory in an isothermal container. The material used in the microbiological analyses was sterilized prior to use (APHA, 2005). Analyses were performed immediately after sampling or samples were properly stored under refrigeration.

Periphyton sampling

Periphyton sampling was performed monthly to determine periphyton biomass in terms of dry matter (DM) weight per unit surface area. On each sampling date six plastic pipe substrates were randomly taken from each pond. Care was taken to avoid contact with the periphyton and consequent loss of material. The pipes used in the sampling were located at approximately 10-20 cm below the surface, and each of the six substrates was analyzed separately in the laboratory. The pipe substrates were replaced after collection of the samples to allow further development and periodical sampling of periphyton. Periphyton material was scraped carefully with a scalpel from the tube, which has a calculated surface area of 157 cm^2. Dry matter (DM) was determined after drying the material at 70 °C, and organic matter (as ash-free dry matter) was determined after burning at 550 °C (APHA, 2005). Phosphorus content was determined in duplicate using the hydrochloric acid (HCl) digestion method by Strickland & Parsons (1965). Total nitrogen content was determined from triplicate samples using the micro-Kjeldhal method according to Umbreit *et al*. (1964). Chlorophyll-a was determined according to Nusch (1980) using warm ethanol 90 % (78 °C) as a solvent and quantified at 665 nm e 750 nm. Indices proposed by Lakatos (1989) were adopted for periphyton classification, using chlorophyll-a contents (%) (Table 1).

Table 1. Classification of the periphyton on the basis of its chlorophyll-a (Lakatos, 1989). *Clasificación del perifiton basada en el contenido de su clorofila-a (Lakatos, 1989).*

Type	Chlorophyll-a Content	(%)
I	autotrophic periphyton	>0.60
II	auto-heterotrophic periphyton	0.25-0.60
III	hetero-autotrophic periphyton	0.10-0.25
IV	heterotrophic periphyton	<0.10

Macrophyte sampling

Sampling of the macrophytes (*E. crassipes*) was performed monthly and taken from an area of 0.5 m^2 with moving quadrants. In the laboratory, plants were carefully washed in distilled water to remove adhered periphyton and also organic and inorganic particulate matter. All plant material in the quadrant was harvested and measured for TP and biomass. After washing, plants were oven-dried (at 103 °C) for biomass determi-

Table 2. Nile tilapia growth performance in the different fishponds and treatments. *Datos de crecimiento de la tilapia del Nilo en los diferentes estanques y tratamientos.*

Treatments[1]	Survival (%)	Initial weight (g/fish)	Final weight (g/fish)	SGR[2] (%/day)	Weight gain (g/fish)	Number of Observations
M	98.7^a	5 ± 3^a	199 ± 103^a	4.4	194^a	10
P	98.7^a	4 ± 3^a	280 ± 113^a	4.8	275^a	10
P+M	100^a	5 ± 3^a	239 ± 111^a	4.6	235^a	10
Control	100^a	5 ± 3^a	239 ± 111^a	3.1	209^a	10

[1] M, macrophyte treatment; P, periphyton treatment; P+M, periphyton and macrophyte treatment.
[2] SGR, specific growth rate.
[3] ns = no significant.
Same letter in the superscript means no differences between treatments after ANOVA test ($p < 0.05$).

nation. Dry matter was ground, sieved through a 0.5 mm mesh and stored in plastic flasks. Plant total phosphorus and nitrogen contents were determined according to Bataglia (1983). Organic matter contents were determined as described in the literature (Westlake, 1963) after burning 0.3 g of dry macrophyte matter for 4 hours in an oven at 550 °C.

Sediments

Vertically mixed sediment samples were taken using a 4-cm diameter PVC core up to approximately 10 cm depth. Samples were taken at sites inside fishponds (FP1 to FP4) and transported to the laboratory in cold boxes. Sediments were air dried, gently disaggregated and dried in a convection oven at 70 °C until completely dry. Determination of organic matter (OM) and amounts of Ca, Cu, Mg, Mn, P, K, Al, Fe, Zn and pH was performed according to the methods described by Raij *et al*. (2001).

Fish harvesting and yield parameters

After 182 days of cultivation, all pipe substrates and macrophytes were removed from the ponds under treatment. The ponds were drained, and the fish were harvested. The mean fish weight at the beginning and end of the experiment was determined. The calculated measurements included the following: survival (%), initial weight (IW, g/fish), final weight (FW, g/fish), weight gain (FW-IW) and specific growth rate ($SGR = 100 \times [\ln(FW) - \ln(IW)/\text{culture period in days}]$).

