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Foliar concentrations of volunteer willows growing on polluted sediment-derived sites *versus* sites with baseline contamination levels†

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Many alluvial soils along navigable waterways are affected by disposal of dredged sediments or overbank sedimentation and contain metal concentrations that are elevated compared to baseline levels. Uptake patterns for metals and other elements by several volunteer *Salix* species growing on these sites were determined during a growing season in field plots and compared with the same species growing on soils with baseline contamination levels. For Cd and Zn, foliar concentrations were clearly higher on dredged sediment landfills. Uptake patterns differed significantly between species. A high uptake of Mn and low uptake of Cu, K and S in *S. cinerea* was attributed to wetland soil chemistry. Site effects on metal uptake were evaluated in more detail for *Salix cinerea* and *S. alba* growing on different sediment-derived sites under field conditions. Foliar Cd concentrations were higher in *S. cinerea* than in *S. alba*. This appeared to be a genetic feature not influenced by soil chemical properties, as it was observed both on clean sites and polluted sediment-derived sites. For *S. cinerea*, soil chemistry was reflected in foliar concentrations, while foliar Cd concentrations and bioavailability were found to be independent of the thickness of the polluted horizon. Dredged sediment landfills and freshwater tidal marshes with comparable Cd soil pollution had significantly different foliar Cd concentrations.

1. Introduction

Salix species naturally invade dredged sediment landfills and are the climax vegetation on freshwater tidal marshes[1] and other sediment-derived substrates contaminated with metals.[2] Uptake of metals in this vegetation is of environmental concern. Based on DTPA extraction, Zn, Cd and Cu in dredged sediment disposal sites were estimated to be highly plant-available.[3] Elevated metal concentrations in the pore water also point towards the possibility of enhanced metal availability for plant uptake on such sites.[4] Overbank deposition of polluted sediments on alluvial soils can result in higher plant metal concentrations.[5]

Willow leaves were observed to be good bioindicators for Cd, Mn and Zn.[2,6] Two evaluation criteria can be used for foliar quality: toxicity towards the plant on the one hand, and bioavailability of metals in the leaves towards higher trophic levels on the other. The adverse effects of metals on willows can be diverse. Pollution causes environmental stress, which, for instance, may be reflected in reduced regrowth after animal browsing.[7] High metal concentrations in the soil solution can cause the inhibition of root elongation and development[8] or reduced transpiration and photosynthesis.[9] Elevated foliar concentrations can directly result in higher body concentrations in herbivorous insects,[10,11] birds[12] and mammals,[13,14] and indirectly influence litter-dwelling organisms[15] due to higher metal concentrations in the forest floor.

The aim of this paper was to determine site effects on metal bioavailability for volunteer willows. In the first part of the study, metal uptake patterns over the growing season were compared between two soil substrates, soils on dredged sediment landfills and soils containing baseline metal concentrations, and between four willow species (Table 1). All sampled trees were volunteer species. The primary goal was to examine for a range of elements whether site or species effect was dominant in determining leaf metal concentrations. The second part further focussed on the uptake patterns of *Salix cinerea* L., a typical wetland willow species, and *S. alba* L., a riparian willow species. Foliar data collected once during the growing season for several sediment-derived sites and control sites with baseline contamination levels were compared to assess the site influence on bioavailability of metals (Table 1). We tested the hypothesis that contamination level as well as soil profile genesis and soil physical properties (grain-size distribution and hydrological regime), generally referred to as site effects, influenced foliar concentrations.

2. Materials and methods

2.1. Study area and sampled plots

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 (mostly a landfill) where several plots were sampled.

† Electronic supplementary information (ESI) available: results for fluctuating asymmetry in the leaves of *S. cinerea* (ESI1, Table 1S) and forest floor quality (ESI2, Table 2S). See http://www.rsc.org/suppdata/em/b3/b314917j/

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Table 1 Schematic summary of the sampled sites for the 2 objectives in this study. DSL: dredged sediment landfills, ISL: infrastructure spoil landfills (= no or low contamination), ALLUV: slightly contaminated alluvial soils, OSZ: overbank sedimentation zones (= polluted floodplains), FTM: freshwater tidal marshes, BAS: sites with baseline metal concentrations.

Part 1: uptake pattern of nutrients and metals over the growing season
sampling: 3-week interval from May (week 18) to November (week 45)

	No or low contamination	Slightly contaminated	Contamination high
Salix cinerea			
ISL (□)	ISL1, ISL2		
DSL (■)			DSL1, DSL2
Salix alba			
ISL (△)	ISL2		
DSL (▲)			DSL3, DSL5, DSL6
Salix viminalis			
ISL (○)	ISL2		
DSL (●)			DSL3, DSL6
Salix caprea			
ISL (◇)	ISL2		
DSL (◆)			DSL4

Part 2: Differences in uptake for different sites
sampling: second half of August

	No or low contamination	Slightly contaminated	High contamination
Salix cinerea			
DSL (■)	DSL7		DSL1, DSL2
Other soils	BAS	ALLUV	OSZ
Salix alba			
DSL (▲)	DSL7		DSL
Other soils	ISL		FTM

