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Long-term impact of primary domestic sewage on metal/loid accumulation in drainage ditch sediments, plants and water: Implications for phytoremediation and restoration

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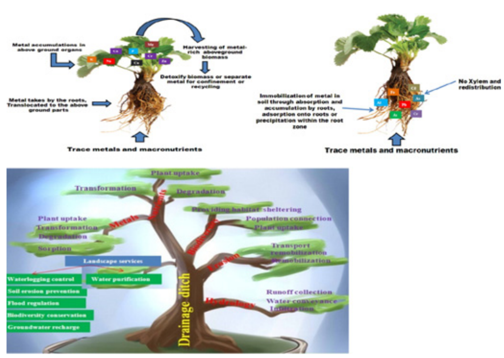
HIGHLIGHTS

- Evaluation of possibilities for phytoextraction and phytostabilization in vegetated drainage ditch plant.
- Significant accumulation of metal/loid in ditch sediment to exceed legal background levels in soil.
- Metal/loid uptake and translocation in ditch plant biomass are limited.
- Seasonal patterns and time of maximum standing stock vary for each metal/loid.

GRAPHICAL ABSTRACT



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ABSTRACT

We evaluate the long-term performance of a vegetated drainage ditch (VDD) treating domestic sewage with respect to heavy metal/metalloid (HM/M) accumulation in sediments, plants and water. VDD sediment contained significantly higher macro and trace elements compared to an agricultural ditch (AD) sediment. However, concentrations of HM/Ms in VDD sediment were below the ranges considered toxic to plants. Most HM/Ms were efficiently removed in the VDD, whereby removal efficiencies varied between 11% for Al and 89% for K. Accumulation of HM/Ms varied among species and plant parts, although sequestration by plants represents only a small proportion (<1%) of the inflow load. Accumulation of Al, As, Cd, Pb, Cr, Fe and Ni in VDD plants were mostly distributed in the roots, indicating an exclusive strategy for metal tolerance. The opposite was found for Zn, Cu, K, Ca, P, K, Na, N and Mg, which were accumulated either in the stems or leaves. Overall, concentrations of metals in sediment showed significant positive correlations with those in ditch plants. None of the studied species were identified as metal hyper-accumulators (i.e. >10,000 mg kg^{-1} of Zn or Mn). Nevertheless, the high translocation factor (TF) values for Mn, Ni, Cu, Zn, Na, Mg, P, K and Ca in the ditch plants make them

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suitable for phytoextraction from water/soil, while the low TF values for Pb, Cd, As, Fe, Cr and Al make them suitable for their phytostabilization.

1. Introduction

Drainage ditches are one of the human-made water bodies often used to collect surface runoff from surrounding plots and roads (Buchanan et al., 2012; Carluer and Marsily, 2004). Their presence grows in proportion to the expansion of anthropogenic land use. They can act as primary conduits of untreated human sewage in the absence of treatment facilities (Kumwimba et al., 2016a, b, c, d; Kumwimba et al., 2015). In recent years, great strides have been made to reduce the discharge of nutrients into the aquatic environment with various countermeasures such as constructed wetlands, retention basins, buffer zones and stiff grass hedges (Bouldin et al., 2004; Cooper et al., 2004; Kröger et al., 2007, 2008, 2011; Moore et al., 2000, 2006; Needelman et al., 2007). Scientists now promote the utilization of ditch systems as innovative and cost effective alternatives for minimizing nutrient runoff from agricultural fields (Cooper et al., 2004; Kröger et al., 2008). There is growing interest in treating domestic sewage through vegetated (eco) ditches as the most attractive alternative to conventional treatments technologies (Kumwimba et al., 2016a, b, c, d; Wu et al., 2014). However, despite their potential importance in treating polluted water, their role in nutrient reduction strategies has only been partially assessed to date and remains a hot topic of interest.

Drainage ditches in North America, Europe and developing countries are mostly applied for the treatment of agricultural runoff pollution and pesticides (Bennett et al., 2005; Bouldin et al., 2004; Chen et al., 2015; Cooper et al., 2004; Flora and Kröger, 2014; Fu et al., 2014; Kröger et al., 2007; Kröger et al., 2008; Moore et al. 2010; Zhang et al., 2013). They have been widely assessed for nitrogen, phosphorus, and suspended solids removal, but information on heavy metals/metalloids (HM/Ms) remains somewhat limited or unknown. Field studies of these systems have been already demonstrated for agricultural wastewaters, domestic sewage and storm water (Kroger et al., 2008; Kumwimba et al., 2016a, b, c, d; Luo et al., 2009; Wu et al., 2014). However, this technique has not yet been sufficiently assessed for the treatment of domestic sewage contaminated with HM/Ms. In addition, no work has been carried out to assess the accumulation of HM/Ms in drainage ditch sediment and release to the water column, apart from two studies that reported the distribution of some metals in sediment and water in different land use areas (Kumwimba et al., 2016c, d; Zhang et al., 2004).

Accumulation of HM/Ms in vegetated ditches treating domestic sewage is not a topic of priority, mainly because the concentrations of HM/Ms are supposed to be significantly lower. Moreover, presently there are no discharge standards for HM/Ms in eco (vegetated) ditches. As a result, HM/Ms have been neglected historically in most treatment ditches. Nevertheless, HM/Ms in domestic sewage may be associated with fine particulate matter, and the long-term deposition of sewage to aquatic ecosystems often results in the accumulation of high levels of HM/Ms in sediments (Rattan et al., 2002) and ditch plant species. Therefore, the reservoirs or lakes into which drainage ditches are diverted must be protected from HM/Ms due to their toxicity, abundance and long-term persistence in the environment.

In aquatic systems, metals have a high affinity for particulate matter and will, therefore, accumulate in surface sediments (Sundaray et al., 2011). Once deposited, however, chemical and biological processes may allow HM/Ms to be desorbed from surface sediments and released into the water column (Li and Davis, 2008). Because of adsorption, hydrolysis and co-precipitation, only <0.1% of free metal ions actually dissolve in the water, and >99.9% are bound to the particles in sediments and soil (Gaur et al., 2005). These HM/Ms are removed partly by accumulation in the biomass of plants. Therefore, sediments may serve as a metal pool that can release metals to the overlaying water via natural and anthropogenic processes, causing potential adverse health effects to the aquatic ecosystems (Singh et al., 1997). Information about the abilities of various ditch plants to take up and translocate metals can give insight into selecting suitable plants for drainage ditch phytoremediation. However, quantitative information comparing the performance of different ditch plant species in eco ditches is rare.

