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Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance

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Abstract

A free water surface wetland was built to treat wastewater containing metals (Cr, Ni and Zn) and nutrients from a tool factory in Santo Tomé, Santa Fe, Argentina. *Eichhornia crassipes* became dominant and covered about 80% of the surface throughout the first year, and decreased progressively until its disappearance. When water depth was lowered *Typha domingensis* steadily increased plant cover and attained 30% of the surface by the end of the study. While *E. crassipes* was dominant, the wetland retained 62% of the incoming Cr and 48% of the Ni. NO_3^- and NO_2^-, were also removed (65% and 78%, respectively), while dissolved inorganic phosphate (i-P_{diss}) and NH_4^+ were not removed. Zn was below 50 $\mu g\, l^{-1}$ in both the influent and effluent. Metal concentration in the sediments did not increase and retention was mediated through macrophytes uptake. During the period of *E. crassipes* decline the wetland retained 49% of the incoming Cr, 45% of Ni, 58% NO_3^-, 94% NO_2^-, 58% NH_4^+ and 47% i-P_{diss}. Cr, Ni and Zn in the bottom sediment increased in the inlet but not in the outlet. Since *T. domingensis* became dominant, retention was 58% Cr, 48% Ni and 64% i-P_{diss}, while 79% NO_3^-, 84% NO_2^- and 13% NH_4^+ were removed. Metals in the bottom sediment increased in the inlet. In spite of the significant growth of *E. crassipes* at the beginning, *T. domingensis* remained after most of the transplanted macrophytes had disappeared. Macrophyte disappearance could be related to the overall toxicity of several environmental constrains as high pH and conductivity, metal concentration, and sulphide presence.

Keywords: Metals; Nutrients; Constructed wetland; Effluents

1. Introduction

Constructed wetlands constitute complex ecosystems, the biological and physical components which interact to provide a mechanical and biogeochemical filter capable of removing many contaminants. They were initially utilized for nutrient removal in domiciliary and municipal sewage, storm water and agricultural runoff displaying a wide range of removal efficiencies (Hammer, 1989; Moshiri, 1993; Kadlec and Knight, 1996; Vymazal et al., 1998; Kadlec et al., 2000). At present, the application of wetlands for industrial wastewater treatment represents a promising alternative. Bahco metallurgic factory constructed a small-scale experimental wetland to assess the feasibility of treating wastewater from the Santo Tomé, Santa Fe (Argentina) tool factory. The wetland, which was 6 m long, 3 m wide and 0.3 m deep, retained 81%, 66% and 59% of the incoming Cr, Ni and Zn, respectively, and removed 84% and 75% of the inorganic nitrogen and dissolved inorganic phosphate (i-P_{diss}) from the incoming wastewater (Maine et al., 2005). However, present experience in Argentina

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remains largely unreported. In principle, the conditions are favorable since low population densities are found together with a large availability of marginal land close to the cities. The central and northern part of the country has mild winters, allowing extended growth periods for the vegetation. Macrophytes are assumed to be the main biological component of wetlands. They not only assimilate pollutants directly into their tissues, but also act as catalysts for purification reactions by increasing the environment diversity in the rhizosphere promoting a variety of chemical and biochemical reactions that enhance purification (Jenssen et al., 1993). *Eichhornia crassipes* (Mart.) Solms. is commonly used in constructed wetlands because of its fast growth rate and large uptake of nutrients and contaminants (Tchobanoglous et al., 1989; Vesk and Allaway, 1997). It attains dense stands in the floodplain wetlands of the Middle Paraná River close to the study site. *Typha domingensis* Pers. is a rooted macrophyte that had demonstrated to be highly efficient in the accumulation of nutrients and contaminants in natural and constructed wetlands (Gersberg et al., 1986; Ellis et al., 1994; Manios et al., 2003).

Because of the high removal efficiency attained in the small-scale experimental wetland a large-scale wetland was constructed at Bahco S.A. for the wastewater treatment of the whole factory. The large-scale wetland has now been in operation for more than two years. Plant growth showed three different periods, the first one dominated by *E. crassipes*, followed by a decline period and a subsequent period of dominance of *T. domingensis*. The removal efficiency and plant growth were studied from the beginning of the wetland operation. The results are compared with those from the previous small-scale prototype and the observed differences are discussed.

2. Study site

A free water surface wetland was constructed at the Bahco Argentina metallurgic plant, located in Santo Tomé, Santa Fe, Argentina (S 31°40′; W 60°47′). It is 50 m long, 40 m wide and 0.5–0.8 m deep, with a central baffle dividing it into two identical sections forcing the effluent to cover double the distance, reaching a 5:1 length–width ratio. Mean wastewater discharge was 100 $m^3\ d^{-1}$ throughout the experiment. Water residence time ranged from 7 to 12 d. The wetland was rendered impermeable by means of bentonite to reach a hydraulic conductivity of $10^{-7}\ ms^{-1}$ (five compacted layers of bentonite – approximate total depth: 0.6 m). One meter of soil was placed on the bentonite layer. Several locally available macrophytes were transplanted into the wetland: *E. crassipes*, *T. domingensis* and *Pontederia cordata* L.

