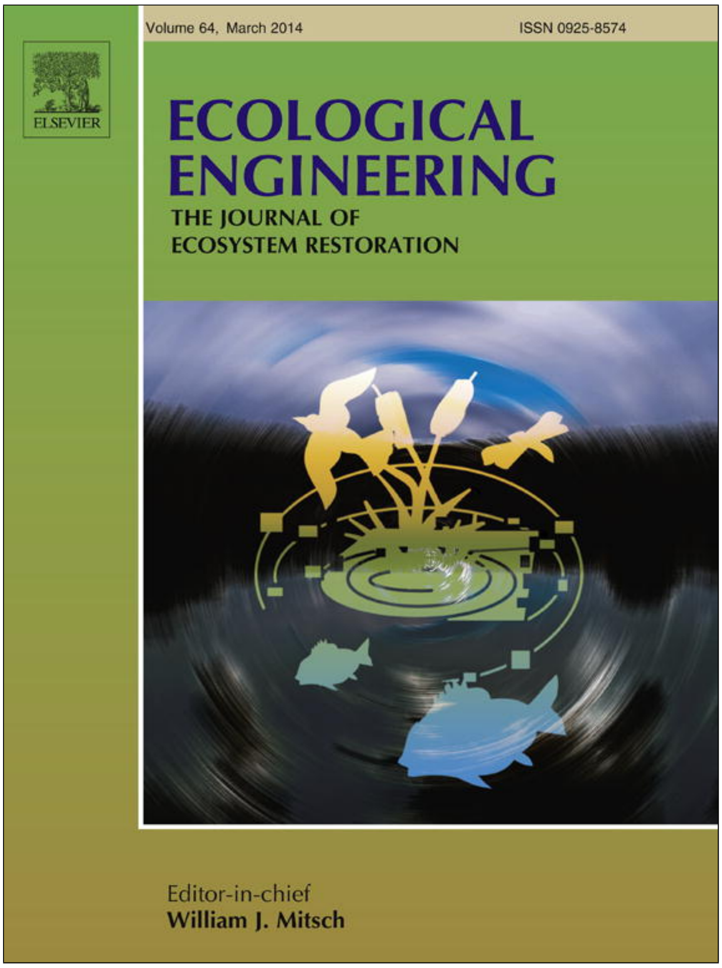
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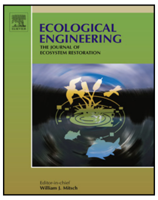

Metal accumulation and distribution in the organs of Reeds and Cattails in a constructed treatment wetland (Etueffont, France)

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ABSTRACT

Concentrations of trace elements were studied in roots, rhizomes, stems, leaves and flowers of cattails (*Typha latifolia* L.) and reeds (*Phragmites australis* L.) and in corresponding samples of water and sediment. Samples were taken from the inflow/outflow points in the fourth of four interconnecting lagooning ponds in constructed treatment wetlands, developed as an integrated pilot system for the treatment of leachates in a domestic landfill site at Etueffont (Territoire de Belfort, France). The elements considered were Ag, Al, As, B, Cd, Cr, Cu, Fe, Mn, Ni, Se, Sn and Zn. The highest average of above-ground water biomasses of *Typha latifolia* and *Phragmites australis* was recorded in fall with 0.85 and 1.13 kg dry weight m^{-2} respectively. The greatest mean concentrations of metals were found during spring in roots and to a lesser extent in the rhizomes of the two species. *T. latifolia* and *P. australis* can be used as bioindicators of Al, As, Fe, Mn, Cu, Cr and Cd and more specifically of Ni and Zn for *T. latifolia* and of B for *P. australis*. These two species may be considered promising alternatives for bioremediation.

1. Introduction

Constructed and artificial wetlands represent one of the alternative methods for purifying waters contaminated by heavy metals (Tam and Wong, 1994; Eger, 1994) before their discharge into rivers. Presenting great ecological and environmental advantages, along with economic and social benefits as well (Herath, 2004), constructed wetlands (CWs) are increasingly used in Europe and North America as an efficient water treatment system for domestic and municipal sewage, agricultural runoff, storm water and landfill leachate (Moshiri, 1993; Martin and Moshiri, 1994; Bulc et al., 1997; Vymazal et al., 1998, 2010; Kadlec et al., 2000; Ibekwe et al., 2003; Tchobanoglous et al., 2003; Hadad et al., 2009). Under variable degrees of chemical pressure, organisms living in CWs must tolerate high levels of heavy metals as well as high organic and nutrient loads (Kabata-Pendias and Pendias, 2001; Schwarzbauer et al., 2002). According to Maine et al. (2006), wetlands allow good retention of heavy metals and several studies from authors worldwide have shown the capacity of macrophytes for accumulation and sequestration of metals in above- (shoot) and below-ground (root) plant parts in relation with water, soil and sediment contents. Their resistance to high pollution levels in water and sediment as well, explain the frequent use of widely distributed species such as *Phragmites australis* L. (common reed), and to a lesser extent *Typha latifolia* L. (cattail) in many temperate aquatic ecosystems for phytoremediation of metals in wetlands (Simpson et al., 1983; Dunbabin and Bowmer, 1992; Kadlec and Knight, 1996; Ye et al., 1997; Mays and Edwards, 2001; Weis and Weis, 2004; Maddison et al., 2005; Southichak et al., 2006; De Feo, 2007; Duman et al., 2007; Bragato et al., 2009; Brisson and Chazarenc, 2009; Grisey et al., 2011; Anjum et al., 2012; Stefanakis and Tsihrintzis, 2012). High biomass production, a fast growth rate and the ability to tolerate and/or accumulate a wide range of pollutants in below- and above-ground organs are required characteristics of plants used to reduce metal concentrations in waters and sediments from natural and artificial wetlands (Reddy and Debusk, 1985; Cooper et al., 1996). Defined as tolerant species (but not hyperaccumulator), these two macrophytes species are therefore widely used for heavy metal removal in constructed wetlands (Dunbabin and Bowmer, 1992; APHA, 1998; Lesage et al., 2007a,b; Southichak et al., 2006; Bragato et al., 2006; Grisey et al., 2011) where significant correlations are observed between concentrations of these elements measured in plant tissues and the surrounding water and sediment (Markert, 1987; Bonanno, 2011).

According to Stoltz and Greger (2002) and Bragato et al. (2006), the lower accumulation of heavy metals in above-ground plant tissues protect the aerial parts and especially the photosynthetic apparatus from phytotoxic effects of some heavy metals such as Cr,

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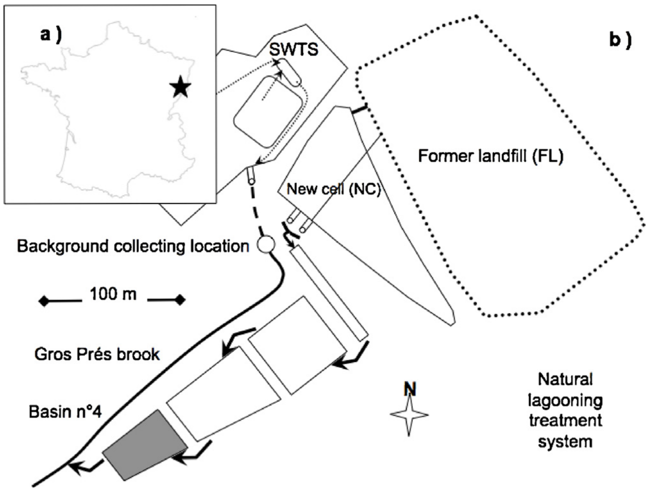

Fig. 1. a – Etueffont landfill location; b – schematic map of the site: former landfill (FL), new cell (NC), fourth pond of the natural lagooning treatment system with collecting points.

Cu and Zn. In addition, translocation of heavy metals from below-to above-ground plant parts (namely in senescing tissues) at the end of the growing season suggests a possible way to eliminate toxic elements by macrophytes (Bragato et al., 2009).

Effects of heavy metals on components of wetland treatment areas are of particular concern at the Etueffont landfill site (Territoire de Belfort, France) which is a pilot site for these studies, especially on macrophytes. The seasonal growth and heavy metal storage dynamics of reeds and cattails growing in the Etueffont lagooning system were investigated for three consecutive seasons in 2008 (Grisey et al., 2011). After the maintenance in fall, 2009 (i.e. clearing and grading) of the fourth pond of the constructed wetland, a mix of reed and cattail plants was planted at a mean density of $1/m^2$. The aim of the present study was to give, through a 2-year study, new insights into the metal storage capacities of the two aquatic macrophytes growing on the inlet and outlet sides of the fourth basin of the Etueffont lagooning system used for landfill leachate treatment. The seasonal growth dynamics of *P. australis* and *T. latifolia* were investigated and metal accumulation (Ag, Al, As, B, Cd, Cr, Cu, Fe, Mn, Ni, Se, Sn and Zn) in the below-ground (root and rhizome) and above-ground plant parts (stem, leaves and flowers) was determined in spring and autumn, along with their concentrations in corresponding water and sediment samples. The impact of the seasonal climatic conditions on heavy metal concentration in water and sediment inflow/outflow was studied and the corresponding accumulation in the above- and below-ground tissues of the two plants. This enabled us to determine the metal removal efficiency of the two aquatic macrophytes for improvement of water quality before discharge into a small stream. Differences in heavy metal accumulation in the two aquatic macrophytes are also discussed.

2. Materials and methods

2.1. Study site

The study was carried out over a 2-year period in the fourth and final pond of the lagooning system constructed for the treatment of leachates at the domestic landfill treatment station of Etueffont (Territoire de Belfort, northeastern France) (Fig. 1). Crushed household waste, collected from 66 municipalities (about 47,650 inhabitants), had been disposed of there, uncompacted, in the open air in a former landfill (between 1976 and 1999) and also at a new site known as the "new cell" (from 1999 to 2002). Composed of about 200,000 tons of waste in a layer 15 m thick when the site was closed in 2002, the landfill was then covered by a 0.4 m thick layer of artificial soil composed of crushed organic waste (paper, wood, lawn cuttings, straw, fabrics) (Khattabi et al., 2006, 2007). A drainage system was installed for downstream collection of leachates and their treatment in four natural lagooning ponds whose characteristics are summarized in Table 1. The water flow entering the lagooning system was adjusted to $59.4\,m^3\,day^{-1}$ during the 2010–2012 monitoring period with a retention time of about 19 days. The present study was carried out in the fourth and final pond, prior to water discharge into Gros Prés Brook, in the fall and spring for 2 consecutive years. The pond itself is situated over an underlying layer of clay 1 m thick that has a slope of 1%. After clearing and grading in fall, 2009, a mixture of cattails (*T. latifolia* L.) and reeds (*P. australis* L.) was planted at a mean density of $1/m^2$ (500 per species).

