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Nitrogen and phosphorus removal and *Typha domingensis* **tolerance in a floating treatment wetland**

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Abstract

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Wy) in the N and P removal from a synthetic runoff effluent and to evaluate

plants were installed. In order to evaluate the plant fole, reacto The aim of this work was to study the efficiency of microcosms-scale floating treatment wetlands (FTWs) in the N and P removal from a synthetic runoff effluent and to evaluate the effluent tolerance of *Typha domingensis*. Each FTW consisted of a raft constructed with a plastic net where *T. domingensis* plants were installed. In order to evaluate the plant role, reactors with FTWs and without FTWs (controls) were used. P and N additions were carried out as follows: 5 mg L⁻¹ P (P5 and P5-control); 10 mg L⁻¹ N (N10 and N10-control); 5 mg L⁻¹ P + 10 mg L⁻¹ N (P5N10 and P5N10-control). Also, a biological control (B-control) without contaminant addition was used. The removal of soluble reactive phosphorus and total phosphorus were significantly higher in the FTWs than in the controls. Ammonium and nitrate concentrations were not significantly different between FTWs and controls at the end of the experiment. However, nitrate concentrations showed significant differences between FTWs and controls during the experiment. N and P were mainly accumulated in plant tissues and not in the sediment. Plants tolerated the effluent conditions and showed a positive growth rate. The use of FTWs is a promising strategy for the sustainable treatment of water bodies affected by runoff waters.

Keywords: Floating wetlands, efficiency, emergent macrophytes, runoff effluent.

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1. INTRODUCTION

Floating treatment wetlands (FTWs) consist of a buoyant artificial medium, which facilitates root development in the water column (Borne, 2014; Tondera et al., 2017; Vymazal, 2007). FTWs employ emergent macrophytes growing in a floating mat on the water surface rather than rooted in the bottom sediment (Headley and Tanner, 2012). These macrophytes are characterized by the presence of large aerenchyma in their roots and rhizomes, which increases their buoyancy potential (Chen et al., 2016). Plant roots provide an extensive surface area for the growth of the attached biofilm and entrapment of suspended particulate matter (Borne, 2014). Since the plants are not rooted into the bottom sediment, they are forced to obtain nutrients directly from the water column, enhancing nutrient accumulation into their biomass (Fonder and Headley, 2010; Tanner and Headley, 2011).

FTWs are appropriate to treat water bodies contaminated with different types of wastewaters (Chen et al., 2016).The use of FTWs has included the treatment of storm water (Tanner and Headley, 2011), sewage (Ash and Troung, 2003; Van de Moortel et al., 2011), piggery effluent (Hubbard et al., 2004), pond water (Kato et al., 2009), urban lake water (Guimarães et al., 2000), dairy manure effluent (Sooknah and Wilkie, 2004), and water supply reservoirs (Garbutt, 2004). Besides, FTWs have been studied for the treatment of urban runoff (Fonder and Headly, 2010; Tanner and Headley, 2011). More recent works have focused on the potential performance improvement of FTW in N removal by

the addition of thiosulfate (Gao et al., 2018), and the assessment of FTW performance to improve water quality in a eutrophic urban pond (Olguín et al., 2017).

The city of Santa Fe (Argentina) is located in the Middle Parana River floodplain. It is surrounded by natural wetlands receiving urban runoff effluents that deteriorate their quality. FTWs could be suitable for the treatment of these urban runoff effluents. The aim of this work was to study the efficiency of microcosms-scale FTWs in N and P removal from a synthetic runoff effluent, and to evaluate the effluent tolerance of *Typha domingensis*.

