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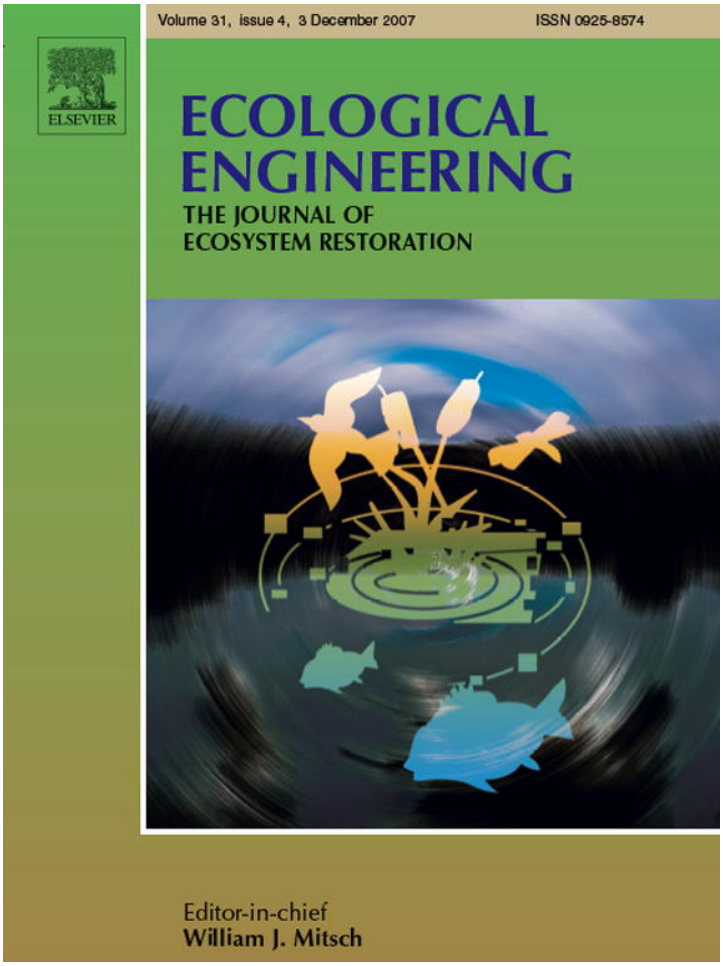

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Phosphorous amount in floating and rooted macrophytes growing in wetlands from the Middle Paraná River floodplain (Argentina)

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ABSTRACT

Biomass, P concentration, P amount and chlorophyll in three floating and three rooted macrophytes growing in wetlands of the Middle Paraná River floodplain measured in winter and summer were compared. Macrophytes were sampled three times in summer and twice in winter, in the period 2002/2004. Although *Pistia stratiotes* was the species with the highest P concentration in leaves and roots, *Typha domingensis*, *Eichhornia crassipes* and *Pontederia cordata* were the most efficient species in P retention in natural wetlands because of their higher biomass. Total P amount in rooted species did not show seasonal variations. However, *T. domingensis* accumulated a greater P amount in its aerial part in summer, whereas in winter it did so in its below-ground parts, indicating an important P dynamic regarding translocation within the plant. In summer, floating species were able to accumulate great quantities of P in a short period due to their high growth rate. In order to optimize and maintain the efficiency of constructed wetlands for P removal throughout the year, a selection of floating and rooted species should be used.

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1. Introduction

Nutrient concentrations in plant tissues are an approximate measure of the consumption of resources, a basic feature to predict the ecological strategy of a species (McJannet et al., 1995). When there is enough room for colonization and abundant availability of nutrients, macrophytes show a high growth rate and an easy propagation explained by their effective agamic or vegetative reproductive mechanism by means of rhizomes and stolons, according to the species.