2.2. Water sampling

One hundred sixty-eight water samples were collected in pond 4 in 150-ml bottles from (a) three locations close to the inflow and (b) three locations at the outflow, as the water ran into the stream, every 2 days for 2 weeks each season, in fall (from 10/25/2010 to 11/07/2010, and from 10/17/2011 to 10/30/2011) and in spring (from 04/18/2011 to 05/01/2011, and from 04/16/2012 to 04/29/2012). After collection, samples were stored at 4 °C for preservation before preparation and analysis. The samples were filtered through a 0.45-μm membrane; 25 ml from each sample were treated with 6 ml of 65% v/v HNO_3 before analysis. Twenty-four samples for background values were collected in Gros Prés Brook on the same dates (Fig. 1). Ambient environmental factors at the wetland study site namely temperature, dissolved oxygen (DO%), pH, and electrical conductivity (portable multiparameter probe WTW, Multiline P3 PH/LF-SET) were determined *in situ* for each sampling campaign.

2.3. Macrophytes (reeds and cattails) and sediment sampling

Two macrophyte species (*T. latifolia* L. and *P. australis* L.), along with water and sediment, were sampled three times. The samples (roots/rhizomes, shoots) were collected from five plots (1 m × 1 m) near the inflow and outflow water collection locations. Five samples of the two species were collected individually, (a) during the spring growth period and (b), at the end of their summer growth period (fall) and were processed separately. Each set of roots, rhizomes, stems and leaves was thoroughly rinsed several times with deionized water before oven drying at 80 °C to constant weight (for 24 h) (Demirezen and Aksoy, 2004; Mishra et al., 2008). The dry samples were powdered in a mortar for analysis. For each plant, a 1-g dry wt. sample was digested with 3 ml HNO_3 and 1 ml H_2O_2 at 105 °C for 3 h in a microwave digestion system, according to the standard NF EN ISO 15587-2 (2002) before analysis by ICP-OES (720-ES, VARIAN).

Sediment was sampled with a sediment corer (10 cm diameter) at about 10–15 cm-deep so as to collect both sludge deposits and a small portion of the underlying clay. After being wet sieved through a 5.0-mm pore-size polypropylene mesh with reagent grade water to separate the sediment-size fraction and eliminate plant fragments, the samples were left to settle and the water was later decanted. The sediment clay-fractions were frozen at −18 °C, according to Annexe A of the standard NF EN 13346. After homogenization using a mortar and pestle, and dry-sieving through a 2.0 mm pore-size polypropylene mesh, 1 g of each sediment sample was digested with 3 ml HNO_3, 9 ml HCl at 105 °C for 3 h in

Table 1
Morphometric characteristics of the fourth pond.

	Ponds			
	1	2	3	4
Length (m)	78	46	66	48
Width (m)	5	43	28	23.5
Depth (m)	0.8	1	1	1
Thickness of sludge (m)	0.09	0.07	0.07	0.05
Area (m^2)	390	1934	1848	1128
Volume (m^3)	312	1934	1848	1128
Average flow rate ($m^3 s^{-1}$)	0.69×10^{-3}	0.69×10^{-3}	0.69×10^{-3}	0.69×10^{-3}
Residence time of water (day)	5	32	31	19

the microwave digestion system. All instruments were cleaned before and after each sample with 10% redistilled HNO_3 and then rinsed with reagent water. The heavy metal concentration in water, plant, and sediment samples was determined by ICP-OES (720-ES, VARIAN). International certified reference materials for the water (NIST-1643-e), plants (INCT-TL-1), and sediments (CRM-029) were analyzed at the beginning and end of each batch of samples for accuracy and precision. Instrument performance during analysis was monitored using an internal standard. For both macrophytes and sediment analysis, internal control standards were analyzed with each sample, and a duplicate was run every 10 samples. The detection limits with ICP-OES were 0.04 mg l^{-1} for Se and Sn; 0.02 mg l^{-1} for Ag, Al, As, B, Fe, Ni and Zn; 0.01 mg l^{-1} for Cd, Cr and Cu. Data outputs were expressed in mg l^{-1} for water, in mg kg^{-1} dry weight for sediment and plant materials.

2.4. Determination of BCF, EC and TLF

The ability of macrophytes to uptake metal with respect to concentration in the surrounding waters in the lagooning system is determined by the biological concentration factor (BCF) (De Bortoli et al., 1968; Zayed et al., 1998). This index is defined as the ratio between metal concentrations in plant and in the residual concentration in water. The translocation properties of macrophytes from sediment to roots and from roots to different plant parts, and the storage capacity within above-/below-ground parts were evaluated by the enrichment coefficient (EC) (Baker et al., 1994; Sasmaz et al., 2008) expressed in the following ratios:

- $[\text{element concentration}]_{\text{root}}/[\text{element concentration}]_{\text{sediment}}$: (ECR),
- $[\text{element concentration}]_{\text{rhizome}}/[\text{element concentration}]_{\text{sediment}}$: (ECRh),
- $[\text{element concentration}]_{\text{stem}}/[\text{element concentration}]_{\text{sediment}}$: (ECS),
- $[\text{element concentration}]_{\text{leaf}}/[\text{element concentration}]_{\text{sediment}}$: (ECL).

Transfer factor (or TransLocation Factor: TLF) was also calculated as the ratio of $[\text{element concentration}]_{\text{leaf}}/[\text{element concentration}]_{\text{root}}$ (Zu et al., 2005; Sasmaz et al., 2008). Transfer factor (Leaf Stem Ratio: LSR) was also calculated as the ratio of $[\text{element concentration of element}]_{\text{leaf}}/[\text{element concentration}]_{\text{stem}}$.

The biological concentration factor (BCF) as well as enrichment coefficient (EC) and transfer factor (TLF) were calculated only for those elements detected in water, sediment and plant organs. All results are summarized in Table 10.

2.5. Statistical analysis

To evaluate statistically significant differences among mean values, one-way analysis of variance with a Tukey post-test was used. In all tests, the significance level for differences in critical values was set at $P < 0.05$. Linear regression was used to evaluate the effect of metal concentration in the water solutions on the mean metal concentration in the aquatic macrophyte plant part biomass. The software used for statistical analysis was Statistica 8.0 (Statsoft, Inc.).

3. Results

3.1. In-/out-flowing water quality

3.1.1. Ambient environmental factors

The pH did not vary noticeably during the experiment (Fig. 2). The highest temperature was recorded in spring, and temperatures did not vary noticeably from inflow to outflow (11.7 °C at inflow). Though freezing conditions never occurred during the study, the water in the lagooning system cooled during fall, with the lowest temperature recorded at the fourth pond's outflow point (4.8 °C). Dissolved oxygen of outflow water was significantly lower ($P < 0.001$) than outflow water during the 2 years. Otherwise mean DO values were highly variable, with the highest values measured in spring. As for the DO, the water's electric conductivity varied from one season to another with the highest values recorded in spring, and generally was significantly reduced at outflow with less than 1000 $\mu S\, cm^{-1}$ (Fig. 2).

3.1.2. Metals in water

All metal concentrations in the 24 samples collected in Gros Prés Brook for background values were near the detection limits of the ICP-OES (data not shown). In the fourth pond of the lagooning system, In-/Out-flowing water was analyzed for metal content (Ag, Al, As, B, Cd, Cr, Cu, Fe, Mn, Ni, Se, Sn and Zn) (Table 2). Significant decreases in concentrations in inflow and outflow waters were observed for Fe, Mn and Zn only. Concentrations of As, Se and Sn recorded in in-/outflow waters were below the detection limit according to sampling season. Mean concentrations of B, Cd, Cr, Cu and Ni were stable throughout the study, with no significant differences between the fourth pond's inlet and the outlet. Except for data collected during spring 2012, the mean concentration of Ag remained constant in the lagoon (at both in- and outflow) in all seasons with values below the detection limits throughout the experiment.

Seasonal decreases in metal content were recorded at in-/outflow with a peak in spring for Al, Fe and Mn. While significant reductions ($P < 0.001$) were recorded for Al (spring 2011) and Fe (fall 2010 and spring 2011), only Mn was significantly reduced compared to the concentration measured at inflow for all of the sampling periods. The small differences in heavy metal removal of Fe between inflow and outflow were not statistically significant. Zn levels were significantly reduced ($P < 0.05$) in comparison to the concentrations measured at inflow for spring 2011 and 2012 only.

Table 2

Heavy metal concentrations (milligrams per liter) in the in/outgoing water flow of the Etueffont fourth pond.

mg l^{-1}	Inlet					Outlet					% reduction/P value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012	P	Fall 2010	Spring 2011	Fall 2011	Spring 2012	P	Fall 2010	Spring 2011	Fall 2011	Spring 2012
Ag	<0.02 a	<0.02 a	<0.02 a	0.074 ± 0.011 b	***	<0.02 a	<0.02 a	<0.02 a	0.060 ± 0.007 b	***	/	/	/	−19*
Al	<0.02 a	0.074 ± 0.020 b	<0.02 a	0.072 ± 0.008 b	***	<0.02 a	0.060 ± 0.007 b	<0.02 a	0.066 ± 0.011 c	***	/	−19***	/	−9 ns
As	<0.02 a	<0.02 a	<0.02 a	<0.02 a	ns	<0.02 a	<0.02 a	<0.02 a	<0.02 a	ns	/	/	/	/
B	0.362 ± 0.050 a	0.398 ± 0.034 a	0.364 ± 0.027 a	0.384 ± 0.028 a	ns	0.322 ± 0.026 a	0.378 ± 0.049 a	0.322 ± 0.031 a	0.328 ± 0.046 a	ns	−11 ns	−5 ns	0 ns	−15 ns
Cd	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0 ns	0 ns	0 ns	0 ns
Cr	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0 ns	0 ns	0 ns	0 ns
Cu	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	0.02 ± 00 a	ns	0 ns	0 ns	0 ns	0 ns
Fe	0.104 ± 0.020 a	0.238 ± 0.062 b	0.106 ± 0.045 a	0.226 ± 0.021 c	***	0.082 ± 0.008 a	0.134 ± 0.042 b	0.098 ± 0.032 a	0.200 ± 0.034 c	***	−21*	−44***	−8 ns	−12 ns
Mn	0.116 ± 0.015 a	0.182 ± 0.023 b	0.170 ± 0.016 b	0.336 ± 0.045 c	***	0.090 ± 0.010 a	0.150 ± 0.016 b	0.108 ± 0.018 a	0.174 ± 0.013 b	***	−22*	−18*	−36***	−48***
Ni	<0.02 a	0.273 ± 0.052 b	<0.02 a	0.258 ± 0.039 b	ns	<0.02 a	0.263 ± 0.049 b	<0.02 a	0.258 ± 0.042 b	ns	/	−4 ns	/	0 ns
Se	<0.04 a	<0.04 a	<0.04 a	<0.04 a	ns	<0.04 a	<0.04 a	<0.04 a	<0.04 a	ns	/	/	/	/
Sn	<0.04 a	<0.04 a	<0.04 a	<0.04 a	ns	<0.04 a	<0.04 a	<0.04 a	<0.04 a	ns	/	/	/	/
Zn	<0.02 a	0.213 ± 0.023 b	<0.02 a	0.192 ± 0.016 b	***	<0.02 a	0.136 ± 0.054 b	<0.02 a	0.110 ± 0.016 b	***	/	−36***	/	−43***

Data are mean ± SD. $n = 6$. Different letters indicate significant differences between the sampling time periods within a sampling location (inlet or outlet).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

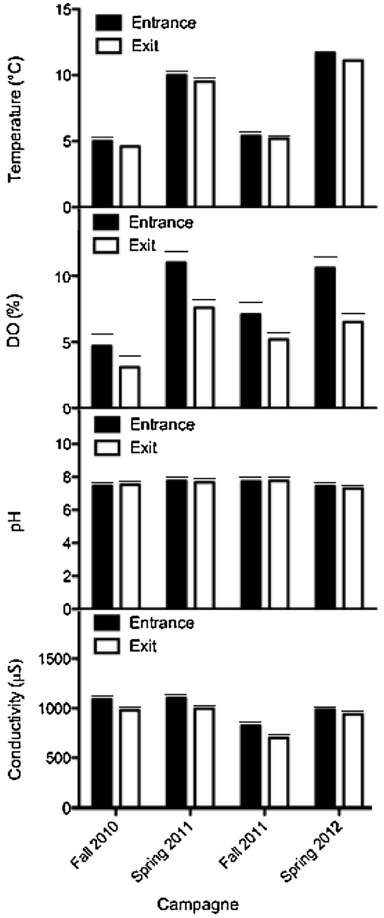

Fig. 2. pH, temperature (degrees Celsius), and conductivity (microsiemens) of the in/outgoing water flow of the Etueffont fourth pond.

