Chemosphere 138 (2015) 405–411

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Influence of *Typha domingensis* in the removal of high P concentrations from water

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HIGHLIGHTS

- High P concentrations were efficiently removed from water.
- Short-term P accumulation was significantly low in vegetated sediments.
- Vegetated and unvegetated sediment presented the same P fractionation pattern.
- Phosphate removal could be enhanced by using wetlands vegetated with *T. domingensis*.

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form 12 June 2015
Accepted 22 June 2015

Keywords:
Phosphorus
Macrophyte
Sediment
Fractionation
Wetlands

ABSTRACT

A greenhouse experiment was conducted to evaluate the removal of high P concentration from water by vegetated and unvegetated wetlands. Reactors containing 4 kg of sediment and two plants of *Typha domingensis* (vegetated treatments) and reactors containing only sediment (unvegetated treatments) were arranged. Reactors were dosed with 100 and 500 mg L^{-1} of P-PO_4. The studied concentrations tried to simulate an accidental dump. Controls without P addition were also disposed. Water samples were collected periodically and analyzed for phosphorus. Sediment (0–3 (surface), 3–7 (medium) and 7–10 cm (deep)) and plant samples (roots, rhizomes, submerged leaves and aerial leaves) were collected at the beginning and at end of the experiment and were analyzed for total phosphorus. P fractionation was performed in the surface sediment layer. Relative growth rate (RGR) was calculated in each treatment considering initial and final plant height. P was efficiently removed from water in both, vegetated and unvegetated treatments. However, the major P removal was achieved in vegetated treatments. *T. domingensis* has a high capacity to tolerate and accumulate high P concentrations, especially in leaves, causing P accumulation in sediment to be significantly low in vegetated treatments. P accumulation was produced in the surface sediment layer (0–3 cm) in all treatments, mainly retained as iron-bound P. Present results point the large removal capacity of phosphate of systems planted with *T. domingensis*. Therefore *T. domingensis* is suitable for phytoremediation practice, being capable to tolerate high P concentration.

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1. Introduction

A large source of phosphate pollution of aquatic systems is due to domestic or municipal wastewater. Phosphorus in large quantities is used in agriculture (fertilizers, animal feeds, and chemical pesticides), food production industry, pharmaceuticals, high-tech electronics and production of metal alloys, etc. Phosphorus is the limiting nutrient of eutrophication in most freshwater aquatic systems. In consequence, effluents containing high P concentration need proper treatment before dumping it to the environment.

Constructed wetlands are widely used for phosphorous removal from domiciliary and municipal sewage, storm water and agricultural runoff and industrial effluent treatment (Calheiros et al., 2007; Di Luca et al., 2011; Gagnon et al., 2013; Kadlec and Wallace, 2009; Maine et al., 2007, 2009; Menon and Holland, 2013; Vymazal, 2007). However, studied P concentrations were often within the range between 0.20 and 10 mg L^{-1}. In wetlands, sediment chemical characteristics are essential for its phosphorus retention capacity. Phosphorus is incorporated into sediments in

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http://dx.doi.org/10.1016/j.chemosphere.2015.06.068
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both inorganic and organic forms including precipitated forms, physically adsorbed onto mineral surfaces, biologically assimilated in cells and in detritus coming from the sediment organisms. Some fractions of phosphorus in sediment can be released to the overlying water, depending on pH, Eh, environmental conditions, etc. The distribution of total phosphorus in the different forms is necessary for the understanding of phosphate exchange mechanisms by sediments and its potential removal from the water column.

Aquatic plants can modify the biogeochemistry of the sediment by altering redox conditions, pH and organic matter content. P may be mobilized or immobilized, depending on the actual combination of factors, and it is difficult to predict the vegetation effects on P mobility under a given set of conditions. Plants use phosphorus for growth but release phosphorus back into the water column due to decomposition and nutrient leaching from plant litter (Kao et al., 2003). Therefore, the accumulation and speciation of P in sediments may differ among vegetated and unvegetated sediments. Studies comparing planted and unplanted systems often lead to controversial results regarding the importance of plants (Baldizon et al., 2002; Calheiros et al., 2007; Marchand et al., 2010; Menon and Holland, 2013). We hypothesized that *Typha domingensis* have an important role in P removal in a wetland receiving high P water loads, as in the case of an accidental P dump.