The wetland receives the wastewater through a PVC pipe provided with a perpendicular distribution pipe with holes poked at regular intervals in order to allow the entrance of water in a wide and uniform way. The effluent, after passing through the wetland, is led by an excavated channel into a 1.5 ha pond (3–5 m depth).

Both wastewater from the industrial processes and sewage from the factory were treated together. It was expected that high nutrient concentrations could increase macrophyte tolerance to the toxic wastewater (Manios et al., 2003). Effluents reached the wetland after a primary treatment (precipitation, sieving and decantation). During the first stage (five months) of the wetland operation only diluted sewage of the factory was poured. The composition of the effluent was 25 $m^3\ d^{-1}$ of sewage + 75 $m^3\ d^{-1}$ of lake water. Later, industrial wastewater and sewage were treated together (25 $m^3\ d^{-1}$ of sewage + 75 $m^3\ d^{-1}$ of industrial wastewater).

Macrophytes in the nearby undisturbed ponds of the Middle Paraná River floodplain were also sampled in order to compare their biomass with those of the studied constructed wetland. *E. crassipes* was sampled in a pond of approximately 0.25 ha with a depth of 50 cm. *T. domingensis* was collected from a floodplain wetland, showing intermittent contact with a Paraná River branch, where it grew in dense monoespecific stands.

3. Material and methods

3.1. Water

Thirty six samples of the influent and effluent were taken from March 2003 until December 2004. Sampling frequency was about every two weeks from March to October 2003 and roughly monthly since then. Samples were taken by triplicate.

Conductivity was measured with an YSI 33 conductometer, 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 APHA (1998); NO_2^- was determined by coupling diazotation 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%), total Kjeldahl nitrogen (TKN) by the Kjeldahl method. i-P_{diss} was determined by the colorimetric molybdenum blue method (Murphy and Riley, 1962). 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 (APHA, 1998). 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), following APHA (1998).

Statistical significance between influent and effluent concentrations was assessed using a mean comparison test ($p < 0.05$).

3.2. Sediment

Cr, Ni and Zn concentrations were determined monthly or bimonthly in the inlet and outlet areas of the wetland. Sediment samples were collected using a 4-cm diameter PVC corer by triplicate. All the samples were transported to the laboratory in the cold. Samples were digested with an $HClO_4$:HNO_3:HCl (7:5:2) mixture. Cr, Ni and Zn were determined in the digests by atomic absorption spectrometry (Perkin–Elmer 5000).

ANOVA analysis was performed to evaluate the influence of time on metal concentrations. Duncan's test was used to differentiate means when appropriate. A level of $p < 0.05$ was used in all comparisons.

3.3. Macrophytes

Macrophytes were sampled monthly with a 0.50 × 0.50 m square sampler following Vesk and Allaway (1997). Four replicates were taken in each sampling. The macrophytes were then harvested and sorted by species in the laboratory, washed, separated between above (steams and leaves) and belowground parts (roots of floating species and roots plus rhizomes of emergent macrophytes). Cr, Ni and Zn in roots and leaves were determined in the same way as in sediment samples. To measure dry weight, plants were dried at 105 °C until constant weight (APHA, 1998). Plant cover was visually estimated measuring the area occupied by each species in the wetland. Statistical significance between plant parameters in natural and constructed wetlands was assessed using a mean comparison test ($p < 0.05$).

4. Results

4.1. Vegetation development

E. crassipes became dominant and covered about 80% of the surface throughout March 2003 to January 2004 and decreased progressively until its disappearance over the following six months (Fig. 1). In September 2003 roughly 20% of the biomass was harvested. In January 2004 the wetland was emptied for a few days, but the plants remained anchored in the mud, without apparent damage. *E. crassipes* decreased progressively since then to attain a very small cover in July 2004. In August 2004, the wetland was emptied again, the few remaining floating macrophytes were harvested and soil was added in two strips of 2 m wide, perpendicular to the water circulation. When the wetland was refilled, the water depth at the strips was 0.3 m, in order to favor *T. domingensis* growth. Both, *T. domingensis* and *E. crassipes* were again transplanted in August 2004. Although *E. crassipes* showed some initial growth, it soon decreased again.

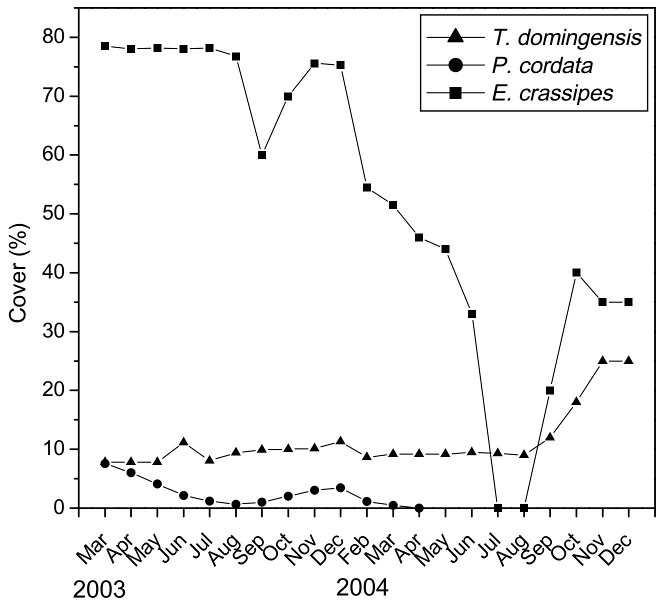

Fig. 1. Macrophyte cover in the wetland throughout the studied period.