3.1.3. *Metals in sediments*

Data recorded during the four sampling periods are shown in Table 3. Significant temporal decreases in metal concentrations at inflow and outflow were observed between fall and spring for As, B, Cd, Cr, Cu, Mn, Sn and Zn. However, no significant differences between seasons were recorded for Ag, Fe and Ni (at inflow or outflow), Al (only at inflow) and Se (only at outflow). Metal content in outflow sediment showed generally lower values than those found at inflow. In-/outflow metal removal in sediment varied from −2 to □54% in fall 2010/2011 and from □2 to □53% in spring 2011/2012. Only Cd, Cu, Sn and Zn were significantly reduced ($P < 0.05$) compared to the concentration measured at inflow, whatever the season. Al and Se concentrations were significantly reduced between inflow and outflow only in spring 2012, as well as As and Cr for spring 2011. No significant removal was recorded for Ag, Fe, Mn and Ni. B was significantly reduced

Table 3

Heavy metal concentrations (milligrams per gram DW) in soil and sediments collected from the study site at inflow and outflow locations throughout the experimental period.

$mg\,kg^{-1}$	In					Out					% reduction/P value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	27,100 ± 3141 a	28,150 ± 4193 a	25,800 ± 3498 a	31,700 ± 4299 a	ns	26,650 ± 2259 ab	29,200 ± 2476 a	22,450 ± 3044 b	25,350 ± 3270 ab	*	−2 ns	4 ns	−13 ns	−20*
As	18.25 ± 2.48 a	32.30 ± 2.71 b	21.30 ± 2.33 a	28.10 ± 4.28 b	***	18.40 ± 1.56 a	19.65 ± 1.44 a	16.85 ± 3.73 a	24.95 ± 2.12 b	***	1 ns	−39***	−21 ns	−11 ns
B	17.10 ± 2.27 a	36.70 ± 3.11 b	29.35 ± 3.98 c	33.15 ± 2.50 c	***	17.50 ± 1.29 a	23.25 ± 2.11 b	27.00 ± 2.29 b	28.35 ± 1.84 c	***	2 ns	−37***	−8 ns	−17***
Cd	0.51 ± 0.01 a	2.25 ± 0.48 b	0.85 ± 0.12 ac	1.10 ± 0.05 c	***	0.50 ± 0.01 a	1.15 ± 0.17 b	0.50 ± 0.01 a	0.87 ± 0.13 c	***	−2**	−49***	−41***	−21**
Cr	56.10 ± 4.76 a	75.30 ± 6.39 b	46.85 ± 5.92 a	51.70 ± 7.31 a	***	53.85 ± 6.10 a	58.95 ± 7.59 a	39.85 ± 6.76 b	47.25 ± 6.69ab	**	−4 ns	−22**	−15 ns	−9 ns
Cu	73.30 ± 8.92 a	219.50 ± 18.61 b	112.10 ± 16.46 c	143.70 ± 5.83 d	***	57.75 ± 4.90 a	104.0 ± 14.71 b	60.05 ± 5.09 a	131.00 ± 6.50 c	***	−21**	−53***	−46***	−9*
Fe	34,250 ± 4644 a	38,800 ± 3289 a	37,800 ± 6409 a	39,850 ± 3378 a	ns	35,000 ± 5528 a	36,250 ± 6146 a	35,300 ± 2993 a	37,700 ± 5955 a	ns	2 ns	−7 ns	−7 ns	−5 ns
Mn	2185 ± 332.8 a	3070 ± 416.3 b	2370 ± 200.9 a	3780 ± 512.6 c	***	1700 ± 380.6 a	2995 ± 219.8 b	2230 ± 258.5 a	3665 ± 355.1 c	***	−22 ns	−2 ns	−6 ns	−3 ns
Ni	39.40 ± 6.00 a	47.60 ± 6.73 a	38.80 ± 5.49 a	38.20 ± 5.82 a	ns	38.40 ± 6.06 a	40.15 ± 6.81 a	34.35 ± 4.86 a	36.10 ± 5.50 a	ns	−3 ns	−16 ns	−11 ns	−5 ns
Se	<1 a	<1 a	<1 a	1.65 ± 0.06 b	***	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	−39***
Sn	7.05 ± 0.60 a	23.45 ± 3.58 b	8.95 ± 0.87 ac	10.65 ± 0.41 c	***	4.50 ± 0.64 a	12.10 ± 1.03 b	5.00 ± 0.01 a	11.45 ± 0.38 b	***	−36***	−48***	−44***	8*
Zn	290.50 ± 24.63 a	458.01 ± 50.13 b	260.03 ± 17.49 ac	225.5 ± 24.71 c	***	158.02 ± 13.39 a	227.51 ± 15.09 b	119.02 ± 18.13 c	116.61 ± 12.74 c	***	−46***	−50***	−54***	−48***

Data are mean ± SD. $n = 6$. Different letters indicate significant differences between the sampling time periods within a sampling location (inlet or outlet).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

($P < 0.001$) compared to the concentrations measured at inflow for spring 2011 and 2012 only.

3.2. Biological parameters

3.2.1. Macrophyte phytomass

The average above-ground biomass of cattail (*T. latifolia*) and common reed (*P. australis*) for the 2 years under study are shown in Fig. 3. The highest shoot biomass of cattail and reed plants was measured at inflow in fall 2011, with 1.13 and 0.85 kg DW m^{-2} respectively. For the two species, above-ground phytomass values at inflow and outflow were not significantly different during the fall and spring seasons for both years. The below-ground biomass of cattail varied from 0.46 to 1.29 kg DW m^{-2} and that of reed from 1.36 to 1.94 kg DW m^{-2} (Figs. 3 and 4). Thus, though the root/rhizome biomass of reed was generally higher than that of cattail, the average below-ground biomass of reed and cattail did not differ significantly. As for location (inflow/outflow), no significant difference was observed for the two plant species in the root/rhizome and shoot biomass as well in relation to seasons confirming uniform growing conditions.

3.2.2. Metal storage in macrophytes and relationships with sediment

Concentrations measured in *T. latifolia* and *P. australis* sampled during the two seasons, for 2 years, are shown in Tables 4–6 for *T. latifolia*, and Tables 7–9 for *P. australis*. In most cases, the concentrations of metals were higher in root/rhizome plant parts than in the different aerial plant parts (leaves, stems and flowers).

T. latifolia: Accumulation of heavy metals and concentrations in roots and rhizomes grown on the inflow side of the lagooning system was significantly higher than in the above-ground phytomass. Ag and Sn remained below the detection limits (BDL) in above- and below-ground plant parts, regardless of the time of collection. Except for these two elements, significant differences in heavy metal concentrations between dates were recorded for roots ($P < 0.001$). Significant variations in rhizome, stem and flower plant parts were recorded for Cr on the inflow side only. Moreover, only Cd and Se did not vary significantly with season in rhizome, stem and flower of *T. latifolia* neither at inflow nor outflow (Tables 4–6). With the exception of As and Ni, metal uptake by *T. latifolia* above-ground phytomass (stems, leaves, and flowers) showed similar significant seasonal variations to those of rhizome plant parts at inflow and outflow. Metal concentrations in below- (roots, rhizomes) and above-ground plant parts were maximal in spring after plant growth, whereas a general decrease occurred in fall before senescence. Ag and Sn excepted, comparison between inflow and outflow plant samples showed that most metal concentrations were significantly lower ($P < 0.05$) in roots of outflow plants, for more than one sampling date, indicating lesser accumulation at ouflow. Similarly to roots, significant differences in metal accumulation ($P < 0.05$) were found in the rhizome, stem and leaves for Al, B, Cu, Fe, Mn, Ni and Zn. Excepted for *T. latifolia* leaves, significant differences were also observed for As and Cr in above- and belowground plant parts. Whatever the season, no significant removal was observed for Ag and Sn by plant roots and rhizome nor by stem, leaves and flowers of *T. latifolia* between inflow and outflow.