Macrophytes assimilate nutrients directly into their tissues, increase environmental diversity in the root zone and promote a series of chemical and biochemical reactions within biogeochemical cycles (Jenssen et al., 1993). Additionally, aquatic plants translocate oxygen from aerial parts into roots and therefore the rhizosphere produces an oxygenated microenvironment which encourages the decomposition of organic matter and bacterial growth (Gersberg et al., 1986). Due to their characteristics, they were used to solve eutrophication problems of freshwater bodies (Aoi and Hayashi, 1996) and to remove pollutants in constructed wetlands (Jenssen et al., 1993; Delgado et al., 1993; Lytle et al., 1998; Maine et al., 2001, 2004a, 2006).

The Middle Paraná River floodplain has many wetlands in which an abundant and varied water vegetation develops. Commonly, floodplain lakes are covered with *Eichhornia crassipes* (Mart.) Solms. and other associated small floating species such as *Salvinia* sp. and *Pistia stratiotes* L. Also, there

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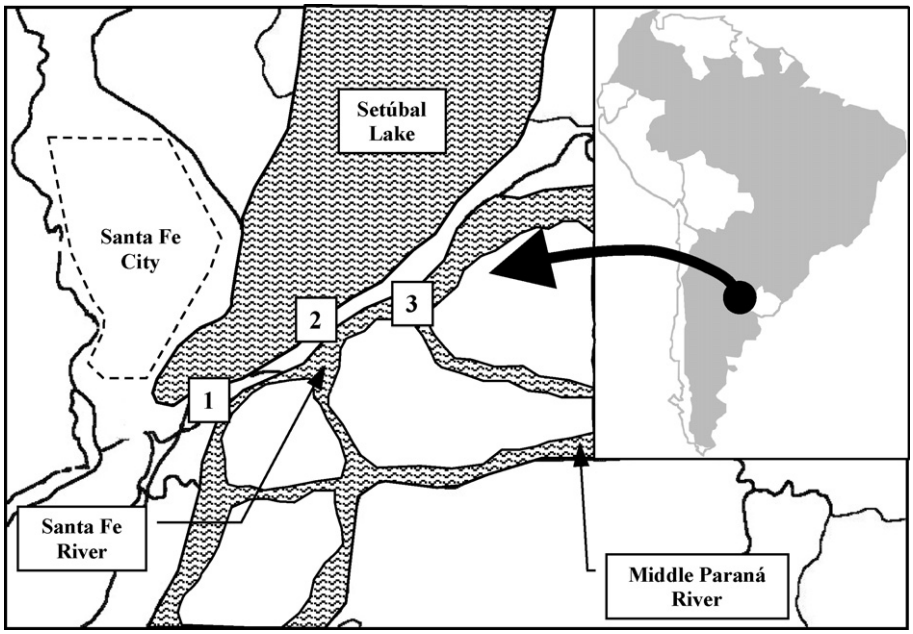

Fig. 1 – Sampling points of the studied species:(1) *P. stratiotes*, *E. crassipes*, *S. rotundifolia* and *P. elephantipes* collection. (2) *T. domingensis* collection. (3) *P. cordata* collection.

are areas mostly covered by rooted species like *Typha domingensis* Pers. or *Panicum elephantipes* Ness. ex Trin. (Mereles, 2004). These and other species have been studied in the Paraná River basin in relation to their productivity (Villar et al., 1996), growth (Lallana, 1981), response to floods (Neiff et al., 2001) and effects of different pollutants (Villar et al., 1998, 1999). Furthermore, the dynamics of nutrients was studied in these environments (Maine et al., 2004b) and the hypothesis of the relationship between nutrient concentration in macrophytes biomass and surrounding water was set forth (Carignan and Neiff, 1992; Feijoó et al., 1996; Maine et al., 1999). Laboratory systems composed of water-floating macrophytes-sediment exposed to elevated phosphorous concentration were used to select the most efficient regional floating macrophyte species in P uptake (Maine et al., 1998), but there are no field studies on the P accumulation in different macrophyte species in natural wetlands. The aim of this work was to compare summer/winter bioaccumulation of P in different free-floating and rooted macrophytes growing in the wetlands of the Middle Paraná River floodplain in Argentina. The results could be used to optimize and maintain the efficiency of constructed wetlands.

