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The Bioaccumulation Performance of Reeds and Cattails in a Constructed Treatment Wetland for Removal of Heavy Metals in Landfill Leachate Treatment (Etueffont, France)

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Abstract The aim of this study was to evaluate and compare the capacities of cattail (*Typha latifolia* L.) and reed (*Phragmites australis* L.) for heavy metal storage in the phytomass. Samples were studied in the fourth of the four interconnected natural lagooning basins of a constructed treatment wetland, developed as an integrated pilot system for the treatment of leachates in a domestic landfill site at Etueffont (Territoire de Belfort, France). The efficiency of the lagooning system was evaluated through physical and chemical parameter measurements over a period of three seasons. Anion/cation and heavy metal concentrations were sampled and analyzed in water flowing into and out of the lagooning basin. Simultaneously, reed and cattail biomass samples (roots/rhizomes, shoots) were collected at both inflow and outflow, and the biomass characteristics were determined. The average above-ground biomass of *T. latifolia* and *P. australis* varied, respectively, from 0.41 to 1.81 kg DW m^{-2} in the fall, 0.31 to 1.34 kg DW m^{-2} in winter, and 0.38 to 1.68 kg DW m^{-2} in spring, with significant seasonal variations. The greatest mean concentrations of heavy metals were found in the below-ground plant parts of the two species during the spring season. The average standing stock of heavy metals was higher in the below-ground than in the above-ground phytomass, whatever the season. With the exception of nickel, heavy metal concentrations in the inflow were correlated to the plant content of both species.

Keywords Landfill leachate · Heavy metals · *Typha latifolia* L. · *Phragmites australis* L. · Phytoremediation

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1 Introduction

Constructed wetlands (CW) designed as natural filter areas for the treatment of water-transported pollutants have been widely used in Europe and North America for landfill leachate or sewage and wastewater management systems, so as to improve water purification before discharge into rivers. Organisms living in CW such as landfill leachate treatment systems undergo variable degrees of chemical pressure and must tolerate high levels of heavy metals and high organic and nutrient loads (Kabata-Pendias and Pendias 2001; Schwarzbauer et al. 2002). Moreover, local climatic conditions as well as hydrology and waste-hiding techniques play an important role in leachate composition as reported both in laboratory experiments (Bookter and Ham 1982; Blaky 1992) and in the field (Kjeldsen et al. 2002).

Effects on aquatic ecosystems are of particular concern at the Etueffont landfill site (Territoire de Belfort, France), especially on phytoplankton (Khattabi et al. 2006), macro-invertebrates (Khattabi and Aleya 2007), and bacteria (Grisey et al. 2010). Other components of wetland treatment areas, for example macrophytes, play an important role in pollutant removal, while the efficiency of different species, such as reeds (*Phragmites australis* L.), cattails (*Typha latifolia* L.) or fescue (*Festuca arundinacea* Schreb.) has been reported (Gray et al. 2000; Karathanasis et al. 2003) in comparative studies. At the Etueffont landfill site, however, these macrophytes had never before been investigated, though their high tolerance to toxic contaminants and their great capacity for heavy metal accumulation in relation to wastewater treatment processes have been studied. Several plant species have been examined in both natural wetlands (Schierup and Larsen 1981; Hocking 1989a, b; Keller et al. 1998; Samecka-Cymerman and Kempers 2001; Windham et al. 2001; Bonanno and Lo Giudice 2010) and constructed ones (Peverly et al. 1995; Kadlec and Knight 1996; Obarska-Pempkowiak and Klimkowska 2000; Vymazal and Krása 2003; Toet et al. 2005; Bragato et al. 2006; Lesage et al. 2006, 2007), with *Phragmites* spp. (common reed) and to a lesser extent *Typha* spp. (cattails), *Juncus* spp. (rush), and *Scirpus* spp. (bulrush) being the most frequent. As shown in previous studies, accumulation of heavy metals in the above- and below-ground plant parts of aquatic macrophytes may be influenced by plant growth dynamics and thus may vary from season to season according to metal levels and availability in the surrounding water and sediment (Larsen and Schierup 1981; Schierup and Larsen 1981; Hardej and Ozimek 2002).

In the present study, the response of two aquatic macrophytes growing in the four basins of the Etueffont lagooning system used to treat landfill leachate was studied for three consecutive seasons at the system's inflow and outflow points. The seasonal growth dynamics of *P. australis* and *T. latifolia* were investigated, and storage of heavy metals (Sb, As, Be, Cd, Cr, Co, Cu, Sn, Li, Mn, Mo, Ni, Pb, Se, Sr, Tl, Ti, V, and Zn) in root/rhizome and shoot tissues in the two macrophytes was determined, along with their concentration in corresponding water and sediment samples. The aim of this study was to investigate the impact of season on heavy metal concentration in water inflow/outflow and the corresponding accumulation in the above- and below-ground plant tissues of the two macrophytes over a 6-month period, from fall to spring, and also to determine the overall cleaning power of aquatic macrophytes in the Etueffont conditions prior to water discharge into a small stream. The differences in heavy metal accumulation by each of the two aquatic macrophytes and plant harvest are also discussed.

2 Materials and Methods

2.1 Presentation of the Study Area

The domestic landfill treatment station (Fig. 1) represents a total area of 4 ha and was used from 1976 to 2002, collecting household wastes from 66 municipalities with a combined population of about 47,650 inhabitants. The landfill was established on schistose ground and operated in the open air by crushing the waste before disposal and leaving it without cover after grinding and before filling. In 2002, the waste layer was 15 m thick and contained 200,000 tons of waste. When the site was closed, it was covered by a layer of artificial soil composed of crushed organic waste (paper, wood, lawn cuttings, straw, fabrics) (Khattabi et al. 2006, 2007). The leachates are collected downstream by a draining system and treated in four natural lagooning basins whose characteristics are summarized in Table 1. During the 2008–2009 monitoring period, inflow was adjusted to 59.4 m^3 day^{-1}. Theoretical retention time was approximately 19 days. The present study was conducted in the fourth and final basin (Fig. 1), prior to water discharge into the stream. The basin itself is found on an underlying layer of clay 1 m thick and has a bed slope of 1%. A mix of reed (*P. australis* L.) and cattail (*Typha angustifolia* L.) plants was planted at a mean density of 1/m^2 in spring 2003 (500 per species).

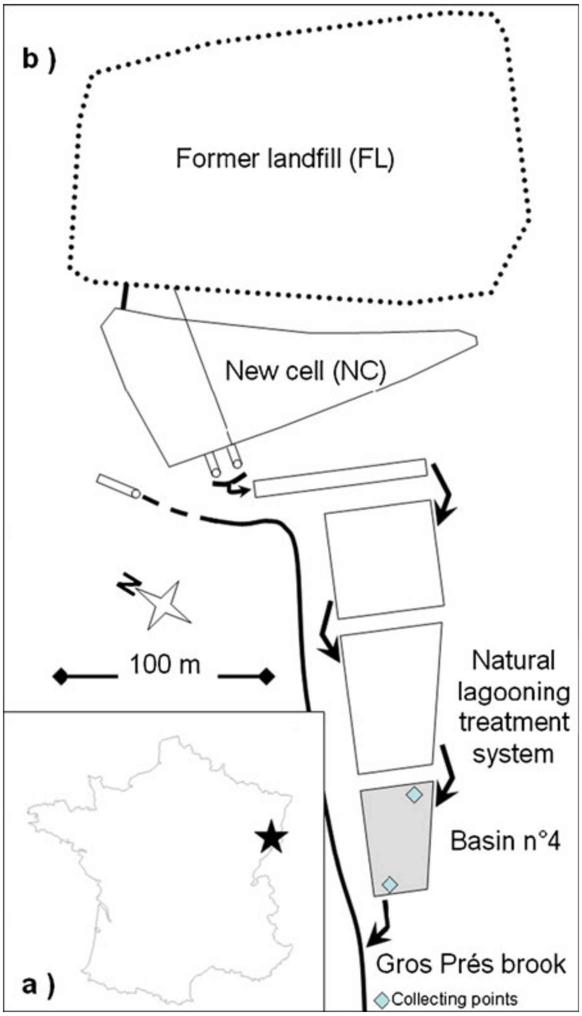


Fig. 1 *a* Etueffont landfill location; *b* schematic map of the site: former landfill (*FL*), new cell (*NC*), fourth basin of the natural lagooning treatment system with collecting points

2.2 Sampling Strategy and Water Analysis

Two hundred forty water chemistry samples were collected in 150-ml and 2-l bottles during the experiment from (a) five points in basin no. 3 as it entered basin no. 4 (inflow) and (b) five points on leaving basin no. 4 as the water moved into the stream (outflow), in November 2008 (fall), February 2009 (winter), and April 2009 (spring), every 2 days during 2 weeks for each season. Samples for background values were collected in the Gros Prés brook on the same dates. All nutrients and heavy metal concentrations were close to detection limits of the ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS) (data not shown). Sediments were sampled with a sediment corer (10 cm diameter) at about 10/15 cm depth in basin 4 so as to obtain both sludge deposits and a small portion of the underlying clay. In each season, samples were taken near the inflow and outflow points. Ambient environmental factors at the wetland study site were determined in situ for each sampling campaign, namely temperature (*T*, degree Celsius), pH, and electrical conductivity of inflow and the outflow with a portable multiparameter probe (WTW, Multiline P3 PH/LF-SET).