P. australis: The highest metal concentrations in *P. australis* were found in roots on the inflow side. As for *T. latifolia* and for all of the seasons studied, Ag and Sn were not detected since values were below the detection limits in above- as well as in below-ground plant parts. Significant differences in all metal concentrations between the four sampling dates were recorded in roots ($P < 0.001$)

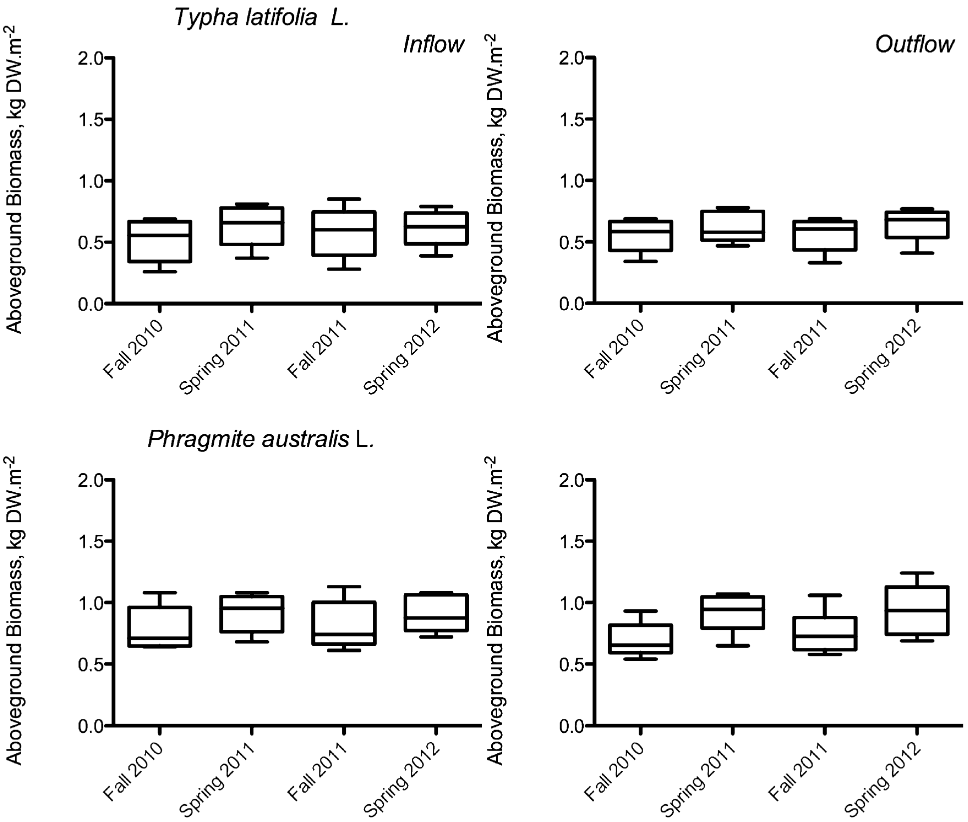

Fig. 3. Biomass of above-ground parts (grams DW per plant) of *T. latifolia* and *P. australis* from the two sampling locations (inflow and outflow) over the entire sampling period ($n = 6$, mean ± SD).

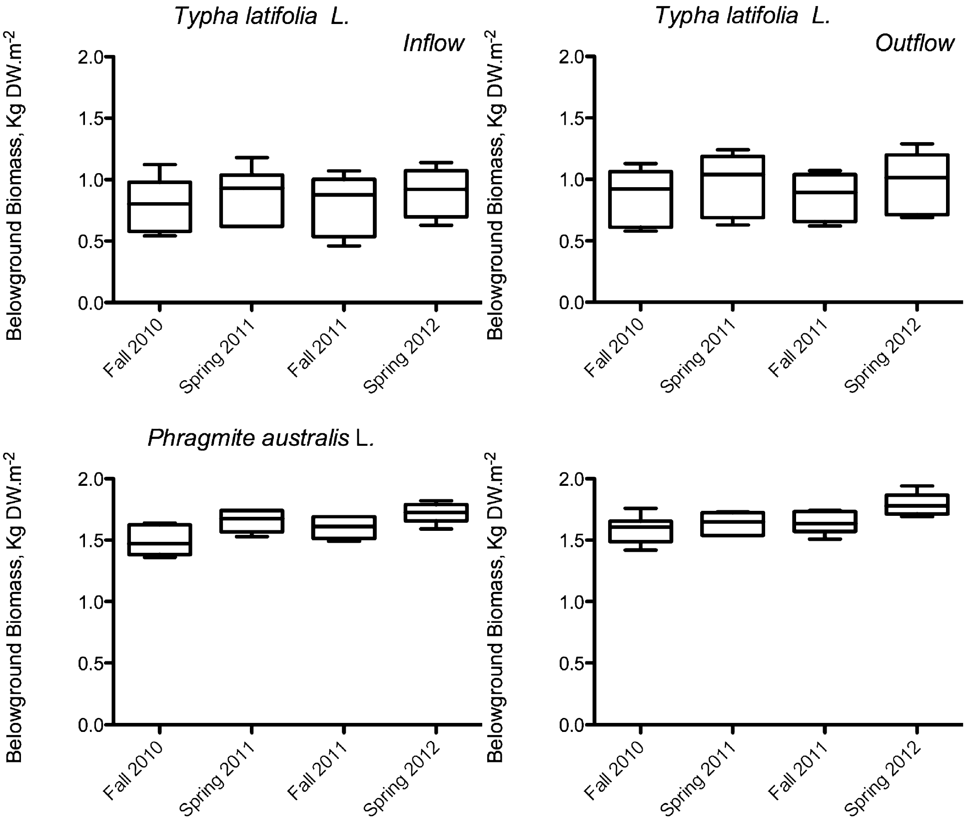

Fig. 4. Biomass of below-ground parts (grams DW per plant) of *T. latifolia* and *P. australis* from the two sampling locations (inflow and outflow) over the entire sampling period ($n = 6$, mean ± SD).

Table 4
Heavy metal concentrations (milligrams per kilogram DW) in the root, rhizome of *T. latifolia* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

T. latifolia	In					Out					% reduction/*P* value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Root														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	3130 ± 362.8 a	6060 ± 702.5 b	1506 ± 198.4 c	10,300 ± 1194 d	***	1830 ± 212.1 a	4590 ± 532.1 b	1104 ± 112.4 c	5520 ± 639.9 d	***	−42***	−24**	−27***	−46***
As	39.22 ± 2.86 a	111.0 ± 8.16 b	71.12 ± 5.23 c	151.2 ± 12.83 d	***	36.78 ± 2.68 a	73.20 ± 5.40 b	69.82 ± 5.07 b	87.42 ± 6.41 c	***	−6 ns	−34***	−3 ns	−42***
B	12.68 ± 1.11 a	26.20 ± 2.22 b	18.17 ± 1.54 c	23.80 ± 2.04 b	***	11.40 ± 0.96 a	14.60 ± 1.26 b	15.30 ± 1.30 b	21.10 ± 1.79 c	***	−10 ns	−44***	−16*	−11 ns
Cd	<0.5 a	1.60 ± 0.09 a	0.56 ± 0.06 b	1.01 ± 0.15 c	***	<0.5 a	0.64 ± 0.10 a	0.53 ± 0.04 a	0.86 ± 0.12 b	***	/	−60***	−5 ns	−15 ns
Cr	9.00 ± 1.02 a	61.30 ± 6.94 b	11.68 ± 1.32 a	30.80 ± 3.48 c	***	8.10 ± 0.92 a	22.60 ± 2.56 b	6.55 ± 0.84 a	17.70 ± 2.01 c	***	−10 ns	−63***	−44***	−43***
Cu	38.30 ± 4.66 a	53.80 ± 6.55 b	29.40 ± 3.58 a	38.32 ± 4.66 a	***	29.20 ± 3.55 a	42.50 ± 5.17 b	27.60 ± 3.36 a	22.83 ± 3.23 a	***	−24**	−21*	−6 ns	−40***
Fe	14,700 ± 2322 a	27,100 ± 4281 b	29,200 ± 4612 b	38,715 ± 6564 c	***	14,400 ± 2275 a	26,900 ± 4249 b	19,900 ± 3143 ab	34,697 ± 5481 c	***	−2 ns	−1 ns	−32**	−10 ns
Mn	9440 ± 1094 a	12,600 ± 1461 a	5047 ± 427.8 b	31,300 ± 3628 c	***	11,000 ± 1275 a	11,900 ± 1379 a	4961 ± 575.0 b	20,000 ± 2318 c	***	−14**	−6 ns	−2 ns	−36***
Ni	12.60 ± 1.99 a	45.30 ± 7.15 b	12.91 ± 2.04 a	30.00 ± 4.74 c	***	11.20 ± 1.77 a	34.20 ± 5.40 b	8.33 ± 1.41 a	29.30 ± 4.63 b	***	−11 ns	−25*	−36**	−2 ns
Se	7.32 ± 0.85 a	8.40 ± 0.97 a	2.00 ± 0.01 b	19.90 ± 2.31 c	***	5.56 ± 0.64 a	8.80 ± 1.02 b	2.00 ± 0.01 c	12.00 ± 1.39 d	***	−24**	5 ns	/	−40***
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	126.0 ± 10.68 b	175.0 ± 14.84 b	54.90 ± 4.66 c	105.3 ± 8.93 a	***	75.40 ± 6.39 a	91.90 ± 7.79 b	45.70 ± 3.88 c	66.80 ± 5.87 a	***	−18**	−28***	−17**	−37***
Rhizome														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	93.50 ± 12.68 a	343.0 ± 46.51 b	35.50 ± 5.29 c	392.0 ± 58.39 b	***	80.30 ± 10.89 a	172.5 ± 25.69 b	25.00 ± 3.72 c	168.8 ± 19.57 b	***	−14 ns	−50***	−30**	−57***
As	4.00 ± 0.34 a	6.52 ± 0.55 b	1.80 ± 0.16 c	7.22 ± 0.29 b	***	<1.5 a	2.24 ± 0.20 b	<1.5 a	4.96 ± 0.51 c	***	−62***	−66***	−17**	−31***
B	9.30 ± 0.79 a	20.20 ± 1.71 b	12.80 ± 0.94 c	16.71 ± 1.42 d	***	9.00 ± 0.66 a	13.10 ± 0.96 b	12.30 ± 1.04 b	15.84 ± 1.43 c	***	−3 ns	−35***	−4 ns	−5 ns
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	1.38 ± 0.18 b	<1 a	1.92 ± 0.27 c	***	<1 a	<1 a	<1 a	<1 a	ns	/	−27**	/	−48***
Cu	3.80 ± 0.54 a	6.28 ± 0.89 b	3.98 ± 0.59 a	4.49 ± 0.63 a	***	1.81 ± 0.29 a	3.30 ± 0.52 bc	2.62 ± 0.42ac	3.96 ± 0.56 b	***	−52***	−47***	−34**	−12 ns
Fe	2520 ± 427.3 ab	3300 ± 559.5 a	1200 ± 203.5 b	11,416 ± 1936 c	***	761.0 ± 129.0 a	844.0 ± 143.1 a	97.20 ± 16.46 b	1513 ± 239.0 c	***	−70***	−74***	−92***	−87***
Mn	334.0 ± 32.32 a	1210 ± 102.7 b	379.0 ± 36.32 a	1155 ± 84.9 b	***	354.0 ± 29.93 a	544.0 ± 52.57 b	395.2 ± 33.62 a	901.8 ± 76.38 c	***	6 ns	−55***	4 ns	−22**
Ni	<1 a	4.12 ± 0.63 b	1.14 ± 0.08 b	1.35 ± 0.19 c	***	<1 a	1.37 ± 0.23 b	<1 a	1.35 ± 0.19 b	***	/	−67***	−12**	0 ns
Se	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	20.26 ± 1.76 a	26.59 ± 1.91 b	19.76 ± 1.71 a	15.36 ± 1.36 c	***	9.68 ± 0.86 a	22.24 ± 0.97 b	10.70 ± 0.92 a	10.52 ± 0.89 a	***	−52***	−16***	−46***	−32***

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

Table 5
Heavy metal concentrations (milligrams per kilogram DW) in the stem and leaf of *T. latifolia* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

