Differences in Cd and Zn bioaccumulation for the flood-tolerant *Salix cinerea* rooting in seasonally flooded contaminated sediments

Bart Vandecasteele[a,*], Gijs Du Laing[b], Paul Quataert[a], Filip M.G. Tack[b]

[a]*Institute for Forestry and Game Management, Ministry of the Flemish Community, Gaverstraat 4, B-9500 Geraardsbergen, Belgium*
[b]*Ghent University, Department of Applied Analytical and Physical Chemistry, Coupure 653, B-9000 Ghent, Belgium*

Abstract

Several authors suggest that a hydrological regime aiming at wetland creation is a potential management option that favours reducing bioavailability for metal-contaminated sites. The hydrological conditions on a site constitute one of the many factors that may affect the availability of potentially toxic trace metals for uptake by plants. Bioavailability of Cd, Mn and Zn on a contaminated dredged sediment landfill (DSL) with variable duration of submersion was evaluated by measuring metal concentrations in the wetland plant species *Salix cinerea* in field conditions. Longer submersion periods in the field caused lower Cd and Zn concentrations in the leaves in the first weeks of the growing season. Foliar Cd and Zn concentrations at the end of the growing season were highest on the initially flooded plot that emerged early in the growing season. Foliar Zn concentrations were also high at a sandy-textured oxic plot with low soil metal concentrations. Zn uptake in the leaves was markedly slower than Cd uptake for trees growing on soils with prolonged waterlogging during the growing season, pointing at a different availability. Zn availability was lowest when soil was submerged, but metal transfer from stems and twigs to leaves may mask the lower availability of Cd in submerged soils. Especially for Cd, a transfer effect from one growing season to the next season was observed: oxic conditions at the end of the previous growing season seem to determine at least partly the foliar concentrations for *S. cinerea* through this metal transfer mechanism. Duration of the submersion period is a key factor for bioavailability inasmuch as initially submerged soils emerging only in the second half of the growing season resulted in elevated Cd and Zn foliar concentrations at that time.

Keywords: Hydromorphic conditions; Oxidation–reduction; Seasonal inundations; Submersion; Metals; Surface ponding

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1. Introduction

Soil pH and oxidation–reduction potential (Eh) are dominant factors determining metal forms and behaviour, and hence mobility and availability.

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[*] Corresponding author. Tel.: +32 54 436168; fax: +32 54 436160.
E-mail address: Bart.Vandecasteele@lin.vlaanderen.be (B. Vandecasteele).

Flooding results in the creation of reducing conditions which may be accompanied by changes in pH, typically towards the more alkaline region. Reduction processes are initiated shortly after flooding. Submersion of well-aerated upland soils resulted in a fast reduction of Mn^{4+} and Fe^{3+} into soluble Mn^{2+} and Fe^{2+} (Peters and Conrad, 1996; Scalenghe et al., 2002). This can be followed by the formation of sulphide precipitates. Flooding of wetlands typically results in a decreased environmental availability of Cd, Zn, Cu and Ni (Gambrell, 1994; Kashem and Singh, 2001). Tack et al. (1998) observed a short period of mobilisation of Cd and Zn and subsequently lower concentrations in the saturation extract of a continuously wet treatment (dfloodedT) of landfilled sediments, compared with the concentrations for an alternating dry and wet regime. Similar observations were reported by Charlatchka and Cambier (2000) for an agricultural polluted soil.

Oxidation–reduction potential measurements allow for a fast although mostly qualitative assessment (Tanji et al., 2003) of reducing conditions developing upon flooding. Submersion resulted in a fast decrease of Eh (Kashem and Singh, 2001; Seybold et al., 2002; Haraguchi, 1991; Tanji et al., 2003), unless nitrate was artificially added (Charlatchka and Cambier, 2000). Water depth and Eh status were the main variables in determining the Zn speciation in a seasonally flooded wetland (Bostick et al., 2001). Hydrology (water level) was observed to be the primary factor that controlled Eh fluctuations (Haraguchi, 1991; Seybold et al., 2002; de Mars and Wassen, 1999), but absolute water table depth had little effect on Eh when water level was beneath the soil depth where Eh was measured (Haraguchi, 1991). On a local scale, spatial variation of Eh may determine vegetation composition (McKee, 1993).

Wetlands with alternating submerged and emerged periods have a specific soil chemistry. Zn speciation reversibly fluctuated in a seasonally flooded wetland: increasing water levels promoted the formation of ZnS and $ZnCO_3$, while dry periods with oxidized soils were dominated by ZnO and Zn adsorbed on hydrated oxides (Bostick et al., 2001). Van den Berg et al. (1998) reported increased Cd and Zn pore water concentrations during the summer in a seasonally inundated wetland polluted with metals. However, pore water concentrations first increased for Zn and afterwards for Cd. Increased Zn mobility was thought to be partly caused by oxidation of labile sulphides, while Cd might be released during biodegradation of organic matter (OM) (Van den Berg et al., 1998). Alternating oxidation and reduction of hydric soils in periodically waterlogged wetlands may cause intense decalcification of the upper soil layers (Van den Berg and Loch, 2000), and thus may affect metal availability.

Hydromorphic conditions result in an increasing oxygen demand for both soil and plant roots (Pezeshki, 2001) and may cause the production or accumulation of phytotoxic compounds (e.g., organic acids, methane, sulphides and reduced Mn and Fe) contributing to root injury, growth reduction and mortality (Kozlowski, 1997; Pezeshki, 2001). Pezeshki et al. (1997) and Pezeshki and DeLaune (1998) reported large differences in plant tolerance and effects on plant physiology and growth to reduced soil Eh conditions. Eh also affects the availability of essential elements and contaminants to plants. Several plant species showed lower Cd and Zn uptake in reduced soils (Gambrell and Patrick, 1989; Gambrell, 1994). As this evidence suggests that flooding and subsequently reduction of contaminated soils may result in lower environmental bioavailability, it may constitute a valid management option for polluted soils.