An experiment was conducted to evaluate the accumulation and distribution of high P concentrations in unvegetated and vegetated wetlands with *T. domingensis*. This species was chosen for this study since it is a widespread and often dominant macrophyte in aquatic natural wetlands systems in Argentina. Furthermore, *T. domingensis* became and remained as the long term dominant specie in a wetland constructed for wastewater treatment in a metallurgic industry in which several locally abundant macrophytes were transplanted (Maine et al., 2007; 2009).

2. Materials and methods

2.1. Experimental design

Sediment, *T. domingensis* and water were collected from an unpolluted pond of the Paraná River floodplain near Santa Fe city, Argentina (31°32′45″S; 60°29′37″W). Only healthy plants of a uniform size and weight were selected. The plants were pruned for their transport to the greenhouse.

Eighteen plastic reactors of 10 L (20 cm diameter and 30 cm height) capacity were disposed outdoors under a semi-transparent plastic roof. After 15 days of acclimation, the plants were pruned again to a height of approximately 20 cm. Nine reactors contained two plants and 4 kg of sediment (10 cm depth, approximately). Another nine reactors without plants (only sediment) were arranged (unvegetated treatments). P solutions (5 L) were added to the reactors to obtain the following treatments: 100 mg L^{-1} P (P100); 500 mg L^{-1} P (P500) and Control (water without the addition of P). The experiment lasted 30 days and it was performed in triplicate.

A stock solution of H_2KPO_4 and water from the sampling site was used to prepare P solutions. The chemical composition of the water from the sampling site used in the experiment was (mean ± standard deviation) (APHA, 1998): pH = 7.8 ± 0.1; conductivity = 223 ± 1 μS cm^{-1}; dissolved oxygen (DO) = 6.71 ± 0.10 mg L^{-1}; soluble reactive phosphorus (SRP) = 0.023 ± 0.002 mg L^{-1}; NH_4^+ = 0.990 ± 0.005 mg L^{-1}; NO_3^- = 0.410 ± 0.005 mg L^{-1}; NO_2^- = non detected (detection limit = 5 μg L^{-1}); Ca^{2+} = 9.8 ± 0.1 mg L^{-1}; Mg^{2+} = 2.2 ± 0.2 mg L^{-1}; Na^+ = 36.8 ± 0.5 mg L^{-1}; K^+ = 16.1 ± 0.5 mg L^{-1}; Fe = 0.291 ± 0.005 mg L^{-1}; Cl^- = 14.6 ± 1.0 mg L^{-1}; SO_4^{2-} = 10.5 ± 1.0 mg L^{-1}; total alkalinity = 104.2 ± 1.2 mg L^{-1}.

Water level in the reactors was maintained by adding water from the sampling site. Temperature ranged from 21.1 to 31.3 °C during the experimental period.

2.2. Chemical analysis

In each reactor, water was sampled at 0, 1, 2, 4, 7, 14 and 28 days. Soluble reactive phosphorus (SRP) was determined by the colorimetric molybdenum blue method (Murphy and Riley, 1962). TP in water samples was determined as SRP, after an acid digestion following APHA (1998).

P concentration in plants and sediment was determined at the beginning and end of the experiment. Sediment samples were collected using a PVC corer of 3 cm diameter. Sediment cores were sliced in situ with a plastic cutter at the following depth layers: 0–3 (surface), 3–7 (medium) and 7–10 cm (deep), and stored at 4 °C until they were analyzed. Redox potential (Eh) (Ag/AgCl electrode) and pH of the bulk sediment layers were measured in situ with an Orion pH/mV-meter in triplicate. Organic matter content (OM) was determined by weight loss on ignition at 550 °C for 3 h. Total P in sediment was determined after acid digestion with $HClO_4$:HNO_3:HCl (7:5:2) mixture followed by SRP determination in the digested samples (Murphy and Riley, 1962). In sediment, P initial concentration was 0.512 mg g^{-1}, organic matter was 5.41%, pH was 7.03 and Eh was 280 mV.