T. latifolia	In					Out					% reduction/*P* value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Stem														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	<1.5 a	51.20 ± 6.95 b	29.60 ± 4.02 c	47.50 ± 5.51 b	***	<1.5 a	25.90 ± 5.90 b	10.55 ± 1.22 c	40.91 ± 5.55 d	***	/	−49***	−64***	−14 ns
As	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	/	/	/
B	11.12 ± 0.10 a	17.40 ± 1.57 b	12.88 ± 1.16 a	18.36 ± 1.56 b	***	12.50 ± 0.92 a	14.90 ± 1.09 b	< 1.5 c	7.88 ± 0.31 d	***	12 ns	−14*	−88***	−57***
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	<1 a	<1 a	3.78 ± 0.54 b	***	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	−74***
Cu	2.60 ± 0.38 a	3.61 ± 0.57 b	1.73 ± 0.26 a	4.77 ± 0.75 c	***	<1 a	2.33 ± 0.37 b	<1 a	3.38 ± 0.41 c	***	−61***	−35**	−42***	−29**
Fe	92.00 ± 15.60 a	122.0 ± 20.69 ac	35.88 ± 5.67 b	132.0 ± 22.38 c	***	32.52 ± 5.15 a	87.30 ± 13.78 b	41.24 ± 7.01 a	104.0 ± 16.42 b	***	−65***	−28*	15 ns	−21 ns
Mn	637.0 ± 46.87 a	1180 ± 86.31 b	864.6 ± 100.1 c	1360 ± 131.6 d	***	467.0 ± 45.19 a	1140 ± 110.5 b	647.6 ± 62.64 c	1460 ± 107.2 d	***	−27***	−3 ns	−25**	7 ns
Ni	<1 a	1.20 ± 0.18 a	<1 a	2.14 ± 0.30 b	***	<1 a	<1 a	<1 a	<1 a	ns	/	−17*	/	−53***
Se	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	41.48 ± 3.52 d	39.11 ± 3.32 b	19.80 ± 2.17 a	19.32 ± 1.62 a	***	12.10 ± 1.02 a	19.80 ± 1.70 b	7.85 ± 0.86 c	19.60 ± 2.14 a	***	−71***	−49***	−60***	2 ns
Leaves														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	41.60 ± 5.37 a	165.0 ± 24.58 b	11.78 ± 1.36 c	66.70 ± 8.60 d	***	<1.5 a	37.50 ± 4.84 b	11.48 ± 1.48 c	23.40 ± 3.49 d	***	−96***	−77***	−3 ns	−65***
As	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	/	/	/
B	7.45 ± 0.63 a	12.50 ± 1.06 a	9.40 ± 0.80 b	10.88 ± 0.98 ab	**	6.32 ± 1.00 a	9.90 ± 0.89 b	<1.5 c	10.58 ± 0.95 b	***	−15*	−21**	−84***	−3 ns
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	/
Cu	3.90 ± 0.57 a	5.380 ± 0.79 b	4.14 ± 0.58 ab	7.46 ± 1.09 c	***	2.60 ± 0.37 a	3.67 ± 0.52 a	< 1 b	6.90 ± 1.01 c	***	−33**	−32**	−76***	−8 ns
Fe	57.90 ± 9.82 a	124.0 ± 19.58 b	132.0 ± 22.38 b	221.0 ± 37.47 c	***	25.40 ± 4.01 a	103.3 ± 13.17 b	80.10 ± 12.64 b	170.6 ± 26.95 c	***	−56***	−17 ns	−39**	−23*
Mn	2120 ± 155.8 a	2800 ± 237.4 b	1870 ± 158.6 a	2724 ± 200.1 b	***	1630 ± 138.2 a	1890 ± 138.7 b	55.40 ± 4.159 c	2268 ± 166.2 d	***	−23***	−33***	−97***	−17**
Ni	<1 a	1.03 ± 0.042 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	−3 ns	/	/
Se	<1 a	2.476 ± 0.39 b	<1 a	<1 a	***	<1 a	<1 a	<1 a	<1 a	ns	/	−60***	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	<1 a	<1 a	<1 a	1.90 ± 0.20 b	***	<1 a	<1 a	<1 a	1.10 ± 0.16 b	***	/	/	/	−43***

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 6
Heavy metal concentrations (milligrams per kilogram DW) in the flower of *T. latifolia* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

T. latifolia	In					Out					% reduction/*P* value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Flowers														
Ag	<5 a	<5 a	<5 a	/	ns	<5 a	<5 a	<5 a	/	ns	/	/	/	/
Al	<1.5 a	16.50 ± 1.91 b	8.20 ± 1.06 c	/	***	<1.5 a	15.80 ± 2.04 b	2.90 ± 0.34 a	/	***	/	−4 ns	−65***	/
As	<1.5 a	<1.5 a	<1.5 a	/	ns	<1.5 a	<1.5 a	<1.5 a	/	ns	/	/	/	/
B	14.02 ± 1.24 a	24.30 ± 2.22 b	12.40 ± 1.14 a	/	***	8.30 ± 0.74 a	21.10 ± 1.91 b	<1.5 c	/	***	−41***	−13*	−88***	/
Cd	<0.5 a	<0.5 a	<0.5 a	/	ns	<0.5 a	<0.5 a	<0.5 a	/	ns	/	/	/	/
Cr	<1 a	10.30 ± 1.30 b	<1 a	/	***	<1 a	<1 a	<1 a	/	ns	/	−90***	/	/
Cu	7.40 ± 1.08 a	5.67 ± 0.83 b	6.57 ± 0.97 ab	/	*	5.80 ± 0.85 a	4.28 ± 0.63 b	<1 c	/	***	−22*	−25*	−85***	/
Fe	42.80 ± 6.75 a	92.20 ± 14.55 b	57.20 ± 9.04 a	/	***	30.70 ± 4.84 a	77.10 ± 12.20 b	53.02 ± 8.34 c	/	***	−28*	−16 ns	−7 ns	/
Mn	882.0 ± 64.92 a	1400 ± 102.8 b	485.0 ± 35.56 c	/	***	733.0 ± 54.04 a	763.0 ± 55.89 a	154.0 ± 11.31 b	/	***	−17**	−46***	−68***	/
Ni	<1 a	5.23 ± 0.80 b	1.10 ± 0.09 a	/	***	<1 a	1.13 ± 0.057 b	<1 a	/	***	/	−78***	−9 ns	/
Se	<1 a	<1 a	<1 a	/	ns	<1 a	<1 a	<1 a	/	ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	/	ns	<5 a	<5 a	<5 a	/	ns	/	/	/	/
Zn	33.50 ± 3.67 a	48.60 ± 5.32 b	22.30 ± 2.44 c	/	***	27.80 ± 3.04 a	19.20 ± 2.10 b	11.90 ± 1.31 c	/	***	−17*	−60***	−47***	/

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

(Ag and Sn excepted). Al, Cr, Fe, Mn, Ni and Zn presented significant seasonal variations ($P < 0.05$) in rhizome of *T. latifolia* both at inflow and outflow (Table 7). Significant seasonal variations in concentrations were recorded for As and Cu at inflow only. As for *T. latifolia*, the maximum concentrations in root and rhizome plant tissues at inflow were measured in spring after plant growth, the lowest occurring in fall before senescence. Metal uptake by *T. latifolia* above-ground phytomass showed significant seasonal variations similar to those of rhizome plant parts at inflow and outflow particularly for Al, Cr, Cu, Fe, Mn, Ni and Zn. Stems and leaves showed similar differences according to season except for the B in leaves which varied significantly with the season. No significant differences between dates were recorded for flowers, except for Al (at inflow and outflow), Mn (only at outflow) and Ni (only at outflow) (Table 9). As observed for *T. latifolia*, comparison between inflow and outflow plant samples showed that most metals decreased both in roots and shoots of outflow-side plants, indicating greater accumulation at inflow. Except in roots, no significant decrease of Cd accumulation for the different sampling seasons was observed. Similarly to roots, significant differences ($P < 0.05$) were found between inflow and outflow in Al, Cr, Cu, Fe, Mn, Ni and Zn in the rhizome, stem and leaves of *P. australis* f (As for rhizome only; B for stem, leaves and flowers only). Cr, Ni and Zn excepted, the same pattern was observed in flowers collected in the pond. Similarly to *T. latifolia* tissues, no significant reduction of Ag and Sn accumulation was observed in above- and below-ground plant parts of *P. australis* between inflow and outflow.

3.2.3. *Biological concentration factor and enrichment coefficient (Table 10)*

Mean values of BCF determined for heavy metals in *T. latifolia* and *P. australis*, summarized in Table 10, increased according to elements in the following order: (B ≈ Cd) < Ni < Zn < (Cr ≈ Cu) < (Mn ≈ Al ≈ Fe). The mean BCF values of Al, Fe and Mn were higher than those of other elements. Ag, As, Se and Sn were not analyzed in the water, since their concentrations were below the method's detection limit, preventing the calculation of their BCF. The ECR were calculated for the two species (Table 10) and varied from 0.00 to 12.06, decreasing in the following order: Al < Cu < Zn < Cr < Ni < Fe < B < Cd < As < Mn < Se. Except for As and Mn, the ECR of *T. latifolia* and *P. australis* were below 1.0 for all of the studied metals, whatever the season (Table 10). ECR of Se was determined for spring 2012 only, with a value over 1.0 for the two species. For both species, the same patterns were observed for ECRh with enrichment coefficients lower in the rhizomes than in the roots for all analyzed elements (EC < 1.0) (Cd and Se not evaluated). The enrichment coefficients calculated for the selected elements in the stems (ECS) and leaves (ECL) of *T. latifolia* were lower than in roots, with ECS and ECL mean values ranging from 0.00 to 0.75 and from 0.00 to 0.97 respectively. The same pattern was observed for *P. australis* with the mean value of ECS and ECL ranging from 0.00 to 0.20 and from 0.00 to 0.60 respectively. EC was not evaluated for Ag and Se as concentrations remained below detection limits. All results are expressed as positive linear correlations between heavy metal concentrations in water (or sediment) and the different plant organs (root, rhizome, stem, leaf and flower) (data not shown).

