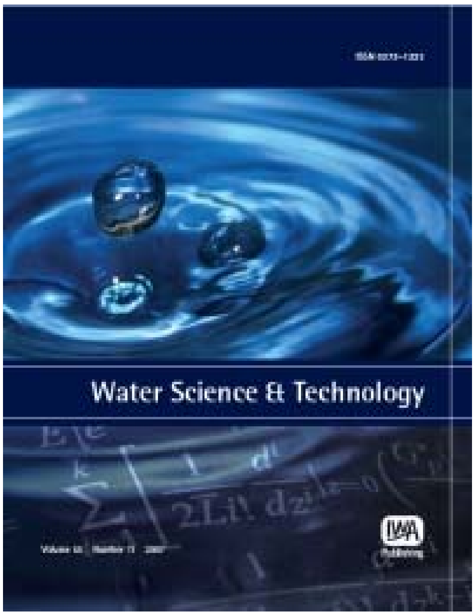

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P distribution in different sediment fraction of a constructed wetland

G. A. Di Luca, M. M. Mufarrege, G. C. Sánchez, H. R. Hadad and M. A. Maine

ABSTRACT

The aim of this work was to study the accumulation and fractionation of P in the inlet and outlet sediment of a constructed wetland for the wastewater treatment of a metallurgic plant in Argentina. It was important to predict whether P could be released into the water again by changing environmental conditions or retained over time. P-fractionation was performed using a sequential extraction method. Sediment cores were sliced at depths of: 0–3; 3–7 and 7–10 cm. Sediment showed high pH values and anoxic conditions. In the inlet area, P was principally bound to the carbonate fraction, whereas in the outlet area, it was mainly bound in the residual fraction. This behavior was justified by the effluent composition, which is rich in Ca^{2+} and Fe^{3+} and presents high values of pH and conductivity. These conditions favor $CaCO_3$ and $Fe(OOH)_n$ precipitation and the subsequent sorption of P to their surface. The sediment active layer involved in the exchange reactions was the superficial one (0–3 cm). The wetland is highly efficient in P retention. P was retained by sediment in fractions that will not release it to the water while chemical and environmental conditions of the system are maintained.

Key words | industrial effluent, phosphorus, sediment, sequential extraction

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INTRODUCTION

Constructed wetlands are aquatic systems that have been demonstrated to be highly efficient in the removal of metals, nutrients, organic matter, etc., from many types of wastewaters (Hammer 1989; Maine *et al.* 2007, 2009; Kadlec & Wallace 2009). Effluent purification includes physico-chemical (adsorption, absorption, precipitation, sedimentation, etc.) and biological processes (bacterial transformation and assimilation through aquatic vegetation) (Brix 1993). Various wetland components play a role in the retention of contaminants, including P. While uptake by plants, periphyton and microbes is important in the removal of P, the sediment compartment represents a major P sink in wetlands (Sakadevan & Bavor 1998). Sorption by sediment is considered 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 they are subjected to prolonged nutrient loading (Richardson & Craft 1993). Nutrients in sediment are in equilibrium between input and binding capacity of the system.

It is important to take into account that sediment could uptake contaminants or could release them when the environmental conditions change. Therefore, it is essential to know the amount of phosphate stored in sediment, and the composition of the different forms of phosphate since the P exchange of sediment is a function of its binding forms (Boström *et al.* 1985). P chemical fractionation allows determining which fraction is potentially involved in the exchange processes.

The studied wetland was constructed at Bahco Argentina metallurgic plant and has been in operation for 7 years. There was a significant increase in P concentration in the inlet sediment. The aim of this work was to study the accumulation of P and the fraction that is potentially involved in the P exchange processes in the sediment of a wetland constructed for the sewage and industrial wastewater treatment of a metallurgical industry.