Statistical analysis

The limnological variables were analyzed using principal component analysis (PCA), a multivariate analysis that can summarize, in a few dimensions, most of the variability of a dispersion matrix of a large number of descriptors (Legendre & Legendre, 1998). For sediment variables, community and fish-harvesting dates, a one-way ANOVA was performed to determine statistical significance between the experimental groups and control ($n = 12$). All analyses were performed using the software Statistica 8.0 (StatSoft, 2007).

RESULTS

After the experimental period of 182 days, the chlorophyll-a contents of periphyton in the P and P+M treatments were classified as heterotrophic (IV) with values below 0.10 % (Table 1). No differences were observed in fish size and weight for the different treatments. Fish from the periphyton treatment (P) presented a higher specific growth rate (SGR) (4.8 %/day) with a weight gain of 275 g. A small mortality rate of approximately 2 % was noted in the macrophytes (M) and periphyton (P) treatments (Table 2).

Water conductivity remained within values suitable for aquaculture (50 μS/cm). The pH of the fishponds ranged from 5.7 to 6.1, and the water temperature fluctuated approximately 24 °C in all ponds studied. As the ponds were exposed to the local weather conditions, and the lowest temperatures were registered in September (local spring) and the highest between November and February (local summer).

The data obtained from the water analysis were submitted to a principal component analysis (PCA), and the results (fishponds and effluent sites) are presented in figure 2. In the fishpond, the control contrasts with other treatments positioned on different sides of PC1. The association of the control fishpond with the variables OP, PT, BOD_5, TC, TAN, COND, TSS and TURB demonstrate the highest values of these variables