3.2.4. *Transfer factor (TLF and LSR) (Table 10)*

Transfer factor was used in order to estimate the ability of the two macrophytes to transfer heavy metals from roots to shoots (leaves) (TLF) as well as from stem to leaves (LSR). The TLF and LSR of *T. latifolia* and *P. australis* are summarized in Table 10. The LSR varied from 0.03 to 7.81 and from 0.12 to 25.56 for *T. latifolia* and *P. australis* respectively. The highest leaf/stem ratio was observed

Table 7
Heavy metal concentrations (milligrams per kilogram DW) in the root, rhizome of *P. australis* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

P. australis	In					Out					% reduction/*P* value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Root														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	1760 ± 204.0 a	5390 ± 624.8 b	1166 ± 150.4 a	6490 ± 451.9 b	***	542.01 ± 62.83 a	4430 ± 659.9 b	348 ± 29.7 c	5520 ± 659.9 b	***	-69^{***}	-18^{*}	-70^{***}	-15^{**}
As	50.40 ± 1.82 a	149.01 ± 12.64 b	85.70 ± 6.31 c	200.90 ± 22.01 d	***	47.12 ± 0.66 a	106.02 ± 7.78 b	69.20 ± 5.86 c	119.21 ± 8.73 d	***	-7^{**}	-29^{***}	-19^{**}	-41^{***}
B	8.00 ± 0.68 a	23.80 ± 2.017 b	8.36 ± 0.18 a	16.30 ± 1.36 c	***	6.30 ± 0.52 a	11.52 ± 0.28 b	7.88 ± 0.28 a	12.90 ± 0.32 c	***	-21^{**}	-52^{***}	-6^{*}	-21^{***}
Cd	<0.5 a	0.89 ± 0.12 b	0.59 ± 0.07 a	1.00 ± 0.15 b	***	<0.5 a	<0.5 a	0.55 ± 0.03 a	0.65 ± 0.10 b	***	/	-44^{***}	−8 ns	-35^{**}
Cr	6.20 ± 0.7022 a	158.01 ± 17.88 b	12.74 ± 1.44 a	31.00 ± 3.51 c	***	4.20 ± 0.48 a	17.80 ± 2.29 b	6.26 ± 0.79 a	17.70 ± 2.28 b	***	-32^{***}	-89^{***}	-51^{***}	-43^{***}
Cu	16.00 ± 1.95 a	40.90 ± 1.36 b	29.70 ± 0.88 c	31.59 ± 2.52 c	***	10.90 ± 1.33 a	38.20 ± 1.36 b	27.60 ± 0.72 c	23.92 ± 2.71 d	***	-32^{**}	-7^{*}	-7^{**}	-27^{***}
Fe	12,100 ± 1911 a	41,800 ± 7088 b	19,900 ± 2812 a	45,586 ± 7201 b	***	7950 ± 1256 a	17,000 ± 2685 b	16,610 ± 1245 b	35,916 ± 5673 c	***	-34^{**}	-59^{***}	-17^{*}	-21^{*}
Mn	16,900 ± 1959 a	18,800 ± 1077 a	31,300 ± 2654 b	23,900 ± 2770 c	***	3950 ± 457.9 a	16,600 ± 1309 b	4696 ± 544.4 a	16,732 ± 1228 b	***	-77^{***}	-12^{*}	-85^{***}	-30^{***}
Ni	10.20 ± 0.70 a	127.01 ± 20.06 b	17.46 ± 0.87 c	33.90 ± 1.63 d	***	9.00 ± 0.53 a	28.70 ± 4.87 b	13.06 ± 1.99 a	30.00 ± 0.94 b	***	-12^{*}	-77^{***}	-25^{**}	-12^{*}
Se	12.60 ± 1.46 a	12.80 ± 0.50 a	10.93 ± 1.38 a	19.93 ± 3.37 b	***	<1 a	11.60 ± 0.32 b	2.05 ± 0.033 a	15.30 ± 1.77 c	***	-84^{***}	-9^{**}	-81^{***}	-23^{*}
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	145.20 ± 13.83 a	171.72 ± 15.09 b	154.80 ± 14.80 a	143.51 ± 15.71 c	***	37.30 ± 3.16 a	52.40 ± 4.44 b	17.46 ± 1.48 c	51.00 ± 4.32 b	***	-74^{***}	-69^{***}	-89^{***}	-64^{***}
Rhizome														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	101.04 ± 13.70 a	66.80 ± 9.06 b	66.70 ± 7.73 c	163.02 ± 24.28 c	***	28.84 ± 4.29 a	35.40 ± 5.27 a	14.80 ± 2.21 b	46.50 ± 6.31 c	***	-71^{***}	-47^{***}	-78^{***}	-72^{***}
As	<1.5 a	<1.5 a	<1.5 a	3.04 ± 0.29 b	***	<1.5 a	<1.5 a	<1.5 a	1.52 ± 0.05 a	ns	/	/	/	-50^{***}
B	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	/	/	/
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	4.92 ± 0.69 b	<1 a	1.25 ± 0.16 c	***	<1 a	2.17 ± 0.28 b	<1 a	<1 a	***	/	-56^{***}	/	-20^{**}
Cu	1.72 ± 0.25 a	2.70 ± 0.43 b	1.45 ± 0.20 a	2.38 ± 0.34 b	***	<1 a	1.21 ± 0.19 a	<1 a	1.16 ± 0.16 a	ns	-42^{***}	-55^{***}	-31^{**}	-51^{***}
Fe	334.03 ± 56.63 a	759.01 ± 128.7 b	393.02 ± 62.05 a	613.01 ± 103.90 b	***	220.03 ± 37.30 a	314.01 ± 53.24 b	299.31 ± 50.76 ab	311.03 ± 52.73 b	*	-34^{**}	-59^{***}	-24^{*}	-49^{***}
Mn	104.03 ± 9.82 a	1170 ± 99.30 b	157.42 ± 14.88 a	554.01 ± 41.60 c	***	47.60 ± 3.912 a	404.4 ± 46.52 b	63.00 ± 5.244 a	370.04 ± 31.48 b	***	-54^{***}	-65^{***}	-60^{***}	-33^{***}
Ni	<1 a	4.140 ± 0.58 b	<1 a	1.21 ± 0.20 a	***	<1 a	2.210 ± 0.3730 b	<1 a	<1 a	***	/	-47^{***}	/	-17^{*}
Se	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	Ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	Ns	/	/	/	/
Zn	18.52 ± 1.57 a	23.08 ± 2.01 b	9.44 ± 1.03 c	13.72 ± 1.18 d	***	11.58 ± 1.05 a	17.20 ± 1.45 b	<1 c	9.72 ± 0.84 d	***	-37^{***}	-25^{***}	-89^{***}	-29^{***}

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

Table 8

Heavy metal concentrations (milligrams per kilogram DW) in the stem and leaf of *P. australis* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

P. australis	In					Out					% reduction/P value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Stem														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	<1.5 a	15.10 ± 2.05 b	4.22 ± 0.49 c	16.50 ± 1.91 b	***	<1.5 a	14.50 ± 0.52 b	4.18 ± 0.57 b	16.10 ± 2.19 b	***	/	−4 ns	−1 ns	−2 ns
As	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	/	/	/
B	<1.5 a	1.62 ± 0.13 b	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	-7^{*}	/	/
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	1.95 ± 0.27 b	<1 a	1.31 ± 0.47 c	***	<1 a	1.86 ± 0.23 b	<1 a	1.30 ± 0.17 c	***	/	−5 ns	/	0 ns
Cu	<1 a	6.83 ± 0.83 c	<1 a	6.39 ± 1.01 c	***	<1 a	1.16 ± 0.15 a	<1 a	3.10 ± 0.49 b	***	/	-83^{***}	/	-52^{***}
Fe	36.30 ± 6.163 a	105.00 ± 16.59 b	76.56 ± 4.76 a	92.20 ± 14.55 a	***	17.90 ± 2.82 a	41.90 ± 7.12 b	64.58 ± 3.57c	89.20 ± 15.11 d	***	-51^{***}	-60^{***}	-16^{**}	−3 ns
Mn	93.00 ± 6.633 a	103.02 ± 9.82 a	154.01 ± 17.90 b	215.4 ± 25.13 c	***	79.00 ± 7.38 a	105.01 ± 7.52 bc	90.60 ± 8.50 ac	109.41 ± 10.88 b	***	-15^{*}	2 ns	-41^{***}	-49^{***}
Ni	<1 a	5.49 ± 0.77 b	<1 a	1.60 ± 0.23 c	***	<1 a	5.23 ± 0.82 b	<1 a	1.19 ± 0.18 a	***	/	−5 ns	/	-26^{*}
Se	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	15.20 ± 1.67 a	86.46 ± 7.33 b	48.60 ± 4.12 c	45.60 ± 4.99 c	***	6.20 ± 0.52 a	36.30 ± 3.05 b	<1 a	10.52 ± 0.89 d	***	-59^{***}	-58^{***}	-98^{***}	-77^{***}
Leaves														
Ag	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Al	<1.5 a	206.0 ± 30.68 b	11.13 ± 1.43 c	36.70 ± 4.74 d	***	<1.5 a	115.0 ± 14.83 b	9.62 ± 1.43 c	29.60 ± 4.02 d	***	/	-44^{***}	−14 ns	-19^{*}
As	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	<1.5 a	<1.5 a	<1.5 a	<1.5 a	ns	/	/	/	/
B	6.40 ± 0.26 a	7.64 ± 0.69 b	5.30 ± 0.50 c	8.46 ± 0.52 b	***	<2.5 a	6.52 ± 0.55 b	3.90 ± 0.14 c	8.16 ± 0.22 d	***	-61^{***}	-15^{*}	-26^{***}	−4 ns
Cd	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	<0.5 a	<0.5 a	<0.5 a	<0.5 a	ns	/	/	/	/
Cr	<1 a	3.780 ± 0.5374 b	<1 a	<1 a	***	<1 a	1.080 ± 0.04637 b	<1 a	<1 a	***	/	-71^{***}	/	/
Cu	4.70 ± 0.66 a	6.23 ± 0.91 b	4.52 ± 0.66 a	7.19 ± 0.18 b	***	3.00 ± 0.44 a	2.84 ± 0.34 a	3.61 ± 0.57 a	6.91 ± 0.09 b	***	-36^{**}	-54^{***}	-20^{*}	-4^{*}
Fe	57.90 ± 9.82 a	124.0 ± 19.58 b	132.0 ± 22.38 b	221.0 ± 37.47 c	***	25.40 ± 4.01 a	103.3 ± 13.17 b	80.10 ± 12.64 b	170.6 ± 26.95 c	***	-56^{***}	−17 ns	-39^{**}	-23^{*}
Mn	1140 ± 132.1 a	1230 ± 90.1 ab	1420 ± 104.4 b	1207 ± 102.3 a	***	610.0 ± 46.9 a	1270 ± 93.5 b	915.2 ± 77.70 b	1360 ± 131.6 b	***	-47^{***}	3 ns	-36^{***}	13 ns
Ni	<1 a	2.78 ± 0.42 b	<1 a	2.14 ± 0.30 c	***	<1 a	<1 a	<1 a	1.23 ± 0.19 b	***	/	-64^{***}	/	-43^{***}
Se	<1 a	<1 a	<1 a	<1 a	ns	<1 a	<1 a	<1 a	<1 a	ns	/	/	/	/
Sn	<5 a	<5 a	<5 a	<5 a	ns	<5 a	<5 a	<5 a	<5 a	ns	/	/	/	/
Zn	18.85 ± 2.18 a	31.60 ± 3.46 b	19.32 ± 1.62 c	23.44 ± 2.07 d	***	15.50 ± 1.70 a	22.98 ± 2.01 b	16.90 ± 1.85 a	14.70 ± 1.25 a	***	−18 ns	-27^{**}	−13 ns	-37^{***}

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 9

Heavy metal concentrations (milligrams per kilogram DW) in the flower of *P. australis* in the inflow/outflow section of the fourth pond at the Etueffont site for the two seasons.

