Volume 37, issue 9, September 2011   ISSN 0925-8574

ECOLOGICAL ENGINEERING
THE JOURNAL OF ECOSYSTEM RESTORATION

Editor-in-chief
William J. Mitsch

Metal retention and distribution in the sediment of a constructed wetland for industrial wastewater treatment

G.A. Di Luca [a,b,*], M.A. Maine [a,b], M.M. Mufarrege [a,b], H.R. Hadad [a,b], G.C. Sánchez [a], C.A. Bonetto [c]

[a] Química Analítica, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santiago del Estero 2829, Santa Fe 3000, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Instituto de Limnología "Dr. Ringuelet", Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Calchaquí Km. 23.5, 1888 Florencio Varela, Buenos Aires, Argentina

ARTICLE INFO

ABSTRACT

A free water surface wetland was built in 2002 to treat wastewater from a tool factory containing metals (Cr, Ni, Zn and Fe), nutrients and organic matter. Until 2006, the last reported period, the wetland retained metals and stored them primarily in the bottom sediment and in the biomass of macrophytes secondarily. The aim of this work was to study metal retention and distribution in the sediment of a constructed wetland for industrial wastewater treatment. Total concentrations and fractions (exchangeable, carbonate-bound, Fe–Mn oxides-bound, organic matter-bound and residual) of metals in sediment were analyzed in this treatment wetland, in order to estimate the fate of metals over time. Metal concentrations were significantly higher in the inlet than in the outlet sediment; concentrations in the latter remained without significant differences throughout the testing period. Metal concentrations and redox potential decreased with depth within the sediment. The lowest metal concentrations and pH and the highest redox values were attained in spring, in agreement with the period of maximum macrophyte growth. Ni and Zn were mainly stored associated with the carbonate fraction; Cr was mainly associated with the Fe–Mn oxides fraction, while Fe was mainly associated with the residual fraction, probably as pyrite. The incoming wastewater composition containing high pH, carbonate, calcium and Fe concentrations favored the observed association in the surface sediment. It would be expected that sediment will continue retaining metals in fractions that will not release them into the water while the chemical and environmental conditions remain unchanged.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Metallurgical industry activities produce wastewater with elevated concentrations of metals. These contaminants in effluents usually exceed discharge levels stipulated in the legislation and can be toxic to a variety of organisms (Allan, 1995; Gao and Bradshaw, 1995). Heavy metals can interact with the sulphydryl groups of amino acids (Salt et al., 1995) and subsequently interfere with enzyme functioning and oxygen-binding processes in organisms (Kelly, 1988).

One technology employed for treating these effluents is treatment wetlands (Hammer, 1989; Vymazal et al., 1998; Kadlec et al., 2000; Maine et al., 2007b, 2009; Kadlec and Wallace, 2009). Constructed wetlands constitute complex systems where the bio-logical, chemical and physical processes interact to produce the removal of many contaminants from water. Effluent purification is performed by mechanisms such as adsorption, precipitation and co-precipitation to the bottom sediment, and uptake by plants (Wood and Shelley, 1999; Yu et al., 2001). Emergent plants not only retain contaminants in their tissues and renew the sources of carbon for degrading bacteria (Dunbabin and Bowner, 1992), but also contribute to wastewater treatment processes in a number of ways, such as favoring the settlement of suspended solids, providing surface area for microorganisms, carrying oxygen from the aerial parts to the roots, creating the proper environment in the rhizosphere for the proliferation of microorganisms and promoting a variety of chemical and biochemical reactions which enhance metal retention by the sediment (Brix, 1994, 1997; Kadlec et al., 2000). Despite the importance of macrophytes for contaminant removal, sediment is the main pollutant storage compartment (Machemer et al., 1993; Wood and Shelley, 1999; Maine et al., 2009).

Several studies reported removal efficiencies of constructed wetlands (Hammer, 1989; Moshiri, 1993; Vymazal et al., 1998; Kadlec et al., 2000; Maine et al., 2006, 2007a,b, 2009; Kadlec and

Wallace, 2009). However, little is known about the long-term fate of metals in wetland sediment. It is well known that metal distribution, mobility and availability in the environment depend not only on their total concentration but also on the association forms in the solid phase to which they are bound. These associations are studied through fractionation or speciation approaches (Tessier et al., 1979; Ure and Davidson, 2001). Different authors emphasized several environmental conditions influencing the extent and chemical form of the metals retained. Sediment redox condition (Guo et al., 1997a,b), pH (Gambrell et al., 1991; Lefroy et al., 1993), organic matter content (OM) and sulfate concentration (Wood and Shelley, 1999; Yu et al., 2001) were studied.

A free-water surface wetland was constructed in 2002 by Bahco Argentina S.A. to treat the wastewater from its metallurgic plant, containing Ni, Cr, Zn and Fe. The wetland removed metals efficiently until the last reported sampling period, in 2006 (Maine et al., 2009), using sediment as the main retention compartment. A significant increase in the metal content of the bottom sediment in the inlet was reported. The aim of this work was to study metal retention and distribution in the sediment of a constructed wetland for industrial wastewater treatment. These results provide an insight of how long retention would persist and if metals would remain bound in the sediment or would eventually be released back to water in response to environmental changes.

2. Study site

The wetland constructed at the Bahco Argentina tool factory is located in Santo Tomé, Santa Fe, Argentina (S 31°40′; W 60°47′). It is 50 m long, 40 m wide and 0.3–0.6 m deep, with a central baffle dividing it into two identical sections. Mean wastewater discharge was 100 $m^3 d^{-1}$. Wastewater and sewage from the factory were treated together. Further details were provided by Maine et al. (2007b). Emergent and floating, locally abundant, macrophyte species were transplanted into the wetland at the beginning of the operation period, in 2003, but only *Typha domingensis* persisted. *T. domingensis* aerial parts are harvested annually to ensure an optimal growth after the winter season.

3. Methods

3.1. Sampling schedule

Water and sediment samples were collected in August (winter) and October (spring) 2007, and May (autumn) 2008. These samples were collected in the inlet (1 m away from the effluent discharge) and outlet (100 m from the effluent discharge) areas of the wetland, by triplicate. Macrophytes were sampled monthly taking four replicates randomly at the inlet in each sampling.