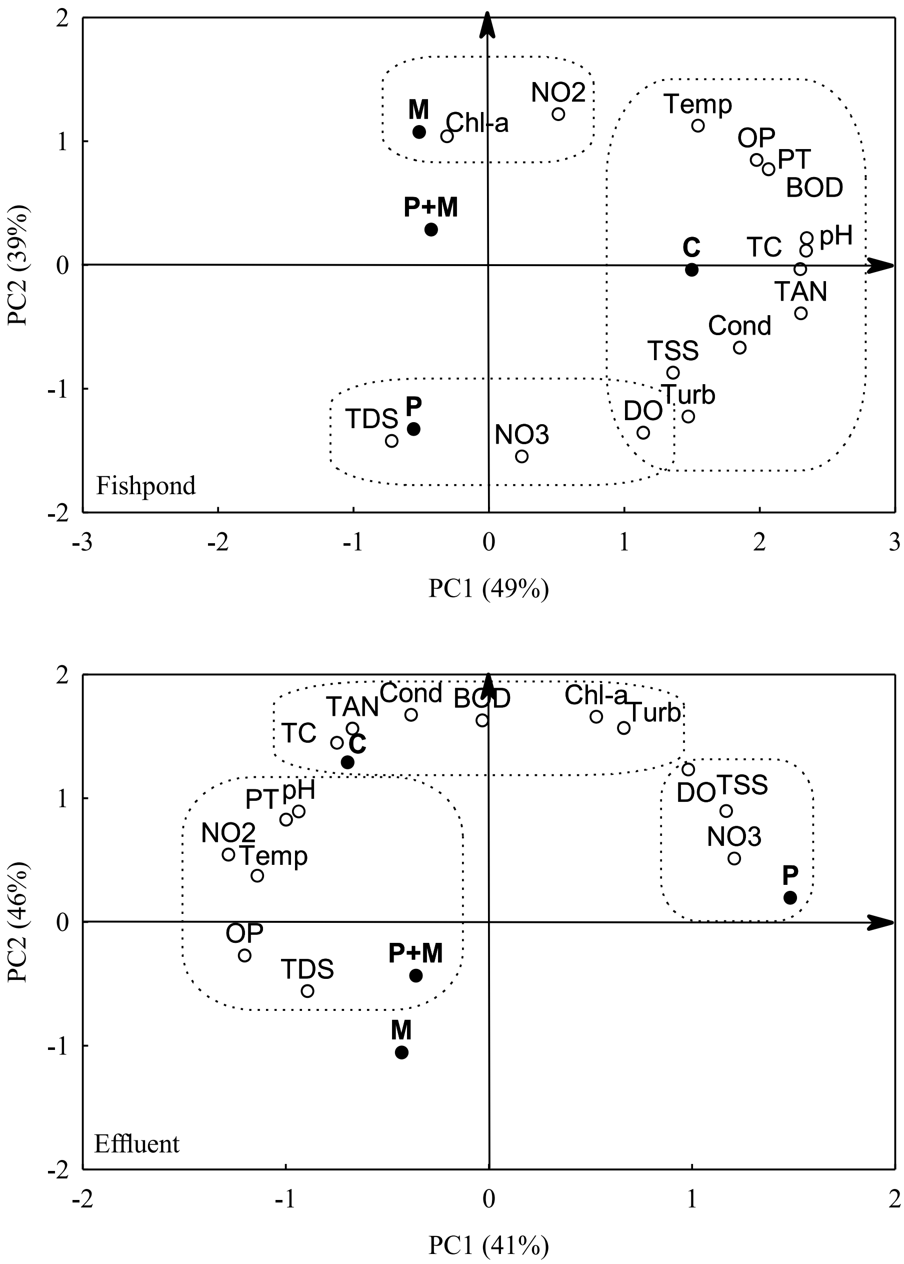

Figure 2. The first two axes from the principal component analysis (PCA) for fishponds and effluents, where: close circle = treatments and control, open circle = water characteristics. *Los primeros dos ejes del análisis de componentes principales (PCA) en los estanques y efluentes, donde: círculo negro = tratamientos y control, círculo blanco = variables de agua.*

in this site. PC2 demonstrated an association of the P treatment with the TDS and NO_3 parameters. Furthermore, PC2 demonstrated an association of the M treatment with the Chl-a and NO_2 parameters. These associations indicated higher values of variables in respective treatments. The opposite was observed for samples collected in the effluent sites, where most of the water parameters were on the negative side, with the exception of five variables that remained positive in the effluent samples (Chl-a, Turb, DO, NO_3 and TSS).

The negative association of the effluent P+M with PC1, thus demonstrating a high level of eutrophication, is noteworthy. Moreover, the control effluent was positively associated with PC2, especially Chl-a, thermotolerant coliforms, BOD_5, TAN, turbidity and conductivity. The P+M treatment in the fishpond and the M treatment in the effluent were not associated with any variables (Fig. 2).

Table 3 summarizes the data collected for sediment analysis. No differences ($p > 0.05$) were observed in pH, P, Ca, Mg or Al. The pH was acidic (varying from 4.8 to 5.0). Calcium was present in a higher quantity than the other nutrients (averaging from 461 to 721 mg/L) during the experimental period. The levels of organic matter and nutrients such as Cu, Fe, Zn and Mn were higher ($p < 0.05$) in the control pond. The P+M treatment presented a higher ($p < 0.05$) concentration K (Table 3).

Macrophyte biomass in the P+M and M treatments exceeded 400 g DW/m^2. No difference ($p > 0.05$) was observed in OM, TP and N contents in plants and periphyton in the treatments (Table 4).

DISCUSSION

The PCA analysis showed the formation of three main clusters. However, the results differed between the water quality in the P+M fishpond and the effluent water from pond M. Such differences may be attributed to the plants, which are highly capable of removing and processing nutrients used in their growth, thus improving water quality.

The highest levels of DO, nitrate, TSS and turbidity in both the fishpond and the effluent sites were observed in the periphyton-treated fishpond. Periphyton-based aquaculture ponds allow the development of attached photosynthetic algae as well as decomposing and nitrifying bac-

Table 3. Values obtained for sediment parameters measured from the different fishponds. *Valores de los parámetros medidos en las muestras de sedimentos de los diferentes estanques.*

Sediment Variables	Treatments M	Treatments P	Treatments P+M	Control	Number of Observations
pH	4.9 ± 0.2^a	4.8 ± 0.2^a	5.0 ± 0.2^a	4.9 ± 0.4^a	24
OM (%)	0.2 ± 0^a	0.2 ± 0^a	0.2 ± 0^a	0.4 ± 0.2^b	24
P (mg/L)	76 ± 13^a	43 ± 24^a	83 ± 12^a	70 ± 38^a	24
K (mg/L)	87 ± 22^{ab}	52 ± 19^b	101 ± 40^a	62 ± 15^b	24
Ca (mg/L)	461 ± 115^a	468 ± 207^a	721 ± 27^a	581 ± 347^a	24
Mg (mg/L)	118 ± 28^a	118 ± 47^a	146 ± 35^a	122 ± 38^a	24
Cu (mg/L)	0.4 ± 0.1^b	0.6 ± 0.1^b	1.0 ± 0.6^b	1.4 ± 0.5^a	24
Fe (mg/L)	34 ± 11^b	36 ± 16^b	70 ± 42^b	85 ± 42^a	24
Mn (mg/L)	0.9 ± 0.4^b	0.9 ± 0.4^b	2.2 ± 0.9^b	3.9 ± 1.7^a	24
Zn (mg/L)	0.6 ± 0.2^b	0.8 ± 0.2^{ab}	1.0 ± 0.3^{ab}	1.3 ± 0.5^a	24
Al (mg/L)	22 ± 20^a	27 ± 24^a	10.8 ± 14.8^a	27 ± 30^a	24

ns = no significant
Same letter in the superscript means no differences between treatments after ANOVA test ($p < 0.05$).