Eco ditches encompass several processes and mechanisms in the removal of contaminants (Fig. S1) such as sorption, sedimentation, evaporation, precipitation, transformation, adsorption, plant uptake, filtration and microbial metabolic activities (Kumwimba et al., 2016a, b). The aquatic plants in ditches play important roles to effectively decrease the flow velocity, increase agrochemical retention, and subsequently provide better conditions for pollutants removal in ditches (Kröger et al., 2007). Accumulation levels of HM/Ms increase with the operational lifetime of the system. Therefore, understanding the differences in HM/Ms accumulation among species is imperative to assess the best species for use in the restoration of polluted aquatic ecosystems and to develop suitable management alternatives.

In this study, accumulation of HM/Ms in vegetated drainage ditch (VDD) sediment and different plant parts (roots, stems and leaves) were assessed in order to determine the biogeochemical processes and mobility as well as removal mechanisms for metals removal in VDD treating primary domestic sewage after 10 years of operation. The possibilities for phytoextraction and phytostabilization in VDD plant communities were also evaluated. The specific objectives of the research presented in this study were to (1) explore the differences in HM/M accumulation by 9 dominant ditch plant species in a VDD impacted by primary domestic sewage; (2) investigate the fate and uptake of domestic sewage-related metal and arsenic in sediments by root and shoot organs of ditch species and (3) examine the enrichment and translocation potentials of these species to metal and arsenic. It is hypothesized that untreated domestic sewage would increase pollutant inputs, increasing the concentrations of HM/Ms in sediment and water above the background concentrations of soils in China. We predict that these higher levels would be reflected in HM/M concentrations in ditch plants. Furthermore, the concentrations of metals are expected to vary between different plant parts, with higher concentrations in the root and shoot parts. For the above reasons, it is very important to comprehensively understand the HM/M contamination status in the agricultural drainage ditch of Sichuan Basin to provide a reference for the large-scale control and management of HM/Ms. This study would provide the latest and most significant information related to the long-term effect of primary domestic sewage on macronutrients and HM/Ms accumulation in drainage ditch sediments, plants and water.

2. Materials and methods

2.1. Description of the study sites, sampling and plant materials

The VDD (Fig. 1) is located in Jieliu catchment (105°27′24″E, 31°16′31″N) in the hilly Sichuan Basin, China. The catchment is a representative headwater urbanized catchment of the Jialing River, which is a first-order tributary of the Yangtze River. The site has a subtropical monsoon climate with a mean annual temperature of 17.3 °C and a mean precipitation of about 826 mm. The average air temperature at the site in January is 2 °C. The lowest and highest average monthly temperatures are −6 °C (in February) and 38 °C (in August). The VDD was designed and built in the downstream section of the catchment for primary domestic sewage treatment. However, it receives storm water, as

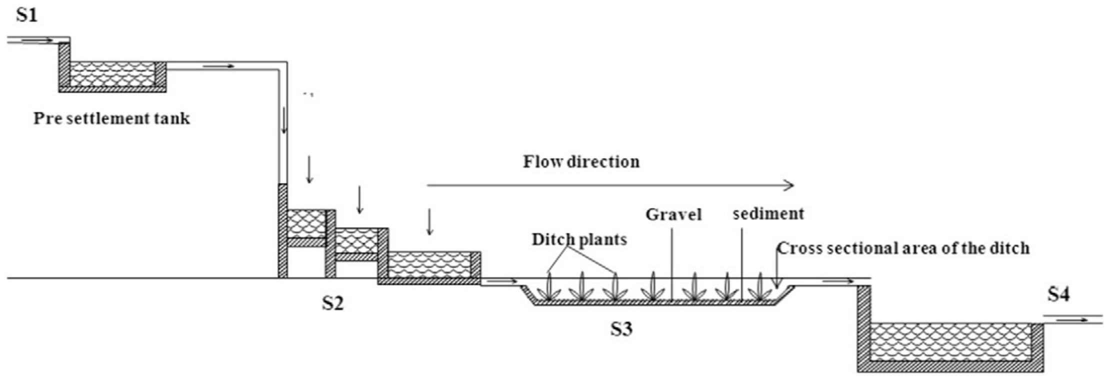

Fig. 1. Overview and sampling points (S1–S4) in the vegetated constructed ditch treatment system. S1-raw influent domestic sewage; S2-artificial wetlands; S3-vegetated constructed ditch and S4-outlet.

well, from catchment runoff, where approximately 1300 people live. The VDD has received domestic sewage from the catchment for over 10 years. Inlet primary sewage is collected in a primary settlement tank consisting of 2 compartments (Fig. 1). Domestic sewage is distributed uniformly across artificial wetlands by means of pipes before flowing into the VDD (Fig. 1). The artificial wetlands are composed of three free water surface (FWS) constructed wetland units. The three FWS units were 2.5 m long, 1.5 m wide and 0.75 m deep, within with *Myriophyllum verticillatum* were planted. The VDD is 300 m long and 2.20 m wide, and has a 0.18 m layer of gravel at the bottom and 0.15 m layer of sediments on the top, where several ditch plants species were planted. The water depth was at approximately 0.25 to 0.40 m. The base width of the VDD and the water level width were 0.9 m and 1.1 m, with a bottom slope of 0.20%. The mean domestic sewage discharge was 15 m^3 d^{-1}. The hydraulic retention time was 12 h. Further details are provided by Kumwimba et al. (2016a). In March 2011, plant species were transferred into the VDD and provided full vegetation cover. The plants were well established in the VDD and provided an estimated 90–100% cover. Site maintenance was undertaken when the VDD became operational in order to guarantee optimal plants growth during the winter months.