2. MATERIALS AND METHODS

2.1. Experimental design

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5.100 mg L²; soluble reactive phosphor *T. domingensis* plants*,* sediment and water were collected from an unpolluted pond of the Paraná River floodplain near Santa Fe City, Argentina. The water physicochemical composition of this pond was (mean ± standard deviation): pH = 7.2 \pm 0.1; conductivity = 223 \pm 1 μ S cm⁻¹; dissolved oxygen (DO) = 6.71 \pm 0.10 mg L⁻¹; soluble reactive phosphorus (SRP) = 0.025 \pm 0.002 mg L⁻¹; NH₄⁺ = 0.790 \pm 0.005 mg L⁻¹; NO₃ = 0.310 ± 0.005 mg L⁻¹; NO₂ = non detected (detection limit = 5 µg L⁻¹); Ca²⁺ = 9.1 ± 0.1 mg L⁻¹; Mg²⁺ = 2.0 \pm 0.2 mg L⁻¹; Na⁺ = 32.8 \pm 0.5 mg L⁻¹; K⁺ = 14.1 \pm 0.5 mg L⁻¹; Fe = 0.291 \pm 0.005 mg L⁻¹; Cl⁻ = 14.6 ± 1.0 mg L⁻¹; SO₄² = 10.5 ± 1.0 mg L⁻¹; total alkalinity = 97.2 ± 1.2 mg L⁻¹. Only healthy plants of a uniform size and weight were selected. Plants were pruned to be carried to the greenhouse.

Plastic reactors (70 L) were installed outdoors under a semi-transparent plastic roof. All reactors contained 4 Kg of sediment and water from the sampling pond. In the reactors, this sediment mass generates a layer of 3-4 cm depth. According to previous studies, this is the layer of sediment involved in the exchange reactions (Di Luca et al., 2011). To evaluate the plant role in contaminant removal, reactors with and without FTWs were used. Each FTW consisted of a raft constructed with a plastic net (surface area: 0.10 m²) where 4 plants were disposed. Buoyancy was provided by a PVC frame (diameter: 12.7 mm). Rafts were designed to allow roots and rhizomes to remain submerged and hanging in the water column while aerial parts are kept above the surface. Reactors without FTWs were installed as controls without plants, only with sediment (Fig. 1).

After an acclimation period of 15 days, during which plants demonstrate a suitable growth in FTWs, they were pruned again to a height of approximately 20 cm, and reactors were drained. Subsequently, 38 L of synthetic effluent containing P and N were added to the reactors with FTWs and controls. One reactor was used as a biological control (with FTW and sediment, and without contaminant addition). Reactors were arranged in triplicate, as follows:

Stock solutions of NH_4NO_3 and KH_2PO_4 and water from the sampling pond were used to prepare the synthetic effluent. P and N concentrations used in this work were chosen due to they were usual concentrations in a peri-urban wetland that receive stormwater, domestic, and sewer flows (data not shown). Water level in the reactors was maintained by adding water from the sampling site. During the experiment, air temperature ranged from 21.1 to 34.5 C°. The experiment lasted 28 days and it was performed in triplicate.

In each reactor water was sampled at 0, 1, 3, 7, 10, 14, 21 and 28 days. Conductivity, pH, soluble reactive phosphorus (SRP), total phosphorus (TP), N-NH₄⁺ and N-NO₃⁻ were measured periodically in all treatments. Sediment and plants were sampled at the beginning and the end of the experiment. Sediment was sampled using a 3-cm diameter PVC corer and plants were separated into roots, rhizomes and leaves. In the sediment, pH and Eh were measured in all treatments. TP and total Kjeldahl nitrogen (TKN) concentrations in plant tissues and sediment were determined.

2.2. Chemical analysis

is were filtered with Millipore filters (0.45 µm). Analytical determination

to APHA (2012). SRP was determined by the colorimetric technique

to and Riley, 1962) (Perkin Elmer Lambda 20 UV-VIS Spectrophoto

by digestion The water samples were kept refrigerated until their analysis. Conductivity, dissolved oxygen (DO) and pH in water were measured with a multi-parameter probe brand WTW, model: Multi 3510 IDS. Water samples were filtered with Millipore filters (0.45 μm). Analytical determinations were carried out according to APHA (2012). SRP was determined by the colorimetric technique of molybdenum blue (Murphy and Riley, 1962) (Perkin Elmer Lambda 20 UV-VIS Spectrophotometer). TP was determined by digestion of the sample with nitric acid and sulphuric acid (APHA, 2012), neutralization and determination of phosphate by the method of Murphy and Riley (1962). NH₄⁺ and NO₃ were determined by potentiometry (Orion ion selective ion electrodes 95-12 and 93-07 respectively, sensitivity: 0.01 mg l 1 N, reproducibility: ± 2%).