3.2. Water

Water characterization was performed following APHA (1998) procedures. Total Fe, Cr, Ni and Zn concentrations were determined by atomic absorption spectrometry (by flame or electrothermal atomization, according to the sample concentration, Perkin Elmer 5000).

3.3. Sediment

Sediment samples were collected using a 3-cm diameter PVC corer. 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 poten-

tial (Eh) and pH of the bulk sediment layers were measured in situ with an Orion pH/mV-meter. OM was determined by weight loss on ignition at 550 °C for 3 h. Particle size was determined according to Stoke's law, in a 1000-mL measuring cylinder beaker with distilled water after destroying the OM and sediment carbonates. 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 $1°2\theta\,min^{-1}$ (10–80). Crystalline phases were identified using DP-D1 system software.

Sediment samples were oven-dried at 45 °C until constant weights were reached and ground using a mortar and pestle. Subsequently, they were sieved through a 53 μm sieve prior to sequential extraction for metals. Each sediment sample was analyzed according to the sequential extraction proposed by Tessier et al. (1979) in order to evaluate the chemical association of metals in sediment. The sequential extraction was divided into five steps:

(a) Exchangeable;
(b) Bound to carbonates;
(c) Bound to Fe–Mn oxides;
(d) Bound to OM;
(e) Residual.

Acid-washed polyethylene centrifuge tubes (50 mL) were used for the extraction, to minimize sediment loss. Separation between each extraction was achieved by centrifugation at 3000 rpm for 20 min. The supernatant was removed with a pipet and analyzed for metals. The residue was washed with distilled water and centrifuged at 3000 rpm for 20 min. This second supernatant was discarded and the residue extracted further. For total and residual metal analyses, samples were digested with a $HClO_4:HNO_3:HCl$ (7:5:2) mixture. The resulting solution from the total and residual digestion and the extracts obtained from the sequential extraction procedure were analyzed for Cr, Ni, Zn and Fe by atomic absorption spectrometry (Perkin Elmer, AAnalyst 300) using an air–acetylene flame.

3.4. Macrophytes

Macrophytes were sampled monthly using a 0.25 m^{-2} quadrate following Vesk and Allaway (1997). Four replicates were taken randomly at the inlet during each sampling. The macrophytes were then separated between above (leaves) and belowground parts (roots plus rhizomes). Cr, Ni and Zn in above and belowground parts were determined in the same way as in the sediment samples. To measure dry weight, plants were dried at 105 °C until constant weights were reached (APHA, 1998). Plant cover was determined by estimating the area occupied by the aboveground plant material in the wetland.

3.5. Statistical analysis

Statistical significance between inlet and outlet effluent concentrations was assessed using a mean comparison test ($p < 0.05$). ANOVA analysis (three ways) was performed to evaluate the influence of time, depth and metal fractions on metal sediment concentrations. Determination of the active layer of the sediment involved in the exchange reactions were performed using two-way ANOVA (depth and sampling date) to evaluate the influence of these factors on the total concentration of metals. To determine if there were significant differences among the sampling dates for metal concentrations in plant tissues (leaves and roots), a two-way ANOVA (sampling dates and plant tissues) was carried out.

One-way ANOVA was performed to determine if there were significant differences among the sampling dates in plant cover. Duncan's test was used to differentiate means where appropriate. A level of $p < 0.05$ was used in all comparisons.

3.6. QA/QC

All glassware was pre-cleaned and washed with 2 M HNO_3 prior to each experiment. All reagents were of analytical grade. All solutions were prepared with Milli-Q water. Certified standard solutions were used. Replicate analyses (at least ten times) of the samples showed a precision of typically less than 8% (coefficient of variation). The detection limits were 30, 20, 3 and 3 $\mu g\,g^{-1}$ for Cr, Ni, Zn and Fe, respectively. The selectivity of each extraction step was performed by DRX analysis. The accuracy of the sequential extraction procedure was also evaluated comparing the sum of concentration determined for five fractions with total concentration data. Mean apparent recovery (%) with a standard deviation value for a total of 36 samples were Cr = 96.8 ± 5.4%, Ni = 98.9 ± 3.0%, Zn = 94.3 ± 5.5% and Fe = 95.6 ± 4.2%.

4. Results and discussion

4.1. Water

Table 1 summarizes the water quality variables measured at the wetland inlet and outlet sampling locations. Analyte concentrations were significantly lower in the outlet than the inlet, with the exception of NH_4^+, indicating high removal efficiency. COD and BOD were reduced by 81 and 90%, respectively from the inlet to the outlet, suggesting extensive mineralization of the high OM load. The OM mineralization released NH_4^+ and CO_2, resulting in the increased NH_4^+ concentration and decreased pH in the outlet. Ca^{2+} and alkalinity were reduced by 62% and 59% in the outlet. The high prevailing pH and CO_3^{2-} concentrations suggest calcium carbonate precipitation in the inlet. Lower pH and undetectable CO_3^{2-} concentrations suggest that calcium carbonate precipitation did not occur in the outlet.

Soluble reactive phosphorus (SRP) and total phosphorus (TP) were reduced by 78 and 52%, respectively, in the outlet. Despite the high mineralization rates, DO was not exhausted and Fe was reduced by 98% in the outlet, suggesting SRP co-precipitation either with Fe or calcium carbonate.

NO_3^- and NO_2^- were depleted in the outlet attaining 85 and 87% removal, respectively. Because NO_3^- and NO_2^- concentrations in the inlet were an order of magnitude higher than NH_4^+, the wetland attained a large inorganic nitrogen removal. SO_4^{2-} concentration was high and variable in the inlet. Concentrations were significantly lower in the outlet and attained a mean removal efficiency of 48%, suggesting SO_4^{2-} reduction and metal sulfide precipitation in the anoxic bottom sediment.

Metal concentrations were significantly lower in the outlet than in the inlet. The highest average retention was attained by Fe (98%), followed by Cr (90%), Ni (59%) and lastly Zn (57%). Zn concentrations fell below the detection limit on one occasion in the inlet and on two occasions in the outlet water, making the removal estimate rather tentative. It is interesting to note that present removal efficiencies were higher than previously reported for all metal and nutrients analyzed (Maine et al., 2007a,b, 2009), even on the basis of a limited number of samples.