Due to the fact that root development was mainly observed in the surface sediment layer, only these sediment samples were analyzed according to the sequential extraction proposed by Golterman (1996). This method uses extractions with chelating compounds which are supposed to react with specific compounds present in the sediment and these are carried out at a pH near that of the sediment. This sequential extraction uses Ca-EDTA + dithionite to extract iron-bound phosphate (Fe(OOH)-P) and then Na_2-EDTA to extract calcium-bound phosphate ($CaCO_3$-P). For the organically bound P-fractions, acid soluble organic phosphate and alkali soluble organic phosphate (org-P-acid and org- P-alk), this sequence is followed by an extraction by H_2SO_4 at 20 °C and then by NaOH at 90 °C, respectively. All extractions were carried out under continuous shaking. The duration of each extraction stage is described by Golterman (1996). SRP was measured in the extracts using the blue-molybdate method with some modifications suggested by Golterman (1996) in order to evaluate the chemical association of P in sediment. Acid-washed polyethylene centrifuge tubes (50 mL) were used for the extraction, to minimize sediment loss. The errors involved were taken as half the distance between the two extreme values. Errors were usually well below 1%, except for P-fractions, in which case errors varied between 4% and 8%. Residual was determined in sediment samples in the same way as plant samples after acid digestion. All determinations were carried out in triplicate.

Plants were sampled and separated into roots, rhizomes, and submerged and aerial parts of leaves. They were washed with tap and distilled water, and subsequently oven dried at 60 °C for 48 h. Dried plant samples were ground and digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture. SRP was measured in the digest using the blue-molybdate method (Murphy and Riley, 1962). In macrophyte tissues, P initial concentration was: roots = 0.781 mg g^{-1}; rhizomes = 0.533 mg g^{-1} and leaves = 1.984 mg g^{-1}.

P amounts (mg) were estimated by multiplying P concentration in plant tissues, sediment (mg g^{-1} dry weight) or in water (mg L^{-1}) by mass (g dry weight) or volume (L).

2.3. Plant study

Plant height was measured and the external appearance of plants was observed daily, to detect possible senescence.

Relative growth rate (RGR) (cm cm^{-1} day^{-1}) was calculated in each treatment considering initial and final plant height, according to:

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

where H_1 and H_2 are the initial and final plant height (cm), respectively and $(T_2 - T_1)$ is the experimental period (days).

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed in relative growth rate among samples and in plant tissues concentrations (aerial parts, submerged parts of leaves, rhizomes and roots). Two-way ANOVA (factors: vegetation and sampling date (for SRP in water); depth and vegetation; vegetation and P fractions) was performed to determine whether significant differences existed in P concentrations in water and sediment. The normality of residuals was analyzed graphically and homogeneity of variances was checked using Bartlett's test. When necessary, data were transformed (log) to achieve homogeneity of variances and normality. Duncan's test was used to differentiate means when appropriate. A level of $p < 0.05$ was used for all comparisons. Calculations were performed using the Statgraphics Plus 3.0 software.

2.5. QA/QC

All glassware was pre-cleaned and washed with 2 N HNO_3 prior to each use. H_2KPO_4 used to prepare phosphorus solution was of analytical grade. Replicate analyses (at least ten times) of the samples showed a precision of typically less than 4% (coefficient of variation). Detection limit was 0.4 μg g^{-1} for P for sediment and plant tissues.

3. Results

Fig. 1 shows P removal percentages from water over time. P was removed from water in all treatments. At the end of the experiment, P removal from water was significantly higher in vegetated than in unvegetated reactors of both P treatments (68.1% and 51.8%, respectively, for P100 and 61.0% and 34.1%, respectively, for P500). P concentration in different plant tissues was determined (Fig. 2a). In both, P100 and P500 treatments, the submerged parts of leaves accumulated the highest P concentration. The higher the P concentration in water, the higher the P concentration in plant tissues. Interestingly, relative growth rates were positive for all treatments with no significant differences regarding the control (Fig. 2b), showing high *T. domingensis* tolerance to the studied P concentrations.

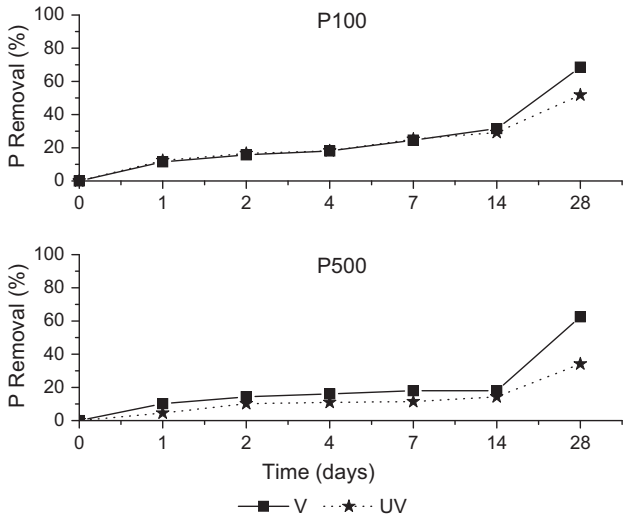

Fig. 1. P removal percentage in vegetated (V) and unvegetated (UV) reactors during the experiment.