TP concentrations in leaves, roots, rhizomes and sediment were determined at the end of the experiment. Plant samples were washed with tap and distilled water, and subsequently plant and sediment samples were oven-dried at 60 °C for 48 h. TP concentrations were determined, after digestion with HCl:HNO₃ (USEPA, 1994), by the colorimetric technique of molybdenum blue (APHA, 2012; Murphy and Riley, 1962) (UV-VIS Spectrophotometer Perkin Elmer Lambda 20). TKN in plant tissues and sediment were determined by the Semi-micro Kjeldahl method according to APHA (2012).

The pH and redox potential (Eh) of the sediment was determined potentiometrically with an Orion pH/mV-meter. Redox potential was measured *in situ,* and pH was measured in a sediment suspension: water 1:2.5.

P and N amounts (mg) were estimated by multiplying P or N concentrations in plant tissues and sediment (mg g^{-1} dry weight) by biomass (g dry weight).

2.3. Plant study

Plant height was measured, and the external appearance of plants was observed daily, to detect possible senescence. Chlorophyll concentration was measured at the beginning and at the end of the experiment. Relative growth rate (RGR) (cm cm⁻¹ day⁻¹) was calculated in each treatment considering initial and final plant height, according to Hunt's equation (1978):

$$
RGR = \frac{\ln H_2 - \ln H_1}{T_2 - T_1}
$$

where H₁ and H₂ are the initial and final plant height (cm), respectively and (T_2-T_1) is the experimental period (days).

Chlorophyll was extracted with acetone for 48 h in cold darkness (3-5°C). The percentage of transmittance of the extracts at 645 and 665 nm was recorded with a spectrophotometer UV-Vis to calculate chlorophyll *a* concentration (Westlake, 1974).

2.4. QA/QC

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All glassware was cleaned and washed with a non-ionic surfactant, then rinsed with distilled water prior to each use. All reagents were of analytical grade. Replicate analyses (at least four times) of the samples showed a precision of typically less than 4% (coefficient of variation).

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to determine significant differences in SRP, TP, and N-NH $_4^{\, +}$ and N-NO₃ concentrations in water, TP and TKN in sediment and plant tissues (roots, rhizomes and leaves) and relative growth rate and chlorophyll *a* concentrations among the different treatments. The normality of residuals was analyzed graphically, and Bartlett's test was used to check the homogeneity of variances (Walpole et al., 1999). Duncan's test was used to differentiate means where appropriate. A level of p<0.05 was used for all comparisons (Walpole et al., 1999).

3. RESULTS AND DISCUSSION

The chemical composition of the synthetic effluents used in the experiment is shown in Table 1.

Table 1. Initial chemical composition of effluents in each treatment (mean±standard deviations).

DO concentrations (mg L⁻¹), pH and conductivity (μ S cm⁻¹) values were significantly higher in the controls than in the FTWs over the experiment (Fig. 2). In the FTWs and controls, DO, pH and conductivity varied over time. Conductivity (μ S m⁻¹) varied between 229 and 265 in FTWs, and between 226 and 420 in controls, while the pH varied between 6.91 and 7.08 in FTW and between 6.90 and 8.32 in controls. However, in FTWs these parameters did not present significant differences between the values at the beginning and the end of the experiment. DO concentrations (mg L^{-1}) varied between 1.77 and 7.79 in FTWs, and between 4.35 and 10.52 in controls. Algal growth was observed in the controls (Fig. 1), but it was not found in FTW, which would be responsible for the higher DO, pH and conductivity in controls. One of the most significant impacts that plant coverage has on any water body, is the reduction of light penetration into the water column, which avoids the growth of photosynthetic algae (Chen et al., 2016; Fonder and Headley, 2010).