2.3 Physicochemical Analyses

After collection, the bottles were stored at 4°C for preservation before preparation and analysis. The samples were filtered by means of a 0.45-μm membrane. Water-soluble ion concentrations were determined by IC. Aqueous extracts were analyzed

Table 1 Morphometric characteristics of the fourth basins

	Basins			
	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	1,934	1,848	1,128
Volume (m^3)	312	1,934	1,848	1,128
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

in a dual column Dionex (Sunnyvale, CA, USA) DX-100 ion chromatograph equipped with a Dionex AS50 autosampler and using isocratic ion analysis and conductivity detection. Background conductivity was suppressed using Dionex Self-Regenerating Suppressors TM, models ASRS-ULTRA (anion suppressor) and CSRS-ULTRA (cation suppressor). Determination of Br^-, Cl^-, F^-, NO_3^-, NO_2^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, K^+, and Na^+ ions was performed using the following setup: firstly, separation of anionic analytes on an AG14 guard column preceded by a Dionex AS14-4 mm analytical column (eluent 3.5 mM Na_2CO_3/1.0 mM $NaHCO_3$, flow rate 1.2 ml min^{-1}) and, secondly, separation of cationic analytes on a CS12-4 mm cation separation column preceded by a Dionex CG12A-4 mm guard column (eluent 20 mM H_2SO_4, flow rate 1.0 ml min^{-1}). Certified standard multi-ion solutions (Combined Seven Anion Standard II, Combined Six Cation Standard II, Dionex) diluted to effective concentrations of 50, 25, 10, 5, 2.5, and 1 ppm standard solutions were used for calibration. Data treatment was achieved with Peaknet software package, version 6. Detection limits obtained with IC were 0.01 for Br^-, Cl^-, and F^-; 0.100 for NO_2^-, NO_3^-, and SO_4^{2-}; 0.01 for Ca^{2+} and Mg^{2+}; and 0.100 for K^+ and Na^+. Data outputs are in milligrams per liter.

2.4 Macrophytes (Reeds and Cattails) and Sediments

Two weed species (*T. latifolia* L. and *P. australis* L.), along with the water, were sampled three times. The reed and cattail biomass samples (roots/rhizomes, shoots) were collected from five plots (1×1 m) near the inflow and outflow points. The weeds were sampled individually, (a) at the end of their summer growth period (fall), (b) at the end of winter (dormant period), and (c) during spring growth, and were processed separately. Each stem and root/rhizome part was thoroughly rinsed several times with deionized water before oven drying at 80°C 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 0.5-g dry wt. sample was pressure digested for 20 min using a mixture of reagent grade: 5 ml HNO_3 (65%) and 1 ml H_2O_2 (VDLUFA 1996) before analysis by ICP-MS.

Sediment samples were wet sieved through a 5.0-mm pore-size polypropylene mesh to separate the sediment-size fraction with reagent water and eliminate plant fragments. Samples were left to settle and the water was later decanted. The clay-fraction sediments were oven-dried to a constant weight at 60°C for 24 h. The samples were later homogenized using a mortar and pestle and dry-sieved through a 2.0-mm pore-size polypropylene mesh. The mortar, pestle, and sieve were cleaned before and after every sample with 10% redistilled HNO_3 and then rinsed with reagent water. A 0.5-g dry wt. of each sediment sample was digested with aqua regia at 95°C in a microprocessor-controlled digestion box for 2 h (DIN ISO 11466 1997). Concentration of heavy metals in water, plant, and sediment samples was determined by ICP-MS using a Perkin Elmer SCIEX ELAN 6100 DRC. International certified reference materials for the water (NRCC-SLRS-3), plants (INCT-TL-1), and sediments (NIST-1643-e) were analyzed at the beginning and end of each batch of samples to assess accuracy and precision. Instrument performance during analysis was monitored using Rh/Ir internal standard. For both macrophyte and sediment analysis, internal control standards were analyzed for every sample, and a duplicate was run for every ten samples. The detection limits obtained with ICP-MS were 0.05 mg L^{-1} for Sb, As, Sn, Pb, Se, and Tl; 0.01 mg L^{-1} for Mo and Mn; 0.001 for Be; 0.005 mg L^{-1} for Cd, Cr, Co, Cu, Li, Ni, Ti, V, and Zn; and 0.0005 mg L^{-1} for Sr. Data outputs are in milligrams per liter.

2.5 Determination Enrichment Coefficient and Transfer Factor

The enrichment coefficient for the roots (ECR) and for the shoots (ECS) and the transfer factor (TF) can be estimated as follows:

ECR Heavy metal concentrations in plant roots/heavy metal concentration in water

ECS Heavy metal concentrations in plant shoots/heavy metal concentration in water

TF Heavy metal concentrations in plant shoots/heavy metal concentration in plant roots

2.6 Statistical Analysis

In order 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 to $P<0.05$.

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/Outflowing Water Quality

3.1.1 Ambient Environmental Factors

During the experiment, the highest temperature at inflow was recorded in spring and the lowest in winter; temperatures did not vary noticeably from inflow to outflow (Fig. 2). In fall and winter, the water cooled, but freezing conditions never occurred and the lowest temperature recorded was 1.3°C at outflow. The pH did not vary noticeably from one period to another. The electric conductivity of water at outflow was significantly reduced compared to conductivity at inflow (Fig. 2).

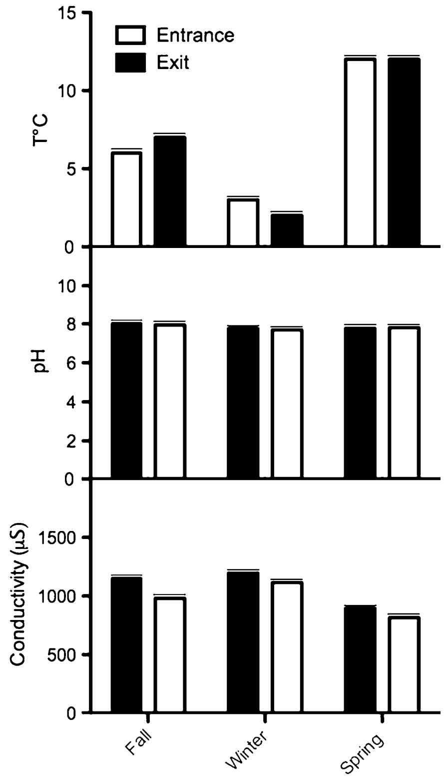


Fig. 2 Temperature (degree Celsius), pH, and conductivity (microsiemens) of the in/outgoing water flow of the Etueffont fourth lagoon

3.1.2 Heavy Metals in Water

In/outflow was also analyzed for heavy metals (Sb, As, Be, Cd, Cr, Co, Cu, Sn, Li, Mn, Mo, Ni, Pb, Se, Sr, Tl, Ti, V, Zn). Data measured at the three dates are shown in Table 2. Significant seasonal variations of heavy metal content were recorded in the in/outflow with a peak in spring for all elements except Cd, Mn, and Ni (peak during winter). Only Se (inflow) and Ti (inflow and outflow) did not vary significantly with the seasons. Thus, the mean concentration of Ti was constant in the lagoon during all seasons (approximately 0.003 mg L^{-1}).

Heavy metal removal measured at the outflow pipe varied according to element and season. Given that chloride is present in water but poorly fixed by organisms and plants, the ratio sum of elements/chloride expresses the entire fixation of heavy metals by the lagooning system. During the fall period, this ratio reached 10 and increased two to three times during winter and spring, indicating that cleaning processes depend on summer period and plant activity. Whatever the season, statistical analysis established that for three elements only (Pb, Se, and Ti), there was no difference between water entering and leaving the lagoon. In fall, only Be, Cd, Mn, Mo, Sr, and Zn were significantly reduced ($P<0.05$) in comparison with the concentration measured at inflow. During the winter period, the small differences in As, Sn, and Li removal between inflow and outflow were not statistically significant, although As, Sn, and Li concentrations in outflow were reduced. Except for Pb, Se, Cr, and Ti, heavy metal content in the water flowing out of the system was significantly lower in spring.

3.1.3 Heavy Metals in Sediments

The mean values of the three collecting dates are presented in Table 3. The heavy metal content in outflow sediments showed lower values than those found in inflow. However, whatever the element, no significant seasonal variations in concentrations were observed between the three seasons.