4.2. Sediment

Mainly clay and sand constituted the wetland sediment, 20 and 70% on average, respectively. The XRD analysis showed that the inlet wetland sediment is composed mainly of quartz with a minor amount of carbonate mineral (calcite), whereas the outlet sediment was composed of quartz (SiO_2) only. Consistent with the measured pH and calcium and carbonate concentrations in water, this suggests that calcium carbonate precipitation was a possible mechanism of contaminant retention in the inlet area.

Table 2 summarizes the measurements performed within the bottom sediment. Redox condition and pH showed a seasonal pattern, pH decreased and redox potential increased in spring (October) in agreement with the highest environment temperatures and the period of active macrophyte growth. Higher temperatures suggest enhanced OM mineralization in the bottom, decreasing sediment pH. Emergent macrophytes influence the biogeochemical cycles of the sediment through the effects on the redox status of the sediment, due to their capacity to transport oxygen from roots into the rhizosphere (Barko et al., 1991; Brix and Schierup, 1990; Jacob and Otte, 2003; Sorrell and Boon, 1992). Oxygen release by roots is likely to be the cause of the higher redox condition measured at all depths in spring.

OM showed a consistent trend to increase throughout the sampling period in the inlet and the outlet, and all depths within the sediment, ranging from 3.1% (0–3 cm, August) to 6.9% (7–10 cm, May). In the inlet, OM was higher at 7–10 cm depth than on the 0–3 cm depth. It seems likely that the higher OM at 7–10 cm in the inlet represents the remaining sediment layers showing older organic accumulation. The OM in the inlet during the first sampling of this study period (August 2007) was similar to the conditions when the wetland started operation (OM in the sediment was 3.5%, mean Eh was 95 mV and pH was 7.7; Maine et al., 2006). On the contrary, sediment pH was higher and redox condition was lower in the present sampling period.

4.3. Metal concentrations in sediment and macrophytes

Metal concentrations were significantly higher in the wetland inlet than in the outlet (Fig. 1). Concentrations in the outlet were not significantly different from the concentrations at the beginning of the operation period, when they were low and uniform throughout the wetland (Cr = 0.025 mg g^{-1}, Ni = 0.022 mg g^{-1}, Zn = 0.005 mg g^{-1} and Fe = 9.70 mg g^{-1}; Maine et al., 2006). Cr, Ni and Zn concentrations decreased with depth at all sites and sampling dates (ANOVA, $p > 0.05$). Similar patterns were repeatedly reported in the literature (Chagué-Goff and Rosen, 2001; Yu et al., 2001; O'Sullivan et al., 2004). Even though Zn concentrations in water fell occasionally below the detection limits, metal concentration in the inlet sediment increased significantly regarding the initial values, emphasizing the high metal retention of the sediment.

Cr, Ni and Zn concentration in the bottom sediment decreased in spring (October) at all sites and depths (Fig. 1), coinciding with the beginning of the vegetation growth period (Fig. 2). Although plants accumulated metals during this period, newly formed biomass during the growing period contributed to dilute the metal concentration in tissues (Maine et al., 2007b).

Throughout the study, metal concentrations were higher in roots and rhizomes than in aerial parts, suggesting little translocation (Fig. 3). Previous studies showed metal uptake by T. domingensis (Maine et al., 2009; Hadad et al., 2010). T. domingensis biomass was higher in the constructed wetland than in nearby natural environments, probably because of the higher temperature and nutrient concentrations which occur in domestic wastewater (Maine et al., 2009; Hadad et al., 2010). Likewise, the depth of T. domingensis roots was greater than the sampled sediment depth. It is therefore suggested that the decreased metal concentrations in the sediment were due to the increased vegetation uptake. Concen-

Table 1
Mean measured variables (as $mg\,L^{-1}$, unless otherwise noted) in the inlet and outlet water of the constructed wetland during the study period (ND = not detected, the values in parentheses are the detection limits).

	August (winter)		October (spring)		May (fall)	
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
DO	3.51	3.76	3.60	3.92	7.59	3.51
pH (units)	10.10	7.50	9.02	8.00	9.90	8.40
Conductivity ($\mu\Omega\,cm^{-1}$)	4850	2140	4200	2330	5000	2820
Temperature ($^\circ$C)	12.8	9.3	27.0	26.8	20.1	16.6
Solids	3488	1417	2777	1329	2450	1697
Alkalinity ($CaCO_3$)	593	315	452	174	988	317
HCO_3^-	ND (0.5)	384	ND (0.5)	212	ND (0.5)	298
CO_3^{2-}	158	ND (0.5)	295	ND (0.5)	132	44
Cl^-	270	153	1061	453	263	192
SO_4^{2-}	1649	554	270	203	751	621
Ca^{2+}	137	61	164	34	119	56
Fe	7.49	0.31	0.78	0.01	34.41	0.17
Cr	0.400	0.045	0.068	ND (0.001)	0.049	0.009
Ni	0.028	0.013	0.028	0.010	0.036	0.014
Zn	0.09	0.04	0.03	ND (0.01)	ND (0.01)	ND (0.01)
SRP	0.161	0.060	0.248	0.096	0.122	0.052
TP	0.444	0.237	0.952	0.252	0.426	0.162
NO_2^-	0.720	0.167	0.719	0.020	0.058	0.047
NO_3^-	33.1	5.9	54.7	5.4	33.1	5.2
NH_4^+	1.65	2.44	1.48	2.04	1.71	2.95
BOD	93.9	25.9	36.9	11.4	290.4	22.5
COD	202.3	29.9	79.7	29.5	702.4	61.5

Table 2
Redox potential (mV), pH and OM (%) at different depths of the inlet and outlet sediment during the study period. Season and measured environment temperature in parentheses.