Fig. 1. a and b: View of the FTWs used in each reactor. c, d and e: View of the treatment reactors with FTWs and control reactors without FTW. d and e: Algal growth in water can be observed in the images d and e.

Fig. 2. DO (mg L-1), pH and Conductivity (µS cm-1) values over the experiment in water of FTWs and controls.

Fig. 3. TP, SRP, NH₄⁺ and NO₃ removal percentages in water of FTWs and controls.

Fig. 3 shows TP, SRP, NH₄⁺ and NO₃ removal (%) in water over the experiment. TP and SRP removal were significantly higher in FTWs than in the controls, reaching a removal of 95%. Weragoda et al. (2012) observed removal efficiencies of SRP over 90% in FTWs containing two species of macrophytes, *Typha angustifolia* and *Canna iridiflora.* There was no significant difference in removal efficiencies between the two macrophyte systems after 50 days. Kansiime et al. (2005) studied nutrient removal performance using FTW microcosms containing *Cyperus papyrus* and *Colocasia*

esculenta plants that were batch-loaded every seven days with secondary-treated sewage in Uganda and reported that FTW removal rates for TN and TP that were 10.4 and 8.8 times higher than pond controls without plants, respectively. The mean TP and SRP removals were approximately 70-80% after 21 weeks of growth, depending on plant species. Borne (2014) reported that the inclusion of a FTW significantly improved P removal efficiency exhibiting 27% lower TP outlet event mean concentrations than a conventional retention pond.

I plant uptake (Bialowiec et al., 2011; Sun et al., 2005). However, nitright
igher in FTWs than in the controls over the experiment. Negative nitrate
incertains and the controls during the first 10 days of the experiment (Ammonium concentrations were not significantly different between FTWs and controls over the experiment, reaching removals of 94-96% at the end of the experiment. Major ammonium removal pathways in constructed wetlands included nitrification, ammonia volatilization, sorption by substrate and plant uptake (Bialowiec et al., 2011; Sun et al., 2005). However, nitrate removal was significantly higher in FTWs than in the controls over the experiment. Negative nitrate removals were observed in the controls during the first 10 days of the experiment (Fig. 3). This nitrate concentration increase suggests that nitrification, but not complete denitrification, occurred in the controls. However, after 21 days nitrate removals in the controls did not show significant differences regarding the FTWs. As it can be seen, nitrate removal was slower in the control than in the FTWs. Floating islands enhance denitrification by producing anoxic conditions through the restriction of oxygen diffusion into the water column. Efficient nitrate removal from wetlands depends on denitrification which is supported by macrophytes supplying organic carbon (Weisner et al., 1994). Organic carbon available to denitrifying bacteria is released from plant litter and from living macrophytes which also offer attachment surfaces for epiphytes which produce additional organic matter (Masters, 2012). Keizer-Vlek et al. (2014) reported that the removal of Total Nitrogen (TN) and TP were higher in FTWs planted with *Typha angustifolia* and *Iris pseudacorus* in comparison with the control, finding the highest TN removal in the systems with *I. pseudacorus* (98%), followed by the systems with *T. angustifolia* (57%).

Chemical characterization of the sediment used in FTWs and controls at the beginning of the experiment was: pH=7.07, OM= 5.41 % and Eh =645 mV. At the end of the experiment OM varied between 9.67 and 11.78 % in FTWs, and between 9.51 and 11.06 % in controls; pH ranged from 6.84 to 7.09 in FTWs and from 7.06 to 7.19 in controls, and Eh ranged from 143.4 to 303.4 mV in FTWs and from 239.9 to 406.8 mV in controls.