Heavy metal removal in the sediment between inflow and outflow varied from −4% to −74%. Only Co, Li, Pb, Se, Tl, Ti, and V did not vary significantly

Table 2 Heavy metal concentrations (milligrams per liter) in the in/outgoing water flow of the Etueffont fourth lagoon

	Inlet				Outlet				% Reduction/P value					
	Season			P value	Season			P value	Season					
mg L^{-1}	Fall	Winter	Spring		Fall	Winter	Spring		Fall		Winter		Spring	
Sb	0.27±0.01 a	0.28±0.01 a	2.42±0.01 b	***	0.25±0.01 a	0.24±0.01 a	2.07±0.00 b	***	−7	ns	−14	**	−14	***
As	2.52±0.07 a	1.54±0.06 b	3.12±0.06 c	***	2.41±0.05 a	1.47±0.04 b	2.61±0.02 c	***	−4	ns	−5	ns	−16	***
Be	1.55±0.07 a	2.58±0.09 b	3.52±0.04 c	***	1.43±0.04 a	2.36±0.05 b	3.44±0.01 c	***	−8	*	−9	*	−2	*
Cd	0.01±0.00 a	0.03±0.00 b	0.01±0.00 a	***	0.01±0.00 a	0.02±0.00 b	0.01±0.00 a	***	0	*	−33	**	0	*
Cr	2.63±0.06 a	2.90±0.04 b	9.72±0.03 c	***	2.51±0.05 a	2.79±0.04 b	9.67±0.01 c	***	−5	ns	−4	*	−1	ns
Co	2.70±0.12 a	2.76±0.09 a	14.55±0.10 b	***	2.51±0.03 a	2.63±0.05 a	12.35±0.07 b	***	−7	ns	−5	ns	−15	***
Cu	5.65±0.11 a	5.77±0.07 a	15.83±0.06 b	***	5.53±0.06 a	5.46±0.05 a	14.36±0.02 b	***	−2	ns	−5	**	−9	***
Sn	0.06±0.00 a	0.06±0.00 a	0.09±0.00 b	***	0.06±0.00 a	0.06±0.00 a	0.08±0.00 b	***	0	ns	0	ns	−11	*
Li	0.06±0.00 a	0.06±0.00 a	0.12±0.00 b	***	0.06±0.00 a	0.06±0.00 a	0.11±0.00 b	***	0	ns	0	ns	−8	*
Mn	0.11±0.01 a	1.51±0.01 b	0.28±0.01 c	***	0.09±0.00 a	1.44±0.00 b	0.18±0.00 c	***	−18	**	−5	***	−36	***
Mo	2.66±0.09 a	2.58±0.08 a	9.54±0.06 b	***	2.49±0.05 a	2.42±0.04 a	9.40±0.01 b	***	−6	*	−6	*	−1	*
Ni	10.11±0.28 a	11.30±0.21 b	10.91±0.13 b	**	9.86±0.10 a	8.93±0.07 b	9.13±0.01 c	***	−2	ns	−21	***	−16	***
Pb	0.08±0.00 a	0.06±0.00 b	0.13±0.01 c	***	0.07±0.00 a	0.06±0.00 b	0.14±0.00 c	***	−13	ns	0	ns	−8	ns
Se	0.26±0.01 a	0.26±0.01 a	0.23±0.04 a	ns	0.25±0.01 a	0.25±0.01 a	0.20±0.01 b	***	−4	ns	−4	ns	−13	ns
Sr	357±12 a	797±11 b	804±15 b	***	310±11 a	708±10 b	756±8 c	***	−13	**	−11	***	−6	**
Tl	0.49±0.01 a	0.48±0.01 a	1.00±0.02 b	***	0.48±0.01 a	0.48±0.01 a	0.88±0.01 b	***	−2	ns	0	ns	−12	***
Ti	0.03±0.00 a	0.03±0.00 a	0.03±0.00 a	ns	0.03±0.00 a	0.02±0.00 a	0.02±0.00 a	ns	0	ns	−33	ns	−33	ns
V	1.18±0.03 a	0.97±0.02 b	0.98±0.03 b	***	1.13±0.04 a	0.89±0.02	0.90±0.01 b	***	−4	ns	−8	**	−8	**
Zn	6.54±0.14 a	11.25±0.12 b	10.04±0.05 c	***	4.49±0.05 a	9.73±0.03	9.77±0.01 b	***	−31	***	−14	***	−3	**

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, and ***P<0.001

Table 3 Mean heavy metal concentrations (micrograms per gram DW) in soil–sediments collected from the study site at inflow and outflow locations throughout the experimental period

μg g^{-1}	Inlet	Outlet	% Reduction	*P* value
Sb	4.92±0.61	2.98±0.73	−39	***
As	46.30±2.51	18.50±3.62	−60	***
Be	8.37±0.74	2.16±0.19	−74	***
Cd	3.30±0.18	0.92±0.04	−72	***
Cr	92.10±4.61	59.30±1.13	−36	***
Co	38.05±4.6	31.70±4.9	−18	ns
Cu	322.30±14.3	91.10±4.1	−72	***
Sn	34.70±1.13	11.30±1.24	−67	***
Li	3.22±0.87	3.04±0.92	−6	ns
Mn	3,890±192.8	2,491±112.3	−36	***
Mo	4.49±0.37	3.12±0.49	−31	***
Ni	56.42±2.18	41.53±2.23	−26	***
Pb	39.17±6.93	35.42±6.42	−10	ns
Se	1.92±0.64	1.84±0.37	−4	ns
Sr	84.60±8.62	68.60±7.52	−19	***
Tl	2.19±0.09	2.11±0.06	−4	ns
Ti	8.64±1.26	8.03±1.12	−7	ns
V	12.69±2.17	11.93±1.93	−6	ns
Zn	657.2±27.1	196.2±23.4	−70	***

Data are mean±SD. $n=15$

*$P<0.05$, **$P<0.01$, and ***$P<0.001$

between inflow and outflow, with removal lower than 18% (Table 3).

3.2 Biological Parameters

3.2.1 Macrophyte Phytomass

The average above-ground biomass of cattail (*T. latifolia*) and common reed (*P. australis*) are shown in Fig. 3. The highest shoot biomass of common reed plants was measured in fall with 1.41 kg DW m^{-2} at inflow of basin no. 4. Above-ground cattail phytomass values between inflow and outflow were not significantly different during the three seasons. The below-ground biomass of cattail varied from 0.58 to 1.38 kg DW m^{-2} and that of reed from 1.72 to 1.84 kg DW m^{-2} (Figs. 3 and 4). Though the average above-ground biomass of reed and cattail did not differ significantly, the reed root/rhizome biomass was higher than that of cattail. No significant difference in the root/rhizome and shoot biomass between seasons and localization (inflow/outflow) was observed for the two plant species, confirming uniform growing conditions.

3.2.2 Heavy Metals in Macrophytes and Relation with Water Content

Concentrations measured in *T. latifolia* and *P. australis* sampled at the three dates are shown in Tables 4 (*T. latifolia* L.) and 5 (*P. australis* L.). In most cases, the concentrations of heavy metals were higher in root/rhizome plant fractions than in aerial plant parts.

T. latifolia The highest concentrations of heavy metals in *T. latifolia* were found in the root/rhizome part on the inflow side of the lagoon. Except for Sb, Cd, Cr, Co, and Li, significant differences between dates in the *T. latifolia* root/rhizome part were recorded (Table 4). The mean concentration of these elements did not vary in the roots whatever the season. For As, Be, Cu, Sn, Mn, Mo, and Pb, concentrations in root/rhizome plant tissues were maximal in spring after plant growth, whereas a general increase of Ni, Sr, Tl, Ti, V, and Zn occurred in early fall before senescence. The uptake of heavy metals by the *T. latifolia* above-ground phytomass showed a similar variation to that of root/rhizome parts with the exception of Cd and Cr which varied significantly with the season, with a maximum concentration in winter for Cd and in spring for Cr. With the exception of the Cr concentration in the root/rhizome plant fraction (significant seasonal variations, $P<0.001$), the same pattern was observed in the root/rhizome and aerial part of *T. latifolia*, at outflow. Comparison between inflow and outflow plant samples showed that most heavy metals decreased both in roots and shoots of outflow plants, indicating a greater accumulation at inflow (Table 6). Whatever the season, no significant removal was observed for As, Ti, and V by plant root/rhizome parts and, for As, by shoot parts between the inlet and the outlet of the fourth basin.