Study site

A free-water surface wetland was constructed at the Bahco Argentina tool factory, located in Santo Tomé, Santa Fe, Argentina (S 31°40′; W 60°47′). It is 50 m long, 40 m wide

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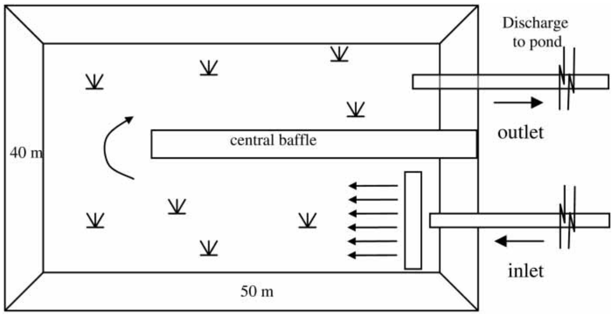

Figure 1 | Layout of the constructed wetland.

and 0.3–0.6 m deep, with a central baffle dividing it into two identical sections forcing the effluent to cover double the distance, reaching a length–width ratio of 5 (Figure 1). Mean wastewater discharge was 100 m^3 d^{-1}. Hydraulic residence time ranged from 7 to 12 d. The wetland was rendered impermeable by means of a bentonite liner to reach a hydraulic conductivity of 10^{-7} m s^{-1} (5 compacted layers of bentonite). A layer of 1 m of soil was placed on top of the bentonite layer. Different locally available macrophyte species were transplanted into the wetland. At the time of the study, approximately 80% of the wetland surface was covered by *T. domingensis*, the aerial parts of which were harvested to ensure an optimal growth annually after the winter season. Wastewater and sewage from the factory were treated together. Effluents reached the wetland after a primary treatment (coagulation, flocculation, precipitation and filtration). The wetland outlet is discharged by a channel into a 1.5 ha pond located within the factory campus. Further details were provided by Maine *et al.* (2007).

METHODS

Water samples and sediment cores were collected in the inlet (1 m away from the effluent discharge) and outlet (99 m from the effluent discharge) areas of the wetland, in triplicate, in August (winter) and October (spring) 2007, May (autumn) 2008 and August (winter) 2009.

In the water samples conductivity was measured with a YSI 33 conductimeter, dissolved oxygen (DO) with a Horiba OM-14 portable meter and pH with an Orion pH-meter. Water samples were filtered through Millipore membrane filters (0.45 μm) for dissolved P and N determinations. Chemical analyses were performed following Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WEF, 1998); NO_2^- was determined by coupling diazotization followed by a colorimetric technique, NH_4^+ and NO_3^- by potentiometry (Orion ion selective electrodes, sensitivity: 0.01 mg l^{-1} of N, reproducibility: ±2%). Soluble reactive phosphate (SRP) was determined by the colorimetric molybdenum blue method (Murphy & Riley 1962). Total phosphorus (TP) was determined after sulfuric acid–nitric acid digestion followed by SRP determination in the digested samples. Ca^{2+} and Mg^{2+} were determined by EDTA titration. Na^+ and K^+ were determined by flame emission photometry. Alkalinity (carbonate and bicarbonate) was measured by HCl titration. Cl^- was determined by the argentometric method. SO_4^{2-} was assessed by turbidimetry. Chemical oxygen demand (COD) was determined by the open reflux method and biochemical oxygen demand (BOD) by the 5-d BOD test. Total Fe, Cr, Ni and Zn concentrations were determined in water samples by atomic absorption spectrometry (by flame or electrothermal atomization, according to the sample concentration, Perkin Elmer 5000).

Sediment samples were collected using a PVC corer with a diameter of 3 cm. 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 (E_h) 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 (Dean 1974). Particle size was determined according to Stoke's law, in a 1,000-ml measuring cylinder beaker with distilled water after destroying the organic matter and the carbonates of the sediment.

Each sediment sample was 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 ($Fe(OOH) \approx P$) and then Na_2-EDTA to extract calcium bound phosphate ($CaCO_3 \approx P$). 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 with H_2SO_4 at 20 °C and then by NaOH at 90 °C, respectively. All extractions were carried out under rotational 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 and Tot-P was determined after acid digestion with $HClO_4$:HNO_3:HCl (7:5:2) mixture followed by SRP determination in the digested samples (Murphy & Riley 1962). X-ray Diffraction Analysis (XRD) (SHIMADZU model XD-D1) was carried out to determine the primary and secondary minerals in the sediment. Working conditions were Cu Kα monochromatic radiation, 40 mA and 30 KV. Samples were run at a speed of 1° 2θ/min (10–80). Crystalline phases were identified using DP-D1 system software.