P concentrations in sediment did not show significant differences between the initial and final values in the FTWs (Table 2), while in the controls final P concentrations in sediment were significantly higher that the initial values. This could indicate that, in systems without macrophytes, sediments replace plants in the role of P removal. Nevertheless, the advantage of macrophytes is the possibility of being harvested, which leads to important removal rates of P in short times (Panigatti and Maine, 2003). In a field-scale FTW application, the sediment is also likely to play an important role in the long-term cycling and storage of contaminants such as P and inorganic fine suspended solids (Tanner and Headley, 2011). At the end of the experiment, in the FTWs and controls N concentrations in sediment did not show significant differences with the initial values (Table 2), indicating that N was not accumulated in sediment. Borne et al. (2013) studied the performance of a FTW in comparison with a control (not vegetated) to remove N of the storm water effluent over one year. Contrary to our results, these authors observed that the N accumulation in sediment contributed to the overall N removal with greater accumulation in the FTW pond than the control pond, being the direct uptake by plants of less importance in these systems. In comparison with our work, the different results may be due to the different time of experimentation.

P and N concentrations increase in plant tissues (Table 2). It is well known that P and N are usually accumulated in aerial parts of higher plants (Marschner, 2012). However, P concentrations were significantly higher in roots and rhizomes than in the aerial parts at the end of the experiment, while N presented the highest concentrations in aerial parts. In FTWs, *T. domingensis* does not grow under

natural conditions, having their roots and rhizomes in direct contact with water. As a consequence, their nutrient uptake and accumulation mechanisms may vary according to its different morphology. Kyambadde et al. (2004) proposed that differences in the structure and development of macrophyte roots have implications for the removal of wastewater contaminants and the uptake of nutrients. These authors concluded that plant uptake and storage was the main factor responsible for P removal in FTWs, contributing with the 88.8 % of TP removal by a FTW system. Van de Moortel et al. (2011) concluded that P removal in FTWs is achieved not only by macrophyte root uptake, but also by accumulation on the bottom sediment. Weragoda et al. (2012) compared the efficiencies of FTWs using *T. angustifolia* or *Canna iridiflora*, observing that *T. angustifolia* has high and steady root growth that allowed better performance than *C. iridiflora*, whose root mat is thick and compact.

Table 2. TP and TKN concentrations (mg g-1) in different plant tissues (roots, rhizomes and leaves) and sediments at the beginning and the end of the experiment (mean±standard deviation). Different letters represent statistically significant differences among treatments.

At the end of the experiment, plant biomass was significantly higher in the treatments with effluent addition than in the B-control. The roots in the treatments with effluent addition were thicker and shorter, with abundant root hairs, in comparison with those of the B-control. These morphological changes favour the uptake of nutrients from water (Bates and Lynch, 1996; Borch et al., 1999; Dinkelaker et al., 1995; Headley and Tanner, 2008; Lorenzen et al., 2001; Miao and Sklar, 1998; Weragoda et al. 2012).

Biomass and concentrations have to be considered to estimate N and P accumulation in plants. P and N amounts (mg) were estimated by multiplying P or N concentrations in plant tissues by biomass (Fig. 4). The final P and N amounts in leaves and roots were significantly higher than their initial values, indicating that P and N were efficiently accumulated by *T. domingensis*. In all treatments P and N amounts were significantly higher in leaves than in roots and rhizomes. In the P5N10 treatment, no significant differences were observed in P amount between roots and rhizomes. In the P5 treatment, a significantly higher P amount in roots than in rhizomes was measured. In the B-control, P amount in leaves and roots was significantly higher than the initial values, while P amount in rhizomes was significantly lower. Since contaminants were not added in this reactor, P accumulated in rhizomes was transported to leaves and roots to ensure the plant growth. Bernard (1999) proposed that translocation of nutrients from rhizomes was estimated to contribute to almost 100% of the total above-ground growth. *Typha* spp. is well known for its high investment in shoots and large biomass

production when grown under high P concentrations (Miao, 2004; Steinbachova-Vojtiskova et al., 2006). The absorption of nutrients is the initial process for the development of the plant. Plants have mechanisms by which nutrients are translocated and accumulated (Marschner, 2012). Plants absorb P, translocate it to the leaves up to the concentration to meet the requirements of chlorophyll synthesis, and then begin to accumulate it in roots and rhizomes (Mufarrege et al., 2016).