E. crassipes biomass was significantly higher in the wetland than in the undisturbed natural environment along 2003 without differences between the inlet and outlet (Fig. 2). Since December 2003 biomass remained lower than in the natural environment showing a decreasing trend parallel to that of plant cover. *P. cordata* developed as an accompanying species attaining a cover lower than 5% along 2003 and disappeared in May 2004 (Fig. 1). *T. domingensis* developed in the borders covering roughly 4–13% of the wetland surface along 2003 (Fig. 1). After August 2004 plant cover steadily increased to attain roughly 30% of the surface at the end of 2004. *T. domingensis* biomass (Fig. 2) was significantly higher in the wetland than in the undisturbed natural wetland during all the studied period. The experimental period was, therefore, divided in the three successive stages described for the vegetation development: *E. crassipes* dominance, *E. crassipes* decline and *T. domingensis* dominance.

4.2. Wetland retention

Table 1 summarizes the mean concentrations of the influent and the effluent of the wetland, and the estimated removal efficiency in each stage. Removal efficiency reported for the first stage was estimated since the mixture of sewage and industrial wastewater was added. Comparing the concentration of variables measured at the inlet and the outlet at the different stages, there are significantly statistical differences, being the concentrations at the outlet significantly lower than at the inlet, except in the case of i-P_{diss} and NH_4^+. DO concentration in the influent showed a large variability being anoxic in many samples. In the effluent, DO concentrations ranged from 1 to 5 mg l^{-1} until June 2003 and became anoxic in most samples since then. BOD and COD decreased by 62–69% and 70–73%,

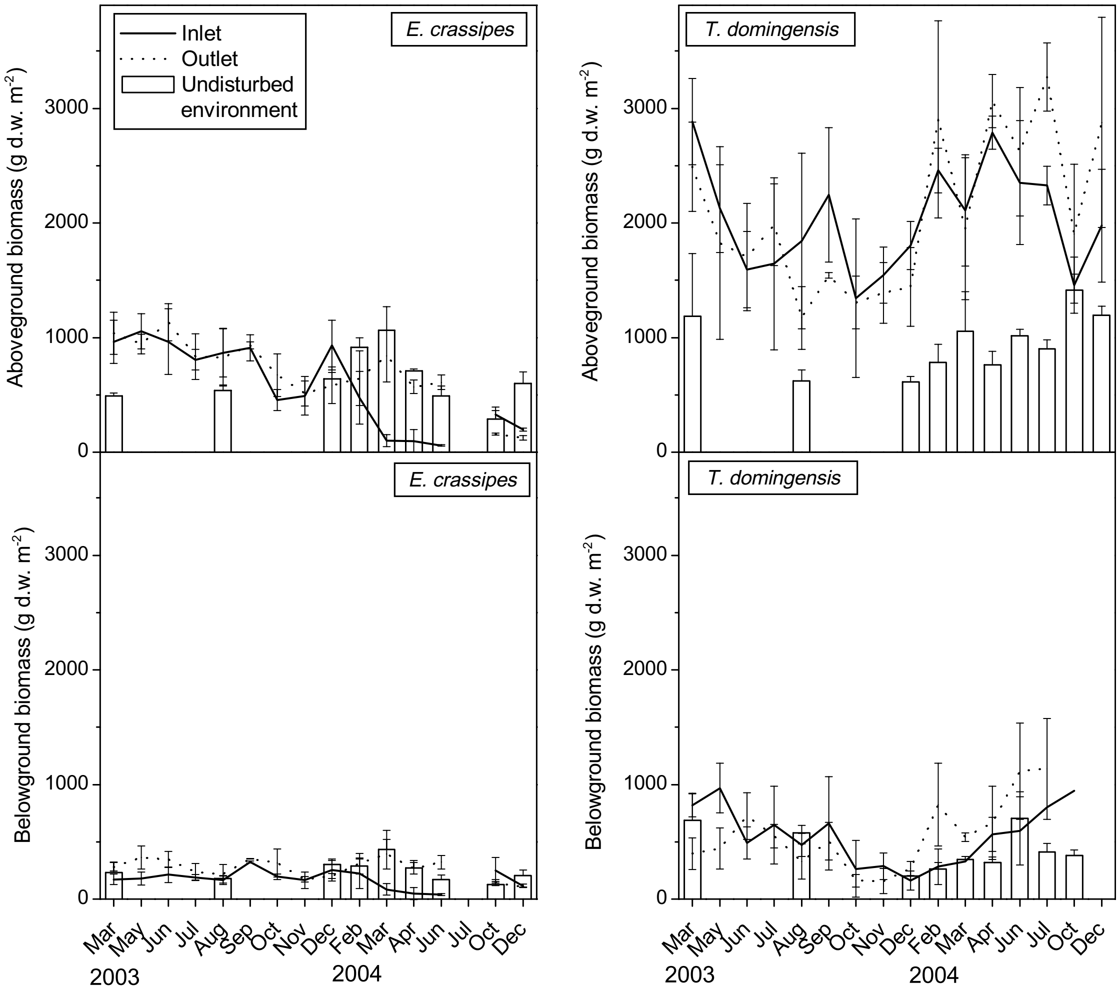

Fig. 2. *E. crassipes* and *T. domingensis* biomass in the wetland compared with a nearby undisturbed natural environment.