The aim of this field-based observational study was to determine whether the duration (period of anoxic soil conditions) of submersion of a polluted dredged sediment landfill (DSL) affects the foliar metal concentration for *Salix cinerea*, a typical wetland willow species. Five plots on clayey soils with high metal levels and variable length of submersion in the first half of the growing season were selected. Foliar metal uptake for these plots was compared with concentrations for a plot on the slightly polluted sandy-textured part of the dredged sediment landfill and with a nearby plot with baseline contamination levels. Foliar samples were collected on a DSL during a growing season, and soil oxidation–reduction condition was determined simultaneously. The advantage of willows for research on metal bioavailability is that they reflect time-integrated accumulated concentrations for the studied soil profiles.

2. Materials and methods

2.1. Soil sampling and measurements

A dredged sediment landfill in Semmerzake near Ghent (Belgium), referred to here as dredged sediment landfill (DSL), was selected for this study. Willows occur as volunteer vegetation on sediment-derived soils such as dredged sediment landfills, overbank sedimentation zones and freshwater tidal marshes (Bal et al., 2001). The volunteer willow vegetation on this polluted DSL was compared with volunteer willows on a nearby infrastructure spoil landfill with baseline metal concentration levels. A plot is defined in this text as a location with relatively homogeneous soil properties where four trees of the same *Salix* species and approximately the same age and diameter were sampled. A site is a larger unit where several plots were sampled. The DSL (13.3 ha) was landfilled between 1992 and 1995, with sediments dredged in the Upper Scheldt, and can generally be characterised as a wetland with stagnant water from late autumn to mid-spring. On this site, five plots (Table 1) on clayey polluted soils were selected representing variable hydrological regimes. Plot 2 was not submerged anymore in week 15. Plot 3, plot 4 and plot 5 were waterlogged during winter, but water level decreased strongly in spring and early summer, while plot 6 in the reed-dominated part was regularly waterlogged in summer as well, depending on the weather conditions. Plot 1 was located on the higher, sandy-textured and least contaminated part of the DSL and was included as a reference. On the infrastructure spoil landfill used as reference site, one plot (Table 1, plot 0) was selected. On each plot, the 0–30 soil horizon was sampled in triplicate. All plots were on calcareous soils.

Time is expressed as the week number during the year. Oxidation–reduction potential was weekly measured between week 15 and week 31 with four replicates on all plots with a combined redox electrode (platina and Ag/AgCl reference electrode; HANNA instruments, HI 3131B) and a WTW multiline P3 meter, and values were reported after correction to the standard hydrogen reference electrode. The combined electrode was pushed into the soil to a depth of 5 cm, and a stable reading was awaited. Oxidation–reduction measurements for plot 3 and plot 0 was not possible from week 22 onwards as the electrode could not penetrate the soil inasmuch as the soil was too dry. Oxidation–reduction measurements were repeated in week 42. Simultaneously with the oxidation–reduction measurements, water level above the soil surface for the waterlogged soils was measured.

2.2. Foliar and stem sampling

S. cinerea was identified based on leaf morphology (Weeda et al., 1999), and the presence and the length

Table 1
Soil properties of the dredged sediment-derived soils (plot 1–plot 6) and the plot on the infrastructure spoil landfill (plot 0) where willow was sampled during the growing season EC—electrical conductivity, OM—organic matter

		Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 0
Clay	%	11 (1)	44 (2)	42 (2)	37 (4)	36 (2)	39 (2)	22 (6)
$CaCO_3$	%	6.9 (0.8)	12.4 (0.4)	11.9 (0.3)	11.6 (0.2)	11.8 (0.2)	11.6 (0.5)	2.3 (1.2)
EC	AS cm^{-1}	176 (128)	226 (15)	360 (78)	603 (585)	1281 (86)	824 (158)	140 (30)
pH–H_2O		7.9 (0.1)	7.6 (0.1)	7.6 (0.1)	7.5 (0.2)	7.3 (0.1)	7.4 (0.1)	7.4 (0.5)
OM	%	4 (3.5)	8.2 (0.3)	8 (0.4)	8.6 (1.7)	8.2 (0.2)	9.3 (0.4)	3.5 (1.4)
Cu	mg kg^{-1} dry soil	49 (10)	195 (23)	147 (11)	184 (13)	190 (5)	168 (2)	17 (9)
Cr	mg kg^{-1} dry soil	165 (5)	479 (22)	488 (23)	410 (60)	398 (22)	522 (22)	57 (6)
Pb	mg kg^{-1} dry soil	66 (8)	150 (9)	139 (6)	140 (12)	141 (3)	165 (11)	26 (9)
Ni	mg kg^{-1} dry soil	20 (11)	43 (1)	42 (1)	38 (5)	37 (2)	43 (3)	24 (6)
Mn	mg kg^{-1} dry soil	335 (130)	772 (69)	744 (47)	644 (36)	615 (42)	815 (66)	1036 (590)
Zn	mg kg^{-1} dry soil	381 (48)	1197 (49)	1165 (101)	971 (148)	1089 (22)	1342 (39)	99 (18)
Cd	mg kg^{-1} dry soil	2.3 (0.5)	12.7 (0.5)	14.2 (0.9)	9.1 (1.2)	9.4 (1.0)	14.7 (2.2)	0.6 (0.1)
Water level	cm	0	0	30	10	15	25	0
Eh	mV	466	464	103	19	113	132	87

Soil metal concentrations are aqua-regia-extractable concentrations (mg kg^{-1} dry soil). Soil oxidation–reduction potential (Eh) on a depth of 5 cm and water height on the soil surface are measured in week 15. Values in parentheses denote standard deviations for three replicates.