2. Materials and methods

2.1. Study sites

Macrophytes, water and sediment were collected from the Santa Fe River floodplain, a secondary branch of the Middle Paraná River system, near Santa Fe city, Argentina (Fig. 1), during the months of December 2002, 2003 and February 2004 (summer), and August 2003 and June 2004 (winter). This region exhibits diverse geomorphological units characterized by streams and shallow lakes (Iriondo, 1989). The macrophyte species studied are shown in Table 1. The sampling points for each species were the same in summer and in winter. *P. elephantipes*, *E. crassipes*, *Salvinia rotundifolia* Willd. and *P. stratiotes* were collected at point 1 (31°38′25″S, 60°39′34″W), from the coastal margin of the Santa Fe River which has a sandy bottom

Table 1 – List of plant species studied

Family	Scientific name	Common name
Floating species		
Araceae	*Pistia stratiotes* L.	Water lettuce
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms.	Water hyacinth
Salviniaceae	*Salvinia rotundifolia* Willd.	Water fern
Rooted species		
Gramineae	*Panicum elephantipes* Ness. ex Trin.	Elephant panicgrass
Pontederiaceae	*Pontederia cordata* L.	Pickerelweed
Typhaceae	*Typha domingensis* Pers.	Cattail

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and approximately 1 m depth. *T. domingensis* and *Pontederia cordata* L. were collected at points 2 (31°38′17″S, 60°38′47″W) and 3 (31°36′49″S, 60°35′09″W) (Fig. 1), respectively, from monospecific stands which were found in wetlands of a muddy bottom, approximately 40 cm deep, without permanent contact with the river.

2.2. Sampling and study of vegetation

Plants were collected with a square frame of 50 cm each side, in five replicates at each sampling. All plants found in squares were harvested following the methodology proposed by Westlake (1974) and APHA (1998). At the laboratory, the plants collected were sorted into aerial parts, submersed parts (roots of floating species) or below-ground parts (rhizomes and roots of rooted species). The roots were carefully rinsed with destilled water to remove sediment residues. In order to measure dry weight, plant material was dried at 105 °C until a constant weight was reached (APHA, 1998). Relative growth rate was calculated according to the equation proposed by Hunt (1978): $R = (\ln W_2 - \ln W_1)/(T_2 - T_1)$, where R = relative growth rate ($g\,g^{-1}\,d^{-1}$), W_1 and W_2 = winter and summer dry weight, respectively, and $(T_2 - T_1)$ = study period.

Chlorophyll was extracted with acetone for 48 h in cold darkness (3–5 °C). The percentage of transmittance of the extracts at wavelengths of 645 and 665 nm was recorded with a spectrophotometer UV–vis (Westlake, 1974). To measure chlorophyll concentration, three samples of each species were collected by hand. For *S. rotundifolia* all the variables were measured taking whole plants, without separating them into aerial and submersed parts, due their small size.

To determine Total P in plants, three samples of each species were collected by hand. Individuals were split into roots and leaves with the aim of comparing accumulation among species and among the different parts of the same species. Plant tissues were digested with $HClO_4$:HNO_3:HCl (7:5:2) mixture, and total phosphorus (TP) was measured as soluble reactive phosphorous (SRP) following Murphy and Riley (1962) (Spectrophotometer UV–vis Perkin Elmer Lambda 20).

In order to measure not only TP concentration in tissues but also the accumulation capacity of each macrophyte, P amount ($g\,P\,m^{-2}$) in aerial part or in submersersed (or below-ground) parts was estimated for each species by multiplying biomass (g dry weight m^{-2}) by P concentration in plant tissue ($g\,P\,g^{-1}$ dry weight). Total P amount ($g\,P\,m^{-2}$) was estimated adding the P amount of aerial and submersed (or below-ground) parts of each species.