P. australis	In					Out					% reduction/*P* value			
	Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012		Fall 2010	Spring 2011	Fall 2011	Spring 2012
Flowers														
Ag	<5 a	/	<5 a	/	ns	<5 a	/	<5 a	/	ns	/	/	/	/
Al	35.00 ± 4.51 a	/	42.50 ± 4.93 b	/	***	26.60 ± 3.43 a	/	20.30 ± 2.35 a	/	***	−24**	/	−52***	/
As	<1.5 a	/	<1.5 a	/	ns	<1.5 a	/	<1.5 a	/	ns	/	/	/	/
B	12.30 ± 1.14	/	13.50 ± 1.23 a	/	ns	10.10 ± 0.92 a	/	10.80 ± 0.98 a	/	ns	−18**	/	−20**	/
Cd	<0.5 a	/	<0.5 a	/	ns	<0.5 a	/	<0.5 a	/	ns	/	/	/	/
Cr	<1 a	/	1.02 ± 0.01 a	/	ns	<1 a	/	1.02 ± 0.01 a	/	ns	/	/	/	/
Cu	6.10 ± 0.90 a	/	5.24 ± 0.77 a	/	ns	3.70 ± 0.54 a	/	4.220 ± 0.62 a	/	ns	−39***	/	−19 ns	/
Fe	112.0 ± 17.71 a	/	144.0 ± 22.73 a	/	ns	114.0 ± 18.00 a	/	108.0 ± 17.04 a	/	ns	2 ns	/	−25*	/
Mn	446.8 ± 32.99 a	/	406.8 ± 30.16 a	/	ns	298.0 ± 21.85 a	/	388.0 ± 28.39 b	/	***	−33***	/	−5 ns	/
Ni	<1 a	/	1.06 ± 0.02 b	/	***	<1 a	/	<1 a	/	ns	/	/	−4 ns	/
Se	<1 a	/	<1 a	/	ns	<1 a	/	<1 a	/	ns	/	/	/	/
Sn	<5 a	/	<5 a	/	ns	<5 a	/	<5 a	/	ns	/	/	/	/
Zn	84.30 ± 9.23 a	/	76.40 ± 8.37 a	/	ns	80.50 ± 8.82 a	/	67.00 ± 7.34 a	/	ns	−5 ns	/	−12 ns	/

Data are mean ± SD. $n = 6$ (in mg kg^{-1} DW). Letters indicates significant differences between periods within a sampling location (for inflow and outflow).

[*] $P < 0.05$.

[**] $P < 0.01$.

[***] $P < 0.001$.

Table 10
Biological concentration factor (mean ± SD), enrichment coefficient (for root, rhizome, stem and leaf), leaf/stem ratio and transfer factor (Leaf/root) of *T. latifolia* and *P. australis* from the Etueffont fourth pond.

	T. latifolia							*P. australis*						
	BCF	ECR	ECRh	ECS	ECL	LSR	TLF	BCF	ECR	ECRh	ECS	ECL	LSR	TLF
Ag	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
Al	102,009 ± 32,252	0.05–0.32	0.00–0.01	0.00	0.00–0.01	0.40–3.22	0.00–0.03	83,219 ± 8513	0.02–0.22	0.00–0.01	0.00	0.00–0.01	1.84–13.64	0.01–0.04
As	ne	2.00–5.38	0.08–0.26	ne	ne	ne	ne	Ne	2.00–7.15	0.06–0.11	ne	ne	ne	ne
B	174 ± 48	0.42–1.06	0.32–0.75	0.34–0.75	0.23–0.55	0.51–1.34	0.46–0.68	68 ± 11	0.23–0.65	ne	0.04	0.09–0.35	4.72	0.32–0.80
Cd	70 ± 19	0.27–1.06	ne	ne	ne	ne	ne	60 ± 30	0.40–1.06	ne	ne	ne	ne	ne
Cr	2314 ± 2244	0.15–0.81	0.02–0.04	0.07	ne	ne	ne	3557 ± 5468	0.11–2.10	0.02–0.07	0.03	0.02–0.05	0.58–1.94	0.02–0.05
Cu	4930 ± 1411	0.17–0.52	0.03–0.05	0.02–0.04	0.02–0.05	1.49–2.39	0.09–0.30	3815 ± 1194	0.18–0.46	0.01–0.02	0.01–0.04	0.03–0.06	0.91–2.45	0.07–0.29
Fe	199,993 ± 45,955	0.41–0.97	0.00–0.29	0.00	0.00–0.02	1.02–7.81	0.00–0.04	184,676 ± 28,133	0.35–1.14	0.01–0.02	0.00	0.00–0.01	1.18–2.47	0.00–0.01
Mn	105,700 ± 36,687	2.13–8.28	0.15–0.39	0.27–0.40	0.02–0.97	0.09–3.49	0.01–0.37	114,774 ± 32,946	2.22–8.28	0.03–0.38	0.03–0.06	0.31–0.60	5.60–12.43	0.04–0.18
Ni	149 ± 34	0.24–0.95	0.03–0.11	0.03–0.06	0.02	0.86	0.02	237 ± 184	0.24–2.67	0.03–0.09	0.03–0.13	0.03–0.06	0.51–1.34	0.02–0.06
Se	ne	12.06	ne	ne	ne	ne	0.29	ne	12.06	ne	ne	ne	ne	ne
Sn	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
Zn	1171 ± 220	0.21–0.57	0.04–0.17	0.04–0.36	0.01	0.03–0.05	0.02	1,229 ± 331	0.37–0.64	0.04–0.08	0.04–0.20	0.06–0.14	0.37–2.50	0.12–0.37

Data are mean ± SD for BCF only.

for Al, Fe and Mn for the two species. The TLF for *T. latifolia* varied between 0.00 and 0.89 and its exclusion capacity was found to be: Fe < Al < Ni < Zn < Cu < Mn < Se < B (not evaluated for Ag, As, Cd, Cr and Sn). The TLF of *P. australis* varied between 0.00 and 0.60, its exclusion capacity being: Fe < Al < Cr < Mn < Ni < Cu < Zn < B (not evaluated for Ag, As, Cd, Se and Sn).

4. Discussion

4.1. Metals and macrophyte phytomass

Despite high values found in the sediment of the Etueffont CW, metals did not affect the growth of the two predominant emergent plant species (*T. latifolia* and *P. australis*), since the average above-ground cattail biomass measured in pond 4 was in accordance with biomass values measured by Maddison et al. (2009, Estonia) or by Wild et al. (2002, Germany) for cattail, but lower than those reported by Toet et al. (2005, Netherlands) and Ennabili et al. (1998, Morocco) for common reeds. Nevertheless, if the average below-ground biomass values were similar to those reported by Maddison et al. (2009) for cattail, lower values than those reported by Ennabili et al. (1998, Morocco), Vymazal (2004, Czech Republic), Toet et al. (2005, Netherlands), Bragato et al. (2006, Italy), Lesage et al. (2007b, Belgium) were found for reed.

4.2. Water and sediment analyses

Concentrations of Ag, Al, Fe, Mn, and Zn in water entering the fourth pond of the Etueffont lagooning system varied significantly during the two study seasons. Data collected were generally lower than (Cd, Cr, Cu, Ni, Zn) (Vymazal et al., 2007) or similar to (Mn, Zn) (Tatsi and Zouboulis, 2002) the average heavy metal concentrations of stabilized leachates entering CW for municipal solid waste landfill treatment reported in the literature. Moreover, for the 2 years, the heavy metal concentrations recorded during the present study were higher in spring than in fall, corroborating the results of Grisey et al. (2011) at the same site. The spring increase in heavy metal in the water entering the fourth pond may be explained by (i) increased spring rainfall, which induced an enhancement of both lixiviation and, to a lesser extent, heavy metal dilution (Khattabi et al., 2007) and (ii) reduced biological activity and reduced fixation of these elements in lagoons 1, 2 and 3 during winter and early spring. Once released into the aquatic environment, metals were partly transferred to sediments by both processes of adsorption onto suspended matter and sedimentation (Zwolsman et al., 1993). Sedimentation of polluted particles resulted in high metal concentrations in CW sediments which are generally considered as a sink (Hart, 1982). Mangani et al. (2005) found that some metals such as Zn and Cu were mainly associated with dissolved fraction, thus the binding of metals to suspended/dissolved organic matter led to precipitation of metals into sediments which may explain accumulations measured in sediments. The concentrations of metals in the sediments collected were within the typical ranges of metal concentrations measured in bottom sediments that are considered European background values by Bowman and Harlock (1998) and Samecka-Cymerman and Kempers (2001) (expressed in mg kg^{-1}: Cu 2–100, Cd 0.1–1, Co 1–25, Ni 0.5–100, Pb 2–80, Zn 10–200, Ni 0.5–100). Moreover, except for Cu whose concentrations are higher than those recorded by Samecka-Cymerman et al. (2004) in Polish horizontal sub-surface flow constructed wetlands (16.2–31.9), metals in Etueffont sediments are lower or in the same range as data collected in horizontal or vertical sub-surface flow constructed wetlands (Germany: Gschlössl and Stuible, 2000; Poland: Samecka-Cymerman and Kempers, 2001;

Czech Republic: Vymazal and Krasa, 2003; Belgium: Lesage, 2006; Lesage et al., 2007a), or in free water surface constructed wetlands (Lesage, 2006). According to Samecka-Cymerman and Kempers (2001), metal concentrations in water and sediments are usually negatively correlated with pH. In addition, sediment structure is also one of the most important factors of soil composition affecting the extent of metals absorbed by plants.

4.3. Root and rhizome metal storage from water and sediments

Plant concentrations appear to reflect the metal content of their surrounding environment, of both water and sediment (Lin and Zhang, 1990; Sawidis et al., 1995), but even more in speciation and changes in bioavailability which depends on numerous environmental characteristics of water and sediment, especially temperature, redox potentiel, pH, water ion content, salinity conditions (Larsen and Schierup, 1981; Schierup and Larsen, 1981; Liang et al., 2003; Sundareshwar et al., 2003; Demirezen and Aksoy, 2004) and interaction between elements (Markert, 1987). For the 2-years, Al, Mn and Fe were stored massively in below-ground plant parts (roots and rhizomes) of *P. australis* and *T. latifolia*. They are mainly extracted from sediments since these elements were at low concentration in input and output waters. Fe and Mn are involved in photosynthetic processes and either strongly absorbed (Fe) or even highly concentrated in roots (Mn) and transfered to rhizomes as indicated by ECR values. Al concentrations in sediments were very high but stored in roots only, indicating that roots exert a strong selection among elements since only Fe and Mn are transferred from root to rhizome, as indicated by low ECRh values for most metals. The spring increase in waters and sediments may be linked to low rates of photosynthesis, element uptake and microbial respiration during winter which can lead to anoxic conditions in the sediment and Al, Fe, and Mn release (Goulet and Pick, 2001). Even though values are not always similar, depending on wetland conditions and climate, the data is in accordance with those reported by Vymazal et al. (2009) for Al and Fe from Czech CW or by Lesage et al. (2007a,b) from a Belgian CW, but greater values were observed for Mn in the Etueffont lagooning system. Generally, average concentrations of Al, Fe and Mn in Etueffont were higher than those reported elsewhere for reed (Surface et al., 1993; Behrends et al., 1994; Zuidervaart, 1996; Eckhardt et al., 1999; Obarska-Pempkowiak et al., 2005) or cattail (Behrends et al., 1994; Carranza-Álvarez et al., 2008; Klink et al., 2012) roots growing in many constructed wetlands.