The nine test species (photographs presented in Fig. S2) were *Cyperus alternifolius* (Cyp), *Colocasia gigantea* (Cog), *Phragmites australis* (Pha), *Iris pseudacorus* (Irp), *Red Canna indica* (Rci), *Thalia dealbata* (Thd), *Acorus calamus* (Acc), *Acorus gramineus* (Acg), and *Hydrocotyle vulgaris* (Hyv). Their main features are reported in supplementary information.

Sampling of VDD sediment, plant biomass and water was conducted at the beginning of the study (May) and after 21 weeks (late August 2015). Plant tissue samples (roots, stems and leaves) of 9 species were collected during May and the late part of the growing season (August) at three random locations within each ± 100 m using 0.50 m $\times$ 0.50 m quadrat survey method to ensure adequate representation in the sample in 3 replicates according to standard MTR methodology (Holmes et al., 1999). Plant species were uprooted by using stainless-steel tools to avoid contamination and carefully washed with pressured water to remove any particles attached to plant surfaces. The sediment and water samples were collected at each sampling point in triplicate along the treatment system (Fig. 1): Inlet (S1), artificial wetlands (S2), vegetated constructed ditch (S3) and outlet (S4). Acid-washed polythene bottles (500 mL) were used as sample containers. At the sampling point, the containers were rinsed twice with the water to be sampled prior to collection. Collected sediment samples were placed in plastic bags and transported to the laboratory for analysis. Samples were prepared according to the protocol described by Bonanno (2013). Plant materials were separated into roots, stems, and leaves in the laboratory. To determine dry weights, all plant materials were oven dried at 60 °C until constant weights. Water physicochemical parameters such as total dissolved solids (TDS), dissolved oxygen (DO), pH, water temperature (T), and electrical conductivity (EC) were recorded in-situ at each site using HACH electrodes.

2.2. Chemical analysis and assessment of plant metal uptake efficiency

Analytical techniques for HM/Ms were carried out according to NEB (1998). Briefly, sediment samples were air-dried at room temperature (20–22 °C) at first and then ground to pass through a (2 mm) sieve to remove organic debris (twigs, roots, etc.). Approximately 0.250 g of samples were weighed into clean Teflon beakers, in which a mixture of concentrated HF-$HClO_4$-HNO_3 (i.e., 10 mL HNO_3, 5.0 mL $HClO_4$ and 5.0 mL HF) were added to the sample and allowed to stand overnight. On the following day, the tubes were heated to 180 °C for 12 h until the solution turned clear. The sample solutions were filtered and adjusted to a suitable volume (50 mL) with double deionized water in a volumetric flask. Concentrations of HM/Ms (Cr, Ni, Cu, Zn, As, Cd, Pb, Al, Fe and Mn) were analyzed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Agilent 7700). Arsenic and cadmium were determined using a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer SIMMA 6000). The calibration curves with $R^2 > 0.999$ were accepted for concentration calculation. The analytical data quality was guaranteed using quality control (QA) and quality assurance (QC) including analysis of reagent blanks, duplicate samples and standard reference materials (GBW 07308) for each batch of samples. The analytical precision for replicate samples was within $\pm 10\%$ and the measurement errors between determined and certified values were <5%. Satisfactory recoveries were obtained for Cr (92.50%), Ni (93.40%), Cu (97.48%), Zn (95.20%), As (98.20%), Cd (99.57%), Pb (100.30%), Al (94.12%), Fe (88.54%) and Mn (91.69%), respectively. Water samples were filtered through a 0.45 μm Millipore membrane filter before HM/Ms analyses by ICP-MS. For HM/M accumulation in plants, the concentration of HM/M was analyzed in roots, stems and leaves of plants. HM/Ms in plant materials were analyzed similarly to the sediment samples. About 1 g of dry plant samples were digested withHNO_3:$HClO_4$ (5:1) in Teflon beakers at 180 °C until the liquid turned clear (8 h). The solutions were analyzed for elemental composition by ICP-MS (Agilent 7700). Standard materials were included for assurance control.

HM/M concentration alone is not enough to assess metal hyperaccumulation in plants, but also the metal concentration in the sediment. Thus, enrichment factor (EF) and translocation factor (TF) must be considered while deciding whether a particular species is a metal hyperaccumulator. Thus, a hyperaccumulator plant should have TF > 1.

Enrichment coefficient (EF), which is calculated as the ratio of HM/M concentrations in the plant to that in the water is a useful indicator to measure HM/M uptake efficiency by plants (Dowdy and McKone, 1997; Branquinho et al., 2007), can be expressed according to Eq. (1):

$$EF = C_{plant\ (DW)}/C_w \quad (1)$$

where, C_{plant} is the total metal concentration in the plant, and C_w is the polluted substrate or contaminated environmental medium (e.g. sewage and sediment) concentrations.

Translocation factor (TF), which is defined as the ratio of HM/M concentration in the aboveground to that in the root (Deng et al., 2004), can be expressed according to Eq. (2):

$$TF = C_{aboveground\ (DW)}/C_{root\ (DW)} \quad (2)$$

where, $C_{aboveground}$ is the concentration of HM/M in the shoot (stem + leaf), and C_{root} is the concentration of HM/M in the root.

Experimental data were analyzed using the statistical software package Statistica for Windows 7.1. Correlations between HM/Ms concentrations in plant parts and in sediment samples were calculated using the Pearson Correlation Coefficient. Data were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests prior to analysis. One-way analysis of variance (ANOVA; LSD test) was used to check the existence of significant differences between the plants regarding the content of trace elements in their different parts; sediment and water. Paired samples *t*-tests were performed to identify significant differences among means.

3. Results and discussion

3.1. Physiochemical characteristics of ditch water and sediment

Over the monitoring period, average pH of the inlet (pH 7.85) and outlet (pH 7.18) indicated that the VDD was weakly alkaline (Table 1). The water temperature ranged from 18 °C to 26 °C. There was considerable variation in DO concentrations at the inlet and outlet (0.45 ± 0.060 and 6.54 ± 0.24 mg L^{-1}, respectively). The mean of EC and TDS varied significantly at the influent and effluent (Table 1). The VDD outflow water quality for TP meets the specified B criteria of Class I in the discharge standards of pollutants for municipal wastewater treatment plants in China (GB18918-2002) of 3 mg L^{-1} d^{-1}, while TN exceeded the limit of 20 mg L^{-1} d^{-1} set in GB18918-2002 (Table 1).