2.3. Sampling and chemical analysis of water and sediment

Water temperature and conductivity were measured *in situ* using a YSI 33 portable conductivity meter. Dissolved oxygen (DO) and pH were also measured *in situ* with a Horiba OM-14 portable meter and an Orion pH-meter, respectively.

Water samples were collected in triplicate at each point, and the samples were kept at 4 °C and later filtered through Millipore membrane filters (0.45 μm) for nutrient determinations. Chemical analysis were performed following APHA (1998); NO_2^- by a coupling diazotized colorimetric method, NH_4^+ and NO_3^- by ion selective electrode potentiometry (Orion ion selective electrodes). Soluble reactive phosphate-phosphorous (SRP) was determined by the colorimetric molybdenum blue method (Murphy and Riley, 1962).

Sediments were sampled with an Eckman dredge and the upper 10 cm were collected in one large visually uniform zone dominated by the studied species. Sediment samples were collected at 3 m intervals along three 15 m transects. Each sample was placed in a plastic bag and stored at 4 °C. TP in sediment was determined in the same way as in plant tissues.

2.4. Statistical analysis

Two-factor variance analysis (ANOVA) was carried out. Factors were: species (6 levels) and seasons (2 levels: winter and summer). The analyzed variables were: chlorophyll concentration, dry weight, P concentration in leaves and roots and total P amount. When there were differences in the levels of each factor, Duncan's Multiple Range Test was applied to differen-

Table 2 – Measured parameters in water and total P in sediment in winter and summer (mean ± s.d.) of the sampling points

Parameter	Winter			Summer		
	1	2	3	1	2	3
Water						
Temperature (°C)	11.1 ± 2.5	12.3 ± 2.5	12.0 ± 2.4	26.4 ± 2.6	27.1 ± 2.6	26.2 ± 3.0
pH	6.8 ± 0.23	7.1 ± 0.35	7.2 ± 0.33	6.89 ± 0.28	6.75 ± 0.26	7.06 ± 0.31
Conductivity ($\mu S\,cm^{-1}$)	175 ± 22	157 ± 7	145 ± 15	222 ± 45	172 ± 18	190 ± 21
DO ($mg\,l^{-1}$)	11.3 ± 0.9	9.4 ± 0.7	11.4 ± 1.1	6.0 ± 0.5	4.9 ± 1.8	5.1 ± 0.4
SRP ($mg\,l^{-1}$)	0.080 ± 0.033	0.066 ± 0.042	0.054 ± 0.014	0.111 ± 0.014	0.097 ± 0.083	0.115 ± 0.058
NH_4-N ($mg\,l^{-1}$)	0.621 ± 0.115	0.443 ± 0.061	0.423 ± 0.145	0.857 ± 0.190	0.547 ± 0.207	0.902 ± 0.312
NO_2-N ($mg\,l^{-1}$)	0.016 ± 0.005	0.022 ± 0.009	0.010 ± 0.005	0.011 ± 0.002	0.019 ± 0.007	0.011 ± 0.003
NO_3-N ($mg\,l^{-1}$)	0.641 ± 0.058	0.412 ± 0.091	0.557 ± 0.049	0.671 ± 0.182	0.463 ± 0.062	0.492 ± 0.077
Sediment						
TP ($mg\,g^{-1}$ d.w.)	0.374 ± 0.045	0.438 ± 0.081	0.338 ± 0.042	0.419 ± 0.092	0.495 ± 0.066	0.372 ± 0.092

(1) *P. stratiotes*, *E. crassipes*, *S. rotundifolia* and *P. elephantipes* collection; (2) *T. domingensis* collection; (3) *P. cordata* collection.

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tiate means. Linear regression and correlation analyses were used to examine relationships among the TP concentrations in leaves and chlorophyll concentrations. A level of $p < 0.05$ was used in all comparisons. Statistical analysis was performed using Statgraphics Plus 3.0 software.