P. australis Accumulation of heavy metals by roots and rhizomes of *P. australis* located on the inflow side of the lagoon was abundant, and values were

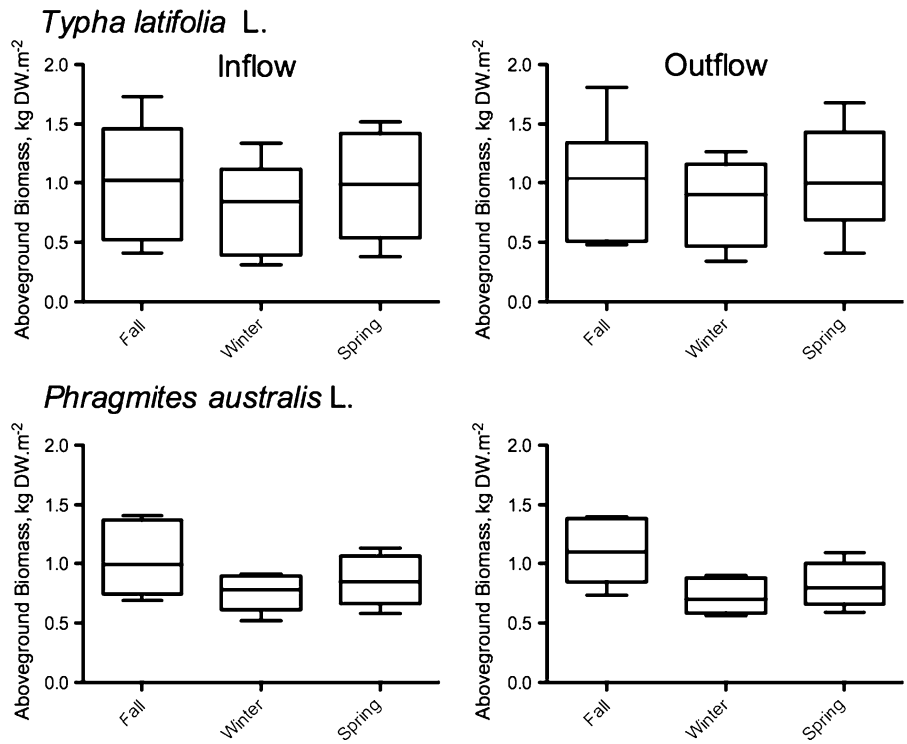


Fig. 3 Biomass of above-ground parts (grams DW per plant) of *T. latifolia* and *P. australis* from the two sampling locations (inflow and outflow) during the entire sampling period (n=6, mean±SD)

significantly higher than in the above-ground phytomass. Table 5 showed that differences between dates were significant in the *P. australis* root/rhizome part except for Sb, Cr, Li, Ni, and Se. For As, Be, Co, Cu,

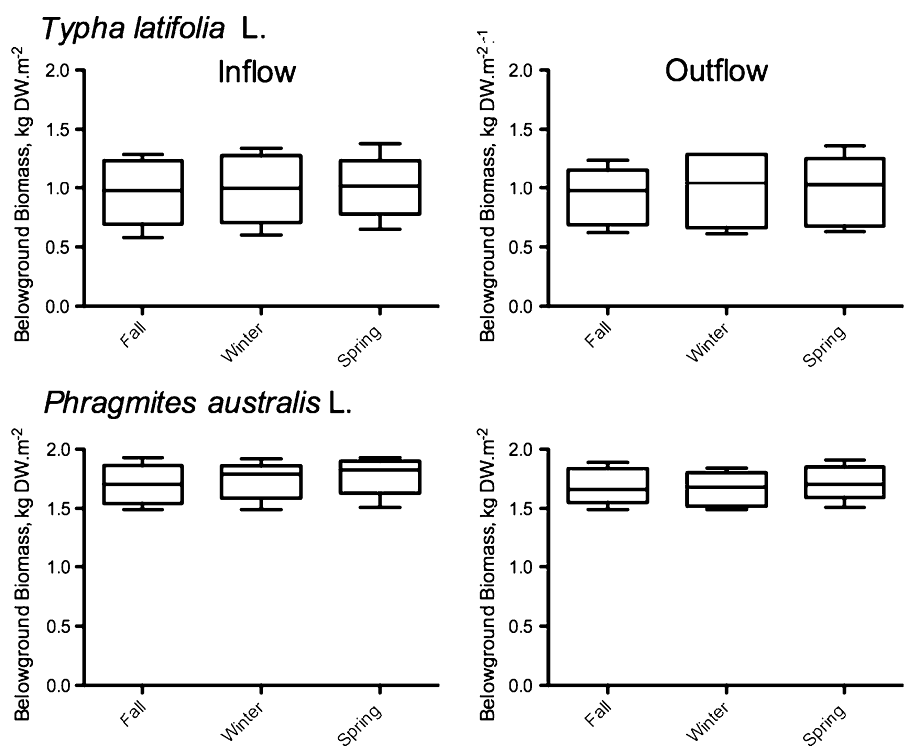


Fig. 4 Biomass of below-ground parts (grams DW per plant) of *T. latifolia* and *P. australis* from the two sampling locations (inflow and outflow) during the entire sampling period (n=6, mean±SD)

Table 4 Heavy metal concentrations (milligrams per kilogram DW) in the root/rhizome and shoot parts of *T. latifolia* in the inflow section of the fourth lagoon at the Etueffont site for the three sampling dates

T. latifolia		Root/Rhizome				Shoot			
		Fall	Winter	Spring	*P* value	Fall	Winter	Spring	*P* value
Sb	In	2.50±0.03 a	2.55±0.12 a	2.44±0.05 a	ns	2.15±0.16 a	2.17±0.08 a	2.13±0.05 a	ns
	Out	2.35±0.16 a	2.37±0.08 a	2.33±0.05 a	ns	1.65±0.08 a	1.65±0.14 a	1.43±0.25 a	ns
As	In	7.79±1.65 ab	5.96±0.86 a	10.23±2.78 b	*	4.89±1.17 ab	3.96±0.78 a	5.99±0.64 b	*
	Out	7.19±0.85 a	5.50±0.65 a	9.31±1.47 b	***	4.30±0.27 a	3.59±0.46 b	5.88±0.32 c	***
Be	In	1.05±0.04 a	2.48±0.01 b	2.49±0.01 b	***	1.01±0.04 a	1.88±0.08 b	1.84±0.10 b	***
	Out	1.01±0.03 a	2.12±0.03 b	2.11±0.04 b	***	0.96±0.03 a	1.46±0.17 c	1.73±0.05 b	***
Cd	In	2.51±0.01 a	2.52±0.02 a	2.51±0.01 a	ns	2.13±0.04 a	2.17±0.03 a	2.10±0.02 b	**
	Out	2.21±0.04 a	2.22±0.04 a	2.21±0.03 a	ns	1.95±0.04 a	1.99±0.01 b	1.92±0.01 a	**
Cr	In	2.23±0.12 a	2.32±0.05 a	2.25±0.06 a	ns	0.54±0.13 a	0.54±0.17 a	0.88±0.13 b	***
	Out	1.51±0.06 a	1.58±0.04 a	1.96±0.07 b	***	0.39±0.06 a	0.50±0.09 a	0.75±0.17 b	***
Co	In	5.38±0.12 a	5.38±0.13 a	5.53±0.14 a	ns	2.79±0.02 a	2.79±0.02 a	2.77±0.02 a	ns
	Out	5.08±0.07 a	5.10±0.08 a	5.14±0.11 a	ns	2.57±0.04 a	2.62±0.15 a	2.65±0.08 a	ns
Cu	In	5.10±1.01 a	3.93±1.27 a	11.29±2.53 b	***	1.99±0.06 a	1.96±0.08 a	3.52±0.35 b	***
	Out	3.36±0.63 a	2.46±0.62 a	6.73±1.09 b	***	1.81±0.19 a	1.71±0.11 a	3.02±0.44 b	***
Sn	In	9.34±0.13 a	9.35±0.05 a	15.12±0.20 b	***	3.90±0.22 a	4.00±0.10 a	5.96±0.14 b	***
	Out	9.00±0.10 a	9.00±0.04 a	14.75±0.17 b	***	3.59±0.27 a	3.59±0.15 a	5.64±0.18 b	***
Li	In	0.79±0.19 a	0.80±0.13 a	0.87±0.03 a	ns	0.57±0.12 a	0.59±0.05 a	0.67±0.08 a	ns
	Out	0.61±0.07 a	0.64±0.04 a	0.67±0.04 a	ns	0.49±0.05 a	0.46±0.05 a	0.50±0.06 a	ns
Mn	In	503.3±13.5 a	483.5±6.4 b	502.3±5.7 a	**	330.5±16.30 b	372.7±12.5 a	330.5±15.3 b	***
	Out	478.4±29.5 a	398.3±19.4 b	421.4±20.7 b	***	291.5±22.40 b	330.0±17.3 a	299.1±9.9 b	*
Mo	In	2.15±0.16 a	4.19±0.49 b	8.85±0.29 c	***	1.10±0.11 a	1.84±0.09 b	3.09±0.13 c	***
	Out	1.86±0.17 a	3.72±0.16 b	8.29±0.28 c	***	0.97±0.04 a	1.34±0.14 b	2.47±0.19 c	***
Ni	In	6.03±0.57 a	4.91±0.34 b	4.95±0.47 b	**	1.39±0.12 a	1.18±0.11 b	1.16±0.13 b	*
	Out	3.91±0.24 a	3.24±0.32 b	3.27±0.42 b	*	1.18±0.08 a	0.89±0.09 b	0.94±0.04 b	***
Pb	In	13.91±0.8 a	13.83±1.3 a	19.10±1.1 b	***	5.1±0.40 a	4.50±0.7 a	6.64±0.6 b	***
	Out	13.42±0.4 a	13.35±0.5 a	17.02±0.9 b	***	4.4±0.20 a	3.82±0.6 a	5.02±0.2 b	***
Se	In	2.40±0.13 a	2.43±0.10 a	2.21±0.09 b	*	1.23±0.10 a	1.17±0.11 a	0.94±0.10 b	**
	Out	2.04±0.07 ab	2.10±0.06 a	1.98±0.08 b	*	1.03±0.07 a	0.96±0.06 a	0.87±0.04 b	**
Sr	In	64.2±2.4 a	56.6±2.4 b	58.3±2.1 b	***	27.6±2.1 a	23.8±1.6 b	23.2±1.7 b	**
	Out	59.9±2.3 a	53.2±1.5 b	54.1±0.8 b	***	23.5±1.6 a	20.2±1.1 b	19.7±0.7 b	***
Tl	In	2.24±0.01 a	2.30±0.11 a	2.03±0.01 b	***	1.48±0.10 a	1.58±0.08 a	1.19±0.11 b	***
	Out	1.98±0.03 a	2.02±0.07 a	1.84±0.04 b	***	1.20±0.04 a	1.18±0.07 a	0.91±0.02 b	***
Ti	In	4.28±0.90 a	3.58±0.38 ab	2.99±0.55 b	*	1.97±0.16 a	1.53±0.18 b	1.42±0.22 b	***
	Out	3.39±0.21 a	3.04±0.17 a	2.44±0.26 b	***	1.53±0.16 a	1.36±0.19 b	1.07±0.07 b	***
V	In	5.35±0.66 a	3.74±0.48 b	4.41±0.32 b	**	1.29±0.21 a	1.08±0.12 ab	0.90±0.12 b	*
	Out	4.70±0.38 a	3.30±0.35 b	3.95±0.33 c	***	0.95±0.05 a	0.87±0.04 ab	0.79±0.07 b	**
Zn	In	45.5±5.0 a	32.1±2.1 b	36.1±4.1 b	***	17.1±1.0 a	13.2±0.7 b	12.9±1.3 b	***
	Out	33.5±2.7 a	30.1±1.6 b	30.8±1.3 ab	*	14.9±1.2 a	11.2±0.7 b	10.4±0.8 b	***