The average concentrations of HM/Ms in water varied from 0.02 to 80 μg L^{-1} in the following order: Mn > Fe > Al > As > Zn > Ni > Cr > Cu > Cd > Pb. The mean values of the ten HM/M did not exceed the Chinese permissible limits in the Discharge Standards for HM/Ms in Wastewater [(GB 8978-1996)] (Table 1). Influent concentrations of all the studied HM/Ms at sample point S1 (Fig. 1) were generally lower than those reported by Tatsi and Zouboulis (2002) and Vymazal (2007) in the stabilized leachates entering constructed wetlands used for municipal solid waste landfill treatment. According to Pescod (1992), threshold levels of HM/Ms in irrigation water leading to plant damage are 2000 μg L^{-1} for Zn, 200 μg L^{-1} for Cu, 5000 μg L^{-1} for Fe, 200 μg L^{-1} for Mn, 200 μg L^{-1} for Ni, 5000 μg L^{-1} for Pb and 10 μg L^{-1} for Cd. This analysis indicated that effluent from the VDD contains permissible concentrations of metals that meet the urban living water standards and agricultural water (Table 1). Mn, Fe and Al showed the highest concentration in the VDD water (inlet). This is probably the reason for the higher concentration of these metals in VDD sediment. Removal efficiencies for the studied HM/Ms in the VDD were quite good and the reduction in concentrations of most of the selected elements was significant (Table 1). These removal efficiencies are higher than those reported by others researchers for the treatment of highway runoff and municipal sewage (Galletti et al., 2010; Hawkins et al., 1997; Lesage et al., 2007b; Maine et al., 2009; Revitt et al., 2004; Terzakis et al., 2008; Vymazal and Sveha, 2012).

In the current study, mean sediment concentrations of Cr (71.4 mg kg^{-1}), Ni (39.9 mg kg^{-1}), Cu (28.4 mg kg^{-1}), Zn (69.5 mg kg^{-1}), As (10.1 mg kg^{-1}), Cd (0.27 mg kg^{-1}), Pb (24.7 mg kg^{-1}) and Mn (838.3 mg kg^{-1}) in the VDD (Table 1) were higher than the background values in soil from the Sichuan Province (CEMS, 1990). However, based on total HM/Ms concentrations in sediment, 60–125 mg kg^{-1} Cu, 70–400 mg kg^{-1} Zn, 100–400 mg kg^{-1} Pb and 3–8 mg kg^{-1} Cd would be considered toxic to plants, according to Kabata-Pendias (2000). The studied HM/M contents in the VDD sediments did not exceed these limits. Independent-samples *t*-test explained that 10 years of VDD operation under primary domestic sewage resulted in an increase in HM/Ms as compared to AD (Table 1 and Table S1). Concentrations of HM/Ms were always higher in the sediments near the inflow of the VDD. Similar observations were also reported by Lesage et al. (2007a, b), Vymazal (2003) and Vymazal et al. (2010a). Statistical analysis displayed the highest concentrations at the inflow with concentrations decreasing with increasing distance downstream.

Table 1
Average inlet and outlet concentrations of monitored physicochemical water quality parameters and HM/Ms and macronutrient during the period of May and August 2015 (mean ± S.D., $n = 20$).

Parameter	[a]Primary domestic sewage			[b]Sediment			[b]Top soil	[c]BSS	[d]IWDS
	Inlet	Outlet	Removal (%)	Inlet	Outlet	Removal (%)			
pH	7.85 ± 0.1	7.18 ± 0.09		7.91 ± 0.09	7.77 ± 0.10		8.05 ± 0.09		
ECmS (cm^{-1})	1351 ± 65	981 ± 53		1429 ± 47	995 ± 78		480 ± 35		
TDS (mg L^{-1})	885 ± 49	589 ± 31		929 ± 37	771 ± 25		312 ± 28		
DO (mg L^{-1})	0.45 ± 0.06	7 ± 0.24							
Ni	6 ± 0.59	5 ± 0.45	13	40 ± 4	27 ± 2	31	14 ± 2	23.9	1
Cu	4 ± 0.25	2 ± 0.11	53	28 ± 3	20 ± 3	31	11 ± 1	19.1	0.5
Cr	7 ± 0.61	3 ± 0.21	51	71 ± 9.24	52 ± 4	27	31 ± 4	50.5	1.5
Zn	20 ± 1.47	3 ± 0.09	83	69 ± 7.24	50 ± 4	29	25 ± 3	51.7	2
Cd	0.2 ± 0.02	0.07 ± 0.00	68	0.27 ± 0.09	0.16 ± 0.03	41	0.13 ± 0.01	0.084	0.1
Pb	0.5 ± 0.02	0.15 ± 0.01	72	25 ± 3	18 ± 4	25	14 ± 3	21.4	1
As	8 ± 0.35	5 ± 0.45	37	10 ± 1	7 ± 0.95	28	5 ± 0.73	9.8	0.5
Fe	64 ± 3	33 ± 6	48	28,474 ± 4089	23,147 ± 2527	19	18,142 ± 1003		
Al	55 ± 5	48 ± 3	11	62,908 ± 5824	55,241 ± 2645	12	41,245 ± 1560		
Mn	80 ± 9	68 ± 6	15	838 ± 116	690 ± 67	18	486 ± 14	648	2
N	65,210 ± 3735	33,140 ± 2105	49	1854 ± 101	921 ± 85	50	452 ± 24		
P	4254 ± 250	2846 ± 120	33	1140 ± 75	684 ± 56	40	324 ± 29		
Na	57,330 ± 4012	43,390 ± 3421	24	12,540 ± 61	8542 ± 601	31	5246 ± 254		
Mg	14,923 ± 1010	10,129 ± 753	32	9340 ± 425	5845 ± 134	37	2546 ± 176		
Ca	90,380 ± 6345	82,451 ± 1597	89	52,140 ± 2508	40,285 ± 1438	23	33,245 ± 2536		
K	17,037 ± 1254	10,067 ± 670	41	18,790 ± 1109	13,547 ± 524	28	7542 ± 408		

[a] Primary domestic sewage (μg L^{-1}).
[b] Sediment and top soil (mg kg^{-1}).
[c] BSS: background of soils in Sichuan in mg kg^{-1}.
[d] IWDS: Integrated Wastewater Discharge Standard of China (GB 8978-1996).