The results obtained in the studied period were compared with the initial values registered at the time the wetland started operation (November 2002) (Maine *et al.* 2007). Initially no statistically significant differences were found in the chemical composition of sediment among the different sampled areas. This is probably due to the fact that during the construction of the artificial wetland, the sediment was removed, mixed and deposited on a bentonite layer. The initial mean concentration of organic matter in sediment was 3.5%. The mean concentration of Ca^{2+} was 1.16 mg g^{-1} dry weight (d.w.) and that of Fe was 19.7 mg g^{-1} d.w. Mean E_h was 95 mV and pH was 7.7. Mean concentration of Tot-P was 0.383 mg g^{-1} d.w. The richest fraction was that of Fe(OOH) ≈ P with 28% of Tot-P, followed by org-P → alk (21%), $CaCO_3 \approx$ P (20%) and org-P → ac (16%) (Maine *et al.* 2007).

Three way ANOVA was performed to evaluate the influence of time, depth and P-fractions on P concentration in the inlet and outlet sediment. Duncan's test was used to differentiate means when appropriate. A level of significance ($p < 0.05$) was used in all comparisons.

RESULTS AND DISCUSSION

Table 1 summarizes the variables measured in the water of the wetland inlet and outlet. Measured concentrations were significantly lower in the outlet than in the inlet, except NH_4^+, indicating high removal efficiency. NO_3^- and NO_2^- were depleted at the outlet attaining a removal of 71–90 and 70–100%, respectively. Organic matter mineralization represents an important source of ammonium, which is not nitrified because low oxygen concentration limited nitrification. Due to nitrate in the incoming water being much greater than ammonium, the overall inorganic N balance showed a net reduction of the incoming inorganic N. Given the observed redox potential it seems likely that denitrification was the major removal process. Mass balances performed in a previous period showed that macrophyte uptake accounted for only a minor proportion (23%) of the N losses (Maine *et al.* 2009). Several different studies have consistently shown denitrification to be a major pathway in wetlands. D'Angelo & Reddy (1993) determined that most of the ^{15}N-nitrate (roughly 90%) applied to sediment-water cores was lost by denitrification. Matheson *et al.* (2002) performed N^{15} balances in wetland microcosms estimating that denitrification accounted for 61% of the nitrate load, 25% were retained in the soil while only 14% was assimilated by the vegetation.

Organic matter mineralization increased CO_2 concentration in water, which, in turn, decreased water pH at the outlet. Ca and alkalinity were reduced by 53–87 and 44–68% at the outlet. The high prevailing pH and CO_3^{2-} concentrations suggest $CaCO_3$ precipitation at the inlet. Lower pH and undetectable CO_3^{2-} concentrations suggest that $CaCO_3$ precipitation did not occur at the outlet. Both SRP and TP were reduced by 57–62 and 47–73% at the outlet. Despite the high mineralization rates, DO was not depleted and Fe was reduced by 92–99% at the outlet, suggesting SRP co-precipitation either with Fe or $CaCO_3$. SO_4^{2-} concentration was high and variable at the inlet. Concentrations were significantly lower at the outlet and attained a mean removal efficiency of 25–74%, suggesting Fe and organic sulfide formation in the anoxic bottom sediment. Metal concentrations were significantly lower at the outlet than at the inlet. The highest average retention was attained by Fe (92–99%), followed by Cr (82–100%), Zn (55–67%) and lastly Ni (54–64%). Both COD and BOD were reduced by 63–91 and 69–92% respectively in the outlet, suggesting extensive mineralization of the high organic matter load.