3. Results

Parameters measured in water and TP concentrations in sediment are shown in Table 2. There were no statistically significant differences in these measured parameters considering the three sampling points, being the DO significantly different between summer and winter ($p = 0.032$).

There were statistically significant differences in all species regarding chlorophyll concentrations in summer and winter ($p = 0.014$) (Fig. 2). *S. rotundifolia*, *P. stratiotes* and *P. cordata* presented chlorophyll concentrations significantly higher than other species ($p = 0.022$). There was no significant correlation between P concentrations in leaves and chlorophyll concentrations ($r = 0.402$, $p = 0.194$).

There were statistically significant differences in the biomass of aerial and below-ground parts between summer and winter ($p < 0.0001$). *T. domingensis*, *P. cordata* and *E. crassipes* presented a significantly greater aerial biomass than the other species in summer as well as in winter, whereas *P. stratiotes* and *S. rotundifolia* showed the smallest aerial biomass ($p < 0.00001$). *P. elephantipes* presented an aerial biomass significantly different from the other species (Fig. 3a). *P. stratiotes* presented a significantly smaller root biomass than the other species, whereas *E. crassipes* showed root biomass values not significantly different from those of the rooted plants ($p < 0.0031$) (Fig. 3b).

For P concentrations in roots and leaves, there were no statistically significant differences between the values recorded in summer and winter. There were no significant differences among the concentrations of P in the leaves of *P. cordata*, *T. domingensis*, *E. crassipes* and *S. rotundifolia*. *P. elephantipes* showed a concentration of P in leaves which was significantly lower than those of the other species and *P. stratiotes* showed a significantly higher concentration ($p = 0.0005$) (Fig. 4a). There

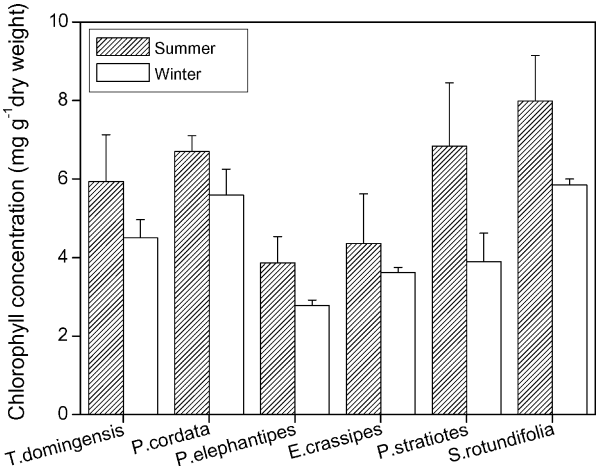

Fig. 2 – Chlorophyll concentration (mg g^{-1} dry weight) of the species studied (mean ± s.d.).

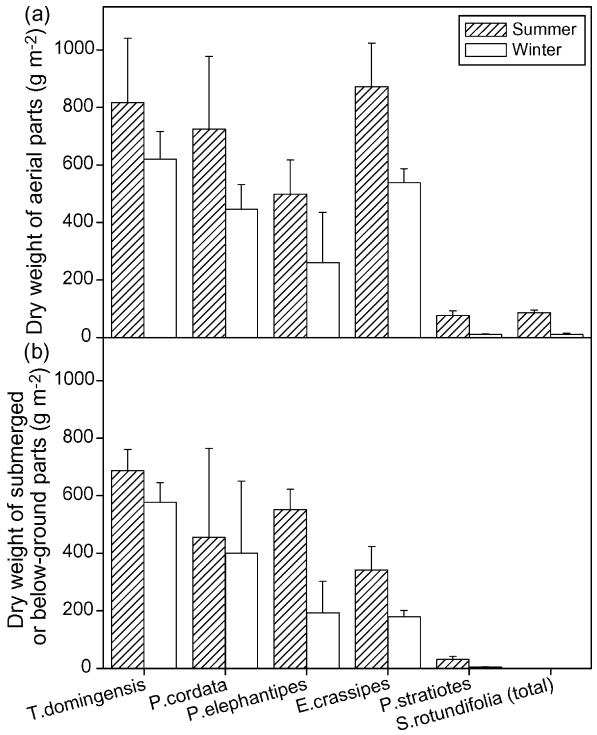

Fig. 3 – Dry weight (g m^{-2}) of aerial parts (a) and submersed (floating species) or below-ground parts (rooted species) (b) of the studied macrophytes (mean ± s.d.).