At the end of the experiment, significantly lower pH and higher Eh values were found in sediments of vegetated treatments compared with unvegetated ones (Figs. 3(a) and 3(b)). The lowest pH and the highest Eh values were measured in the surface layer (0–3 cm). The mean values of pH of superficial sediments were: P100V: 6.73, P100UV: 7.19, P500V: 6.80, and P500UV: 6.95. The mean Eh values were: P100V: −61 mV, P100UV: −100 mV, P500V: −156 mV, and P500NV: −179 mV.

Total P concentration in sediment was significantly higher in unvegetated than in vegetated treatments. P accumulation was produced in the surface sediment layer (0–3 cm), in both, vegetated and unvegetated reactors (Fig. 4). P concentration in the deeper layers did not show significant differences regarding the concentration in the controls.

Fig. 5 shows P fractionation in the surface sediments of all treatments. In the control treatments, P was mainly found in the $CaCO_3$-P fraction (33% and 26% in vegetated and unvegetated treatments, respectively). However, in P100 and P500 treatments, distribution of P in chemical phases changed since P was accumulated mainly in Fe(OOH)-P fraction in all treatments (Fig. 5). The presence of the plants modifies pH and redox conditions and also changed P fractionation in the sediment (Fig. 5). The percentages of the P in Fe(OOH)-P fraction was significantly higher in vegetated than in unvegetated treatments. Besides, P in the residual fraction was significantly lower in the vegetated sediments than in the respective unvegetated sediments from both treatments, which is compatible with higher P availability. The lowest P concentration was found in Org. acid-P fraction in all treatments.

In order to compare the accumulation in sediment and plants, P concentration were converted into P amount (mg). Thus, not only concentration but also each compartment mass was considered. P mass balances in the different treatments are shown in Fig. 6. P content in water, roots, rhizomes, leaves, submerged part of leaves, and three layers of sediment were considered. Sediment was the compartment that accumulated the highest P content in all treatments, mostly in the surface layer (0–3 cm). In both, P100 and P500 treatments, higher P amount in superficial unvegetated than in vegetated sediment was found. In P500 treatments, higher P amounts were found in the 3–7 cm layer of vegetated than unvegetated treatments, probably due to the fact that the roots of *T. domingensis* were mainly contained within this sediment layer, acting as a P pump. Also, significantly higher amounts of P in water were found in unvegetated treatments than in vegetated ones.

4. Discussion

Vegetated treatments presented higher P removal from water than unvegetated treatments, suggesting that *T. domingensis* is directly involved in P removal. Present results are consistent with those reported by Huett et al. (2005) who reported that *Phragmites australis* vegetated systems removed significantly higher TP than unvegetated systems and Soto et al. (1999) who reported high P removal from water in *Scirpus lacustris* vegetated treatments.

Despite P is an essential nutrient for plant growth, at high concentrations it become an environmental contaminant because it triggers eutrophication. Eutrophication may lead to widespread