Sampling	Area	Depth (cm)	Redox potential (mV)	pH	OM (%)
August (winter $T = 12\,^\circ$C)	Inlet	0–3	−92.2	9.10	3.6
		3–7	−130.8	9.11	3.1
		7–10	−159.8	9.13	4.4
	Outlet	0–3	−319.7	9.31	4.6
		3–7	−363.0	9.42	4.2
		7–10	−363.0	9.42	3.1
October (spring $T = 28\,^\circ$C)	Inlet	0–3	−81.0	8.84	5.2
		3–7	−120.3	8.89	5.0
		7–10	−129.6	8.92	5.3
	Outlet	0–3	−246.6	9.03	5.6
		3–7	−250.8	9.10	5.7
		7–10	−251.0	9.13	5.3
May (fall $T = 19\,^\circ$C)	Inlet	0–3	−98.5	9.56	6.5
		3–7	−118.2	9.27	6.1
		7–10	−151.9	9.14	6.9
	Outlet	0–3	−366.4	9.29	6.1
		3–7	−373.4	9.16	5.7
		7–10	−414.7	8.94	6.1

trations in the bottom sediment increased again in the fall (Fig. 1), denoting a continued retention throughout the summer and eventual release coinciding with vegetation senescence. In order to compare the removal rates in sediment and plants, metal concentrations were converted into metal standing stock ($mg\,m^{-2}$). Thus, we have considered not only the concentration but also the amount of Cr, Ni and Zn in each compartment according to the initial and final amounts of Cr, Ni and Zn in the sediment (considering a total depth of 10 cm and taking into account the concentrations obtained at each studied layer) and in *T. domingensis* (taking into account leaf

Table 3
Cr, Ni and Zn retained by sediment and *T. domingensis* ($mg\,m^{-2}$) during the studied period.

|	*T. domingensis*		Sediment		
	Leaves	Roots	0–3 cm	3–7 cm	7–10 cm
Initial Cr amount	25.3	209.4	10044.8	8798.3	4668.0
Final Cr amount	47.9	212.1	11511.2	10557.9	7038.7
Cr retained	22.6	2.7	1466.4	1759.6	2370.7
Initial Ni amount	21.1	185.5	5865.5	5303.4	3812.6
Final Ni amount	47.9	191.9	8138.4	7576.3	6574.3
Ni retained	26.8	6.4	2272.9	2272.9	2761.7
Initial Zn amount	25.3	56.0	904.3	684.3	366.6
Final Zn amount	47.9	60.5	1955.2	1393.1	928.7
Zn retained	22.6	4.5	1050.9	708.8	562.1
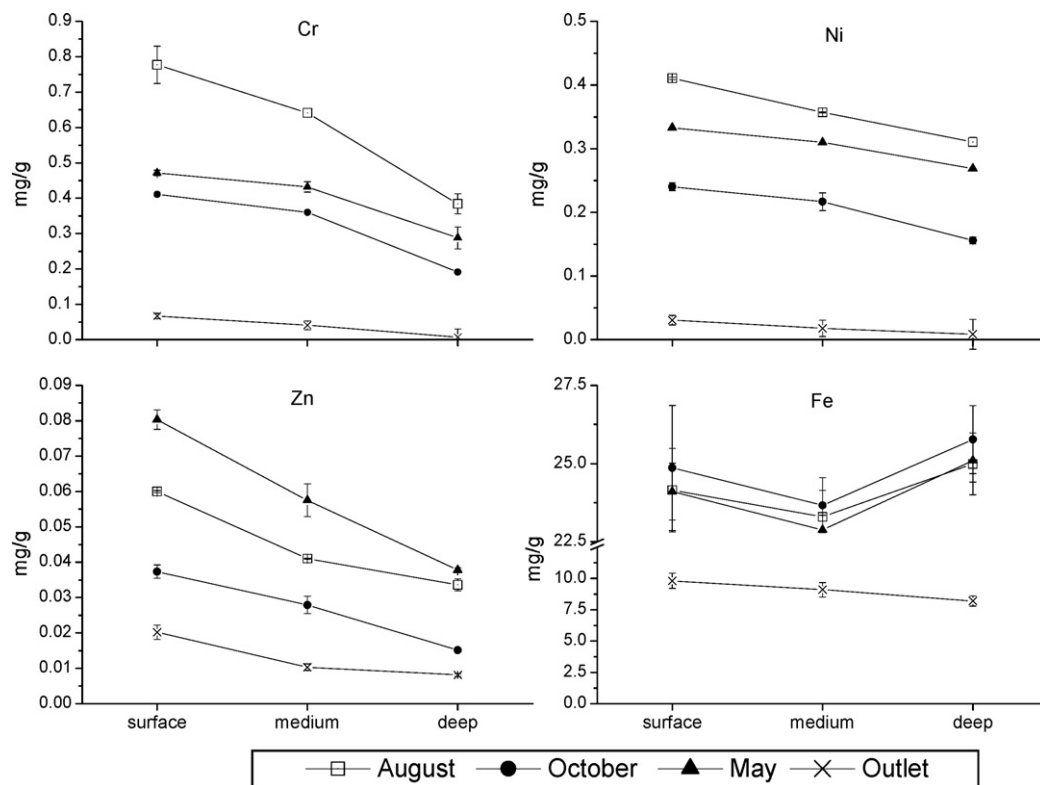
Fig. 1. Sediment total metal concentrations at 0–3 cm (surface), 3–7 cm (medium) and 7–10 cm (deep) during different sample dates in the inlet area (August, October, May).

and root biomass and concentration) (Table 3). The Cr, Ni and Zn retained during the experiment is the difference of Cr, Ni and Zn amount between the initial (August 2007) and final (May 2008) conditions. The amount of metals in the sediment decreased with depth in both, initial and final conditions. However, the amount of Fe retained during the studied period increased with depth. This behavior is probably due to the higher Fe concentrations in the effluent over the other studied metals (Table 1). Consequently its accumulation occurs in deeper layers. In *T. domingensis*, roots accumulated higher amount of metals than leaves (ANOVA, $p > 0.05$).

According to the amount of Cr, Ni and Zn in the different compartments of the system, it can be concluded that the sediment is the main metal storage compartment which is in agreement with the results obtained in natural systems (Maine et al., 1999). As metal concentrations increase at all studied depths compared with initial concentrations, it can be concluded that the active layer is larger than the deepest sampled layer (7–10 cm).