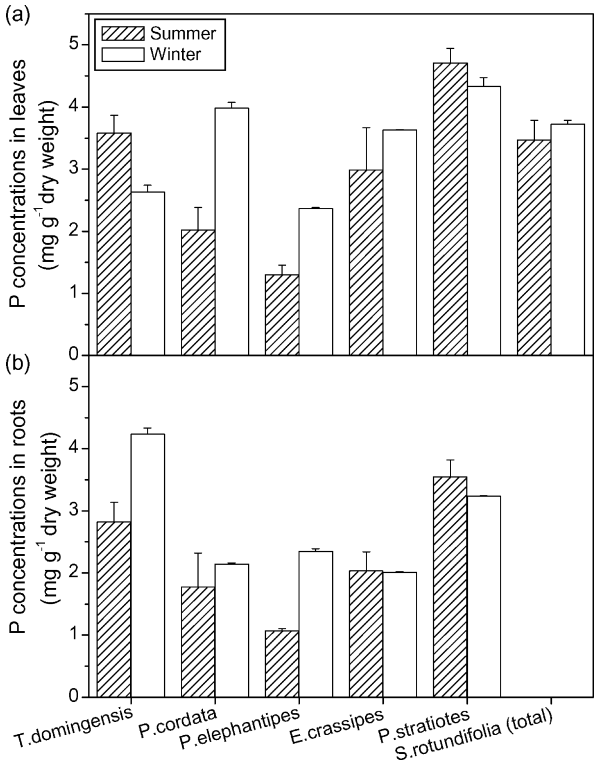

Fig. 4 – P concentration (mg g^{-1} dry weight) in leaves (a) and roots (b) of the studied macrophytes (mean ± s.d.).

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Table 3 – Total P amount and relative growth rate (*R*) of the macrophytes studied (mean ± s.d.)

Macrophytes	P amount ($g\,m^{-2}$)						R ($g\,g^{-1}\,d^{-1}$)
	Winter			Summer			
	Aerial parts	Submersed or below-ground	Total	Aerial parts	Submersed or below-ground	Total	
T. domingensis	1.63	2.44	4.07 ± 0.6	2.93	1.94	4.87 ± 0.5	0.0251
P. cordata	1.78	0.856	2.63 ± 0.7	1.47	0.806	2.27 ± 0.5	0.0441
P. elephantipes	0.618	0.455	1.07 ± 0.3	0.647	0.591	1.24 ± 0.3	0.0761
E. crassipes	1.95	0.361	2.31 ± 0.6	2.61	0.694	3.31 ± 0.7	0.0479
P. stratiotes	0.046	0.013	0.059 ± 0.003	0.360	0.110	0.47 ± 0.15	0.1814
S. rotundifolia	0.041	–	0.041 ± 0.003	0.298	–	0.298 ± 0.15	0.1878

were no significant differences in P concentration in roots among the species, except for *P. stratiotes* and *T. domingensis*. These species presented values significantly higher than those recorded for the other species ($p = 0.0001$) (Fig. 4b).

For rooted species there were no significant differences between total P amount for summer and winter (Table 3), whereas floating species showed higher values in summer ($p < 0.001$). *T. domingensis* reached the significantly highest P amount, followed by *E. crassipes* and *P. cordata* (Table 3). *T. domingensis* presented a significantly higher P amount in aerial parts in summer than in winter, whereas in winter the P amount was significantly higher in the below-ground parts, even though there were no significant differences in the total P amount in summer or winter. *P. cordata* and *P. elephantipes* did not show significant differences in P amount in aerial and below-ground parts in summer compared to winter. Floating species showed a significantly higher P amount in aerial and submersed parts in summer than in winter. *P. stratiotes* and *S. rotundifolia* presented a relative growth rate significantly higher than the other species ($p < 0.001$) (Table 3).