respectively, in the effluent. Water pH decreased from 7.2 to 10.8 in the influent to 6.9–8.1 in the effluent without differences among the several vegetation stages. Mean Ca concentrations decreased in the effluent, being retention larger in the samples in which the pH of the incoming water was higher (9.2–10.2). On the contrary, concentrations were often higher in the effluent when the pH of the incoming water was lower (7.2–7.6).

i-P_{diss} concentrations in the influent showed a large variation range. Concentrations were lower when water pH was above 9. i-P_{diss} was 5% higher throughout the *E. crassipes* dominance but was removed for 47% and 64% through the *E. crassipes* decline and *T. domingensis* dominance, respectively. Similarly, NH_4^+ concentration was 27% higher in the outlet during *E. crassipes* dominance but decreased 58% and 13% during the following *E. crassipes* decline and *T. domingensis* dominance periods, respectively. NO_3^-, NO_2^-, and SO_4^{2-} were removed 58–79%, 78–94% and 31–39%, respectively, at the effluent irrespective of plant cover.

Total alkalinity decreased in the effluent along the *E. crassipes* dominance and was larger in the effluent in the following periods. Fe, Ni and Cr were retained by 79–80%, 45–48% and 49–62%, respectively, without differences in the three vegetation stages. Zn concentration was below 50 $\mu g\, l^{-1}$ in both influent and effluent along the study period.

4.3. Metal concentration in plants and sediment

Metal concentration in plant tissue was significantly higher in roots than in leaves (Fig. 3). Large temporal variations were observed in roots along the studied period. Maximum Ni and Zn tissue concentrations in *E. crassipes* were determined in the last sampling before its disappearance in July 2004 and Cr in the sampling previous to the last. All three metals attained higher concentrations in *T. domingensis* roots in August, December 2003 and December 2004.

During the *E. crassipes* dominance phase, Cr, Ni and Zn concentrations in bottom sediments did not change significantly ($p < 0.05$) (Fig. 4). Some higher concentrations were observed in the inlet area in March–June 2004 during the *E. crassipes* decline stage and yet significantly higher concentrations were determined in the inlet during the *T. domingensis* increasing stage ($p < 0.05$).

Table 1
Mean water composition of the influent and the effluent of the wetland through the three successive stages of vegetation development and the comparison with the measured variables in the pilot-scale wetland (Maine et al., 2005)

Variables	Constructed wetland									Pilot-scale wetland		
	E. crassipes dominance			*E. crassipes* recede			*T. domingensis* dominance					
	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)
pH	8.8 ± 1.1	7.3 ± 0.3		8.8 ± 1.2	7.7 ± 0.2		8.0 ± 0.8	7.7 ± 0.2		10.1 ± 1.54	7.98 ± 0.599	
Conductivity	2958 ± 2181	1327 ± 638		2371 ± 1035	1664 ± 349		3468 ± 1462	2175 ± 194		5130 ± 1465	3120 ± 1506	
DO (mg l^{-1})	2.6 ± 2.5	1.4 ± 1.8		1.0 ± 2.4	1.2 ± 3.1		0.7 ± 1.5	0.2 ± 0.3		6.2 ± 3.1	3.2 ± 1.7	
BOD (mg l^{-1})	43 ± 25	13 ± 10	65	53 ± 30	12.0 ± 7.9	69	42 ± 16	15 ± 4.6	62	136 ± 108	15 ± 9.8	78
COD (mg l^{-1})	200 ± 140	41 ± 20	70	164 ± 174	29.0 ± 24.9	71	101 ± 62	31 ± 13.3	73	276 ± 160	40 ± 16	78
i-P_{diss} (mg l^{-1} P)	0.19 ± 0.15	0.19 ± 0.13	−5	0.11 ± 0.12	0.03 ± 0.02	47	0.17 ± 0.08	0.03 ± 0.01	64	0.55 ± 0.69	0.14 ± 0.15	75
NO_3^- (mg l^{-1} NO_3^-)	17 ± 18	3 ± 2	65	13.5 ± 11.1	3.4 ± 3.1	58	12 ± 8.3	2.5 ± 0.7	79	20 ± 19.8	1.5 ± 0.87	88
NO_2^- (mg l^{-1} NO_2^-)	0.77 ± 1.01	0.08 ± 0.21	78	0.77 ± 0.66	0.04 ± 0.09	94	0.44 ± 0.64	0.06 ± 0.05	84	2.2 ± 3.15	0.15 ± 0.24	85
NH_4^* (mg l^{-1} NH_4^*)	2 ± 2.5	2.2 ± 3.7	−27	2.0 ± 1.1	1.1 ± 1.2	58	3.6 ± 2.9	2.9 ± 1.9	13	2.2 ± 1.4	2.1 ± 2.7	13
SO_4^{2-} (mg l^{-1})	985 ± 736	423 ± 211	37	647 ± 650	473 ± 210	31	1661 ± 1043	740 ± 228	39	1440 ± 470	929 ± 415	35
$CaCO_3$ (mg l^{-1})	472 ± 297	247 ± 83	32	300 ± 149	325 ± 63	–	206 ± 120	278 ± 27	–	519 ± 95	671 ± 106	–
Ca^{2+} (mg l^{-1})	162 ± 165	44 ± 14	41	91 ± 45	85 ± 44	19	182 ± 118	88 ± 25	36	171 ± 78	97 ± 62	41
Fe (mg l^{-1})	14.4 ± 17.6	0.35 ± 0.34	79	2.8 ± 3.6	0.12 ± 0.11	79	2.4 ± 0.7	0.09 ± 0.04	80	9.09 ± 10.7	0.21 ± 0.19	82
Cr (μg l^{-1})	14 ± 3.3	2.0 ± 0.9	62	4.0 ± 2.2	2.0 ± 0.4	49	13 ± 24	5.0 ± 1.1	58	127 ± 195	13 ± 25	81
Ni (μg l^{-1})	15 ± 13	8.0 ± 5.8	48	10 ± 7.8	4.0 ± 1.0	45	47 ± 41	27 ± 5.0	48	181 ± 211	51 ± 63	66