Data are mean±SD. n=6 (in milligrams per kilogram DW). Letters indicate significant differences between periods within a sampling location (for inflow and outflow)

*P<0.05, **P<0.01, and ***P<0.001

Table 5 Heavy metal concentrations (milligrams per kilogram DW) in the root/rhizome and shoot parts of *P. australis* in the inflow section of the fourth lagoon at the Etueffont site for the three sampling dates

P. australis		Root/rhizome				Shoot			
		Fall	Winter	Spring	*P* value	Fall	Winter	Spring	*P* value
Sb	In	2.42±0.12 a	2.43±0.04 a	2.73±0.31 a	ns	1.90±0.05 a	1.99±0.18 a	2.04±0.10 a	ns
	Out	2.10±0.12 a	1.98±0.04 a	2.21±0.19 a	ns	1.51±0.04 a	1.50±0.07 a	1.59±0.07 a	ns
As	In	131.0±31.3 a	126.8±27.3 a	250.9±52.0 b	***	82.9±6.3 a	76.4±7.8 a	144.9±13.4 b	***
	Out	121.2±19.1 a	108.3±11.8 a	228.6±47.8 b	***	85.8±12.0 a	72.6±7.5 a	134.00±8.9 b	***
Be	In	1.08±0.05 a	2.50±0.01 b	2.50±0.01 b	***	0.98±0.04 a	1.81±0.12 a	1.84±0.10 b	***
	Out	1.06±0.05 a	2.15±0.19 b	2.22±0.16 b	***	1.04±0.06 a	1.57±0.06 b	1.59±0.08 b	***
Cd	In	2.53±0.05 a	3.56±0.50 b	2.62±0.14 a	***	1.94±0.05 b	2.81±0.20 a	1.74±0.08 b	***
	Out	1.98±0.06 a	2.56±0.15 b	2.17±0.07 c	***	1.55±0.06 a	1.73±0.11 b	1.39±0.06 c	***
Cr	In	3.02±1.49 a	3.10±1.40 a	5.05±1.15 a	ns	0.91±0.15 a	0.86±0.09 a	1.02±0.15 a	ns
	Out	1.66±0.27 a	1.76±0.24 a	2.11±0.36 a	ns	0.72±0.13 a	0.65±0.04 a	0.77±0.13 a	ns
Co	In	27.8±3.9 a	27.6±3.8 a	39.3±3.5 b	***	16.8±1.6 ab	15.8±0.8 b	19.3±1.9 a	**
	Out	20.7±0.7 a	21.5±1.5 a	32.6±2.0 b	***	11.7±1.4 a	11.2±1.9 a	13.6±0.6 b	*
Cu	In	24.24±1.79 ab	22.66±3.62 a	34.16±9.72 b	*	8.04±0.35 a	7.66±1.13 a	11.43±2.88 b	*
	Out	19.01±2.04 a	17.44±2.27 a	25.46±2.96 b	***	7.43±0.49 a	7.33±1.02 a	10.93±2.34 b	***
Sn	In	2.33±0.08 a	2.37±0.05 a	7.92±1.09 b	***	1.02±0.03 a	1.14±0.06 a	2.67±0.15 b	***
	Out	2.15±0.03 a	2.25±0.07 a	6.10±0.43 b	***	0.96±0.05 a	1.04±0.03 a	2.07±0.16 b	***
Li	In	2.47±0.12 a	2.48±0.07 a	3.51±1.12 a	ns	1.87±0.19 a	1.97±0.16 a	2.14±0.24 a	ns
	Out	2.38±0.06 a	2.43±0.07 a	3.52±0.64 b	***	1.81±0.12 a	1.75±0.11 a	1.96±0.08 b	*
Mn	In	619.6±81.4 ab	796.3±181.2 a	502.0±5.5 b	**	454.9±32.6 a	468.0±17.4 a	407.9±25.3 b	**
	Out	482.2±17.4 a	538.4±17.2 b	485.2±11.1 a	***	353.6±19.4 a	428.0±16.4 b	348.1±23.0 a	***
Mo	In	2.11±0.12 a	3.43±1.28 ab	5.63±2.26 b	*	0.88±0.08 a	1.19±0.27 a	1.82±0.57 b	**
	Out	2.01±0.05 a	2.98±0.82 b	4.34±1.29 c	**	0.97±0.07 a	1.14±0.18 a	1.63±0.44 b	**
Ni	In	14.56±3.29 a	11.62±2.31 a	11.92±2.32 a	ns	2.82±0.36 a	2.45±0.38 a	2.41±0.44 a	ns
	Out	8.23±1.22 a	7.21±1.14 a	7.23±1.29 a	ns	1.68±0.25 a	1.45±0.35 a	1.41±0.21 a	ns
Pb	In	25.53±4.71 a	21.91±5.65 a	37.11±7.28 b	**	11.02±3.14 a	8.61±3.77 a	18.74±4.85 b	**
	Out	21.78±2.06 a	19.34±2.49 a	31.62±4.14 b	***	7.44±1.65 a	5.68±1.48 a	13.85±3.22 b	***
Se	In	3.56±1.64 a	2.73±0.93 a	2.05±0.58 a	ns	1.12±0.31 a	1.06±0.12 a	0.95±0.17 a	ns
	Out	3.05±0.59 a	2.59±0.62 ab	1.97±0.18 b	*	0.83±0.08 a	0.88±0.06 a	0.80±0.04 a	ns
Sr	In	50.22±16.83 a	62.65±7.58 ab	72.90±4.54 b	*	21.40±4.03 a	23.24±2.10 a	31.98±2.47 b	***
	Out	54.68±5.91 a	63.44±5.34 b	67.22±3.46 b	**	20.02±2.32 a	22.90±1.48 a	29.65±1.53 b	***
Tl	In	2.35±0.02 a	2.45±0.12 b	2.04±0.01 a	***	1.82±0.034 a	1.82±0.03 a	1.69±0.01 b	***
	Out	2.32±0.01 a	2.20±0.11 b	2.01±0.01 a	***	1.71±0.087 a	1.64±0.07 ab	1.55±0.07 b	*
Ti	In	6.42±1.21 a	9.97±2.37 b	3.33±1.13 c	***	2.12±0.06 a	2.65±0.13 a	1.43±0.18 b	***
	Out	5.68±0.51 a	9.26±0.83 b	2.66±0.26 c	***	2.16±0.09 a	2.11±0.09 a	1.19±0.10 b	***
V	In	12.00±2.23 a	7.41±1.54 b	11.45±2.48 a	**	4.09±0.54 a	4.10±0.29 a	3.12±0.37 b	**
	Out	11.22±1.18 a	7.80±1.54 b	10.93±1.65 a	**	3.76±0.30 a	3.77±0.21 a	2.94±0.16 b	***
Zn	In	68.1±11.4 a	99.4±16.1 b	82.8±23.8 ab	*	23.2±3.7 b	35.0±3.9 a	31.9±2.8 a	***
	Out	63.5±9.4 a	96.0±6.9 b	75.1±8.8 a	***	20.4±2.7 b	34.3±3.4 a	30.6±2.6 a	***

Data are mean±SD. $n=6$ (in milligrams per kilogram DW). Letters indicate significant differences between periods within a sampling location (for inflow and outflow)