of the *striae* on 1-year-old branches (Meikle, 1984). *Striae* are longitudinal lines underneath the bark. *S. cinerea* can be distinguished from *S. aurita* and *S. x multinervis* based on the presence of solely long *striae* on 1-year-old branches (Meikle, 1984). To account for the variability associated with sampling, four *S. cinerea* L. trees of approximately the same age and diameter were sampled within a circle with a diameter of 10 m on each plot. Foliar samples were taken with a 3-week interval between week 18 and week 42 during the growing season 2003. For plot 3, foliar samples were previously collected with the same methodology between week 24 and week 42 in 2001 and 2002, allowing for analysis of temporal and spatial variability. Our sampling strategy for leaves focused on individual trees. For foliar sampling, at least four branches from different heights and positions in the crown were sampled by means of an extension crosscut saw (Blair, 1995). Approximately 1000 cm^3 of leaf samples were collected at each sampling location. Leaves were not washed as washing procedures have to be avoided for food web research purposes (Ernst, 1990) and seem to produce misleading results due to incomplete removal of metals on the leaf surface and partial leaching of metals from the leaf tissues (Kozlov et al., 2000). Luyssaert (2001) showed that foliar Cd concentrations of trees grown at a dredged sediment landfill in the vicinity of the sites studied here are chiefly determined by soil pollution. Absolute contribution of aerial deposition on oleander leaves in a Mediterranean area with dry hot summers was in the range of 0.25 mg Cd kg^{-1} dry weight (DW) and 7 mg Zn kg^{-1} DW for the most affected sites (Aksoy and Öztürk, 1997). Baseline concentrations for washed willow leaves are in the range 0.2–3.4 mg Cd kg^{-1} DW and 110 and 560 mg Zn kg^{-1} DW (Severson et al., 1992), thus importance of aerial contamination of willow leaf surfaces is relatively low. For each tree, two willow cuttings from 1-year-old dormant stems were collected in week 49 (December 2003). Trees on plot 1 were severely affected by phyllophagous insects between week 18 and week 24, but regrowth of leaves allowed for continuous sampling.

In week 33, *S. cinerea* was sampled with the sampling strategy (four trees per plot) described above on reference sites grouped according to soil contamination level (Table 2). Trees were sampled at eight plots on sites (one plot per site) with baseline contamination levels (REF1), at six plots on a dredged sediment landfill with low metal concentrations (REF2) and at six plots on sites (one plot per site) with slightly contaminated soils (REF3).

2.3. Chemical analyses

Foliar and stem samples were dried for 7 days at 40 8C, mechanically ground (Pulverisette 14, Fritsch, Idar-Oberstein, Germany) and stored in dark vials before analysis. Total foliar N was measured by the Kjeldahl method (Van Ranst et al., 1999). Total foliar and stem element concentrations are extracted with HNO_3 (p.a. 65%) and H_2O_2 (ultrapure) in a 3:1 ratio using microwave digestion and measured with ICP-AES (Varian Liberty Series II, Varian, Palo Alto, CA). The accuracy of the foliar and stem element analysis was checked using BCR 60 (Aquatic plant) for Cd, Cu, Mn and Zn and CRM 100 (Beech leaves) for S. Soil total concentrations of Cd, Cr, Cu, Ni, Pb, Mn and Zn are actually pseudototal aqua-regia-extractable concentrations measured with ICP-AES after microwave digestion. Soil pH_{H2O} and electrical conductivity (EC) were measured in a 1:5 soil to water suspension. Organic matter (OM) was determined by the method of Walkley–Black, assuming that this method measures 75% of the total organic matter. $CaCO_3$ content was

Table 2
10th–90th Percentile range for soil properties of the reference plots where *Salix cinerea* was sampled in week 33 EC—electrical conductivity, OM—organic matter, NA—not assessed

		REF1	REF2	REF3
Clay	%	11–33	34–48	17–44
$CaCO_3$	%	0.4–9.2	2.6–5.5	1.7–4.3
EC	AS cm^{-1}	75–140	695–2040	100–312
pH–H_2O		6.05–8.59	4.87–7.45	6.88–8.44
OM	%	1.1–6.3	8.8–24.4	1.8–11.8
Cu	mg kg^{-1} dry soil	4–21	28–85	8–59
Cr	mg kg^{-1} dry soil	21–62	53–82	77–108
Pb	mg kg^{-1} dry soil	7–66	26–55	13–173
Ni	mg kg^{-1} dry soil	7–34	36–41	14–40
Mn	mg kg^{-1} dry soil	166–1130	NA	173–665
Zn	mg kg^{-1} dry soil	41–135	211–528	118–576
Cd	mg kg^{-1} dry soil	b0.3–0.7	0.7–1.2	0.8–4.6

REF1: sites with baseline contamination levels, REF2: dredged-sediment derived soil with low metal concentrations, REF3: slightly contaminated soils. Soil metal concentrations are aqua-regia-extractable concentrations (mg kg^{-1} dry soil).

determined by back-titration with 0.5 M NaOH of an excess of H_2SO_4 added to 1-g air-dried sediment. The grain size distribution of the soil samples was determined using laser diffractometry (Coulter LS200, Miami, FL), with the clay fraction defined as the 0–6-**A**m fraction (Vandecasteele et al., 2002).