teria, microorganisms known to produce oxygen and assimilate inorganic nutrients (Özkan *et al.*, 2010). The higher levels of turbidity in the periphyton-containing ponds (P and P+M) were attributed to the detached periphyton and to the phytoplankton, thus affecting the whole pond system (Azim *et al.*, 2003).

In this study a higher ammonia concentration was observed at the fishpond treated with periphyton in comparison with the fishpond with macrophytes, but it was lower than the control. Lower ammonia (193 μg/L) contents in the macrophyte-treated fishpond and its effluent could be attributed to the biomass of the macrophytes themselves that compete with phytoplankton and consequently absorb more nutrients from the water.

Both treatments that included macrophytes (M and P+M) presented lower mean values for nitrate in the water and the highest total ammonium nitrogen (TAN) values in the plant biomass. Inorganic nitrogen is associated with rapid growth rates in plants. Nitrate serves as the main source of inorganic nitrogen resources for higher plants (Li & Wang, 2011).

The highest phosphorus contents were observed in both plant and periphyton biomass with a simultaneous decrease in available phosphorus in the water column during the experiment period. This observation demonstrates the link between the highest phosphorus content in plants and periphyton biomass and its dependence on several factors including mainly water column phosphorus concentration, forms of available phosphorus and growth stage.

The higher concentrations of thermotolerant coliforms in the control fishpond and their lower concentrations in the M fishpond were observed in samples obtained from both the fishponds and the effluent water. This phenomenon might be linked to the antimicrobial properties of macrophyte exudates. Nonetheless, the same result was not observed in the P+M treatment. Ksoll *et al.* (2007) reported that the abundances of fecal coliforms and *Escherichia coli* in periphyton communities were positively correlated with increases in water temperature, an important regulator of growth. Moreover, Nirmai-Kumar & Oomem (2009) argued that the highest values of total coliforms occur due to the high supply of oxygen and the presence of moderate amounts of nutrients such as phosphate, sulfate and nitrate. In the present study, the low contents of nutrients and dissolved oxygen observed resulted in a low total thermotolerant coliform count.

Although identical amounts of fertilizer were used in all experimental ponds, the control fishpond presented higher levels of ammonia and total phosphorus contents in the water as well as higher concentrations of organic matter, Cu, Fe, Mn and Zn in the sediment.

In our study, the chemical processes occurring in the sediment affected the water quality and consequently improved the biological communities. High levels of Cu, Zn, Al and Fe and low pH in sediment did not directly affect the biological treatments (P, M and P+M) because the presence of phosphorus in the sediment and in the water helped to decrease the sensitivity of the natural periphyton to such metals. Importantly, Serra

Table 4. Values obtained for biological variables of macrophyte and periphyton from the different treatments. *Valores de las variables biológicas obtenidos de las muestras de macrófitas y perifiton en los diferentes tratamientos.*

Community	Treatments	Variables				Number of Observations
		Biomass (gDW/m^2)	TP (mgP/gDW)	N (mgP/gDW)	OM (%)	
Macrophyte	P+M M	415 ± 213^a	4.5 ± 1.0^a	33.5 ± 7.8^a	82^a	6
		444 ± 182^a	4.3 ± 1.4^a	38.2 ± 3.2^a	80^a	6
Periphyton	P+M P	0.7 ± 0.4^a	6.5 ± 4.0^a	1.1 ± 0.3^a	8^a	6
		1.2 ± 1.0^a	6.7 ± 2.8^a	1.4 ± 0.8^a	11^a	6

ns = no significant
Same letter in the superscript means no differences between treatments after ANOVA test ($p < 0.05$).

et al. (2010) suggested that the association with phosphates causes precipitation and a consequent decrease in metal availability.