3.2. Plant biomass, height and seasonal pattern of metal uptake

Plants biomass increased significantly throughout the growing season, which then remained steady during the late summer (data not shown). Species *Thd* and *Pha* recorded the highest mean increase in shoot height (352 and 340 cm, respectively) followed by *Cyp*, *Rci* and *Irp*, whose mean increase in shoot height were about 260, 235 and 168 cm, respectively in August (Table 2). Similarly, the total biomass of the nine ditch plant species varied among species. The total dry weight (DW) biomass of the plant species ranged from 75 to 3203 g m^{-2} in the in the order of Rci > Cyp > Thd > Irp > Cog > Pha > Acg > Acc > Hyv > Aph > Myv (Table 2). Furthermore, the average aboveground biomass of *Canna indica* (Rci), *Cyperus alternifolius* (Cyp) and *Thalia dealbata* (Thd) were highest at 2843, 2457 and 2137 g m^{-2}, respectively, which were higher than values reported in earlier studies (Vymazal and Kröpfelová, 2005a). There were significant differences in root biomass (DW) among the 9 ditch plants ($p > 0.05$) (Table 2). Species Irp had the largest dry root biomass (1071 g m^{-2}), which was 44 times that of Hyv (24.36 g m^{-2}), which had the smallest root biomass (Table 2).

The criteria for selecting best plants for phytoextraction vary, but generally include fast growth rate, high plant biomass, ability to grow in other areas, easy harvesting and accumulation of a range of HM/Ms in their aboveground shoot, or any other harvestable plant part and appropriate characteristics to survive under high pollutant levels and high pollutant removal capacity (Vymazal, 2011a, b). Nevertheless, no species has been reported, so far, that satisfies all these traits. However, a fast-growing non-accumulator species can be engineered to achieve some of the properties mentioned above (Clemens et al., 2002). In the current study, plant species well-adapted to aquatic conditions as well as operational conditions site. About 70–95% increase in the plant biomass was recorded at the end of the experimental period, and thus, as mentioned before, are preferred in VDD for phytoremediation goals.

The HM/M contents in plant parts combined with biomass of various plant parts (per unit area and expressed as mass per unit area, mg m^{-2}) (Brezinová and Vymazal, 2015; Johnston, 1991) allowed us to compare the standing stocks of HM/Ms in whole plants and to compare between species and different seasons. Taking into account the standing stocks for all plant species in the current study (Fig. 2), the average standing stock of metals in the nine species varied from 0.0146 to 199 mg m^{-2} in the order Al > Fe > Mn > Zn > Cr > Pb > Cu > Ni > As > Cd. The seasonal variation in metals standing stock exhibited the highest values in August than May due to the high biomass. The findings of the current study demonstrated that the seasonal patterns of HM/Ms distribution in the whole plant structure of all species differ among elements and plant species. Similar results were found by other researchers who reported that seasonal patterns and time of maximum standing stock for each element may vary (Brezinová and Vymazal, 2015). The range of standing stocks of Cr, Pb, Cu and Cd (0.14–0.2, 0.18–0.23, 0.16–0.11 and 0.00967–0.0146 mg m^{-2}, respectively) found in this study were similar to that reported in previous studies (Bernard and Lauve, 1995; Kufel, 1991; Lesage et al., 2007b; Maddison et al., 2009; Peverly et al., 1995; Vymazal et al., 2010b; Windham et al., 2003). The peak standing stocks of Zn and Ni (0.73 and 0.10 mg m^{-2}) found in the current study were lower than those (1.1 and 0.57 mg m^{-2}) reported by Lesage et al. (2007b) and Maddison et al. (2009). Biomass is commonly recognized as an important factor in determining the value of metal standing stock in the system (Richardson and Vymazal, 2001). In spite of the low biomass observed in May, Al, Mn and Fe still showed high accumulation in the spring season, suggesting high uptake rate and subsequent translocation from sediment to plant. This study indicates that judicious ditch plants harvesting (i.e. at the appropriate time) would be a reasonable drainage ditch management option to increase the mitigation amount of HM/M (except Al, Mn and Fe) estrated during the growing season.

Table 2
Leaves, stems, roots and total biomass (g m^{-2}, DW) of nine species, as well as heights (cm) after 21 weeks.

Species	Leaf biomass		Stem biomass		Root biomass		[a]Total biomass		[a]Height	
	Mean	[b]SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cyp	331	26	2127	225	423	50	2880	294	260	7
Cog	245	30	798	89	498	42	1541	191	145	6
Pha	279	15	625	56	84	6	989	100	340	10
Irp	818	34			1071	85	1889	156	168	13
Rci	820	24	2024	209	359	35	3201	275	235	16
Thd	295	17	1843	142	363	30	2501	215	352	14
Acc	403	23			315	40	718	65	135	8
Acg	445	17			404	45	848	105	55	3
Hyv	62	8			24	1	87	9	46	2

[a] Note: shoot height and total biomass values were calculated by net change = final value − mean initial starting value.
[b] Standard deviation of the mean.

Table S2 illustrates the percentage of HM/Ms load sequestered by plant biomass. As reported in the results, the percentages of most studied HM/Ms load sequestered by plant biomass are <1%. The highest amounts were measured for Al and Fe in Rci species, which amounted to 28.8% and 19.48%. Similar observations were also reported by Lesage et al. (2007a), who found 0.36, 0.25, 0.26, 0.46 and 0.9% of the inflow Cd, Pb, Ni, Cu, and Zn load sequestered in the plants. Fibbi et al. (2012) documented 0.38% of the inflow Cr load accumulated in the plants. Peverly et al. (1995) also found < 1% of the inflow Cd load sequestered in the biomass of *Phragmites australis* in a wetland receiving landfill leachate. On the other hand, 54.6% of the inflow cadmium load accumulated in the plant biomass (Vymazal and Krása, 2005b). Vymazal and Březinová (2016) concluded that the amount of HM/Ms sequestered in the plants is variable and represents, frequently, only a small fraction of the inflow load.