2.4. Statistics

Effect of tree, year and week and interaction between these factors on foliar Cd and Zn concentrations for plot 3 (sampled between week 24 and week 42 in 2001, 2002 and 2003) were tested in a split-plot ANOVA model with tree included in the error term as the same trees were sampled during the 3 years. The results for the model were found to be robust for square root transformation and both the definition of week as continuous or factor variable. The reported model used the untransformed Cd and Zn concentrations, and week was defined as a continuous variable. Foliar data for week 33 for the six plots on the DSL were grouped and were compared with the results for the three reference groups (REF1, REF2 and REF3) with ANOVA. The plots were nested in the factor dgroupT. The Sidak method was applied for multiple comparison. Effect of plot, tree and week and interaction between these factors on foliar concentrations for seven plots sampled between week 24 and week 42 were tested in a split-plot ANOVA model with tree included in the error term as the same trees were sampled during the growing season. Measurements for week 18 and week 21 were excluded inasmuch as not all trees could be sampled for plot 1 as they were severely affected by phyllophagous insects. The Sidak method was applied for multiple comparison after aggregation of the data per plot. Bioconcentration factors (BCF) for Cd and Zn on a dry weight (DW) base were defined as the ratio [foliar concentration/total soil concentration (aqua regia)].

3. Results

3.1. Hydrological regime

Redox measurements throughout the observation period reflect the changes in submersion state. Plot 2 and plot 0 were not any more submerged in week 15 (3 weeks before the start of the foliar sampling campaign), while plot 4, plot 5 and plot 6 remained submerged until week 24. Plot 3 initially showed the highest water level, but water disappeared faster than on the other plots. It was submerged until week 19 (Fig. 1a). Oxidation–reduction potential values in the topsoil (Fig. 1b) were lower than 200 mV for plot 4, plot 5 and plot 6. The initial low Eh for plot 3 and plot 0 increased strongly in week 20 to levels typical for oxic (aerated) soils. Eh values of 350–400 mV are the boundary between presence and absence of oxygen (Pezeshki, 2001). Eh values for the submerged plots were decreasing until week 24 (end of the submersion) and then increased towards 200–250 mV, values still pointing at prevailing anaerobic conditions. Plots 4, 5 and 6 were subjected to more prolonged periods of waterlogging. Eh values at plots 4, 5 and 6 until week 29 point at reductive dissolution of Mn and Fe and formation of sulphides (Peters and Conrad, 1996; Bostick et al., 2001). A decrease in Eh just after emergence was also reported by Haraguchi (1991) for a submerged floating peat mat both in field and in pot experiments. Haraguchi (1991) attributed this decrease to prolonged water saturation and restricted oxygen diffusion combined with strong organic

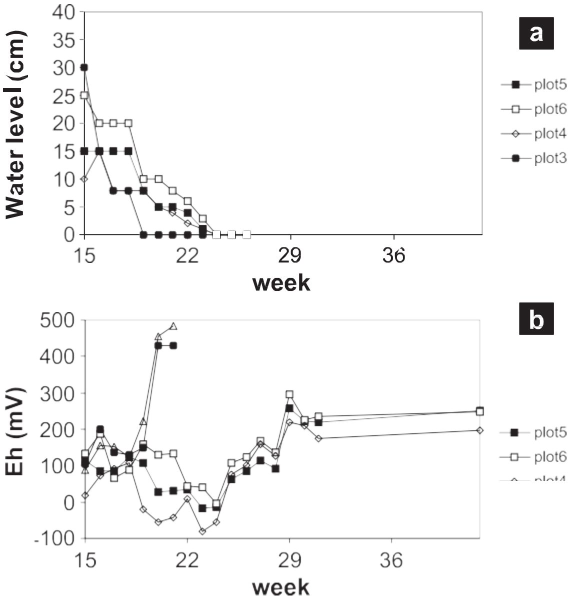

Fig. 1. Evolution of (a) the water height on the soil surface and (b) the weekly measured soil oxidation–reduction potential on a depth of 5 cm for the six sampled plot.

matter decomposition (Haraguchi, 1991). The observed Eh patterns over the growing season may vary with soil depth (Seybold et al., 2002) and may be modified by the presence of adult root systems (McKee, 1993). Eh measurements in the field are only indicative: there is a strong variability within soil aggregates, and Eh may change as a function of depth. After emergence of plot 4, plot 5 and plot 6, soil was still waterlogged, while plot 3 emerged earlier and was fast transformed to an aerated soil. After emergence, soils showed variable rates of oxidation, visualised in differences in topsoil structure. A crumbly soil structure due to soil drying accelerates oxidation, while a waterlogged muddy structure allows the anaerobic soil conditions to prevail. The summer of 2003 was exceptionally hot and dry. This may explain why plot 6, found still being submerged in August 2002, was already emerged in June 2003.

3.2. Temporal and spatial variability

Four trees on plot 3 were sampled between week 24 and week 42 in 2001, 2002 and 2003. “Week” and “year” significantly affected foliar Cd and Zn concentrations (Fig. 2, Table 3). Although all factors were highly significant in explaining variability, variance associated with “week” and “tree” was higher than variance for the sampling year (Table 3). Cd concentrations were highest for tree 1, especially in 2003. Concentration patterns (Fig. 2) of Cd and Zn differ in that Cd initially tends to remain constant or decrease slightly, while Zn concentrations increased right from the beginning of the observation period.

Nondestructive foliar samplings allow for in situ biomonitoring using willows. Current data illustrate the large variability between individual trees on the same stand. It is thus necessary that several trees be sampled on each plot in monitoring programs to assess temporal and spatial variability. However, trees must be identified, and the same trees must be sampled to assess changes in foliar concentrations with time.

3.3. Baseline concentrations in week 33

Foliar concentrations measured in week 33 on the DSL were compared with concentrations measured in the same period on reference sites (Fig. 3). Differences between the groups were highly significant for Cd and Zn ($p<0.001$). Multiple comparison revealed significantly higher foliar Cd concentrations for the DSL than for the REF3 plots and significantly higher foliar Cd concentrations for the REF3 plots than for the REF1 and REF2 plots. Foliar Zn concentrations were significantly lower for the REF1 plots than for the other groups. Foliar Cd concentrations in week 33 were thus elevated for the DSL, but Zn concentrations were not. High foliar Cd (Punshon and Dickinson, 1997) and Zn (Álvarez et al., 2003) accumulations in *S. cinerea* were also observed for oxic contaminated soils.