4. Discussion

Hadad and Maine (2001) reported that when the concentration of P in water increases, the concentration of P in leaves and the concentration of chlorophyll in *P. stratiotes* and *S. herzogii* also increases, suggesting that a greater availability of P in water favors the processes inherent to chlorophyll synthesis. The lack of correlation found among the chlorophyll concentration and the concentrations of P in leaves could be explained by the fact that the concentrations of SRP and other nutrients in water did not vary significantly throughout the study period, and the increase in chlorophyll in summer would be mainly due to higher temperatures and photoperiod, similarly to the changes observed in biomass, representing a maximum productivity during the summer months. Pietro et al. (2006), in a microcosms study, found that P bioaccumulation was proportional to initial SRP concentrations and was weakly correlated with solar irradiance and water temperature, probably because they studied a submersed macrophyte. In our work, the water temperature and solar irradiance were obviously higher in summer than in winter, obtaining higher P amount in aerial parts of most of the studied species.

In natural water bodies, most of the P load is deposited in sediment where the adsorption to metalic oxides was identified as one of the main reactions (Lijklema, 1977). In agreement with results of previous experiments carried out in the Middle Paraná River (Maine et al., 1992, 1996), it may be stated that $Fe(OOH) \approx P$ is the key fraction for the dynamics of P in aerobic aquatic systems. Water-sediment P exchange reactions are fundamentally governed by the granulometry and the mineralogical composition of the sediment, the presence of microorganisms, pH, redox potential, temperature and water stir (Holdren and Armstrong, 1980; Bates and Neafus, 1980; Boström et al., 1982; Boström, 1984; Maine et al., 1992, 1996; Golterman, 2004). The adsorptive capacity of the sediment for phosphate varies mainly due to changes in redox conditions related to the temperature cycle and the course of organic deposition (Lijklema, 1993). In our work, significant differences were registered in DO and temperature between summer and winter, while TP concentrations in sediment were not significantly different. Reina et al. (2006) found that the concentration of organic matter was significantly higher in the top sediment of sites covered by emergent vegetation than in their adjacent open-water sites. In summer, plant growth accumulates organic matter in bottom sediments, which decreases DO and redox potential of the sediment releasing P to the water column. Increased nutrients can also increase the growth of planktonic and benthic algae in wetlands, which, in turn, may compete with and inhibit macrophyte growth (Willis and Mitsch, 1995).

The species that presented the highest biomass, such as *T. domingensis* and *E. crassipes*, were the main P-bioaccumulators. Maine et al. (2006) reported that in a constructed wetland *E. crassipes* carried out the highest P-removal in the studied period, due to its high productivity. Greenway (2003) reported that emergent species of a constructed wetland of Australia had lower nutrient concentration but a greater biomass and were able to store more nutrients per unit area of wetland. Keddy et al. (1994) studied the responses and competitive effects of 20 plant species in natural wetlands. Their results indicated that the species of a greater competitive capacity were the perennial rhizomatous plants of a considerable individual biomass. In other studies, *Typha angustifolia* was one of the predominant species due to its capacity to dominate areas of dense vegetation (Weiher and Keddy, 1995; Mal et al., 1997), and *Typha latifolia* was placed within the highest competitive hierarchy (Gaudet and Keddy, 1995).