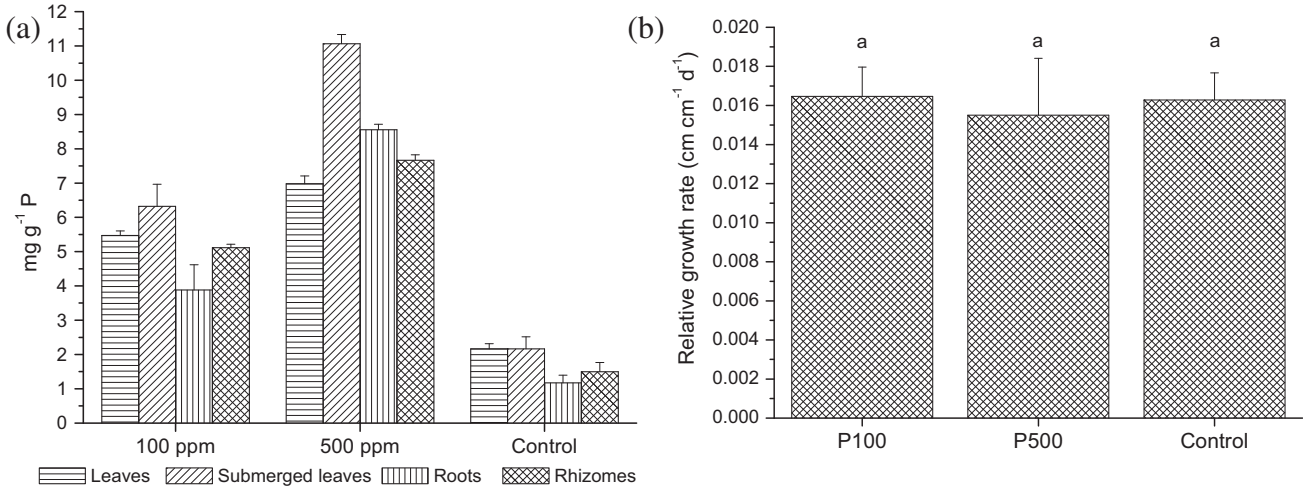

Fig. 2. (a) P concentration (mg g^{-1}) in *T. domingensis* tissues (leaves, submerged leaves, roots and rhizomes) in the different treatments. (b) Relative growth rates (cm cm^{-1} d^{-1}) obtained at the end of the experiment compared with the control. Different letters represent statistically significant differences among the treatments. Bars represent standard deviations.

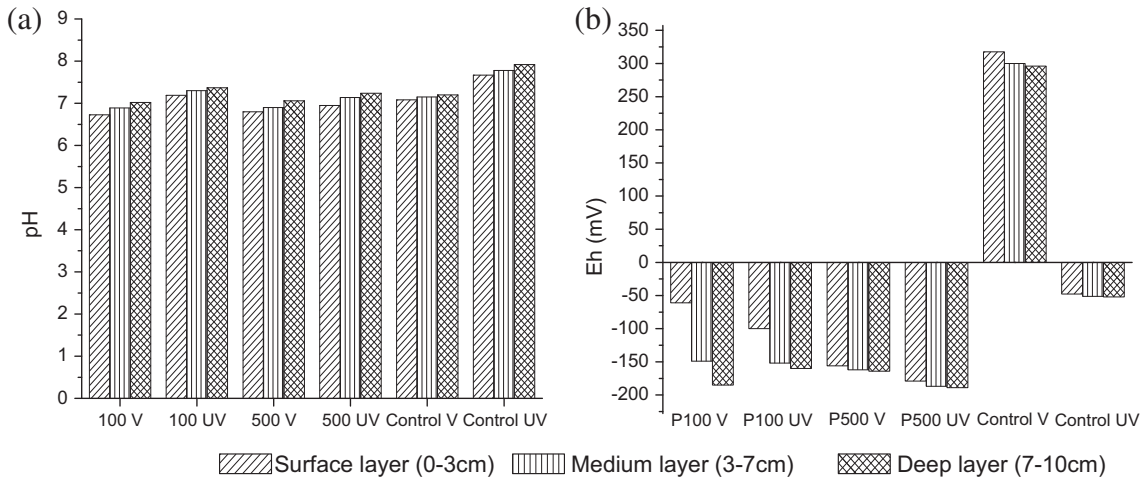

Fig. 3. pH (a) and Eh (mV) (b) values measured in different sediment layers of vegetated (V) and unvegetated (UV) treatments.

hypoxia and anoxia, habitat degradation, alteration of foodweb structure, loss of biodiversity, and increased frequency, spatial extent, and duration of harmful algal blooms (Di Luca et al., 2014; Kowalkowski et al., 2014; Schindler and Vallentyne, 2008; Schindler, 2012; Smith and Schindler, 2009; Steinbachová-Vojtíšková et al., 2006). The high P concentrations assayed in this work are representative of raw effluents of some industries. Emergent macrophytes have a great potential to store P. It is often assumed that vegetation rooted in sediment obtains most of its P needs from the sediment porewater and translocates it to above-ground parts to support active vegetative growth (Reddy et al., 1999). Present results show that when *T. domingensis* is exposed to high P concentration, the submerged parts of leaves accumulated the highest P concentration, suggesting that P is not only traslocated from roots, but also sorbed by leaves due to direct contact with water. Panigatti and Maine (2002) studied P dynamics in natural wetlands with *Paspalum repens* using ^{32}P, reporting similar results.