3.4. Uptake patterns

Uptake patterns over the growing season (Fig. 4) demonstrate increasing trends for Cd, Zn, Fe and Mn, while S concentrations decrease in the first weeks of the growing season and increase afterwards. Cu concentrations decrease in the first weeks of the growing season and increase marginally towards harvest. S concentration increases strongly from week 30 on and is particularly high for plot 5 and to a lesser extent for plot 4 at the end of the growing season. Differences in foliar concentrations between plots were highly significant for Cd ($p<0.01$), Zn ($p<0.001$), Cu ($p<0.001$), Mn ($p<0.001$) and S

Table 3
ANOVA descriptives for foliar Cd and Zn concentrations (mg kg^{-1} DW) on plot 3, where four trees were sampled between week 24 and week 42 in 3 consecutive years

	Factor	Mean square	Pr (F)
Cd	Error: tree		
	Residuals	1197	
	Error: within		
	Week	2677	0.000
	Year	147	0.021
	Week:year	29	0.065
	Residuals	36	
Zn	Error:tree		
	Residuals	1130226	
	Error: within		
	Week	16359463	0.000
	Year	396656	0.001
	Week:year	55698	0.323
	Residuals	48545	

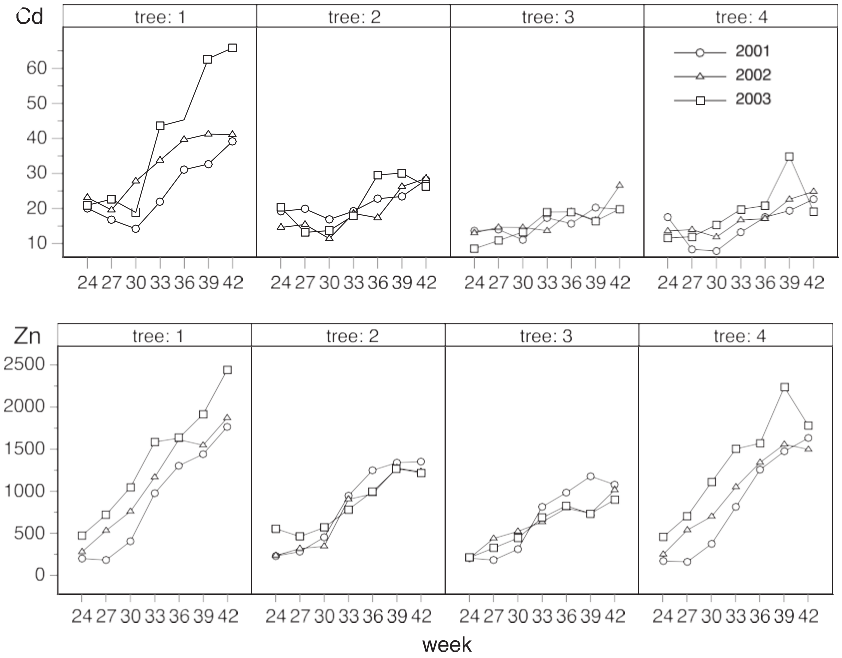

Fig. 2. Foliar uptake patterns for Cd and Zn (mg kg^{-1} DW) during the growing season (week 24 to week 42) for four *S. cinerea* trees (tree 1, 2, 3 and 4) on a dredged sediment-derived soil (plot 3) in 3 consecutive years.

(*p*b0.01) but not for Fe (*p*=0.079). Cd concentrations for plot 3 and plot 0 were significantly higher, respectively, lower than for the other plots of the DSL. Zn concentrations for plot 1 and plot 3, Cu concentrations for plot 1 and S concentrations for plot 5 were significantly higher than for the other plots.

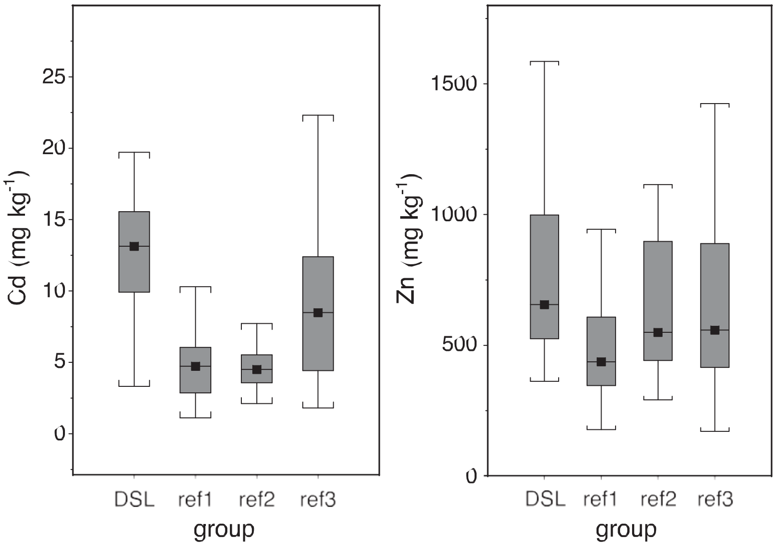

Fig. 3. Boxplots comparing the foliar concentrations for Cd and Zn (mg kg^{-1} DW) in week 33 on the six plots on a dredged sediment landfill (DSL) with three reference data sets. REF1: plots with baseline contamination levels, REF2: plots on a dredged sediment landfill with low metal concentrations and REF3: plots on slightly contaminated soils. Black squares indicate the median value.