Fe loads were larger than those of Cr, Ni and Zn. It was largely retained within the wetland resulting in significantly higher concentrations in the inlet sediments. However, no significant dif-
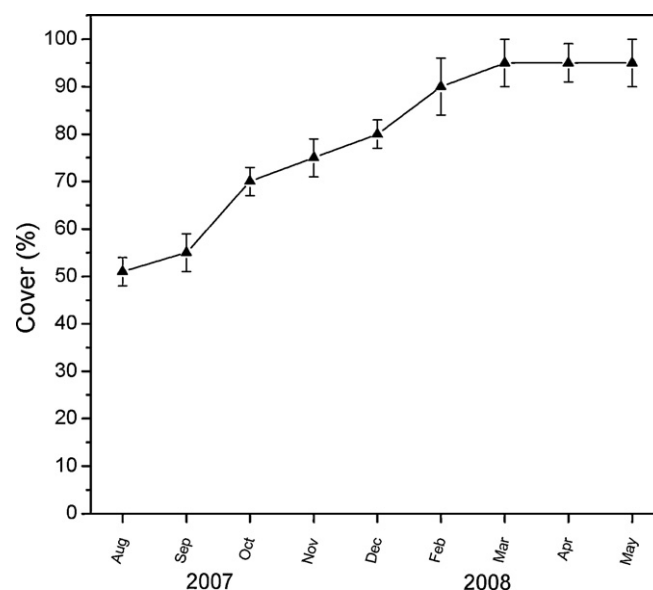

Fig. 2. Cover of *T. domingensis* in the wetland throughout the study period.
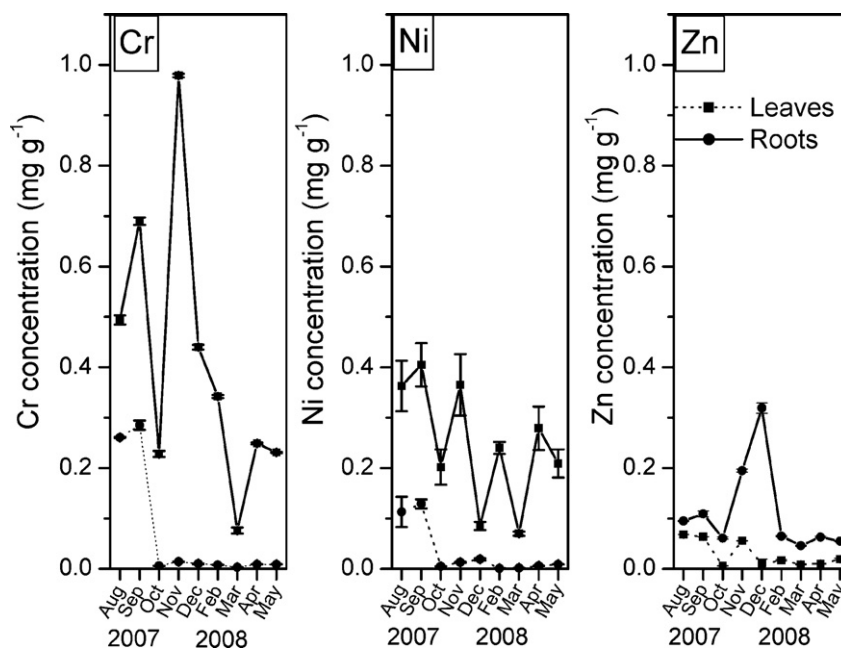
Fig. 3. Cr, Ni and Zn concentration in roots and leaves of *T. domingensis* throughout the study period.

ferences were observed among depths or sampling dates (Two-way ANOVA, $p > 0.05$).

4.4. Partitioning of metals in sediment

Fig. 4 shows metal partitioning in the bottom sediment of the wetland inlet at different depths and sampling dates. Metal concentrations in the different fractions were statistically different among sampling dates and depths (ANOVA, $p > 0.05$). Metal concentrations in the bottom sediment of the wetland outlet were not significantly different from the initial values at the beginning of the operation period. In this area, metals were largely associated with the residual fraction (data not shown).

Cr was mainly associated with Fe–Mn oxides (50–70%) and, to a lesser extent, with the residual (8–35%) and carbonate (8–25%) fractions. Cr(VI) is easily reduced to Cr(III) by OM (Losi et al., 1994; Fendorf, 1995). The high OM and low DO concentrations led to the absence of Cr(VI) in the wetland water throughout the sampling period. The low solubility of Cr(III) produces its precipitation as $Cr(OH)_n$ (mostly $Cr(OH)_3$). Cr speciation in the sediment surface showed the influence of the overlaying water composition: in the last sampling (May), Fe and DO concentrations in inlet water were the highest of the sampled periods in agreement with the lowest Ca and carbonate concentrations (Table 1). Consistently, the fraction bound to Fe–Mn oxides at the surface sediment was the largest and the fraction bound to carbonates was the lowest (Fig. 4). A trend to increase the Fe–Mn bound Cr at the expense of the carbonate bound Cr was observed in the superficial sediment throughout the sampling period (Fig. 4). This trend was blurred deeper in the sediment, where the residual and organic fractions were often higher, coinciding with the lower redox potentials, implying further and slower diagenetic processes within the sediment. Guo et al. (1997b) studied the distribution of the different fractions within a redox gradient. As the redox potential decreased, the Fe–Mn oxides bound fraction decreased and the organic bound fraction increased. At about -130 mV the organic bound fraction replaced the Fe–Mn oxides bound as the dominant fraction. In the present study, the organic bound fraction remained small, probably because of the comparatively high redox potential in the superficial

sediment which was always above the threshold value reported by Guo et al. (1997b). In the present study, the residual fraction was the second largest.

Ni was mainly bound to the carbonate fraction, attaining 35–50% of the total, followed by the residual (22–35%) and Fe–Mn oxides bound fractions (20–30%), respectively. Guo et al. (1997a) studied Ni speciation in a wetland sediment at neutral pH and different redox potentials. At a high redox potential, the Ni precipitate adsorbed onto Fe–Mn oxides. When the redox potential decreased, the concentration of soluble Ni decreased noticeably, due to its precipitation as carbonates. This process was favored in the present study by the high alkalinity and pH prevailing in the wetland inlet. A trend to decrease the carbonate bound Ni accompanied by an increase in the residual fraction was noticed in the superficial sediment throughout the study period. Temporal trends were blurred deeper in the sediment. The residual Ni fraction was the second largest. High percentages of Ni in the residual fraction have been reported by Stone and Marsalek (1996), Banerjee (2003) and Lee et al. (2005). The Fe–Mn oxide bound fraction was the third important fraction. Jenne (1968) suggested that adsorption by Mn oxides is an important Ni fate in soils.