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In summer as well as in winter, floating species showed higher P concentrations in leaves than rooted species because P was translocated after being absorbed by roots directly from water. Except for *T. domingensis*, emergent species reached greater P concentrations in leaves and a lower aerial biomass in winter than in summer. This agrees with what was reported by Bernard (1999) for emergent macrophytes used in artificial wetlands to treat polluted water, and by Villar et al. (1996) for the species *Cyperus giganteus* in the delta floodplain of the Lower Paraná River. However, McJannet et al. (1995) found no significant correlation between biomass and nutrient concentration. It is worth highlighting that the capacity of P retention of the studied rooted species did not show seasonal variations as it can be observed in the values of total P amount (Table 3). P amount in aerial parts of *P. elephantipes* and *P. cordata* did not present significant differences between summer and winter, because when biomass decreases the P concentration increases and when biomass increases the P concentration decreases. The same fact can be seen for below-ground parts. For *T. domingensis*, the highest P concentration in leaves was reached in summer, whereas in winter the highest P concentration was found in roots. Analysing the P amount in below-ground parts in *T. domingensis* it is observed that it was significantly higher in winter. A reason for this could be that when the aerial biomass of this species decreases in winter, P is translocated to below-ground parts to be accumulated for the next growth season. P amount in aerial parts in *T. domingensis* was higher in summer since both aerial biomass and P concentration increases, showing an important dynamics of P being translocated within the plant. The behaviour of this rooted species corresponded to a growth strategy consisting in that roots and rhizomes stored a big amount of P in winter to reach a remarkable biomass development in summer. Bernard and Lauve (1995) proposed that in spring and summer shoots grow rapidly, partly through translocation of reserve food and nutrient elements from rhizomes, estimated to contribute to almost 100% of the total spring above-ground growth. Also, reallocation of biomass between compartments is essential for surviving water level changes. Species that can maintain allocation to shoots without an adverse effect on total or below-ground biomass are at a distinct advantage (Mars et al., 1999).

The different propagation form and the morphology of the plants studied are important characteristics in the acquisition and accumulation of nutrients. Free-floating species reproduce principally by extending stolons on the surface of water. *P. elephantipes* reproduces mainly by occupying submersed and aerial spaces since it does not have underground rhizomes. On the other hand, *T. domingensis* and *P. cordata* have a vegetative reproduction by extension and ramification of their rhizomes, thus occupying aerial and underground spaces (Bernard, 1999). The difference in the behaviour of *T. domingensis* and *P. cordata* could have been due to the fact that the former has larger rhizomes than the latter, which allows for a high accumulation of nutrients and an effective vegetative propagation for the colonization of space.

Although the importance of macrophytes for P retention in aerobic aquatic systems was shown, Panigatti and Maine (2003) demonstrated that sediment is the main P compartment. This could indicate that in systems without macrophytes, sediment replaces 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. Adler et al. (1996) proposed a wetland system that better exploits the nutrient uptake mechanisms of plants. P stored within plant biomass was periodically harvested. Because the mechanism of P uptake was not based on the soil matrix, P could not be reversibly desorbed and sites for P sorption could not be saturated. Another advantage of macrophytes is their ability to cover the surface of water. When the water surface is not covered by plants, light penetrates into the water, providing adequate conditions for algal growth.

5. Conclusions

Taking into account the high relative growth rate showed by the small floating species (*S. herzogii* and *P. stratiotes*), their high total P amount observed in summer is not surprising. These species were able to accumulate significant amounts of P in a short time during the summer months, but due to the decrease in biomass in winter, their accumulation capacity would decrease, whereas the rooted species could maintain their capacity of P accumulation throughout the year for they retain it within their below-ground parts during winter.

At a constructed wetland for wastewater treatment, where P is one of the contaminants to eliminate (sewage, municipal, agroindustrial waste, etc.), in order to maintain the efficiency of the system throughout the year, the use of floating as well as rooted species may be recommended. During the winter months the rooted species could remove P, whereas in summer the small floating species would be the ones responsible for P removal. In order to achieve a complete P removal from the system, the harvest of the floating species during the summer months is proposed. Their population would soon be recovered due to their high relative growth rate. Also, as harvesting a complete emergent plant is a complicated operation, the aerial parts of *T. domingensis* could be harvested taking advantage of its capacity to accumulate P in its aerial part during the summer months.

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