During the growth of periphyton on plastic substrates, the external mostly autotrophic portion can be dislodged, leaving behind the heterotrophic inner layer (Milstein *et al.* 2008). The periphyton that remains attached on the substrates, such as nitrifying bacteria, are mainly linked with nitrogen flow, whereas the role of periphyton is not limited to the filtering of nutrients but rather extends to active processing (Lakatos & Biró, 1989; Milstein, 2012).

Phosphorus cycling in aquatic systems is attributable to both biotic and abiotic processes, but specific measurements can be challenging (Wolfe III & Lind, 2010). It is known that periphyton can play an active role in phosphorus absorption in many ecosystems. Periphyton leads to a net increase in short-term nutrient flow from the water column into the sediments in small artificial systems, such as fishponds. Periphyton can also directly influence many sediment properties, with the most prominent effects being observed in metal-phosphate chelation and the co-precipitation of Ca and magnesium phosphates (Dodds, 2003). In this study, all ponds presented high Ca and Mg contents; however, higher values were observed in the periphyton associated with macrophyte (P+M) treatment.

It has been suggested that the pH of the media plays a role in the toxicity of some metals on periphyton, especially in acid conditions. In this study, the lower pH in the sediment (between 4.6 and 5.3) and water (between 5.2 and 6.7) suggests that better fertilization practices are necessary to avoid acidic pH.

The best growth rate performance of Nile tilapia occurred in the fishpond with the P treatment, where fish achieved an average final weight of 280 ± 113 g, which is considered a good size for fish consumption (Freato *et al.*, 2012).

Ponds used in this study have been used for over 20 years without any careful consideration with regards to fertilization and fish feed management. It is relevant to mention that the initial step of draining the ponds is of utmost importance to avoid possible cross-contamination and/or the presence of substances that might influence the study. However, a pond's architecture consists of walls covered in concrete and a bottom exposed to the soil (sediments). The accumulation of sediments in the bottom might result in accumulating organic and inorganic matter over the years, making it difficult to accurately assess the direct effects of the biological treatments used in this study.

Considering all the factors mentioned above, the differences in the physical and chemical properties of the water column and sediment between the treatments showed an improvement in the overall water quality, when the treated fishponds were compared with the control fishpond. The biological treatments had a positive effect in reducing conductivity, orthophosphates, total phosphorus, turbidity and thermotolerant coliforms in the water column and also assisted in decreasing the organic matter, Cu, Fe, Mn and Zn in the sediment. High levels of Al and low pH in the sediment may have caused unfavourable conditions in the water column and may have ultimately had an impact on fish production, suggesting the need to pay more attention to these variables. Future studies will aim to evaluate the effects of such biological treatments on the water quality of small fishponds, from which effluents are often directly discharged into larger ponds.

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REFERENCES

APHA. 2005. Standard methods for the analysis of water and wastewater. American Public Health Association, Washington, DC.

AZIM, M. E., A. MILSTEIN, M. A. WAHAB & M. C. J. VERDEGAM. 2003. Periphyton-water quality relationships in fertilized fishponds with artificial substrates. *Aquaculture,* 228: 169–187.

BATAGLIA, O. C., A. M. C. FURLANI, J. P. F. TEIXEIRA, P. R. FURLANI & J. R. GALLO. 1983. Plants chemical analyses (Boletim Técnico 78). Instituto Agronômico, Campinas (portuguese).

BOYD, C. E. & C. S. TUCKER. 1992. *Water quality and pond soil analyses for aquaculture*. Auburn, USA.

DODDS, W. K. 2003. The role periphyton in phosphorus retention in shallow freshwater aquatic systems. *Journal of Phycology*, 39: 840–849.

DRENNER, R. W., D. J. DAY., S. J. BASHAM., J. D. SMITH & S. I. JENSEN. 1997. Ecological water treatment system for removal of phosphorous and nitrogen from polluted water. *Ecological Applications*, 7 (2): 381–390.

FREATO, T. A., R. T. F. FREITAS, M. E. S. G., PIMENTA, G. R. OLIVEIRA, R. V. R. NETO & B. O. MATTOS. 2012. Evaluation of Nile tilapia strains cultivated in cages under different feeding programmes. *Revista Brasileira de Zootecnia*, 41(6): 1332–1336.

GOLTERMAN, H. L., R. S. CLYMO & M. A. M. OHNSTAD. 1978. *Methods for physical and chemical analysis of freshwater*. Blackwell Scientific Publication, Oxford.

KISS, M. K., G. LAKATOS, G. BORICS, Z. GIDÓ & C. DEÁK. 2003. Littoral macrophyte-periphyton complexes in two Hungarian shallow waters. *Hydrobiologia*, 506–509 (1-3): 541–548.