3.3. Metal/metalloid storage in ditch plant parts

HM/M concentrations in the different VDD plant parts are listed in Table 3. Results show that for each species, HM/M concentrations in the root, stem and leaf were variable and there were significant, consistent and regular patterns in the distribution of most HM/Ms within these three plant parts. In terms of roots, all the studied plants had the highest concentrations of Al, Cd, Pb, As (except species Hyv), Cr (except Acg and Hyv), Fe (except Acg and Hyv) and Ni (except Acc, Acg and Hyv), whereas species Pha had the lowest Cd concentration (Table 3). Overall, the concentrations of HM/Ms are higher in roots than both stems and leaves (Lesage et al., 2007a) and follows the order of root > leaf > stem (Bonanno and Lo Giudice, 2010; Galletti et al., 2010; Tam and Wong, 1996; Vymazal et al., 2009). Most of the studied species in this study also follow this trend rule. However, concentrations of Cu, Cr (in species Acg and Hyv), Mn (in species Cyp, Cog, Pha, Rci, and Tha), Fe (in species Acg and Hyv) and Ni (in species Acc, Acg and Hyv) were always higher in leaves than in roots and stems. Moreover, the concentrations of Zn in all the studied species (except species Acg and Acc) found in the either stems or leaves were higher compared to the roots.

Over 10-years of VDD operation, high accumulation of Al, Fe and Mn were recorded in different parts of the investigated plant species. These elements are mostly adsorbed from the VDD sediments since concentrations were low in the VDD water. As reported by Albers and Camardese (1993), HM/M concentrations in plants can be >100,000 times higher than in water. Moreover, Kabata-Pendias (2011) reported that concentrations of Al in plants displayed phytotoxic effects in the ranges of 1000–3000 mg kg^{-1}. In this study, the Al content in all the studied species was lower than these values, indicating that these species developed various mechanisms of tolerance to toxicity. In spite of the high concentrations in various plant organs as compared to the other HM/Ms, Al and Fe exhibited the lowest translocation factors, thus, proving the least mobile elements.

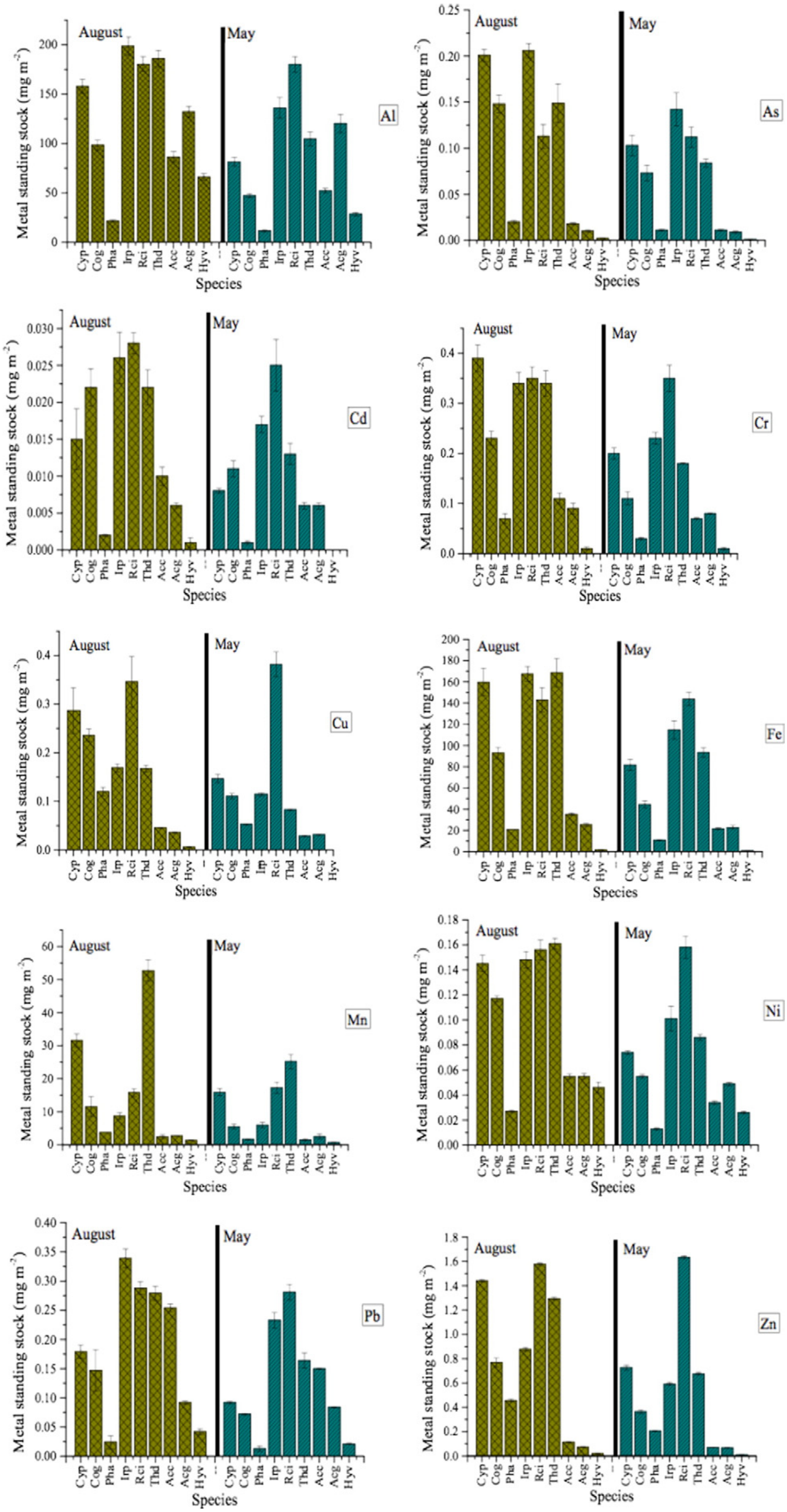

Fig. 2. Average standing stocks of HM/Ms during the monitored period in VDD (May–August 2015, mean ± S.D., $n = 6$).