*$P<0.05$, **$P<0.01$, and ***$P<0.001$

Table 6 Heavy metal removal between the inlet and the outflow section of the fourth lagoon at the Etueffont site for the three sampling dates

Removal	*T. latifolia*						*P. australis*					
	Root/rhizome			Shoot			Root/rhizome			Shoot		
	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring
Sb	−7 *	−6 ns	−5 *	−23 ***	−24 ***	−33 ***	−13 *	−19 **	−19 ***	−21 ***	−25 ***	−22 ***
As	−8 ns	−8 ns	−9 ns	−12 ns	−9 ns	−2 ns	−7 ns	−15 ns	−9 ns	3 ns	−5 ns	−8 ns
Be	−4 ns	−15 ***	−15 ***	−5 ns	−22 ***	−6 ns	−2 ns	−14 ***	−11 **	6 ns	−13 ***	−14 **
Cd	−12 ***	−12 ***	−12 ***	−8 ***	−8 ***	−9 ***	−22 **	−28 ***	−17 *	−20 ***	−38 ***	−20 ***
Cr	−32 ***	−32 ***	−13 ***	−28 ns	−7 ns	−15 ns	−45 ns	−43 ns	−58 **	−21 ns	−24 *	−25 **
Co	−6 ***	−5 **	−7 ***	−8 **	−6 *	−4 *	−26 **	−22 *	−17 *	−30 ***	−29 ***	−30 *
Cu	−34 ns	−37 ns	−40 ***	−9 ns	−13 ns	−14 *	−22 ns	−23 ns	−25 ns	−8 ns	−4 ns	−4 ns
Sn	−4 **	−4 **	−2 **	−8 ns	−10 *	−5 ns	−8 ns	−5 ns	−23 ns	−6 ns	−9 ns	−22 **
Li	−23 **	−20 ns	−23 *	−14 *	−22 ns	−25 ns	−4 ns	−2 ns	0 ns	−3 ns	−11 ns	−8 ns
Mn	−5 ns	−18 ***	−16 ***	−12 **	−11 **	−10 *	−22 **	−32 ***	−3 *	−22 ***	−9 **	−15 ***
Mo	−13 ns	−11 ns	−6 *	−12 ns	−27 ***	−20 ***	−5 ns	−13 ns	−23 ns	10 ns	−4 ns	−10 ns
Ni	−35 ***	−34 ***	−34 ***	−15 *	−25 **	−19 *	−43 ***	−38 *	−39 *	−40 ***	−41 **	−41 **
Pb	−4 ns	−3 ns	−11 *	−14 ns	−15 ns	−24 ***	−15 ns	−12 ns	−15 ns	−32 ns	−34 ns	−26 ns
Se	−15 ***	−14 ***	−10 **	−16 **	−18 **	−7 ns	−14 ns	−5 ns	−4 ns	−26 ns	−17 ns	−16 ns
Sr	−7 *	−6 ns	−7 *	−15 **	−15 *	−15 *	9 ns	1 ns	−8 ns	−6 ns	−1 ns	−7 ns
Tl	−12 ***	−12 ***	−9 ***	−19 ***	−25 ***	−24 ***	−1 ns	−10 ns	−1 ***	−6 ns	−10 ***	−8 ***
Ti	−21 ns	−15 ns	−18 ns	−22 **	−11 ns	−25 *	−12 ns	−7 ns	−20 ns	2 ns	−20 **	−17 *
V	−12 ns	−12 ns	−10 ns	−26 *	−19 **	−12 ns	−6 ns	5 ns	−5 ns	−8 ns	−8 ns	−6 ns
Zn	−26 ***	−6 ns	−15 ns	−13 *	−15 *	−19 **	−7 ns	−3 ns	−9 ns	−12 ns	−2 ns	−4 ns

*$P<0.05$, **$P<0.01$, and ***$P<0.001$

Sn, Mo, Pb, and Sr, the maximum concentrations in root/rhizome plant tissues at inflow were measured in spring after plant growth, whereas a general increase of Ti and V occurred in early fall before senescence. The highest Cd, Mn, Tl, and Zn concentrations were measured in winter. Similarly to root and rhizome parts, significant differences ($P<0.05$) between seasons in heavy metal storage were found in the aerial organs of *P. australis* except for Sb, Cr, Li, Ni, and Se.

With the exception of the Li and Se concentration in the root/rhizome plant fraction (significant seasonal variations, $P<0.05$), the same pattern was observed in the root/rhizome and aerial part of *P. australis* at outflow from basin no. 4. Comparison between inflow and outflow plant samples showed that most heavy metals decreased both in roots and shoots of outflow plants, indicating a greater accumulation at inflow (Table 6). Whatever the season, no significant uptake was observed for As, Cu, Li, Mo, Pb, Se, Sr, and V by plant root/rhizome and shoot plant parts between the inlet and outlet of the fourth basin.

3.2.3 Enrichment Coefficient for the Roots and Enrichment Coefficient for the Shoots (Table 7)

Enrichment coefficients are very important factors, indicating the phytoremediation ability of the two species for heavy metals in water. In essence, the ECR is the slope of the linear correlation between the heavy metal concentrations in the water and in root/rhizome and in shoot parts. Constant slope supports a causal relation. Therefore, constant ECR values reflect the similar changes in concentration between plant and water while large changes show a disconnection. Except for Be, Cr, Cu, Mo, Ni, and Sr, the ECR of *T. latifolia* were above 1.0, whatever the season (Table 7). Except for Cu, similar values were recorded for *P. australis*. For both species, the same patterns were observed for ECS with enrichment coefficients lower in the shoots than in the roots for all analyzed elements.

3.2.4 Transfer Factor (Fig. 5)

Transfer factor was used in order to estimate the ability of the two macrophytes to transfer heavy metals from roots to shoots. The TF for *T. latifolia* varied between 0.20 and 0.95. Excluder ability was in the following order: Cd>Sb>Be>Li>Mn>As>Tl>Co>Cu>Se>Ti>Mo>Sn>Sr>Zn>Pb>Cr>Ni>V. The TF of *P. australis* varied between 0.19 and 0.98. Excluder ability was in the following order: Be>Tl>Sb>Mn>Cd>Li>As>Co>Sn>Sr>Pb>Mo>V>Cu>Se>Zn>Ti>Cr>Ni.

4 Discussion

4.1 Water and Sediment Analyses

Concentrations of nutrients and heavy metals in water entering the fourth basin varied significantly during the three-season study period and were generally lower than (Cd, Cu, Pb, Zn) or similar to (Cr, Ni) the average inflow concentrations of heavy metals in CW for municipal wastewater treatment reported in the literature (Vymazal et al. 2007). Seasonal variations may be explained by rain which increased both lixiviation and, to a lesser extent, the dilution of heavy metals. The highest concentrations were found in spring when rainfall is maximal and the lowest in fall and winter (Khattabi et al. 2007).

The concentrations of heavy metals in the sediments collected at both inflow and outflow (after previous filtration through the three upstream basins) were within the range of what are considered to be European background values (Samecka-Cymerman and Kempers 2001). The plant concentrations appear to reflect changes in water concentrations, but the sediment does not. The plants exposure is mainly through the roots able to absorb both water and dissolved elements. Although the levels remained relatively constant in the sediments during the experimental period, their availability may be modified by changes in redox, pH, and salinity conditions (Liang and Wong 2003). Therefore, the high As, Cu, Sn, Mo, and Pb concentrations in the roots/rhizomes of *P. australis* and *T. latifolia* in spring may be the result of the increased bioavailability of these metals.