Conductivity is expressed in μmho cm^{-1}.

Table 2 compares the metal retention percentages of sediment and macrophytes in the successive vegetation stages. During *E. crassipes* dominance, macrophytes account for most of the retained metals. During the *E. crassipes* decline stage, metals were accumulated in the sediment. In the *T. domingensis* dominance stage, metals accumulated both in sediment and macrophytes, but their concentration was higher in sediment.

5. Discussion

5.1. Vegetation development

The development of the vegetation in both the small-scale prototype (Hadad et al., 2006) and the large-scale wetland showed the same pattern but occurred at a different time scale. In the former, *E. crassipes* and *Pistia stratiotes* L. started active growth attaining roughly 60% cover in three months. However, they soon started to decay and disappeared from the wetland in the sixth month. In the latter, *E. crassipes* covered most of the surface for almost a year. The following *E. crassipes* decline stage took another six months. After emptying the wetland and adding more soil, *E. crassipes* again started active growth attaining 40% cover but soon started to decline again (Fig. 1). Metal and nutrient concentration, pH and conductivity in the incoming wastewater were higher in the small-scale prototype. Table 3 shows Cr, Ni and Zn concentrations in *E. crassipes* tissues in the last sampling before disappearance in both wetlands and in *T. domingensis* at the end of the studied period in the small-scale wetland and at the maximum concentration in the large-scale wetland. Cr, Ni and Zn concentrations were higher in the roots and lower in the leaves of the large-scale wetland than in the pilot scale wetland, except for Zn in the roots of *E. crassipes*. Metal translocation from roots to leaves was larger at the most toxic condition prevailing in the small-scale wetland, and lower at the less toxic condition in the large-scale wetland.

Newly formed biomass during the growing period contributed to dilute the metal concentration in tissues. However, metal concentration decreased simultaneously with *T. domingensis* belowground biomass from September to December, suggesting selective allocation of metals to senescent tissues as observed by Villar et al. (1999) in natural floodplain marshes of the Paraná River.

Metal concentration in the incoming wastewater (Table 1) as well as plant tissue (Table 3) measured in this study looks modest as compared with concentrations reported in the literature. Ellis et al. (1994) reported an order of magnitude higher Zn concentration in *Typha latifolia* roots (700 μg g^{-1} dry weight) in a natural wetland receiving storm water loads in London. Manios et al. (2003) compared chlorophyll concentration of plants exposed to different metal concentrations, reporting threshold damage for a mixture of Cd, Cu, Ni, Pb and Zn of 4, 80, 40, 40 and 80 mg l^{-1}, respectively. Zn concentration in plant tissue was