Table 3
Metal/metalloid concentration (μg kg^{-1} DW, $n = 3$) in different plant parts of species in the VDD.

Species		Al	Cr	Mn	Fe	Ni	Cu	Zn	As	Cd	Pb
Cyp	Leaf	10,132	42.1	13,243	31,241	31.2	174.2	651	41.24	1.83	28.45
	stem	47,880	134.7	11,170	49,890	44.55	98.95	482	60.23	3.69	40.97
	Root	124,800	213	8035	102,200	94.7	123.7	477.1	139	15.74	195
Cog	Leaf	11,390	38.68	10,900	13,710	49.9	239	646.3	30.39	6.20	22.02
	Stem	41,770	111.9	5896	41,080	69.17	158.8	476.1	36.07	5.19	39.71
	Root	125,200	270.7	8371	114,000	100.5	102.3	462.3	224.9	31.96	220.2
Pha	Leaf	14,400	44.83	5275	14,800	24.98	181	438	18.8	1.79	18.79
	Stem	11,540	61.5	2980	14,630	20.51	102.5	506.4	11.28	2.19	11.49
	Root	122,200	208.5	3820	89,710	82.05	59.09	197.5	93.18	4.51	141.2
Irp	AG	61,880	122.2	3166	47,350	55.06	93.91	541.4	25.08	11.2	60.95
	BG	138,200	223.5	5693	120,100	96.44	86.27	404.9	173.3	15.32	270.1
Rci	Leaf	44,520	93.83	10,120	40,190	43.17	227.2	437.4	28.12	4.315	39.38
	Stem	91,340	182.1	6913	70,970	80.34	154.7	894.3	54.65	10.78	153.2
	Root	122,500	188.9	6470	96,530	91.35	112.4	447.7	91.25	40.65	205.5
Thd	Leaf	19,430	54.66	70,020	29,090	61.26	166.3	470.8	40.06	3.369	40.01
	Stem	67,720	137.8	15,630	63,880	64.2	53.46	588.2	46.17	4.112	66.59
	Root	153,000	179.4	8891	116,400	67.34	43.26	196.9	144.2	37.06	398.6
Acc	AG	63,080	90.02	3192	32,930	81.08	80.63	204.6	12.99	5.12	41.15
	BG	193,600	226.3	3668	69,830	70.91	41.41	96.62	39.87	25.98	754.3
Acg	AG	125,400	109	2591	30,710	80.25	54.4	84.54	8.391	4.178	79.35
	BG	189,200	92.21	3966	28,900	46.64	28.89	90.53	14.52	10.99	140.2
Hyv	Leaf	133,200	107.1	7976	18,270	644.2	68.71	278.6	29.33	2.265	93.31
	Root	162,100	104.1	21,800	11,690	58.18	38.21	39.28	2.903	16.31	1007

3.4. Metal uptake and transfer in nine ditch plant species

The enrichment factor (EF) refers to the degree of HM/M transfer from growth media to the plant. In general, high EFs of plant species for the selected HM/Ms indicates that those HM/Ms would be accumulated in the plants. Mean values of EF values measured for HM/Ms in the selected species (Table 4) decreased according to HM/M in the following order: Pb > Al > Fe > Cd > Mn > Zn > Cu > Cr > Ni > As. The mean EF values of Pb (9421), Al (2036) and Fe (793) in the species were higher than those of other HM/Ms.

The translocation factor (TF) was used to estimate HM/M mobility. TF values for each HM/M in various plant species are shown in Table 4. The TFs varied from 0.05 to 11.07 for all the studied species. According to the TF values, HM/Ms such as Cr (except in Rci, Thd, Acg and Hyv), Fe (except in Rci, Acg and Hyv), As (except in Hyv), Al (except Rci), Cd and Pb were largely retained in roots, as displayed by general TF values < 1. Exceptions occurred for Cu, Zn, Ni (except Cyp, Pha and Irp), Mn (except in Irp, Acc, Acg and Hyv), which were higher than unity. Taking all the species together, the relative orders of HM/Ms transfer from roots to aboveground plant parts were Zn > Cu > Mn > Ni > As > Cr > Fe > Al > Cd > Pb.

Phytoremediation is an emerging low-cost and environmentally friendly method that cleans up polluted environments. This technique has been utilized in contaminated areas using phytoextraction and phytostabilization processes (Wong, 2003). Phytostabilization is the use of plant species to immobilize pollutants and store them in below-ground biomass and/or sediment (Weis and Weis, 2004; Yoon et al., 2006). Generally, phytostabilization is applied for stabilization of pollutants in polluted water and soil, by preventing pollutants migration to groundwater or their transfer into the food chain, in contrast to phytoextraction in which plants can be used to remove metals from the water/soil and concentrate them in harvestable plant parts. In the end, these plants must be harvested and disposed of or incinerate to recycle the pollutants. In general, the most effective phytoremediation species, referred to as hyper accumulators are classified on the basis of pollutant concentrations in the aboveground parts, translocation ability, enrichment factor (Wei et al., 2006) and metal tolerance potential. However, the most important characteristic of hyperaccumulators is that the plant species should tolerate very high concentrations of HM/Ms (i.e. >10,000 mg kg^{-1} of Zn or Mn; >1000 mg kg^{-1} of Pb or Cu and Ni, and > 100 mg kg^{-1} of Cd). In the current work, none of the studied species from the VDD reached the metal hyperaccumulator threshold levels (Baker and Brooks, 1989; Lasat, 2002; Prasad et al., 2006) and regardless of the high biomass production of most of the ditch plant species, only a small fraction of the HM/Ms mostly present in the water or sediment were accumulated in the plant biomass. It should be noted that the lower values found for the HM/Ms

Table 4
Enrichment coefficient (EF) and transfer factor (TF) for ditch plant species.