Mainly clay and sand constituted the wetland sediment, 20 and 70% on average, respectively. The XRD analysis showed that the crystalline phases identified in the inlet area of the wetland corresponded to $CaCO_3$ (PDF # 5-586) and SiO_2 (PDF # 33-1161) (Figure 2(a)), consistently with the measured pH, calcium and carbonate concentrations in water, suggesting calcium carbonate precipitation as a possible mechanism of contaminant retention in the inlet area. In the outlet sediment the only crystalline phase identified was SiO_2 (PDF # 33-1161) (Figure 2(b)). The absence of Fe(OOH) peaks indicated that these oxides were present in amorphous or pour crystalline phases.

Redox potential, pH and organic matter content in the bottom sediment are shown in Table 2. Redox condition and pH showed a seasonal pattern, pH decreased and redox potential increased in spring (October) in agreement

Table 1 | Mean measured variables in the inlet and outlet water of the constructed wetland during the study period

	August 2007		October 2007		May 2008		August 2009	
	Inlet	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**
DO ($mg\,l^{-1}\,O_2$)	3.50	3.76	3.60	3.90	7.60	3.50	2.30	1.10
pH	10.1	7.50	9.02	8.00	9.9	8.4	11.4	7.9
Conductivity ($\mu mho\,cm^{-1}$)	4,850	2,140	4,200	2,330	5,000	2,820	6,000	2,570
Temperature (°C)	12.8	9.3	27.0	26.8	20.1	16.6	14.5	12.0
Solids ($mg\,l^{-1}$)	3,488	1,417	2,777	1,329	2,450	1,697	5,479	1,683
Alkalinity ($CaCO_3$) ($mg\,l^{-1}$)	593.2	314.9	452.0	173.7	988.2	317.2	403.2	224.0
HCO_3^- ($mg\,l^{-1}$)	ND(0.5)	384.2	ND(0.5)	211.9	ND(0.5)	297.7	ND(0.5)	273.3
CO_3^{2-} ($mg\,l^{-1}$)	158.2	ND(0.5)	295.0	ND(0.5)	131.8	43.9	107.5	ND(0.5)
Cl^- ($mg\,l^{-1}$)	269.8	152.6	1,060.7	453.4	262.7	191.7	386.9	213.0
SO_4^{2-} ($mg\,l^{-1}$)	1,648.7	553.6	270.4	203.5	751.4	620.8	3,003.2	781.0
Ca^{2+} ($mg\,l^{-1}$)	136.9	61.2	163.8	34.3	118.8	55.8	617.5	81.8
Mg^{2+} ($mg\,l^{-1}$)	15.0	14.0	14.9	14.4	5.6	5.5	ND(0.5)	14.8
Na^+ ($mg\,l^{-1}$)	967.7	426.7	874.2	395.3	812.6	54.7	1,231.1	165.8
K^+ ($mg\,l^{-1}$)	14.3	14.0	12.1	14.1	17.5	14.7	15.6	15.4
Fe ($mg\,l^{-1}$)	7.49	0.306	0.78	0.013	34.4	0.173	2.35	0.19
Cr ($mg\,l^{-1}$)	0.400	0.045	0.068	ND(0.001)	0.049	0.009	0.079	0.014
Ni ($mg\,l^{-1}$)	0.028	0.013	0.028	0.010	0.036	0.014	0.029	0.012
Zn ($mg\,l^{-1}$)	0.09	0.04	0.03	ND(0.01)	ND(0.01)	ND(0.01)	ND(0.01)	ND(0.01)
SRP ($mg\,l^{-1}$)	0.160	0.060	0.248	0.096	0.122	0.050	0.135	0.058
TP ($mg\,l^{-1}$)	0.444	0.237	0.952	0.252	0.426	0.162	1.06	0.330
NO_2^- ($mg\,l^{-1}$)	0.720	0.167	0.719	0.020	0.158	0.047	1.263	ND(0.005)
NO_3^-($mg\,l^{-1}$)	33.1	5.9	54.7	5.4	33.0	5.22	33.5	9.8
NH_4^+ ($mg\,l^{-1}$)	1.65	2.44	1.48	2.04	1.71	2.95	0.71	3.31
BOD ($mg\,l^{-1}$)	93.9	25.9	36.9	11.4	290	22.5	78.6	20.9
COD ($mg\,l^{-1}$)	202.2	29.9	79.7	29.5	702	61.5	115.2	37.5

ND = not detected, the values in parentheses are the detection limits.