The N amounts in rhizomes and roots did not show significant differences among N10, P5N10and Bcontrol. In the B-control, the amount of N in leaves was significantly lower than that of the treatments with N addition. The availability of N for roots is a decisive factor for plant growth. Plant harvesting is a method that eliminates contaminants from the system. Harvesting depends on the contaminant accumulation in plant tissues and plant growth. Gao et al. (2018) proposed that further study is required to investigate the impact of shoot harvesting on the N removal performance in FTWs.

Fig. 5 shows N and P standing stocks in the different treatments. It could be seen that plant tissues accumulated significant amounts of N and P at the end of the experiment. In the case of N, the decrease in the total amount of N at the end of the experiment may be due to the loss of N by volatilization, either of NH₃ in the controls (justified by the pH increase) and of N₂ from the denitrification by the biofilm in the roots of plants (Zhao et al., 2012). In the case of P, although sediment was the main responsible for P accumulation in control treatments, plants accumulated it at a greater extent in FTWs, achieving greater removal from water. These results highlight the role of plants in nutrient removal from water.

Fig. 5. P and N total amounts (mg) in *T. domingensis* **tissues, water and sediment obtained initially and at the end of the experiment in the different treatments and controls.**

The same of the second term of the second term in the second secondary and the second second second second terms of the second second second to the experiment in the different treatments and conduction of the second of the Plant height increased during the experiment in all treatments, resulting in positive growth rates (Fig. 6) and reflecting *T. domingensis* tolerance to effluents. The highest growth rate was obtained in the P5N10 treatment, while the lowest rate was recorded in the B-control. *T. domingensis* present adaptations to survive in habitats with high nutrient concentrations (Miao and Zou, 2012). Among others, this macrophyte presents high growth rate, high nutrient sorption capacity, high concentrations of foliar nutrients, and ease of harvest of its biomass, becoming a suitable species to be used in FTWs. In the N10 treatment, growth rate was significantly higher than that of P5 treatment. This result agrees with Escutia-Lara et al. (2009), who studied the effect of N and P on the multiplication of rhizomes of *T. domingensis* plants. N is an integral component of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones and secondary metabolites (Bonilla, 2008). The increase in plant height can result in considerable amounts of biomass, which correspond to significant nutrient removal from water. At the end of the experiment, aerial and submerged biomass increased significantly in all treatments, with growth rates significantly higher than those of B-control. Chlorophyll concentration increased in all treatments and in the B-control at the end of the experiment (Fig. 6). The highest concentrations of this pigment were observed in the treatments with addition of N (Bonilla, 2008).

Fig. 6. Relative growth rate (cm cm⁻¹ d⁻¹) and chlorophyll concentrations (mg g⁻¹) of *T. domingensis* **measured in the different treatments and in the B-control. Different letters represent statistically significant differences among treatments. Bars represent standard deviations.**

Plant growth and nutrient availability are not always directly correlated (Canfield and Hoyer, 1988; Feijoo et al., 1996; Kern-Hansen and Dawson, 1978). Indeed, if plant development results from the mobilization of energy reserves and structural materials accumulated in different storage organs, nutrient-uptake is likely to exceed the rate of utilization in growth, allowing reserves to accumulate (Chapin and Van Cleve, 1989; Grime, 1988). This "luxury" uptake may be later beneficial for plants, if water nutrient concentrations diminish.

4. CONCLUSIONS

SRP, TP, ammonium and nitrate were efficiently removed from water during the experiment, demonstrating the efficiency of the FTW. N and P were mainly accumulated in plant tissues and not in the sediment.

T. domingensis is a suitable species to be used in FTWs, since it tolerated effluent conditions and accumulated N and P in its tissues.

The use of FTWs is a promising strategy for the treatment of water bodies affected by runoff waters. However, larger-scale and longer-term studies are necessary to evaluate FTWs sustainable treatment performance.

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Highlights

- Treatment of synthetic runoff effluent was evaluated with *T. domingensis* FTWs
- P was efficiently removed by FTWs and plants tolerated the effluent conditions
- Nitrate removal was higher in FTWs than in the controls along the experiment
- N and P were mainly accumulated in plant tissues and not in the sediment
- FTWs are a promising strategy to treat water bodies affected by runoff

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