In the first part of this study, uptake patterns for nutrients and metals over the growing season were assessed (Table 1). Selected sites were dredged sediment landfills (DSL), and infrastructure spoil landfills (ISL). Both the DSL and ISL were established by hydraulic filling, but the latter consists of pure mineral and thus uncontaminated soil material from river profile broadening. Eight plots on DSL and five plots on uncontaminated ISL (Table 2) with volunteer willows were sampled in 2002 with a 3-week interval between week 18 (first week of May) and week 45 (first week of November). The sampled species were: *S. alba*, *S. caprea* L., *S. cinerea* and *S. viminalis* L. For 1 site on an ISL, four species were found relatively close to each other. On the D3 plot, only three *S. alba* trees could be sampled.

In the second part of this study, uptake of metals and nutrients by volunteer *S. cinerea* and *S. alba* were compared for several sites (Table 1) based on foliar samples collected in the second half of August. Selected sites were DSL, overbank sedimentation zones (OSZ), and freshwater tidal marshes (FTM) representing polluted sites, and infrastructure spoil landfills (ISL) and alluvial soils (ALLUV) representing sites with baseline contamination levels. Soils on all sites were thus relatively recent in age. All sampled trees were volunteer with exception of *S. alba* specimens sampled at FTM, for which the origin is not known with certainty. The advantage of studying naturally established plants is that they are assumed to be adapted to the site conditions studied.

The sampled sites for *S. cinerea* (Table 3) can be classified in four groups: (a) dredged sediment landfills (3 sites: DSL1 (4 plots, containing plot D1), DSL2 (5 plots, containing plot D2) and DSL7 (6 plots)), (b) sites with polluted floodplain soils referred to as overbank sedimentation zones (OSZ) (5 plots along the Upper Scheldt), (c) sites with slightly contaminated alluvial soils along several rivers and brooks in the province of East-Flanders (ALLUV, 4 plots) and (d) sites with baseline contamination levels (BAS) including infrastructure spoil landfills (5 plots). Baseline contamination levels are defined here as normal baseline concentrations in Flanders[16] and are not influenced by contamination due to overbank sedimentation. The DSL7 site (26.5 ha) was used between 1990 and 2000 for sediment disposal from maintenance dredging works in the catchment of the river Yzer and in the polder canals. The DSL1 site (12.5 ha) was landfilled between 1976 and 1983 with sediments dredged in the Leie near Deinze. The DSL2 site (13.3 ha) was landfilled between 1992 and 1995 with sediments dredged in the Upper Scheldt. Both the DSL2 and DSL1 site were used as one basin, while the DSL7 site was subdivided in 12 smaller entities which were landfilled separately. The DSL2 and DSL1 sites can be characterised as wetlands with stagnant water during late autumn, winter and spring. The subsites at the DSL7 landfill had clear texture gradients with the clayey part being wetland, and the sandy part being well-drained.

S. cinerea allowed for comparison of different sediment-derived sites, but was not observed on FTM. *S. alba* specimens were therefore sampled on (a) freshwater tidal marshes along the Sea Scheldt between Wetteren and Temse (11 plots), (b) the relatively uncontaminated DSL7 site (3 plots), (c) several polluted recent DSL in the Scheldt catchment (11 plots, referred as DSL), and (d) infrastructure spoil landfills (6 plots).

Sampled sites are indicated in Fig. 1. Site characteristics are summarised in Table 2 and 3. Metal concentrations in most sediment-derived sites were elevated compared to normal baseline concentration levels in Flanders (90% percentile values between 0.6–2; 37–77 and 56–100 mg kg^{-1} for Cd, Cr and Zn,

Table 2 Soil properties of the dredged sediment landfills (DSL) and infrastructure spoil landfills (ISL) used as reference where willows were sampled during the growing season. Soil metal concentrations are *aqua regia* extractable concentrations (mg kg^{-1} dry soil). Values in parentheses denote standard deviations (3 replicates)