The major P accumulation in the superficial sediments found in this work, is in agreement with that reported by several authors (Di Luca et al., 2011; Maine et al., 2007; Reina et al., 2006). After the experiment, unvegetated sediments accumulate higher P concentration than vegetated sediments. Some authors found an opposite situation, i.e. higher P concentration in vegetated than in unvegetated sediments. Reina et al. (2006) studied the effect of emergent macrophytes (*Juncus subulatus, Scirpus maritimus* and *Phragmites australis*) on the sediment P composition of an eutrophic shallow marsh on the NE margin of Doñana (SW Spain) and reported that total P was significantly higher in the top sediment of the sites covered by *J. subulatus* and *S. maritimus* than in their adjacent open-water sites. They suggested that litter on the top sediment of areas vegetated by *J. subulatus* and *S. maritimus* explains these differences. Gagnon et al. (2013) studied the effect of *P. australis, Typha angustifolia* and *Scirpus fluviatilis* on sludge dewatering and fate of pollutants in sludge treatment wetlands (STWs), finding that phosphorus was mainly retained in the sludge in the planted system (52–68%) and, to a lesser extent, in the unplanted control (46%). The sludge cake of the planted systems had a higher mass and nutrient content than the unplanted STWs, possibly due to the presence of plant litter in the sludge cake. This exposes the influence of plant species in the P uptake.

Vegetated sediments presented significantly lower pH values and significantly higher Eh values than unvegetated sediments. Emergent macrophytes alter local sediment pH conditions through assimilation/production of anions/cations by root exudates and stabilize and oxidize the bottom sediment by oxygen translocation from their aerial parts to the roots and then the rhizosphere (Brix,

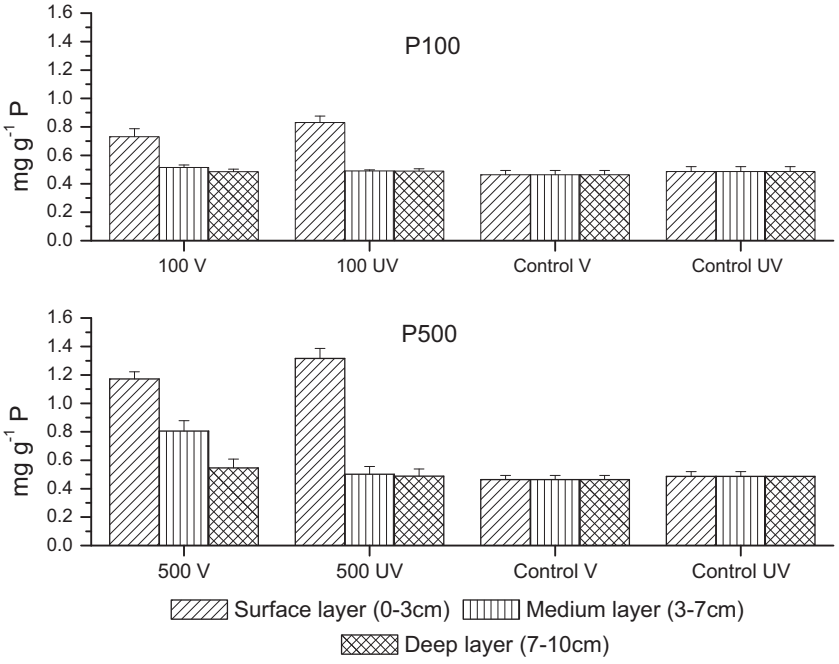

Fig. 4. Total P concentration (mg g^{-1}) in different sediment layers of vegetated (V) and unvegetated (UV) treatments.