Species	Al		Cr		Mn		Fe		Ni		Cu		Zn		As		Cd		Pb	
	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF	EF	TF
Cyp	1128	0.47	23	0.83	135	**3.04**	955	0.79	10	0.81	65	**1.4**	83	**2.37**	10	0.73	79	0.35	4407	0.36
Cog	1101	0.42	25	0.56	105	**2.01**	879	0.48	12	**1.18**	83	**3.9**	82	**2.43**	12	0.31	161	0.36	4699	0.28
Pha	914	0.21	18	0.51	50	**2.16**	621	0.33	7	0.55	57	**4.8**	59	**4.78**	5	0.32	31	0.89	2858	0.21
Irp	1853	0.45	30	0.55	55	0.56	1308	0.39	13	0.57	45	**1.1**	74	**1.34**	12	0.14	147	0.73	8276	0.23
Rci	1595	**1.11**	27	**1.46**	98	**2.63**	1082	**1.15**	12	**1.35**	82	**3.4**	92	**2.97**	7	0.91	206	0.37	6635	0.94
Thd	1482	0.57	22	**1.07**	394	**9.63**	1090	0.8	11	**1.86**	45	**4.1**	65	**5.38**	10	0.61	165	0.20	8420	0.27
Acc	2377	0.33	28	0.41	43	0.87	803	0.47	13	**1.14**	30	**1.9**	23	**2.12**	3	0.33	173	0.20	19,886	0.05
Acg	2913	0.66	18	**1.18**	41	0.65	466	**1.06**	11	**1.72**	21	**1.9**	14	**1.01**	1	0.58	84	0.38	5489	0.57
Hyv	2734	0.82	18	**1.03**	186	0.37	234	**1.56**	59	**11.1**	26	**1.8**	25	**7.09**	2	**10.1**	103	0.14	27,508	0.09
Mean	2036	0.48	23	0.84	115	2.44	793	0.78	16	2.25	48	2.7	54	3.27	6	1.56	125	0.40	9421	0.33

Values in bold represent high translocation factor.

concentrations in the aboveground parts, as against characteristic hyperaccumulators, might be due to lower concentration of HM/Ms in the assessed VDD waters or sediments. Nevertheless, the ability of these species to accumulate HM/Ms from sediments or sewage and translocate them to the aboveground biomass might be useful for phytoextraction. Both EF and TF can be utilized to investigate plant species' potential for phytoremediation goal.

The highest TFs recorded for As, Zn, and Ni were 10.1, 7.09 and 11.07, respectively. TFs for Cu and Mn in species Thd were 4.13 and 9.63, respectively. The ditch plant species were categorized into two classes according to their TF values. Class 1 is composed of tolerant species for which TF < 1. These include species such as Cyp, Cog, Pha, Irp, Rci, Thd, Acc, Acg, Hyv for As, Cd and Al. The apparent advantage of this phenomenon (TF < 1) is that although long-term sewage application resulted in high accumulation of HM/Ms in sediment, the same cannot be proportionately transported to the food chain. Class 2, on the other hand, is composed of species that showed TF values > 1 (1.06–11.07). These include species Cyp, Cog, Pha, Irp, Rci, Thd, Acc, Acg, Hyv for Cu, Zn (except Acg), Ni (except Cyp, Cog, Pha, Irp), Mn (except Irp, Acc, Acg and Hyv). These findings demonstrate that plants adopted an accumulation strategy with regard to some metals. According to Baker (1981), a TF > 1 indicates a very efficient ability to transport pollutants from roots to aboveground parts (i.e. accumulator). It is worth noting that TFs for Al (in only Rci), Cr (Rci, Acg, Hyv), Mn (Cyp, Cog, Pha, Rci, Thd), Fe (Rci, Acg, Hyv), Ni (Cog, Rci, Thd, Acc, Acg, Hyv) and As (in Hyv) were >1. Consequently, they are suitable plants for phytoextraction of these metals. However, the TF should not be considered as the only feature of accumulator plants, but also species with the ability to accumulate high elemental contents in their shoot tissues can be good candidates for phytoextraction. The EF is another important factor when considering the phytoremediation potential of species. In general, plant species with greater EF than one combined with less TF than one can be suitable for phytostabilization of soil/water contaminated with metals/metalloids (Fitz and Wenzel, 2002; Yoon et al., 2006). The EFs of all metals were higher (EF > 1) in the studied plants. The low TFs for Pb, Cd, Fe, Cr and Al make them suitable for their phytostabilization. Pb (with the highest EF values) was the most transferred element into the plant root. This was followed by Al and Fe. The accumulation of these metals by the selected plants indicates their potential usefulness for the sequestration metals from polluted environments. The outcome of enrichment factors found in this study indicate potential values of the selected species as efficient phytoremediation plants for the sequestration of the studies metals/metalloids particularly Pb, Al and Fe. These plants can be useful for phytoextraction in water environments since the plants used in phytoextraction processes must display values of TF higher than unity, and produce high quantities of biomass.

4. Conclusions

This is the first study to reveal metals/metalloids accumulation in vegetated (Eco) drainage ditch plants impacted by long-term diffuse with untreated domestic sewage. After 10 years of operation, the VDD sediment accumulated significant amounts of metals/metalloids associated with untreated domestic that otherwise could have been released directly into streams and lakes. The selected ditch plant species demonstrated some abilities of accumulating metal/metalloid. However, metal bioaccumulation in ditch plants was limited in most species and rather accumulated mainly in ditch sediment. The TF > 1 of Al (in only Rci), Cr (in Rci, Acg, Hyv), Mn (in Cyp, Cog, Pha, Rci, Thd), Fe (in Rci, Acg, Hyv), Ni (in Cog, Rci, Thd, Acc, Acg, Hyv) and As (in Hyv) make them suitable for its phytoextraction from water, while the TFs < 1 for Al, Fe, Pb, Cd, As and Cr make them suitable for phytostabilization. Correlation analyses demonstrate that the concentrations of metals in ditch plant species mostly depend on metal sediment concentrations. Although metal concentrations do not pose a significant threat yet, the accumulation of Mn, Cu, Cr, Zn, Pb, Cd, Ni and As in ditch sediments needs to be monitored periodically in comparison with the corresponding background concentrations in the soils of the Sichuan Basin.

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Appendix A. Supplementary data

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