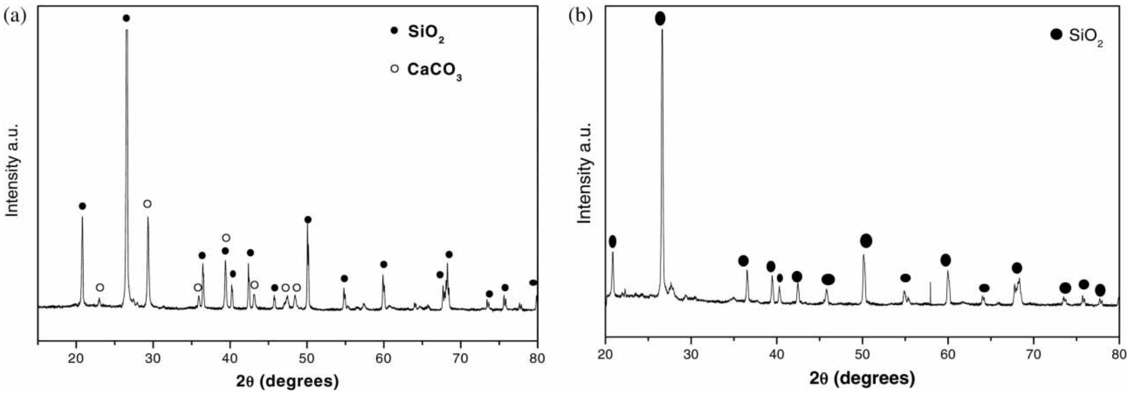

Figure 2 | XRD spectra for the inlet (a) and the outlet (b) sediment of the wetland.

with the highest measured temperatures and the period of active macrophyte growth. Higher temperatures enhanced organic matter mineralization in the bottom, decreasing sediment pH. Faster macrophyte growth likely enhanced oxygen released in the rhizosphere increasing redox values. Emergent macrophytes transfer oxygen from the

Table 2 | Redox potential, pH and organic matter of the inlet and outlet sediment

Sampling	Area	Depth (cm)	Redox potential (mV)	pH	OM (%)
August 2007	Inlet	0–3	−92.2	9.10	3.64
		3–7	−130.8	9.11	3.07
		7–10	−159.8	9.13	4.43
	Outlet	0–3	−319.7	9.31	4.61
		3–7	−363.0	9.42	4.19
		7–10	−363.0	9.42	3.10
October 2007	Inlet	0–3	−81.0	8.84	5.16
		3–7	−120.3	8.89	5.04
		7–10	−129.6	8.92	5.33
	Outlet	0–3	−246.6	9.03	5.56
		3–7	−250.8	9.10	5.70
		7–10	−251.0	9.13	5.32
May 2008	Inlet	0–3	−98.5	9.56	6.50
		3–7	−118.2	9.27	6.15
		7–10	−151.9	9.14	6.88
	Outlet	0–3	−366.4	9.29	6.07
		3–7	−373.4	9.16	5.75
		7–10	−414.7	8.94	6.11
August 2009	Inlet	0–3	−97.3	7.86	6.09
		3–7	−131.5	8.10	5.42
		7–10	−235.0	8.31	7.47
	Outlet	0–3	−338.4	8.08	8.02
		3–7	−323.3	8.00	7.70
		7–10	−335.6	7.82	7.85

aerial parts to roots modifying the sediment redox conditions (Dunbabin *et al.* 1988). Organic matter showed a consistent trend to increase throughout the sampling period at the inlet and outlet, and all depths within the sediment. At the inlet, organic matter was higher at 7–10 cm depth than at 0–3 cm depth. Redox condition and organic matter at the inlet in the first sampling (August 2007) were similar to the conditions when the wetland started operations. On the contrary, sediment pH was higher than the initial value (Maine *et al.* 2007).