Toxic elements such as Cd, Cr, Cu, Ni and Zn were in low concentrations or even at detection limits in water while stored in sediments. Their availability in sediments explains their uptake by roots of both plants though with a limited transfer to rhizomes, similarly to Al as confirmed by ECRh. The roots of these macrophytes acted as filters for potentially toxic elements. For example, Vardanyan and Ingole (2006) showed that aquatic macrophytes accumulated more Cr in roots than in other plant organs. Though the levels of Cr in Etueffont macrophytes were significantly higher than those reported for reeds by Vymazal et al. (2007, 2009) in the Czech Republic or by Baldantoni et al. (2004) and Ranieri (2005) in Italian wetlands, they were generally lower than those reported by Carranza-Álvarez et al. (2008) in a Mexican artificial lagoon or by Szymanowska et al. (1999) in western Poland for cattails. High Cr concentrations in roots as well as high ECR suggest that, similarly to Aksoy et al. (2005b), *P. australis* may be a more efficient accumulator of heavy metals in root/rhizome parts than *T. latifolia*.

The levels of Cu and Zn in *P. australis* roots and rhizomes are significantly higher than those reported by Eckhardt et al. (1999) and Aksoy et al. (Aksoy et al., 2005a,b) but in the same value range recorded at the same site in 2009 before the clearing of the ponds (Grisey et al., 2011). Though higher values were recorded for root system, the Cu and Zn concentrations in rhizomes in spring were similar to those found in constructed and natural wetlands by Samecka-Cymerman and Kempers (2001), Lesage et al. (2007a,b) as well as by Vymazal et al. (2007, 2009). Nevertheless concentrations of the two elements were substantially lower than those reported for reeds by Mungur et al. (1994) from a natural wetland or by Windham et al. (2003) from a contaminated marsh.

The levels of Cu and Zn in fall in *T. latifolia* below-ground organs are similar to those reported by Maddison et al. (2009) in cattail in Estonian semi-natural and constructed wetlands, or by Grisey et al. (2011) at the Etueffont landfill leachate treatment site in 2009. However, the concentration values in roots and rhizomes from cattail in the Etueffond CW measured during the spring period are higher than those observed by Tanner (1996) in New Zealand and by Klink et al. (2012) in Poland.

The levels of Cd in *P. australis* and *T. latifolia* below-ground plant parts are lower than those reported by Grisey et al. (2011) at the same site. Moreover, though root contents were lower than those observed in Poland in *T. latifolia* by Klink et al. (2012) or by Samecka-Cymerman and Kempers (2001) in *P. australis* collected in anthropogenic lakes, concentrations were twice as high as those reported by Surface et al. (1993), Eckhardt et al. (1999) or by Vymazal et al. (2007) in the same plant organs. Therefore, similarly to Iannelli et al. (2002) for *P. australis* and by Klink et al. (2012) for *T. latifolia*, reeds and cattails of the Etueffont lagooning system have a high capacity for accumulation in roots associated with a strong limitation of transport toward rhizomes and upper parts as confirmed by ECL and TLF values (ne).

Similarly, for both cattail and reed, we measured a Ni storage in roots and a low storage in rhizomes (ECRh 0.03–0.11). Ni concentrations were higher than those reported by Ranieri (2005) and Vymazal et al. (2009), and lower than those reported by Sasmaz et al. (2008).

According to our data and to the literature, bioaccumulation in rooted macrophytes such as *T. latifolia* and *P. australis* (especially in roots) depends more on heavy metal concentrations in sediment than those in wetland waters, which implies relevant availability in sediment and poor selectivity of the cortex parenchyma with large intercellular air spaces (Sawidis et al., 1995). Taken as a whole these results are typical of tolerant plants which tend to restrict root-shoot transfer and limit accumulation in above-ground biomass while hyper accumulators tend to facilitate uptake and translocation (Ahmad et al., 2010).

4.4. Storage in aerial parts

In accordance with previous studies of metallophytes (Baker et al., 1994; Singh and McLaughlin, 1996; Demirezen and Aksoy, 2006; Vardanyan and Ingole, 2006; Bonanno, 2011; Klink et al., 2012) data collected in the Etueffont CW during the 2-year study showed for both species a predominant immobilization in the roots rather than a transfer to the above-ground plant parts (stems and leaves) for metals such as Al, As, Cd, Cr and Ni as shown by low TLF values. This metal sequestration in roots and exclusion from above-ground tissues behave as a metal tolerance strategy with filtering action in order to protect rhizomes and shoots from metal-induced injuries as demonstrated for cadmium (Taylor and Crowder, 1983) and zinc (Ellis et al., 1994; Du Laing et al., 2003; Klink et al., 2012) in *T. latifolia*, or for copper in *P. autralis* by Fürtig et al. (1999). Fe and Zn are poorly stored in aerial parts (TLF < 0.03) while we recorded a transfer of Mn from rhizomes to stems and leaves though TLF value are below 0.37. The leaves of *P. australis* and *T. latifolia* represented the second site of accumulation of Mn after the root with concentrations greater than those reported by Eckhardt et al. (1999)

or by Lesage (2006) for reed, and by Obarska-Pempkowiak et al. (2005) for cattail. These findings are also in agreement with those of Demirezen and Aksoy (2006) and Klink et al. (2012) who reported that Mn can easily move within plants and accumulate mainly in above-ground plant organs and that its concentration is higher in roots than in sediments.

These elements, though necessary for photosynthesis as oligoelements, have been shown: (i) to inhibit at high concentration levels plant rooting and growth (Chaney, 1989; Šottníková et al., 2003), (ii) to disturb nutrient uptake and sulphate assimilation (Baszynski et al., 1980; Baszynski, 1986), and (iii) to interfere with photosynthetic processes, such as chlorophyll content and photosynthesis (Baszynski et al., 1980; Memon et al., 2001), CO_2 fixation (Bienfait, 1988) or pigment apparatus (Baszynski, 1986; Stiborova et al., 1986). Toxic levels also have deleterious effects on enzyme activity or protein function (Baszynski, 1986; Sheoran et al., 1990; Du Laing et al., 2009; Bonanno and Giudice, 2010), carbohydrate metabolism (Alloway, 1990; Borkert et al., 1998) and affect electron transport (Greger and Ögren, 1991) and water balance (due to alteration of plasma membrane properties) (Sanità di Toppi and Gabbrielli, 1999).

Boron behaves similarly to Fe, but to a much lower extent in root/rhizome, though it showed high mobility in the above-ground parts for reed and cattail, as underlined by TLF values ranging from 0.16 to 0.80 and 0.40–0.89 for *P. australis* and *T. latifolia* respectively. However, TLFs indicate that boron storage by *P. australis* was restricted to below-ground plant parts, especially in roots, as shown by the TLF below 1, which were lower than values reported by Bonanno (2011) in Sicilian natural wetlands (1.47).

4.5. Phytoremediation potential of *P. australis* and *T. latifolia*

Phytoremediation, using *P. australis* and *T. latifolia* as tolerant species, is well-founded and eco-friendly in the restoration of water quality and sediment properties in constructed wetlands (Vymazal et al., 2009; Grisey et al., 2011; Anning et al., 2013). In the present study, as observed by Windham et al. (2003), the enrichment coefficients for the stems and leaves of the two species were generally low, indicating a low transfer rate into the above-ground plant biomass during the four collecting periods. These results are supported by TLFs below 0.1, characteristic of exclusion species, with a reduced ability for heavy metal uptake, transport, and storage in above-ground parts (Peverly et al., 1995; Karpiscak et al., 2001; Weis et al., 2004), most likely due to inefficient metal transporter systems (Zhao et al., 2002). According to Zu et al. (2005), differences in TLF values indicate that, though each heavy metal has specific phytotoxic effects on macrophytes, restriction of translocation from below- to above-ground plant parts is believed to be one of the tolerance mechanisms developed by non-hyperaccumulator species to cope with metal stress induced by surrounding water and sediment, therefore avoiding negative effects on the photosynthetic tissues. Since root exclusion is an energy requiring a mechanism, a decrease in photosynthetic activity would reduce metal exclusion by *T. latifolia* and *P. australis* roots, thus leading to an increase in heavy metal transfer to the plant's aerial tissues. This has been already observed in senescing aerial plant parts of *P. australis* collected at the end of fall (December) in a CW of the Venice Lagoon watershed (Bragato et al., 2006) which was considered as a potential means to eliminate the accumulated toxic metals from the plant. Despite positive results obtained in the Etueffont lagooning system, with maximum values close to 1 in fall for many elements (Cd, Sb, Be, Li, and Mn), neither *T. latifolia* nor *P. australis* efficiently transferred heavy metals from root to shoot and therefore cannot be considered as hyper accumulators. Moreover, element interactions in plants may occur in conflicting or synergetic processes which may affect metal uptake and translocation between plant parts. Accumulation of heavy metals may also be affected by several factors in addition to their concentration in water and sediment: plant growth dynamics and physiology in relation with season, heavy metal uptake, translocation and compartmentalization, species-specific capacities for storage and detoxification and heavy metal tolerance capacity of root-cell walls (Bargagli, 1998; Cardwell et al., 2002; Mishra et al., 2008).

In agreement with the literature, significant correlations of heavy metal concentrations between organs, water and sediment indicate that metal accumulation in *P. australis* and *T. latifolia* reflects the temporal fluctuations of elements in water and sediment (Bargagli, 1998; Zakova and Kockova, 1999; Vardanyan and Ingole, 2006). The two plant species can thus provide quantitative assessment of environmental quality.

5. Conclusion

In the fourth pond of the Etueffont lagooning system, high amounts of heavy metals found at the inflow of this constructed wetland system are efficiently removed through the aquatic macrophyte bioaccumulation process. Despite differences in metal accumulation ability in the above- and below-ground tissues of *T. latifolia* and *P. australis*, concentrations of studied heavy metals decreased in the following order: roots > rhizomes ≥ leaves > stems > flowers. The significantly different levels of heavy metals found in the different plant parts imply low metal mobility from roots to rhizomes and from roots to stems, leaves or flowers. Though macrophyte growth is not affected by metal concentrations in water and sediment (tolerance to high phytotoxic concentration), leachate composition plays an important role in the uptake of heavy metal by plant roots. The positive correlations found in this study between metal concentrations in plant organs and those in water and sediment indicate that *T. latifolia* and *P. australis* root tissues reflect the cumulative effects of environmental pollution from water and/or sediment, thus registering metal temporal fluctuations. These plant species are therefore useful tools for biomonitoring programs aiming to provide quantitative assessment of the environmental quality of waters and sediments.

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