KOROLEFF, F. 1976. Determination of nutrients, in: Grasshoff, E., Kremling, E. *Methods of sea water analysis*. Verlag Chemie Wenhein. New York.

KOSÁROS, T., D. GÁL, F. PEKÁR & G. LAKATOS. 2010. Effect of different treatments on the periphyton quantity and quality in experimental fishponds. World Academy of Science, Engineering and Technology, 40 (1): 363–366.

KSOLL, W. B., S. ISHI, M. J. SADOWSKY & R. E. HICKS. 2007. Presence and sources of fecal coliforms in epilithic periphyton communities of Lake Superior. *Applied and Environmental Microbiology*, 37 (12): 3771–3778.

LAKATOS, G. 1989. Composition of reed periphyton (biotecton) in the Hungarian part of Lake Fertö. *BFB-Bericht*, 71: 125–134.

LAKATOS, G. & P. BIRÓ. 1989 Study on chemical composition of reed-periphyton in Lake Balaton. *BFB-Bericht*, 77: 157–164.

LEGENDRE, P. & L. LEGENDRE. 1998. *Numerical Ecology*. Elsevier Science B.V., Amsterdam.

LI, W. & J. WANG. 2011. Influence of light and nitrate assimilation on the growth strategy in clonal weed *Eichhornia crassipes*. *Aquatic Ecology*, 45: 1–9.

MEI, X. & X. ZHANG. 2013. Periphyton biomass response to phosphorus additions in an aquatic ecosystem dominated by submersed plants. *Journal Environmental Protection*, 4 (1): 83–90.

MILSTEIN, A., Y. PERETZ & S. HARPAZ. 2008. Comparison of periphyton grown on different substrates as food for organic tilapia culture. *The Israeli Journal of Aquaculture-Bamidgeh*. 60(4): 243–252.

MILSTEIN, A. 2012. Periphyton-based aquaculture: underwater hard surfaces in ponds promote development of natural food for fish. *Indian Journal of Social and Natural Sciences*. 1(1): 93–99.

NIRMAI-KUMAR, J. I. & C. OOMMEN. 2009. Influence of limiting factors on phytoplankton and coliform population in an inundated, isolated wetland. *Journal of Wetlands Ecology*, 3(1): 43–55.

NUSCH, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv für Hydrobiologie*, 14: 14–36.

ÖZKAN, K., E. JEPPESEN, L. S. JOHANSSON & M. BEKLIOGLU. 2010. The response of periphyton and submerged macrophytes nitrogen and phosphorus loading in shallow warm lakes: a mesocosm experiment. *Freshwater Biology*, 55(2): 463–475.

RAIJ, B. V., J. C. ANDRADE, H. CANTARELLE & J. A. QUAGGIO. 2001. *Chemical analysis to evaluation of sediments fertility.* Instituto Agronômico, Campinas (portuguese).

SERRA, A., H. GUASH, W. ADMIRAAL, H. G. VAN DER GEST & S. A. M. VAN BEUSEKOM. 2010. Influence of phosphorus on copper sensitivity of fluvial periphyton: the role of chemical, physiological and community-related factors. *Ecotoxicology*, 19(4): 770–780.

SIPAÚBA-TAVARES, L. H., A. F. BARROS & F. M. S. BRAGA. 2003. Effect of macrophyte cover in the water quality in fishpond. Acta Scientiarum Biological Sciences, 25(1): 27–36.

STATSOFT, Inc. 2007. STATISTICA (Data Analysis Software System), version 8. www.statsoft.com.

STRICKLAND, J. D. H & T. R. PARSONS. 1965. *A manual of sea water analysis*, (second ed. Bulletin 125). Fisheries Research Board of Canada.

UMBREIT, W. W., R. H. BURRIS & J. F STAUFFER. 1964. *Manometric methods applicable to the study of tissue metabolism*. Ed. Burgess Publishing Company.

VAN DAM, A., M. C. M. BEVERIDGE, M. E. AZIM & M. C. J. VERDEGEM. 2002. The potential of fish production based on periphyton. *Reviews in Fish Biology and Fisheries*, 12(1): 1–31.

WESTLAKE, D. F. 1963. Comparisons of plant productivity. *Biological Reviews*, 38(3): 385–425.

WOLFE III, J. E. & O. T. LIND. 2010. Phosphorus uptake and turnover by periphyton in the presence of suspended clays. *Limnology*, 11(1): 31–37.