P concentration in sediment was significantly higher at the wetland inlet than at the outlet (Figure 3). A significant increase in P concentration was found in the inlet sediment along the studied period. Concentration in the outlet sediment was not significantly different either along the studied period or with the concentration at the beginning of the operation period. First order area or volumetric models were used to explain an exponential decrease in pollutant concentrations to a background value approaching zero along the transect from the wetland inlet to the wetland outlet (Kadlec & Wallace 2009). P concentration decreased significantly with depth at the inlet on all sampling dates. Similar patterns were repeatedly reported in the literature (Nielsen & Andersen 2005; Reina *et al.* 2006; Maine *et al.* 2007). The superficial layer accumulated a significantly high P amount, so it could be proposed that the sediment active layer involved in the exchange reactions was the superficial one (0–3 cm).

The P-fractional composition showed that the sediment was dominated by the inorg-P fractions in all cases. For the five fractions studied (Fe(OOH) ≈ P, $CaCO_3$ ≈ P, org-P → acid, org-P → alk and residual fractions), statistically significant differences were found among the sampled zones, depths, and samplings dates, as well as meaningful interactions among the three factors (ANOVA, three ways).

In the inlet sediment, $CaCO_3$ ≈ P fraction was significantly higher than the others. This could be explained by the high pH of this area and by the high Ca^{2+} and CO_3^{2-} loads in the effluent which lead a P co-precipitation with $CaCO_3$. In all samplings measured pH varied from 9.0 to 11.4, CO_3^{2-} concentrations averaged 6.1 mmol/L, and Ca concentrations were near 6.5 mmol/L. With these values as input and at 25 °C, calcite saturation indices (SI = log (IAP/Kps)) varied from 2.74 (pH 9) to 2.54 (pH 11.4), with a mean of 2.64 at the average pH of 10.2. This positive saturation index means that calcite was supersaturated, and hence its precipitation was thermodynamically feasible. Furthermore, the presence of $CaCO_3$ was corroborated in the XDR analysis. The second P-fraction was Fe(OOH) ≈ P. Golterman *et al.* (1998) found that phosphate adsorption to iron colloids decreases while increasing pH, whereas adsorption to calcareous matter shows the opposite trend. Regarding initial values, in the inlet sediment the $CaCO_3$ ≈ P fraction increased about 20%, mainly at the expense of Fe(OOH) ≈ P fraction. Therefore, results suggest that $CaCO_3$ ≈ P represents the main precipitating mechanism in this area.

In the outlet area the main P sediment fraction was the residual one, while Fe(OOH) ≈ P represented the second fraction that accumulated P significantly. In this area pH and E_h were significantly lower than the values prevailing in the inlet area. It is widely recognized that E_h influences on P cycling in environments subjected to frequent or permanent submergence. It is generally thought that low redox potential in sediment increases available P levels. Nevertheless, the idea that the release of P under anaerobic conditions comes predominantly from the chemical reduction of FeOOH was accepted for years; Golterman (2001) argued that the reducing capacity in lakes is too small to reduce a considerable quantity of Fe(OOH) present, and that Fe(OOH) ≈ P is a stronger complex than Fe(OOH), so that it takes more energy to reduce Fe(OOH) ≈ P than the excess Fe(OOH) itself. Several other studies found increased P sorption after flooding and