Zn was mainly bound to the carbonate fraction (50–65%) and, to a lesser extent, to the residual (10–40%) and Fe–Mn oxides bound (5–18%) fractions. Extensive Zn-carbonate precipitation seems to be a consequence of the high pH and carbonate concentration prevailing in the incoming wastewater. Zn retention as Zn bound to carbonates has repeatedly been reported in the literature (Stone and Marsalek, 1996; Banerjee, 2003; Lee et al., 2005). Nuttall and Younger (2000) studied the removal process of Zn from an alkaline wastewater in a Zn and Pb abandoned mine and suggested that precipitation observed corresponded to $ZnCO_3$ when the pH ranged between 7.8 and 8.5 and to Zn oxides when the pH values were higher. Chagué-Goff (2005) studied a wetland for domiciliary wastewater treatment where lower water pH prevented carbonate precipitation, being the residual, organic and Fe–Mn oxides the main Zn fractions. O'Sullivan et al. (2004) found that Zn precipitated as insoluble ZnS in anaerobic and alkaline substrates of constructed wetlands for treating mine wastewater and they determined it in the residual fraction. Guo et al. (1997b) reported that in neutral
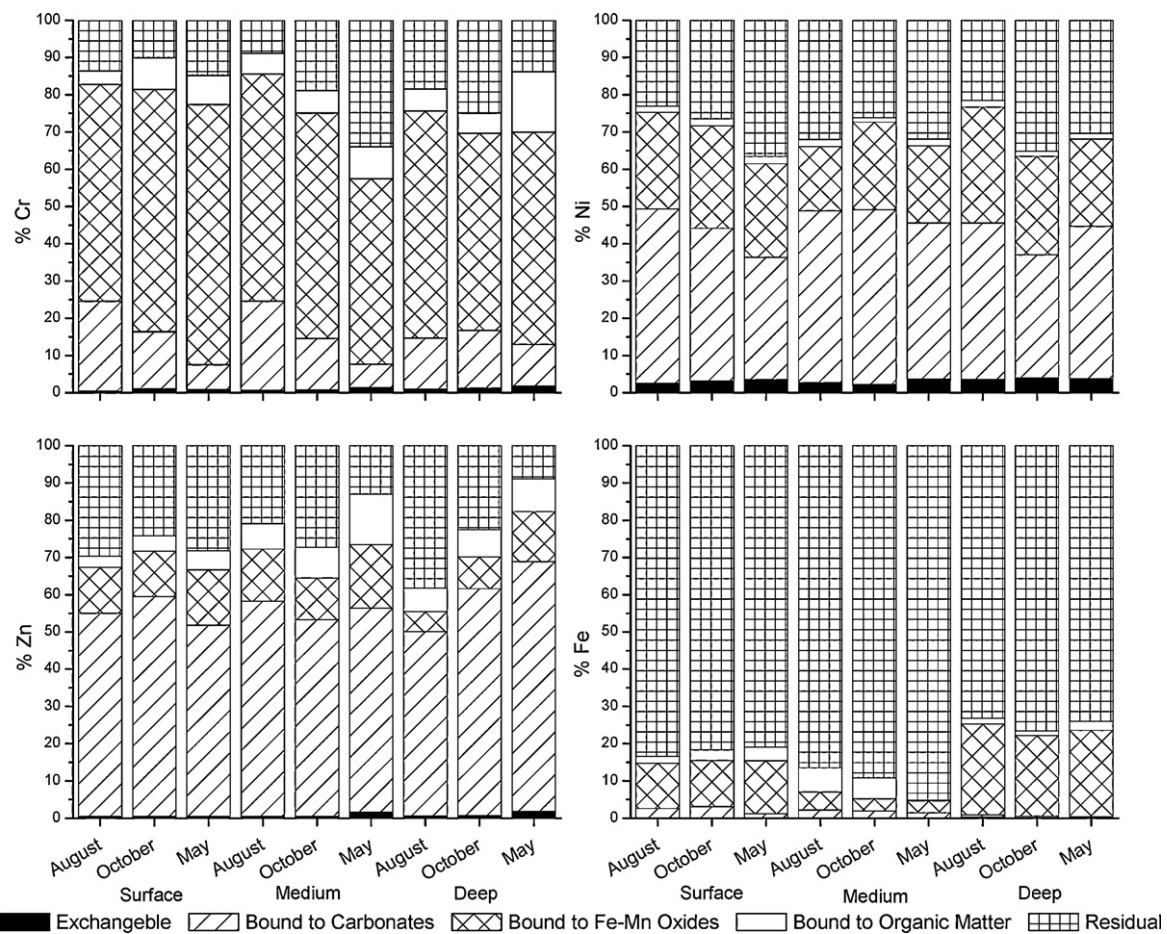
Fig. 4. Exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to OM and residual fractions at different depths (surface, medium and deep) and sampling dates (August, October and May) in the inlet area.

sediment as Eh decreased, the Zn fraction associated with Fe–Mn oxides was replaced by the Zn fractions associated with carbonates in the Eh range observed in the present study. At redox values of about −170 mV the ZnS fraction replaced the carbonate as the main fraction. In the present study, Zn was associated with the residual fraction (ZnS) in a lesser extent probably due to the comparatively high Eh values recorded in the inlet sediment, always larger than the threshold observed by Guo et al. (1997b) (Table 2). Consistently, the Zn fraction associated with OM and ZnS was larger and the Fe–Mn oxides bound fraction was smaller in the deeper sediment layers (Fig. 4) where the redox conditions were lower (Table 2).

Fe was accumulated largely in the residual fraction (74–90%), suggesting relatively low mobility and availability of this element. The behavior of this metal shows clear differences with the others. Our results are consistent with those of O'Sullivan et al. (2004) and Chagué-Goff (2005) who found that the sulfide-bound Fe is retained within the residual fraction suggesting that Fe is mostly present in mineral/detrital form. Once the first four fractions have been removed, the residual solid should contain mainly primary and secondary minerals, which may hold metals within their crystal structure (Tessier et al., 1979). Simultaneous SO_4^{-2} and Fe removal and low DO concentration in water suggest insoluble FeS_2 formation within the sediment. Microbial production of sulfide takes place in wetlands, as long as SO_4^{-2} is present in the inlet water and plants provide the organic substrate for the growth of the sulfate-reducing bacteria (Machemer and Widelman, 1992; Sobolewski, 1996; Chagué-Goff and Rosen, 2001; O'Sullivan, 2001; O'Sullivan et al., 2004). Because of the high SO_4^{-2} concentration in the incom-

ing wastewater, most of the OM mineralization might take place at the expense of biological SO_4^{-2} reduction as observed in coastal marine sediment where S^{-2} released by SO_4^{-2} reduction subsequently reacts with Fe to form FeS minerals (Giblin, 1988). Several monosulfide minerals precipitated early are later converted to pyrite (Giblin, 1988). FeS formation depends on the rate of Fe(II) and S^{2-} supply. The lower concentrations of Fe than SO_4^{-2} in the incoming wastewater and the almost complete Fe retention within the wetland (Table 1) suggest that Fe availability limits FeS formation in the wetland. Under the reducing and anoxic conditions in wetlands, metal removal through precipitation with sulfides can represent a long-term removal process, as this phase is most stable under reducing conditions.