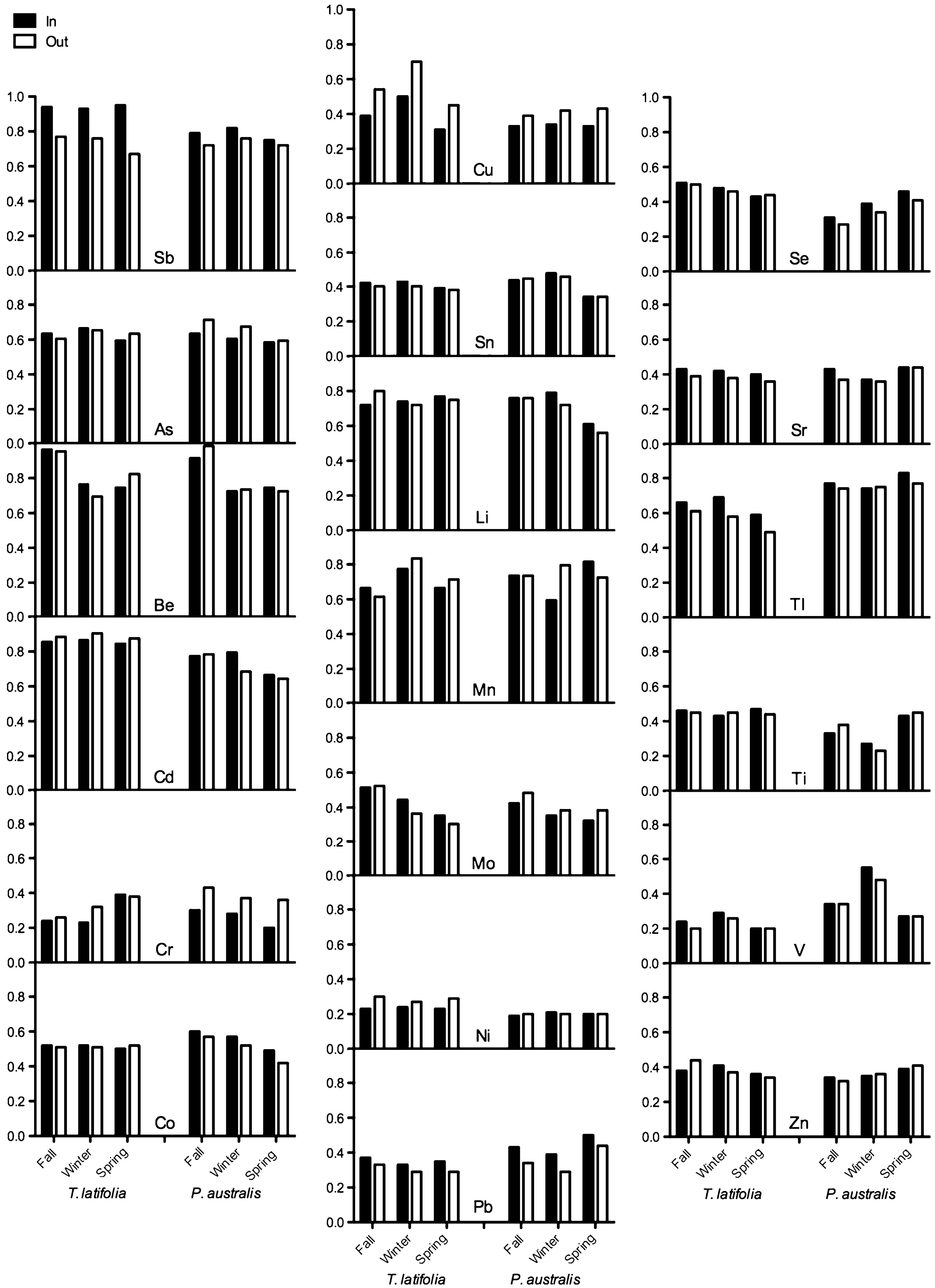
4.2 Heavy Metals and Plant Biomass

Despite the high values of all the heavy metals analyzed in the water inflow, they did not affect the growth of the two predominant plant species (*T. latifolia* and *P. australis*), since the average above-

Table 7 Enrichment coefficient for the root/rhizome and shoot parts of the two species for the three sampling dates

		T. latifolia						*P. australis*					
		EC root/rhizome			EC shoot			EC root/rhizome			EC shoot		
		Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring
Sb	In	9.26	9.11	1.01	8.70	8.46	0.96	8.96	8.68	1.13	7.04	7.11	0.84
	Out	8.60	9.04	1.03	6.60	6.88	0.69	8.40	8.25	1.07	6.04	6.25	0.77
As	In	3.09	3.87	3.28	1.94	2.57	1.92	51.98	82.34	80.42	32.90	49.61	46.44
	Out	2.98	3.74	3.57	1.78	2.44	2.25	50.29	73.67	87.59	35.60	49.39	51.34
Be	In	0.68	0.96	0.71	0.65	0.73	0.52	0.70	0.97	0.71	0.63	0.70	0.52
	Out	0.71	0.90	0.61	0.67	0.62	0.50	0.74	0.91	0.65	0.73	0.67	0.46
Cd	In	251.00	84.00	251.00	213.00	72.33	210.00	253.00	118.67	262.00	194.00	93.67	174.00
	Out	221.00	111.00	221.00	195.00	99.50	192.00	198.00	128.00	217.00	155.00	86.50	139.00
Cr	In	0.85	0.80	0.23	0.21	0.19	0.09	1.15	1.07	0.52	0.35	0.30	0.10
	Out	0.60	0.57	0.20	0.16	0.18	0.08	0.66	0.63	0.22	0.29	0.23	0.08
Co	In	1.99	1.95	0.38	1.03	1.01	0.19	10.30	10.00	2.70	6.22	5.72	1.33
	Out	2.02	1.94	0.42	1.02	1.00	0.21	8.25	8.17	2.64	4.66	4.26	1.10
Cu	In	0.90	0.68	0.71	0.35	0.34	0.22	4.29	3.93	2.16	1.42	1.33	0.72
	Out	0.61	0.45	0.47	0.33	0.31	0.21	3.44	3.19	1.77	1.34	1.34	0.76
Sn	In	155.67	155.83	168.00	65.00	66.67	66.22	38.83	39.50	88.00	17.00	19.00	29.67
	Out	150.00	150.00	184.38	59.83	59.83	70.50	35.83	37.50	76.25	16.00	17.33	25.88
Li	In	13.17	13.33	7.25	9.50	9.83	5.58	41.17	41.33	29.25	31.17	32.83	17.83
	Out	10.17	10.67	6.09	8.17	7.67	4.55	39.67	40.50	32.00	30.17	29.17	17.82
Mn	In	4,575.45	320.20	1,793.93	3,004.55	246.82	1,180.36	5,632.73	527.35	1,792.86	4,135.45	309.93	1,456.79
	Out	5,315.56	276.60	2,341.11	3,238.89	229.17	1,661.67	5,357.78	373.89	2,695.56	3,928.89	297.22	1,933.89
Mo	In	0.81	1.62	0.93	0.41	0.71	0.32	0.79	1.33	0.59	0.33	0.46	0.19
	Out	0.75	1.54	0.88	0.39	0.55	0.26	0.81	1.23	0.46	0.39	0.47	0.17
Ni	In	0.60	0.43	0.45	0.14	0.10	0.11	1.44	1.03	1.09	0.28	0.22	0.22
	Out	0.40	0.36	0.36	0.12	0.10	0.10	0.83	0.81	0.79	0.17	0.16	0.15
Pb	In	173.88	230.50	146.92	63.75	75.00	51.08	319.13	365.17	285.46	137.75	143.50	144.15
	Out	191.71	222.50	121.57	62.86	63.67	35.86	311.14	322.33	225.86	106.29	94.67	98.93
Se	In	9.23	9.35	9.61	4.73	4.50	4.09	13.69	10.50	8.91	4.31	4.08	4.13
	Out	8.16	8.40	9.90	4.12	3.84	4.35	12.20	10.36	9.85	3.32	3.52	4.00
Sr	In	0.18	0.07	0.07	0.08	0.03	0.03	0.14	0.08	0.09	0.06	0.03	0.04
	Out	0.19	0.08	0.07	0.08	0.03	0.03	0.18	0.09	0.09	0.06	0.03	0.04
Tl	In	4.57	4.79	2.03	3.02	3.29	1.19	4.80	5.10	2.04	3.71	3.79	1.69
	Out	4.13	4.21	2.09	2.50	2.46	1.03	4.83	4.58	2.28	3.56	3.42	1.76
Ti	In	142.67	119.33	99.67	65.67	51.00	47.33	214.00	332.33	111.00	70.67	88.33	47.67
	Out	113.00	152.00	122.00	51.00	68.00	53.50	189.33	463.00	133.00	72.00	105.50	59.50
V	In	4.53	3.86	4.50	1.09	1.11	0.92	10.17	7.64	11.68	3.47	4.23	3.18
	Out	4.16	3.71	4.39	0.84	0.98	0.88	9.93	8.76	12.14	3.33	4.24	3.27
Zn	In	6.96	2.85	3.60	2.61	1.17	1.28	10.41	8.84	8.25	3.55	3.11	3.18
	Out	7.46	3.09	3.15	3.32	1.15	1.06	14.14	9.87	7.69	4.54	3.53	3.13

EC enrichment coefficient


In
Out
Sb
As
Be
Cd
Cr
Co
Cu
Sn
Li
Mn
Mo
Ni
Pb
Se
Sr
Tl
Ti
V
Zn
Fall
Winter
Spring
T. latifolia
P. australis

◀ **Fig. 5** Transfer factor of *T. latifolia* and *P. australis* for the three sampling dates

ground cattail biomass measured in basin 4 were similar to those reported by Wild et al. (2002) in Germany (1.3–1.45 kg DW m^{-2}), or found in the three wetlands in Estonia (0.27–1.58 kg DW m^{-2}) (Maddison et al. 2009). Nevertheless, these biomass are lower than those reported in the study by Toet et al. (2005) on Texel Island, the Netherlands (2.09 kg DW m^{-2}), by Ennabili et al. (1998) on the Tingitan Peninsula (Morocco) (2.16 kg DW m^{-2}), or by Fernandez and De Miguel (2005) in Lorca (Murcia, Spain) (2.23 kg DW m^{-2}). The cattail root/rhizome biomass values were similar to those recorded by Romero et al. (1999) in the Ebro Delta in Spain (0.7–1.6 kg DW m^{-2}) and by Maddison et al. (2009) (0.6–1.3 kg DW m^{-2}), but much smaller than those measured by Ennabili et al. (1998) (3.5 kg DW m^{-2}).