For plots 1–3, Cd concentrations slightly decrease between week 21 and week 30. The initially high foliar concentrations might indicate metal transfer from stems to foliage in the early growing season for the plots with the highest Cd availability, and the subsequent decrease might be a result of dilution by growth. Foliar Cd concentrations seem to increase during oxidation of polluted soils. Cd concentration strongly increased for plot 3 from week 33 on, 13 weeks after the strong increase in Eh (Fig. 1). Oxidation of the sediment topsoil resulting in a higher S availability due to the oxidation of labile sulphides to sulphates might be the reason for the high S foliar concentrations for plot 4 and plot 5 in the second half of the growing season. Furthermore, foliar Fe and Mn concentrations increased during lowering of the water level and oxidation of the soils. The soils on plots 3, 4, 5 and 6 emerged between week 19 and week 24 (Fig. 1), whereas the foliar Fe and Mn concentrations started to increase after week 24 (Fig. 4). As the same is observed for S, the increasing Fe and Mn availability could be related to the oxidation of precipitated Fe–Mn–sulphides to sulphates and their resulting mobilisation and increase of bioavailability. A similar increase is observed for Zn. This can also be explained by the oxidation of labile ZnS (Van den Berg et al., 1998). Zn concentrations are especially high for plot 1 and plot 3 in the second part of the growing season. Foliar Cu concentrations are highest for the sandy-textured plot 1. This is the plot with the lowest soil Cu concentration but also with the lowest OM content (Table 1). OM is known to largely reduce Cu availability (Tyler et al., 1989). BCF for Cd and Zn was highest for plot 1, some lower for plots 0 and 3 and lowest for plot 2, 3, 4 and 6 (Fig. 5). Willows on uncontaminated and slightly contaminated soils typically have high BCF for Cd and Zn (Granel et al., 2002; Meers et al., 2003), while lower BCF for Cd and Zn on polluted DSL were found for other

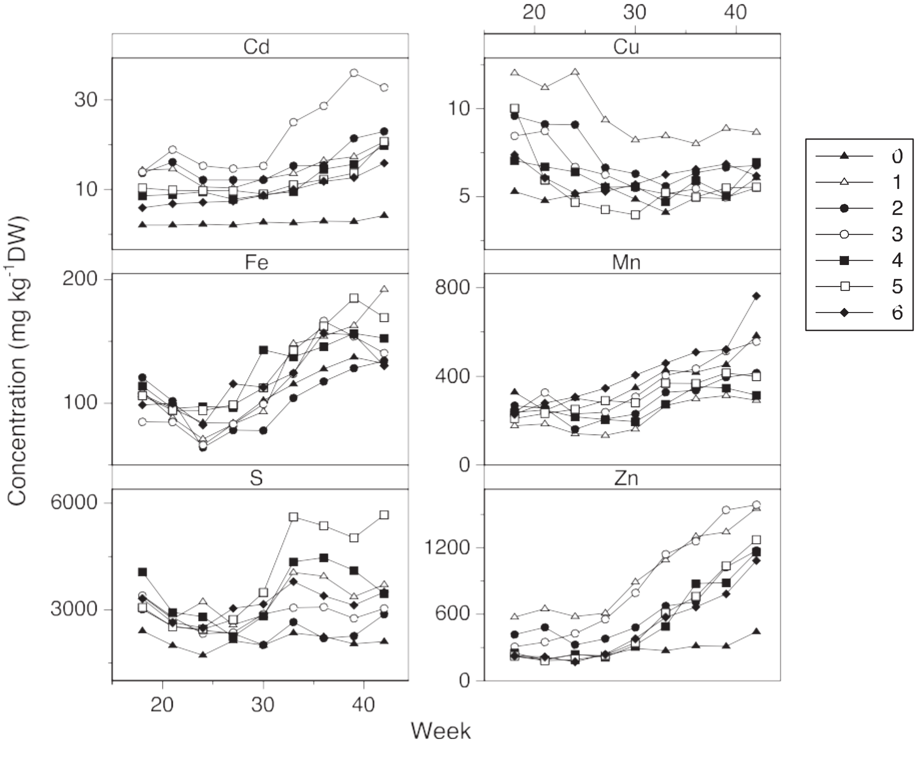

Fig. 4. Average foliar uptake patterns for Cd, Zn, Cu, Mn, Fe and S (mg kg^{-1} DW) during the growing season (week 18 to week 42) for *S. cinerea* (four trees per plot) on a dredged sediment landfill (plot 1–plot 6) and an uncontaminated infrastructure spoil landfill (plot 0).

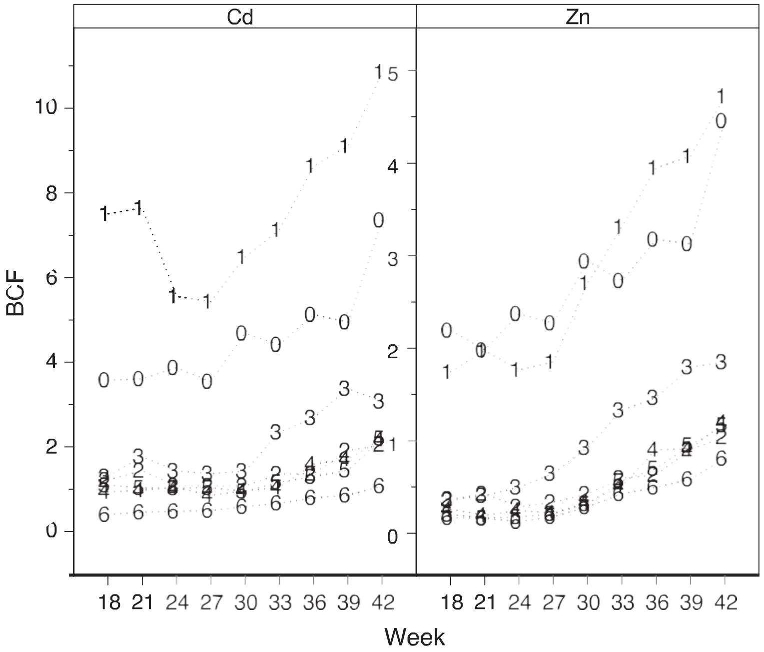

Fig. 5. Evolution of the bioconcentration factor (BCF) for Cd and Zn in the leaves of *S. cinerea* during a growing season for six plots on a dredged sediment landfill and one plot on an infrastructure spoil landfill with baseline contamination levels.

willow species as well (Vandecasteele et al., 2004). The higher BCF for plots 0, 1 and 3 could also be a result of the oxic soil conditions or earlier soil emergence and thus higher Eh, which could result in the higher Cd and Zn availability.