Metal ions can also be strongly bound as insoluble associations with OM, in particular with components of the humic fraction (Machemer et al., 1993; Yu et al., 2001). The relative magnitude of the organic bound fraction increased as redox potentials decreased (Guo et al., 1997a,b). In the present study, OM accumulation in the inlet remained comparatively modest, and the redox potentials remained comparatively high. Therefore, the OM represented a minor fraction.

Although the sequential extraction method was not developed to assess metal availability, it is recognized that the sequence of the extraction can be seen as an inverse scale of the relative availability of metals since the strength of the extraction reagents used is progressively increased from the exchangeable to the residual fraction (Tessier et al., 1979; Stone and Droppo, 1996; Banerjee, 2003). Following Lee et al. (2005), we assume that

the exchangeable and carbonate fractions are the most bioavailable fractions. Thus, the comparative availability of metals in the studied wetland decreased in the following order: Zn > Ni > Cr > Fe. These results are in agreement with those reported by Lee et al. (2005) who found that the comparative mobility and bioavailability of the metals in a sediment tends to decrease in the following order: Zn > Ni > Cd > Pb > Cu > Cr and by Irvine et al. (2009), who reported the following order of comparative bioavailability Zn > Cd > Mn > Pb > Cu > Fe.

5. Conclusions

The constructed wetland continued removing metals, nutrients and OM after 5 years of operation. Metals have been accumulating in the inlet sediment, while the outlet concentrations have remained at the amounts obtained at the beginning of the operation period. If retention depends on the substrate fixation capacity, for example the total amount of active sites in the sediment, it would be suggested that there is still a long effective life time because the inlet sediment continues to retain metals, and has apparently not yet reached saturation. However, wetland removal capacity seems more related to the incoming wastewater composition and the macrophyte development than to substrate nature: in the inlet, Ni and Zn were mainly bound to the carbonate fraction; Cr was mainly bound to the Fe–Mn oxide fraction; while Fe was mainly bound to the residual fraction. Such a pattern was determined by the high pH, Ca^{2+}, carbonate and Fe concentration of the wastewater that caused $CaCO_3$ and $Fe(OOH)_n$ precipitation and the subsequent metal sorption to their surfaces. High SO_4^{2-} concentrations and anoxic conditions in the sediment favor metal-sulfide formation. The exchangeable fraction of the studied metals, the most labile and readily bioavailable, showed a negligible concentration (less than 5% of the total) in all cases. Metals retained by sediment fractions will not release them into the water, while the chemical and environmental conditions remain unchanged. Consequently, the removal would be sustainable as long as the incoming wastewater composition is maintained.

The active layer of the sediment involved in the exchange reactions was unexpectedly deeper than the deepest sampled sediment layer (10 cm). Moreover, since most contaminants were retained in the upper layer, retention capacity could eventually be renewed by removing the upper substrate layer.

Acknowledgements

References

Allan, R., 1995. Diffuse sources of pollution by toxic metals. In: Salomons, W., Forstner, U., Hyman, D. (Eds.), Heavy Metals: Problems and Solutions. Springer-Verlag, Berlin, Germany, pp. 33–52.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. Amer. Publ. Health Assoc., New York.

Banerjee, A.D.K., 2003. Heavy metal levels and solid phase speciation in street dusts of Delhi, India. Environ. Pollut. 123, 95–105.

Barko, J.W., Gunnison, D., Carpenter, S.R., 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquat. Bot. 41, 41–65.

Brix, H., Schierup, H.H., 1990. Soil oxygenation in constructed reed beds: the role of macrophyte and soil–atmosphere interface oxygen transport. In: Cooper, P., Findlater, B. (Eds.), Constructed Wetlands in Water Pollution Control. WRc Swindon, Wiltshire, UK, pp. 53–65.

Brix, H., 1994. Functions of macrophytes in constructed wetlands. Water Sci. Technol. 29 (4), 71–78.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol. 35 (5), 11–17.

Chagué-Goff, C., 2005. Assessing the removal efficiency of Zn, Cu, Fe and Pb in a treatment wetland using selective sequential extraction: a case study. Water Air Soil Pollut. 160, 161–179.

Chagué-Goff, C., Rosen, M., 2001. Using sediment chemistry to determine the impact of treated wastewater discharge on a natural wetland in New Zeland. Environ. Geol. 40, 1411–1423.

Dunbabin, J., Bowmer, K., 1992. Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. Sci. Total Environ. 111, 151–168.

Fendorf, S., 1995. Surface reactions of chromium in soils and waters. Geoderma 67, 5–71.

Gambrell, R., Wiesepape, J., Patrick Jr., W., Duff, M., 1991. The effects of pH, redox, and salinity on metal release from a contaminated sediment. Water Air Soil Pollut. 57–58, 359–367.

Gao, Y., Bradshaw, A., 1995. The containment of toxic wastes: II. Metal movement in leachate and drainage at Parc lead–zinc mine, North Wales. Environ. Pollut. 90 (3), 379–382.

Giblin, A., 1988. Pyrite formation in marshes during early diagenesis. Geomicrobiol. J. 6, 77–97.

Guo, T., Delaune, R., Patrick, W., 1997a. The effect of sediment redox chemistry on solubility/chemically active forms of selected metals in bottom sediment receiving produced water discharge. Spill Sci. Technol. Bull. 4 (3), 165–175.

Guo, T., Delaune, R., Patrick, W., 1997b. The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium and zinc in estuarine sediment. Environ. Int. 23 (3), 305–316.