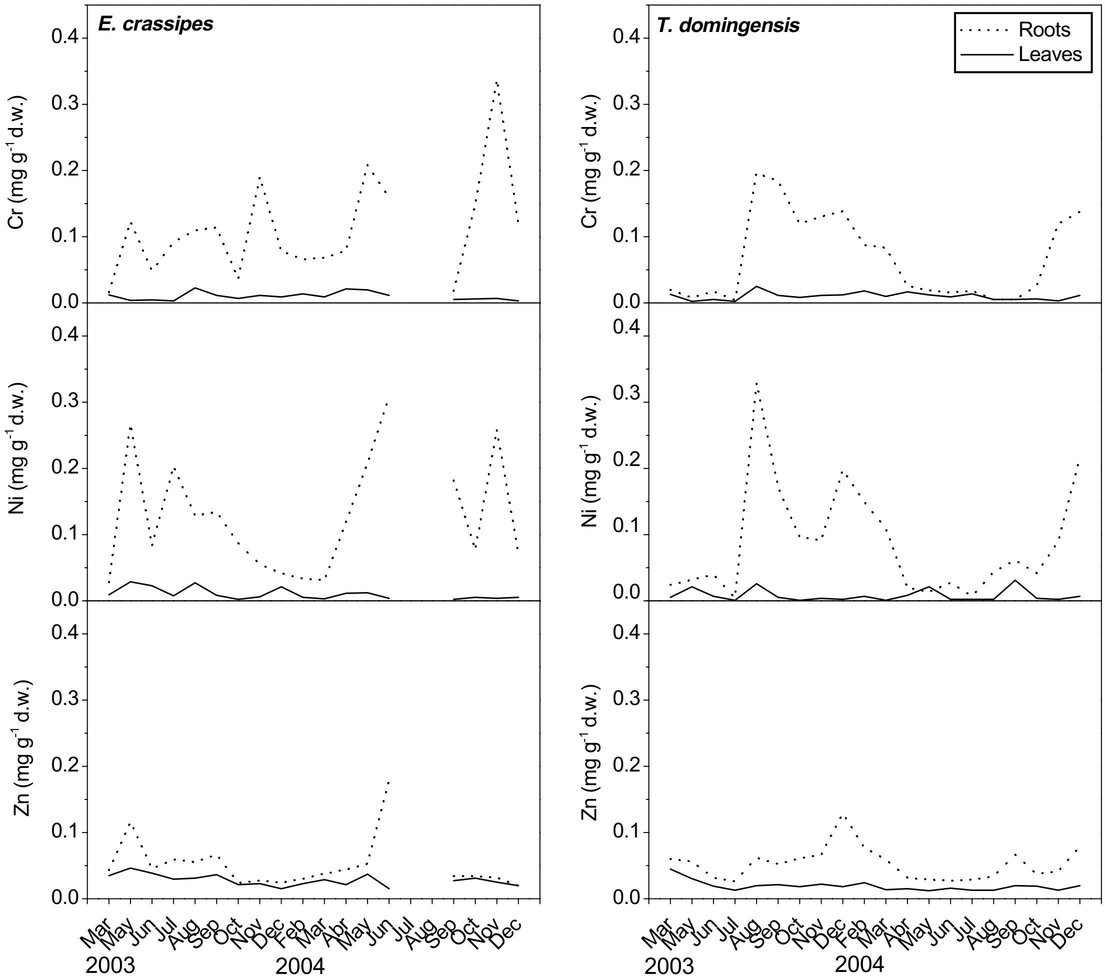

Fig. 3. Cr, Ni and Zn concentrations in roots and leaves of *E. crassipes* and *T. domingensis* during the studied period.

392 μg g^{-1} in roots and 61 μg g^{-1} in leaves. However, Ni concentration in plant tissue amounted to 55 and 28 μg g^{-1} in roots and leaves, respectively, lower than in our study. Soltan and Rashed (2003) studied *E. crassipes* tolerance to increasing concentrations of metal mixtures. A mixture of 3 mg l^{-1} of Cr, Ni, Zn, Cd, Co, Cu, Mn and Pb was not toxic and resulted in Cr tissue concentrations of 1950 μg g^{-1} in roots and 58 μg g^{-1} in leaves. Ingole and Bhole (2003) reported that *E. crassipes* exposed at concentrations of 5 mg l^{-1} of Cr, Ni, Hg, Pb, As and Zn, attained a normal plant growth. Damage became evident at concentrations greater than 10 mg l^{-1}.

Tolerance thresholds for pH and conductivity were experimentally determined for *E. crassipes*, *P. stratiotes* and *Salvinia herzogii* De la Sota (Hadad et al., 2006). *E. crassipes* tolerance thresholds for pH and conductivity lay between 9 and 10 and 3–4 mS cm^{-1}, respectively. Soltan and Rashed (2003) reported growth inhibition for *E. crassipes* at a pH of 9.5. In most samples in the small-scale wetland, conductivity and pH were higher than the tolerance thresholds (Maine et al., 2005) while in the large-scale wetland pH and conductivity surpassed the threshold in 30% of the samples. Other conditions prevailing in the large-scale wetland might contribute to the observed disappearance of most transplanted macrophytes. Soluble sulphide is highly toxic for macrophytes (Koch and Mendelssohn, 1989). A decrease of SO_4^{2-} concentrations in the outlet and sulphide smell in most samples suggests sulphide release from the wetland. The long term development of most transplanted macrophytes was impaired not only due to a single toxic element but rather to the overall toxicity produced by the synergetic effect of the contaminants.

Present results suggest that floating macrophytes in general and *E. crassipes* in particular do not represent an advisable macrophyte for its utilization in industrial wastewater treatment. On the other hand, *T. domingensis* seems the best alternative for the given conditions since it survived until the end of the studied period in both wetlands without any symptom of toxicity being observed and attaining a biomass significantly higher in the wetland than in the nearby undisturbed floodplain environment. Among the emergent macrophytes, *Schoenoplectus* spp. and *Typha* spp. were noted to develop a higher tolerance to metals than other macrophytes (Cardwell et al., 2002), suggesting that tolerance is related to plant phenology, vigour and growth.