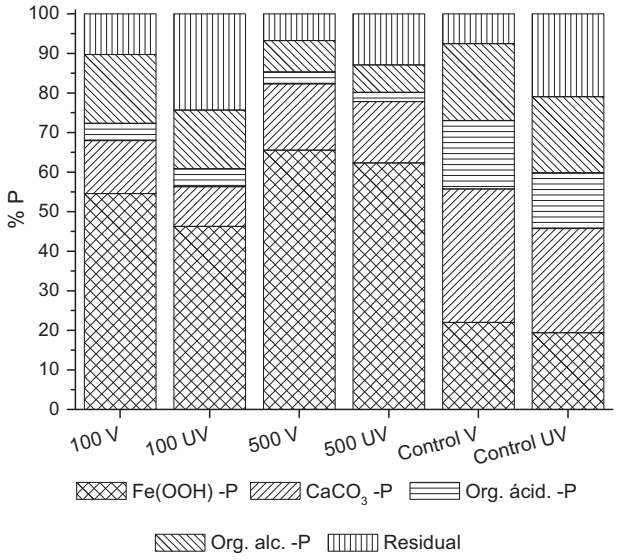

Fig. 5. P fractionation in surface sediment layer (0–3 cm) of vegetated (V) and unvegetated (UV) treatments.

1994; Dunbabin et al., 1988; Jacob and Otte, 2003). Organic material associated with plant growth, senescence and root exudates directly affects microbial activity, increases sediment oxygen demand, and decrease sediment pH. Since the acidic zone of the root may coincide with the location of radial oxygen loss (ROL), the net pH decrease may result from the combined effect of iron oxidation and root hydrogen release. Negrin et al. (2011) argued that the low pH values in the vegetated sediment in the lower marsh of the Bahía Blanca Estuary could reflect local CO_2 production via aerobic oxidation of organic matter in the rhizosphere. On the other hand, the root tissues release protons to the porewater to maintain electro-neutrality after taking up ammonium ions.

It is known that Eh is one of the variables influencing the biogeochemical cycling of P in soils/sediments. Since Mortimer's work (1941), it is thought that low redox potential in sediment increases P availability due to iron bound P de-sorption. The idea that the release of P under anaerobic conditions comes predominantly from the chemical reduction of FeOOH was accepted for years. However, Golterman (2001) argued that the reducing capacity in lakes is too small to reduce a considerable quantity of the Fe(OOH) present, and that Fe(OOH)-P is a stronger complex than Fe(OOH), so that more energy is required to reduce Fe(OOH)-P than the excess Fe(OOH) itself. In agreement, several studies found increased P sorption after flooding and a decrease in available P caused by submergence (Da-Peng and Yong, 2010; Di Luca et al., 2011; Oxmann et al., 2009). Increased P sorption is related to amorphous and poorly crystalline oxides and hydroxides of iron (Zhang et al., 2003). Based on the difference in P associated with Fe compounds, the distinction between P bound by amorphous or crystalline Fe oxides is significantly important in order to predict the P release potential (Da-Peng and Yong, 2010). These controversial results may be partly explained by an initial release of P during the transformations of Fe followed by re-adsorption on amorphous or poorly crystalline Fe-oxides and mixed Fe(II)Fe(III)-hydroxy compounds (Gale et al., 1994; Moore and Reddy, 1994). It is also possible that occluded Fe(III) covered with organic matter (mainly humic compounds) may be protected from reduction (Peng et al., 2007). Overall, available evidence suggest that not all Fe(OOH)-P could be released under anoxic conditions.

Previous works showed that the sediment is the main accumulation compartment of phosphorus (Maine et al., 2009), in agreement with our results. Adsorption by sediment is considered as one of the dominant long-term P storage mechanisms in wetlands. The magnitude of P sorption is finite and limited, as sediment sorption sites could become saturated when subject to prolonged nutrient loading. However, if physical and chemical characteristics of the effluent provide appropriate conditions for P removal from water (high pH, Fe, alkalinity and Ca^{2+}, ionic concentrations), the wetland would be expected to continue retaining P in the long-term (Maine et al., 2009). Nevertheless, the advantage of macrophytes is the possibility of being harvested, which leads to important removal rates of contaminants in short periods of time.