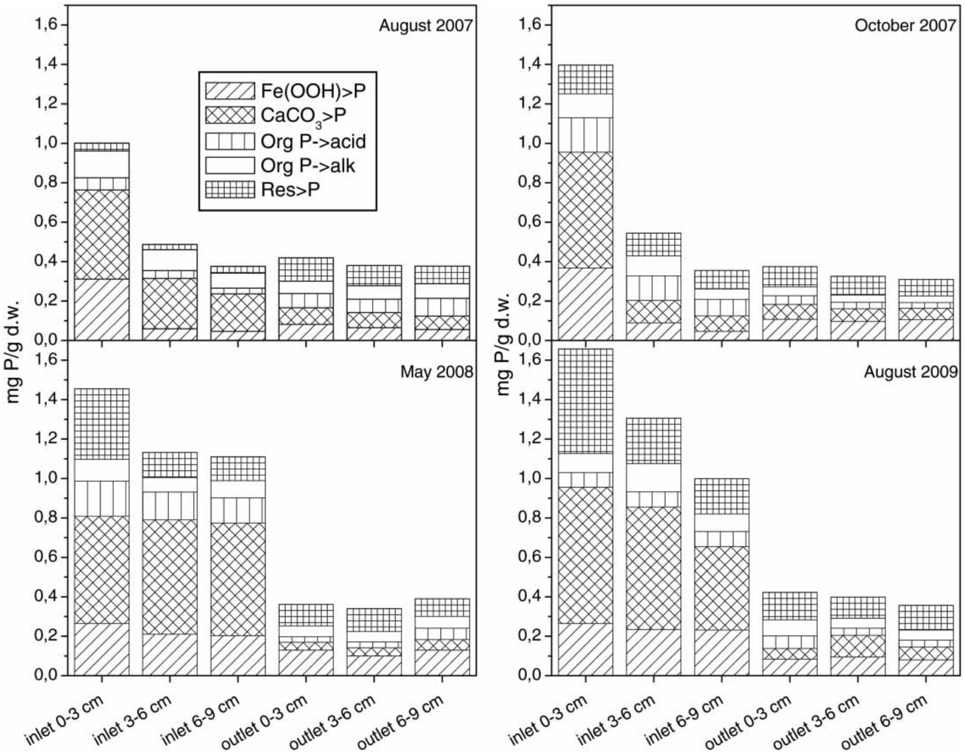

Figure 3 | Fe(OOH) ≈ P, $CaCO_3$ ≈ P, org-P → acid, org- P → alk and Residual P fractions at different depths (0–3; 3–6 and 6–9 cm) and samplings (August and October 2007; May 2008 and August 2009) in the inlet and outlet area.

decrease in available P caused by submergence (Oxmann *et al.* 2009; Da-Peng & Yong 2010). Increased P sorption is related to amorphous and poorly crystalline oxides and hydroxides of iron (e.g. 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 & 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). It is also possible that occluded Fe^{3+} covered with organic matter (mainly humic compounds) may be protected from reduction (Peng *et al.* 2007). All these show that not all Fe(OOH) ≈ P could be released under anoxic conditions.

Although, TP concentration in the outlet sediment did not show significant differences with the initial values, P speciation changed. Regarding initial values, residual P increased its proportion at the expense of the org-P → alk fraction. The org-P → alk fraction is composed of phytates that form complexes highly resistant to bacterial action (De Groot & Golterman 1993). Nevertheless, several bacterial strains contain phytase, which may be involved in the production or mineralization of phytate (Golterman 2001). Gächter & Meyer (1993) also established that bacteria mineralize org-P compounds more efficiently under eutrophic rather than oligotrophic conditions. Golterman *et al.* (1998) suggested that phytate mineralization might be an important mechanism for anoxic P-release from sediment. Taking into account the low P concentration in water in this area, it could be proposed that the P from org-P → alk fraction was not released into the water column but it was occluded in the sediment matrix as residual P, the most stable of the P fractions. The fact that the major amount of P is extracted as residual P shows that the system functioned as a long-term P-sink.

CONCLUSIONS

P was mainly accumulated in the first three centimetres of the inlet sediment while the outlet concentrations remained without differences regarding the initial ones, indicating that the retention capacity of the sediment in this area was not yet utilized.

P was mainly associated with inorganic forms in the sediment of both areas of the wetland. It was mainly bound to the

$CaCO_3 \approx P$ fraction in the inlet area while in the outlet, the major fractions were residual and $Fe(OOH) \approx P$.

The longer-term bioavailability of sediment P can be estimated from our sequential extraction results. If we consider that $Fe(OOH) \approx P$ and $CaCO_3 \approx P$ fractions are the most unstable fractions since they could release P if E_h or pH changes, we found that, on average, 52% of the total P was in these fractions. But we have to note that if the effluent composition is maintained (high pH, Fe, Ca^{2+} and ionic concentrations), it would be expected that these fractions continue accumulating P and will not release it. Consequently, the wetland will continue retaining P in the bottom sediment and will function as a long-term P-sink.

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