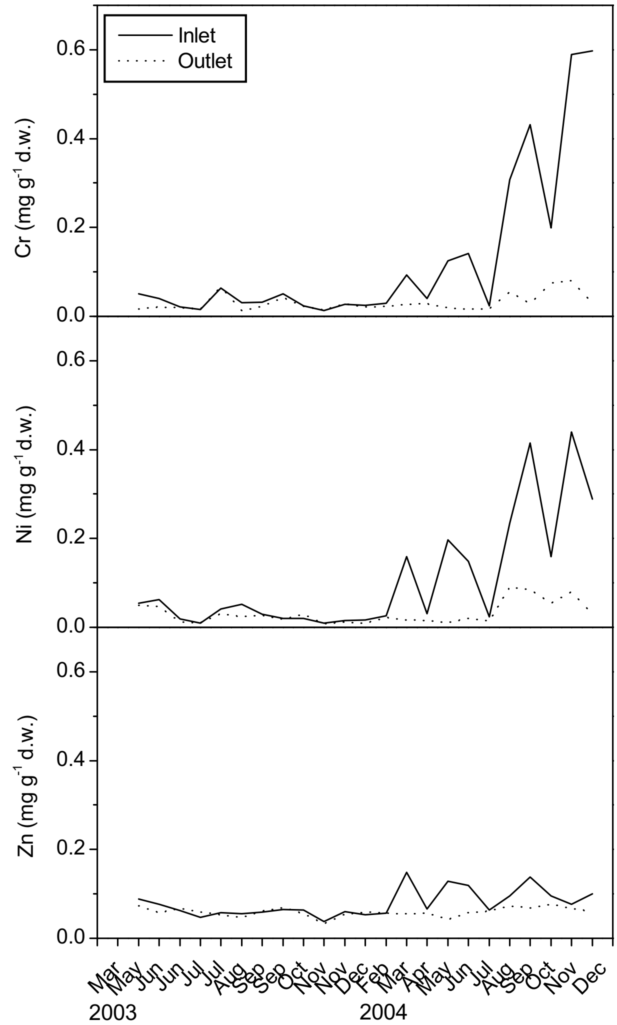

Fig. 4. Cr, Ni and Zn concentrations in sediment in the inlet and outlet area during the studied period.

5.2. Wetland retention

Large organic matter mineralization, as revealed by the decreased BOD and COD in the effluent, resulted in DO depletion from the first months of operation. Organic matter mineralization represented a large contribution of CO_2. CO_2 concentration increase, denitrification and sulfate reducing processes probably caused the pH decrease from 7.2 to 10.2 in the influent to 7.0–8.3 in the effluent. Alternative Ca^{2+} retention or release accompanying pH fluctuations in the incoming wastewater suggest alternative $CaCO_3$ precipitation or dissolution governed by the influent wastewater composition.

Table 3
Maximum metal concentration in plant tissue in the pilot-scale wetland (Maine et al., 2005) and in the large wetland

Macrophytes		Pilot-scale wetland		Large wetland	
E. crassipes		$\mu g\ g^{-1}$	Roots:Leaves	$\mu g\ g^{-1}$	Roots:Leaves
Cr	Leaves	27		23	
	Roots	102	3.8	208	9.1
Ni	Leaves	93		29	
	Roots	130	1.4	308	10.6
Zn	Leaves	91		46	
	Roots	183	2.0	179	3.9
T. domingensis					
Cr	Leaves	36		25	
	Roots	110	3.1	196	7.8
Ni	Leaves	32		31	
	Roots	70	2.2	329	10.6
Zn	Leaves	39		24	
	Roots	67	1.7	129	5.4
Range			1.4–3.8		3.9–10.6

As the wetland water remained anoxic most of the time, it was not surprising that NO_3^-, NO_2^-, and SO_4^{2-} were removed throughout the study period irrespective of the dominant vegetation. Moreover, the incoming NO_3^- and SO_4^{2-} represented an O_2 load one and two orders of magnitude larger than the DO of the incoming wastewater. Because of the high SO_4^{2-} concentration in the incoming wastewater, most of the organic matter mineralization took place by biological SO_4^{2-} reduction as observed in coastal marine sediment where SO_4^{2-} reduction is responsible for 25–79% of the total organic matter mineralization (Giblin, 1988). H_2S released by SO_4^{2-} reducing bacteria reacts with detrital Fe in sediment to form FeS minerals. Several monosulphide minerals early precipitated are later converted to pyrite (Giblin, 1988). FeS formation depends on the rate of Fe(II) and sulphide 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 constructed wetland. Giblin and Howarth (1984) observed that episodic Fe shortages limited pyrite formation and increased sulphide concentration in pore water depressing *Spartina* spp. growth in salt marshes of the eastern United States. Similarly, it seems plausible that the comparatively small Fe supply to the constructed wetland enhances sulphide production and contributes to the overall toxicity of the wastewater.

Mass balance suggested that N retained by plants represented a minor fraction of the N removed from the incoming wastewater in the small-scale wetland (Maine et al.,

Table 2
Metal distribution (%) in macrophytes and sediment in the wetland during the three studied stages

Stages	Cr		Ni		Zn	
	Sediment	Macrophytes	Sediment	Macrophytes	Sediment	Macrophytes
E. crassipes dominance	11	89	7	93	1	99
E. crassipes decline	99	1	97	3	99	1
T. domingensis dominance	73	27	54	46	69	31

2005). Denitrification may represent the major removal process given the observed DO depletion. Consistently, several different studies have shown denitrification to be a major pathway in wetlands. D'Angelo and Reddy (1993) determined that most of the $^{15}N–NO_3^-$ (roughly 90%) applied to sediment-water cores were lost by denitrification. Reddy et al. (1989) measured large denitrification rates in the rhizosphere of emergent macrophytes of deltaic marshes. Matheson et al. (2002) performed ^{15}N balances in wetland microcosms estimating that denitrification accounted for 61% of the NO_3^- load, 25% was retained in the soil, and 14% was stored in the vegetation biomass. Minzoni et al. (1988) measured large N losses through denitrification in rice field enclosures, and Golterman et al. (1988) confirmed the results by mass balances performed in experimental plots.