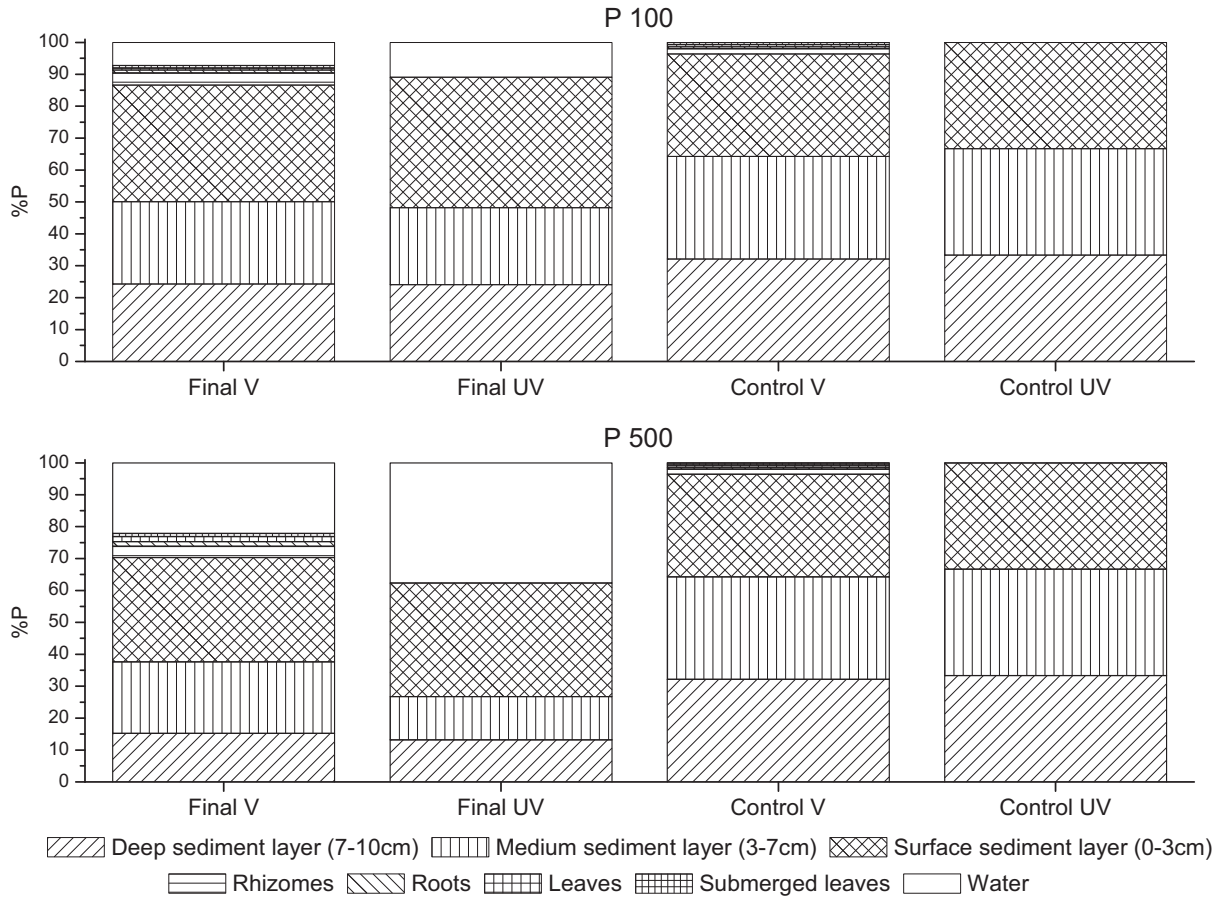

Fig. 6. P mass balance (water, sediment and plant tissues) in different treatments.

5. Conclusions

The highest P removal from water and the lowest P content in the sediments of vegetated treatments point the important contribution of vegetation uptake and retention.

P was accumulated mostly in the surface layer (0–3 cm). This fact suggests a low mobility of P or that the sorption sites of this layer were not saturated at the assayed conditions. In all cases, P was mainly accumulated in the Fe(OOH)-P fraction, which remained stable under experimental conditions. Nevertheless, the presence of *T. domingensis* influences the accumulation and speciation of P in sediments. Unvegetated sediments accumulated higher P concentration and its accumulation was higher in less available fractions than vegetated sediments.

Present results point the large P removal capacity from water of systems planted with *T. domingensis*. Therefore *T. domingensis* is suitable for phytoremediation practice, being capable to tolerate high P concentration. In the case of an accidental dump of high P concentrations, constructed wetlands may retain P in sediments, minimizing the environmental impact. P removal from water could be enhanced by using vegetated wetland systems planted with *T. domingensis*.

Acknowledgements

The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional del Litoral (UNL)-CAI+D Project and Agencia de Promoción Científica y Tecnológica for providing funds for this work.

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