4. Discussion

There is a distinct difference in the first weeks of the growing season between emerged clayey plots (plots 2 and 3) on the one hand and the submerged

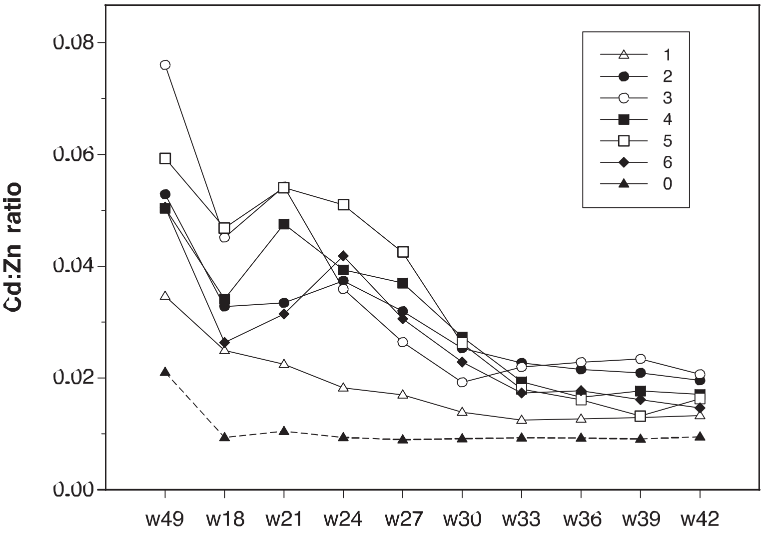

Fig. 6. Cd/Zn ratio in stem cuttings (taken in week 49) and leaves (sampled between week 18 and week 42) for six plots on a dredged sediment landfill and one plot on an infrastructure spoil landfill with baseline contamination levels (Table 1).

clayey plots (plots 4, 5 and 6) on the other, with higher foliar Cd and Zn concentrations for the emerged plots (Fig. 4). The assessment of the submersion effect is not straightforward as no plots on clayey soils were completely aerated or submerged during the whole year. The studied landfill site might be the subject of gradually increasing oxidation of the upper soil layer. Plot 3 was still submerged in week 24 of 2001, but, in 2002, the plot was already emerged in week 24, and, in 2003, the plot emerged in week 18. This long-term process may as well affect foliar concentrations.

Cd/Zn ratios in leaves (Fig. 6) clearly decreased during the growing season. All plots, except plot 1 and plot 0, have a pronounced increase of the Cd/Zn ratio in the first weeks of the growing season and a decrease afterwards. This graph illustrates that Cd is more available for leaves than Zn in the early growing season. Fig. 6 also demonstrates that the Cd/Zn ratio in the stems (sampled in week 49) matches the foliar