NH_4^+ showed a different behaviour at the different phases of the vegetation development. *E. crassipes* produce a large amount of detritus, which decomposes in the anoxic bottom reducing the redox potential in the water column. Organic matter mineralization represents a source of NH_4^+, which is not nitrified because of DO depletion and therefore NH_4^+ was often higher in the effluent than in the influent. During the *E. crassipes* decline period, decreased plant cover likely allowed planktonic and periphytic growth and higher O_2 contribution from the atmosphere to the water column. Emergent macrophytes are known to release O_2 from the roots producing a strong positive effect on nitrifying bacteria in the rhizosphere (Bodelier et al., 1996). ANAMMOX process (acronym for: ANaerobic AMMonia Oxidation; Sliekers et al., 2002) has been shown to be a quantitatively important pathway in reactors (Sliekers et al., 2002), wastewater (Strous et al., 2002) and marine sediments (Thamdrup and Dalsgaard, 2002). The simultaneous occurrence of partial NH_4^+ oxidation and denitrification likely accounted for the observed NH_4^+ removal through the *E. crassipes* decline and *T. domingensis* dominance phases. The pattern of NH_4^+ removal as *T. domingensis* cover increased is consistent with the observed NH_4^+ removal in the small-scale prototype in which *T. domingensis* was dominant (Maine et al., 2005).

The low i-P_{diss} concentrations in the influent in coincidence with high pH, Ca^{2+} and carbonate suggest co-precipitation with $CaCO_3$. An increase of the calcium-bound P fraction in the bottom sediments at the inlet was observed in the small-scale wetland prototype (Maine et al., 2005) as well as in the large-scale wetland (Maine et al., 2007).

As pH decreases, i-P_{diss} sorption to carbonates decreases while adsorption to Fe oxy hydroxides increases (Golterman, 1995). However, DO depletion prevented adsorption to iron, resulting in often higher i-P_{diss} concentrations in the effluent throughout the period of *E. crassipes* dominance. Mineralization of organic matter contributed to the observed increased concentration in the effluent. During the *E. crassipes* decline and the *T. domingensis* dominance stages i-P_{diss} was retained within the wetland. As previously stated, enhanced phytoplanktonic and periphytic growth and O_2 transfer from the atmosphere as well as O_2 release by emergent macrophyte roots probably created niches of high redox potential where i-P_{diss} might have been adsorbed onto Fe oxy hydroxides and later settled on the bottom sediment.

Cr, Ni and Zn concentrations in the bottom sediment did not increase through the *E. crassipes* dominance phase in spite of the fact that between influent and effluent concentrations revealed wetland retention. *E. crassipes* covered 80% of the wetland and was responsible for the high metal-removal. Floating macrophytes may easily be harvested, leading to fast metal removal rates. Through the *E. crassipes* decline and *T. domingensis* dominance stages, a trend to increase bottom sediment concentration became evident. In the *E. crassipes* decline stage, sediment accounted for 97–99% of the metal removal, while in the last stage the sediment was the responsible for 54–73%. Although Zn concentration in the influent and effluent remained below detection limits in most samplings, sediment accumulation during the *E. crassipes* decline and *T. domingensis* dominance suggest that the wetland effectively retained Zn at the later stages.

6. Conclusions

The large-scale constructed wetland retained metals efficiently. Three successive phases of vegetation development were observed and three different patterns of metal retention were attained. During the *E. crassipes* dominance, metals were retained in the macrophyte biomass, during *E. crassipes* decline period metals were retained in the sediment and in the *T. domingensis* dominance period, metals were retained mainly in sediment but, a 27% Cr to 46% Ni was also retained in the macrophyte biomass. NO_3^- and SO_4^{2-} were removed in the three vegetation stages. i-P_{diss} and NH_4^+ were not efficiently removed during the *E. crassipes* dominance and were retained in the following stages.

In spite of the significant growth of *E. crassipes* at the beginning, a dense stand of *T. domingensis* remained after most of the transplanted macrophytes had disappeared. Macrophyte disappearance could be related to the overall toxicity of several environmental constrains as high pH and conductivity, metal concentration, and sulphide presence.

Because of its higher tolerance and efficient nutrient and metal removal *T. domingensis* is the best choice to treat wastewater of high pH and conductivity with heavy metals, a common result from many industrial processes. *T. domingensis* development could be favored by regulating water level, attaining best growth at 30 cm depth. The high biomass attained without any symptom of toxicity and the comparatively low metal concentration in plant tissue in *T. domingensis* after two years operation suggest that the wetland could continue in operation for a long time representing a long term sustainable wastewater treatment.

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