The average above-ground biomass values of common reeds in the Etueffont lagooning system were lower than those reported by Lesage et al. (2006; Halic Region, Turkey), Vymazal (2004; Czech Republic), Ennabili et al. (1998; Morocco), Bragato et al. (2006; Venice Lagoon), and Toet et al. (2005; the Netherlands)—1.5, 2.09, 2.3, 2.5, and 2.85 kg DW m^{-2}, respectively—but were similar to those found in northern countries, i.e., in Estonia (Maddison et al. 2009) and Germany (Wild et al. 2002) (1.3–1.45 kg DW m^{-2}). The average below-ground biomass values of reeds in the studied areas were similar to those reported by Maddison et al. (2009) (1.60–1.69 kg DW m^{-2}) in Estonia. These discrepancies can be linked to the different climatic conditions prevailing in the various monitoring stations under study.

4.3 Heavy Metal Storage in Macrophytes

The levels of Cu and Zn in *T. latifolia* in fall and winter are similar to those reported by Maddison et al. (2009) in *T. latifolia* in Estonian semi-natural and constructed wetlands, or by Karpiscak et al. (2001) in *Scirpus* spp. in CW in Arizona (USA) or by Tanner (1996) in *Bolboschoenus fluviatilis* of the Waikato River (New Zealand). Copper and zinc levels in above- and below-ground parts of *T. latifolia* measured during the spring period are higher than concentrations observed by Tanner (1996). However, Cu and Zn contents in *T. latifolia* collected in the Etueffont CW are lower than contents in *Typha domingensis* (Cardwell et al. 2002) or in *P. australis* (Maddison et al. 2009). The levels of Zn, Pb, and Cd in *P. australis* above-ground parts are similar to those reported by Samecka-Cymerman and Kempers (2001) in anthropogenic lakes in western Poland while the investigated aquatic macrophytes contained higher levels of Mn and Cu. On the other hand, Cr and V concentrations in the aerial parts of *P. australis* reported by the same authors were higher than our measurements from three seasons at Etueffont.

Our data, collected during the three-season study in the Etueffont CW, are in accordance with previous works. Macrophytes store preferentially heavy metals in roots than in stems and leaves as shown in previous studies (Schierup and Larsen 1981; Peverly et al. 1995; Cardwell et al. 2002; Stoltz and Greger 2002; Demirezen and Aksoy 2004; Weis et al. 2004; Weis and Weis 2004; Duman et al. 2007; Mishra et al. 2008). Heavy metal storage in helophytes varies considerably according to plant parts as well as to type of element (Larsen and Schierup 1981; Schierup and Larsen 1981; Stoltz and Greger 2002; Carranza-Alvarez et al. 2008). This may depend on seasonal plant growth dynamics and low ability transfer from roots toward the aerial parts for storage and detoxification (Cardwell et al. 2002; Mishra et al. 2008).

Great variability is thus found in the literature concerning heavy metal concentrations in above- and below-ground parts of aquatic macrophytes in wetland systems (Zayed et al. 1998; Cardwell et al. 2002). The heavy metal concentrations in *P. australis* used for phytoremediation in natural or constructed wetlands are relatively low in the above-ground parts, as reported in many studies (Ye et al. 1997, 2003; Vymazal and Krása 2003; Obarska-Pempkowiak et al. 2005; Lesage et al. 2006; Vymazal and Kröpfelová 2008; Maddison et al. 2009). It has also been shown that *P. australis* was found to accumulate heavy metals in root/rhizome parts more efficiently than *T. latifolia*, similar to the findings of Aksoy et al. (2005) or Maddison et al. (2009). Seasonal trends of heavy metals observed with *P. australis* and *T. latifolia* in the Etueffont CW cannot be compared to other studies of natural or constructed wetlands having different wetland plant species and generalized according to Weis and Weis (2004). Thus, Duman et al. (2007)

showed greater heavy metal storage in the fall and winter seasons in macrophyte roots, especially for *P. australis*, as senescence and the death of aerial parts of plants are generally associated with remobilization of cell materials and probably of heavy metals. Conversely, these authors described a decrease in heavy metal storage in spring and winter that may be due to a dilution effect by root growth or a transfer linked to the growth of aerial parts. In addition, it has been established that the translocation of useful elements from root/rhizome to shoots depends on numerous biotic and abiotic factors and varies according to plant species, as well as environmental conditions, the latter noticeably influencing the temperature, redox, pH, water ion content, or salinity conditions (Liang and Wong 2003; Demirezen and Aksoy 2004). Similarly, these variations can also induce changes in the availability of heavy metals to aquatic macrophytes. Thus, the large increase in heavy metal accumulation we recorded in spring in the root/rhizome of both plant species, and less in shoots, can only be attributed to an increase in element concentrations in the inflow influenced by environmental conditions. Several authors (Peverly et al. 1995; Weis et al. 2004) have reported the restricting capacity of *Phragmites* spp. in heavy metal transport to the above-ground parts, functioning mostly as excluders. If shoot concentration is low in comparison to root/rhizome plant parts, restricting upward transport, the efficiency of plant restriction can vary with the season and may be affected by photosynthetic activity (Bragato et al. 2006). Moreover, our data suggest that the ability to exclude metals varies widely according to element and plant. As in Windham et al. (2003), the root/rhizome ratios (data not shown) for Cr, Cu, Pb, and Zn were generally low, indicating a low rate transfer into the above-ground plant biomass for the two plants. In addition, *T. latifolia* and *P. australis* are able to increase Sb, Cd, Sn, Li, Se, and V content into the senescing aerial tissues of the plant in early fall. There is no physiological explanation excepted that is probably a way to eliminate sequestered toxic heavy metals.

4.4 Phytoremediation Potential of *T. latifolia* and *P. australis*

The ECR were higher than in the ECS, indicating the high capacity of *T. latifolia* and *P. australis* roots for heavy metal accumulation. Cd, Mn, Pb, and Zn ECR and ECS in *T. latifolia* were higher than 1.0 exceeding the ranges previously reported by Sasmaz et al. (2008) for ECR (Cd 0.78–3.95, Mn 1.30–4.51, Pb 0.58–2.25) and ECS (Cd 0.30–2.38, Mn 1.18–5.30, Pb 0.26–2.25), while Cr, Co, Cu, Ni, and Zn ECR and ECS of *T. latifolia* were nearly similar.

Transfer factors of heavy metals, from water to root/rhizome as well as from root/rhizome to shoot, are determining criteria for the selection of plants for phytoremediation. On the other hand, the TF of heavy metals from root to shoot parts (i.e., phytoremediation potential of the two macrophytes) were lower than 1.0 for the two species. Etueffont TF were similar (Cr (0.15–0.92), Co (0.16–0.70), Cu (0.33–0.83)) or lower (Mn (0.81–1.62), Ni (0.32–0.99), Pb (0.33–1.00), Zn (0.45–1.01)) to those reported by Sasmaz et al. (2008) in *T. latifolia* in Turkey while Cd TF was higher.

As shown by Peverly et al. (1995) and Weis et al. (2004), TFs below 1.0 are representative of metal excluder species, whereas TFs above 1.0 are observed in accumulator species, which shows a high ability for heavy metal uptake, transport, and storage in above-ground parts (Brown et al. 1994). It is admitted that TFs lower than 1.0 indicate an inefficient ability to transport metal from root/rhizome to shoot parts, most likely due to inefficient metal transporter systems (Zhao et al. 2002). The differences in TF values indicate that if each heavy metal has different phytotoxic effects on macrophytes, restriction of upward movement from root/rhizome to shoot is one of the tolerance mechanisms developed to cope with metal stress (Zu et al. 2005). 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), *T. latifolia* and *P. australis* did not effectively transfer heavy metals from root to shoot and cannot be considered as hyper accumulators. Therefore, the aerial biomass harvest did not constitute an effective option for heavy metal removal from CW.

5 Conclusion

This study demonstrated that heavy metals entering the constructed wetland system are efficiently removed from the water flow through aquatic macrophyte species uptake and accumulation processes and that the outflow water can thus be released into the stream. In addition, the results showed that leachate composition plays an important role in heavy metal uptake in plants, without affecting the growth of the two predominant plant species (*T. latifolia* and *P. australis*). However, the evolution of concentrations in the fourth basin in the future may lead to phytotoxic effects on macrophytes. Data comparison of heavy metal accumulation in plants showed that, from season to season, cattails are not as efficient as reeds in trapping heavy metals. Moreover, considering that heavy metals are preferentially accumulated in the root/rhizome plant parts of the two tested macrophyte species and to a lesser extent in the aerial parts, the harvest of the aerial biomass has only a minor influence on heavy metal removal from CW and hence is a transitional option before the complete harvesting of macrophytes and the dredging of the wetland. Despite the fact that data are not shown, the two species fixed efficiently nitrogen during the growth season. Our results give new insight into the use of macrophyte species for toxic element storage and for their increased use in self-cleaning processes.

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