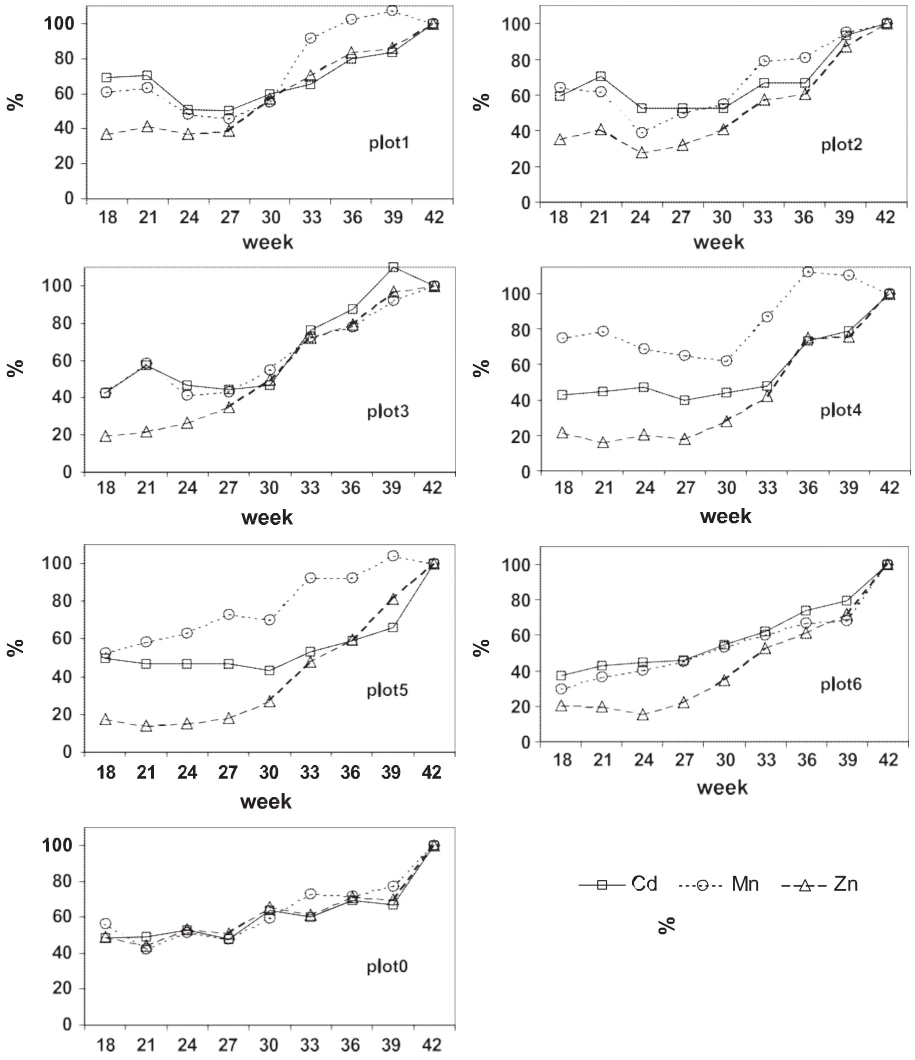

Fig. 7. Relative uptake of Cd (5), Zn (D) and Mn (o) during the growing season for *S. cinerea* on six plots (1–6, see Table 1) on a dredged sediment landfill and one plot on an infrastructure spoil landfill with baseline contamination levels. Values are averages for four individually sampled trees and are expressed relative to the concentration measured in week 42.

ratio better in the early growing season than in the late growing season.

Cd, Zn and Mn concentrations expressed relatively towards the concentration in week 42 (Fig. 7) indicate large differences in accumulation dynamics for Cd and Zn. Relative concentrations increase proportionally for Cd and Zn for plot 0, but the Zn concentration increase is much slower for plot 2, plots 4–6 (proportional to Cd from week 36 or later on) than for plot 1 and plot 3 (proportionality already met in week 30). The initial Cd and Zn concentration as percentage of the concentration in week 42 is relatively low for plots 3–6, while foliar concentrations for plot 1, plot 2 and plot 0 are relatively high in the early growing season (Fig. 7).

As opposed to Zn, foliar Cd concentrations were relatively high in the early growing season even for submerged soils. This might indicate a higher Cd availability in reduced soils but contradicts other observations. In general, Cd and Zn exhibit a similar environmental behaviour in sediment-derived soils (Gambrell et al., 1991), which is reflected in similar fractionation and extractability (Singh et al., 1998). Van den Berg et al. (1998) reported that Zn pore water concentrations increased earlier than Cd in an emerged wetland. We, in contrast, observed higher Cd foliar concentrations early in the growing season.

Foliar metal concentrations in the field might be influenced by metal uptake in the previous growing season due to transfer of elements from the stems to the leaves. Landberg and Greger (1994) found a significantly positive correlation between initial Cd concentrations in field-collected cuttings of diverse *Salix* species before a hydroponical experiment with a 1-AM treatment and Cd concentrations in the shoots after the experiment. Foliar concentrations in willows are thus partly determined by Cd transfer from stems to leaves. The results of the foliar samplings suggest that Cd is strongly transferred from stems to leaves in the early growing season, while stem cuttings collected at the end of the growing season contained high Cd concentrations (Table 4). Cd/Zn ratio in the stems is two to four times as high as Cd/Zn ratio in leaves (Fig. 6). Strong positive correlation was observed between Cd concentrations in the stems (collected in week 49) and in leaves collected in week 18 (R^2=0.849, n=28, pb0.001) or week 42 (R^2=0.702, n=28, pb0.001). Positive correlation was markedly lower for Zn between stems and leaves and in week 18 (R^2=0.302, n=27, p=0.005) or week 42 (R^2=0.187, n=27, p=0.024) and not significant for Mn between stems and leaves and in week 18 (R^2=0.114, n=28, pb0.09) or week 42 (R^2=0.062, n=28, p=0.189).

5. Conclusions

Variability in foliar Cd and Zn concentrations was lower between sampling years than between individual trees and sampling period for a plot sampled in 3 consecutive years. Tree individuality must therefore be acknowledged for when using willow leaves for biomonitoring.

Bioconcentration for Cd, Cu and Zn in the leaves was highest for the sandy-textured oxidised soil with relatively low contamination levels. Foliar Cd and Zn concentrations were highest for the soil initially submerged but with the shortest submersion period and the most rapid emergence and oxidation.

Results indicate that variable hydrological circumstances in the field may result in elevated foliar concentrations for Cd and Zn in willows on polluted sites. Submersion and waterlogging in the first weeks of the growing season resulted in normal foliar Zn concentrations, but emergence then sharply increased foliar concentrations to levels comparable with the plots already emerged at the beginning of the growing season. Hydrological conditions in the previous growing season seem to determine at least partly the

Table 4
Cd, Zn and Mn concentrations in stem cuttings collected in week 49 on the six plots on a dredged sediment landfill and one plot on an infrastructure spoil landfill with baseline contamination levels

		Plot 0	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
Cd	mg kg^{-1} DW	2.9 (1.2)	16.2 (11.2)	15.2 (8.9)	18.9 (7.8)	12.5 (5.2)	13 (4.1)	7.9 (3.5)
Zn	mg kg^{-1} DW	158 (110)	440 (225)	275 (63)	242 (59)	244 (68)	234 (82)	156 (26)
Mn	mg kg^{-1} DW	94.6 (10.6)	55.1 (7.1)	78 (28.5)	130.1 (83.7)	63.4 (17.2)	53.5 (9.7)	67.9 (24.4)

Values in parentheses denote standard deviations for four replicates.

foliar Cd concentrations for *S. cinerea* through metal transfer from stems to leaves. Hydrological regime aiming at wetland creation is a potential management option for reducing bioavailability and thus for establishing a safe management of wetlands polluted with metals as long as submersion can be maintained until the end of the growing season.

Acknowledgements

This study was funded by the Waterways and Marine Affairs Administration (AWZ) of the Ministry of the Flemish Community. We are grateful to Carine Buysse, Els Mencke, Anya De Rop, Athanaska Verhelst and An Capieau for the careful and precise analyses.

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