Hadad, H.R., Mufarrege, M.M., Pinciroli, M., Maine, M.A., 2010. Morphological response of Typha domingensis to an industrial effluent containing heavy metals in a constructed wetland. Arch. Environ. Contam. Toxicol. 58, 666–675.

Hammer, D.A., 1989. Constructed Wetlands for Wastewater Treatment. Lewis Publishers Inc., Chelsea, MI.

Irvine, K.M., Perrelli, M.F., Ngoen-klan, R., Droppo, I.G., 2009. Metal levels in street sediments from a industrial city: spatial trends, chemical fractionation, and management implications. J. Soils Sediments 9, 328–341.

Jacob, D., Otte, M., 2003. Conflicting processes in the wetland plant rhizosphere: metal retention or mobilization? Water Air Soil Pollut. 3, 91–104.

Jenne, E.A., 1968. Controls on Mn, Fe, Co, Ni Cu and Zn concentrations in soils and water: the significant role of hydrous Mn and Fe oxides. In: Gould, R.F. (Ed.), Advances in Chemistry Series. Trace Inorganics in Water, vol. 73. American Chemical Society Publications, Washington, DC, pp. 337–387.

Kadlec, R.H., Wallace, S.D., 2009. Treatment Wetlands, second ed. CRC Press, Boca Raton, FL.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P., Haberl, R., 2000. Constructed Wetlands for Pollution Control: Processes, Performance Design and Operation. IWA Specialist Group on Use of Macrophytes in Water Pollution Control. IWA Publishing.

Kelly, M., 1988. Mining and the Freshwater Environment. Elsevier, London, UK.

Lee, P.K., Yu, Y.H., Yun, S.T., Mayer, B., 2005. Metal contamination and solid phase partitioning of metals in urban roadside sediments. Chemosphere 60, 672–689.

Lefroy, R., Samsoir, S., Blair, G., 1993. The dynamics of sulfur, phosphorus and iron in flooded soils as affected by changes in Eh end pH. Aust. J. Soil Res. 32, 493–508.

Losi, M.E., Amrhein, C., Frankenberger Jr., W.T., 1994. Environmental biochemistry of chromium. Rev. Environ. Contam. 136, 91–121.

Machemer, S., Widelman, T., 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J. Contam. Hydrol. 9, 115–131.

Machemer, S., Reynolds, J., Laudon, L., Wildeman, T., 1993. Balance of S in a constructed wetland built to treat acid mine drainage, Idaho Springs, Colorado, USA. Appl. Geochem. 8, 587–603.

Maine, M.A., Suñé, N., Panigatti, M.C., Pizarro, M., Emiliani, F., 1999. Relationships between water chemistry and macrophyte chemistry in lotic and lentic environments. Arch. Hydrobiol. 145, 129–145.

Maine, M.A., Suñé, N., Hadad, H., Sánchez, G., Bonetto, C., 2006. Nutrient and metal removal in a constructed wetland for waste-water treatment from a metallurgic industry. Ecol. Eng. 26, 341–347.

Maine, M.A., Suñé, N., Hadad, H., Sánchez, G., 2007a. Temporal and spatial variation of phosphate distribution in the sediment of a free surface water constructed wetland. Sci. Total Environ. 380, 75–83.

Maine, M.A., Suñé, N., Hadad, H.R., Sánchez, G., Bonetto, C., 2007b. Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance. Chemosphere 68, 1105–1113.

Maine, M.A., Suñé, N., Hadad, H., Sánchez, G., Bonetto, C., 2009. Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J. Environ. Manage. 90, 355–363.

Moshiri, G.A., 1993. Constructed Wetlands for Water Quality Improvement. CRC Press, Boca Raton, FL.

Nuttall, C., Younger, P., 2000. Zinc removal from hard, circumneutral mine waters using a novel closed-bed limestone reactor. Water Res. 34, 262–1268.

O'Sullivan, A., 2001. Constructed Wetlands for Passive Biological Treatment of Mine Tailings Water at Tara Mines, Ireland. Ph.D. Thesis. Department of Botany, Division of Biosciences, University College Dublin, Ireland.

O'Sullivan, A.D., Moran, B.M., Otte, M.L., 2004. Accumulation and fate of contaminants (Zn, Pb, Fe and S) in substrates of wetland constructed for treating mine wastewater. Water Air Soil Pollut. 157, 345–364.

Salt, D., Blaylock, M., Kumar, N., Dushenkov, V., Ensley, B., Chet, I., Raksin, I., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13, 468–473.

Sobolewski, A., 1996. Metal species indicate the potential of constructed wetlands for long-term treatment of metal mine drainage. Ecol. Eng. 6, 259–271.

Sorrell, B.K., Boon, P.L., 1992. Biogeochemistry of billabong sediments II. Seasonal variations in methane production. Freshwater Biol. 27, 435–445.

Stone, M., Droppo, I.G., 1996. Distribution of lead, copper and zinc in size-fractionated river bed sediment in two agricultural catchments of southern Ontario, Canada. Environ. Pollut. 3, 353–362.

Stone, M., Marsalek, J., 1996. Trace metal composition and speciation in street sediment: sault Ste. Marie, Canada. Water Air Soil Pollut. 87, 149–169.

Tessier, A., Campbell, P., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51 (7), 844–851.

Ure, A.M., Davidson, C.M., 2001. Chemical speciation in soils and related materials by selective chemical extraction. In: Ure, A.M., Davidson, C.M. (Eds.), Chemical Speciation in the Environment. Blackie, Glasgow, pp. 265–321.

Vesk, P., Allaway, W., 1997. Spatial variation of copper and lead concentrations of water hyacinth plants in a wetland receiving urban run-off. Aquat. Bot. 59, 33–44.

Vymazal, J., Brix, H., Cooper, P.F., Green, M.B., Haberl, R., 1998. Constructed Wetlands for Wastewater Treatment in Europe. Backhuys Publishers, Leiden.

Wood, T., Shelley, M.A., 1999. Dynamic model of bioavailability of metals in constructed wetland sediments. Ecol. Eng. 12, 231–252.

Yu, K.-C., Tsai, L.-J., Chen, S.-H., Ho, S.-T., 2001. Chemical binding of heavy metals in anoxic river sediments. Water Res. 35 (17), 4086–4094.