Site	Plot	Sampled species	Clay (%)	$CaCO_3$ (%)	SOM (%)	pH_{H_2O}	Mn	Cu	Cr	Zn	Cd
ISL1	I1	*S. cinerea*	22 (6)	2.3 (1.2)	3.5 (1.4)	7.4 (0.5)	1036 (590)	17 (9)	57 (6)	99 (18)	0.6 (0.1)
ISL2	I2	*S. cinerea*	12 (1)	6.5 (0.8)	2.1 (0)	8.3 (0.2)	208 (14)	6 (1)	41 (3)	50 (16)	0.6 (0.2)
	I3	*S. viminalis*	14 (5)	7.6 (2.8)	2.1 (0.5)	8.3 (0.1)	228 (92)	6 (4)	34 (14)	42 (16)	<0.5
	I4	*S. alba*	11 (1)	5.7 (0.4)	2 (0)	8.5 (0.1)	172 (7)	4 (1)	31 (4)	36 (6)	<0.5
	I5	*S. caprea*	9 (0)	4 (0.5)	2.1 (0.4)	8.2 (0.2)	156 (6)	3 (1)	29 (2)	37 (11)	<0.5
DSL1	D1	*S. cinerea*	39 (2)	8.5 (1.6)	8 (0.9)	7.4 (0)	407 (33)	171 (17)	292 (26)	1766 (175)	15 (1.4)
DSL2	D2	*S. cinerea*	33 (2)	9.9 (0.3)	8.2 (0.5)	7.2 (0.1)	744 (47)	104 (4)	374 (11)	858 (33)	10.7 (0.6)
DSL3	D3, D4	*S. alba*, *S. viminalis*	30 (10)	7.7 (0.9)	5.3 (2.1)	7.6 (0.3)	336 (68)	120 (57)	166 (73)	864 (448)	7.3 (4.2)
DSL4	D5	*S. caprea*	31 (1)	2 (1)	5.8 (0.1)	7 (0.1)	524 (7)	44 (0)	69 (4)	2143 (37)	6.8 (0.1)
DSL5	D6	*S. alba*	31 (4)	8 (0.6)	6.7 (0.8)	7.3 (0.2)	317 (28)	107 (26)	132 (29)	830 (236)	4.2 (1.4)
DSL6	D7, D8	*S. alba*, *S. viminalis*	40 (1)	7.2 (1)	11.6 (0.3)	7.1 (0.1)	464 (38)	208 (5)	199 (13)	1602 (77)	8.1 (2.6)

Table 3 Average soil characteristics for the sampled sites for *S. cinerea* and *S. alba*. Values indicated in bold are significantly different (Dunnett test with a 0.95 confidence level, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) from the BAS (*S. cinerea*) and ISL (*S. alba*) sites used as reference. Soil metal concentrations are *aqua-regia* extractable concentrations. Number of replicates (n) is indicated on the first row (ALLUV: alluvial soils, OSZ: contaminated overbank sedimentation zones, DSL: dredged sediment landfills, FTM: freshwater tidal marshes)

	S. cinerea							*S. alba*				
	BAS	ALLUV	OSZ	DSL1	DSL2	DSL7	p	ISL	FTM	DSL7	DSL	p
Soil samples (*n*)	18	14	19	12	12	24		15	48	16	33	
Plots	5	4	5	5	4	6		6	3	11	11	
Clay (%)	19.7	**31.8**	**33.8**	**36.1**	**38.3**	**40.0**	***	19.1	**39.6**	**39.8**	**38.1**	***
Cd/mg kg^{-1} dry soil	0.6	**2.3**	**21.0**	**12.8**	**11.9**	0.8	***	1.21	**8.68**	0.72	**7.67**	***
Zn/mg kg^{-1} dry soil	89	337	**1215**	**1425**	**1137**	315	***	130	**900**	290	**1427**	***
Cu/mg kg^{-1} dry soil	14.7	35.8	**73.2**	**132.4**	**168.8**	**45.5**	***	17	**137**	41	**182**	***
Mn/mg kg^{-1} dry soil	540	473	435	417	**724**	NA	*	301	NA	NA	372	
Ca/mg kg^{-1} dry soil	9177	13773	12173	**38907**	**56283**	NA	***	29502	NA	NA	35537	
Mg/mg kg^{-1} dry soil	3065	**4677**	3303	**6201**	**4788**	NA	***	3900	NA	NA	**5240**	*
P/mg kg^{-1} dry soil	670	1115	**1585**	**3093**	**4036**	**1738**	***	658	**4711**	**1685**	**4017**	***
S/mg kg^{-1} dry soil	379	**1199**	**2022**	**1707**	**2902**	**7287**	***	342	**2619**	**7562**	**3439**	***
SOM (%)	3.7	**10.6**	**13.4**	**8.4**	**8.6**	**15.2**	***	2.7	**8.8**	**11.2**	**8.1**	***
$CaCO_3$ (%)	1.9	2.4	**4.1**	**7.9**	**11.6**	**3.7**	***	6.5	**8.5**	**4.9**	**8.5**	***
pH_{H_2O}	6.9	7.1	7.2	7.3	7.4	**6.3**	***	8.1	**7.5**	**6.8**	**7.2**	***
pH_{CaCl_2}	6.1	6.5	6.7	**6.9**	**7.2**	6.0	***	7.5	**7.0**	**6.7**	**6.9**	***
EC/μS cm^{-1}	115	**263**	**473**	**504**	**757**	**1291**	***	162	**318**	**1729**	**1174**	***

respectively[16]). Sediment-derived sites are also expected to be polluted with PAHs, PCBs and pesticide residues, but systematic survey data are currently not available.

2.2. Tree and soil sampling

S. cinerea was identified based on leaf morphology,[17] and the presence and the length of the *striae* on one-year old branches.[18] *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 one year-old branches.[18] *S. alba* was mainly determined based on leaf morphology and pubescence.[17,18] In contrast to other *Salix* species, *S. cinerea* is uncommonly used for willow cultures and selection programs.

The standard sampling strategy focused on individual trees or shrubs. To account for the variability associated with sampling, four different trees or shrubs of the same plant

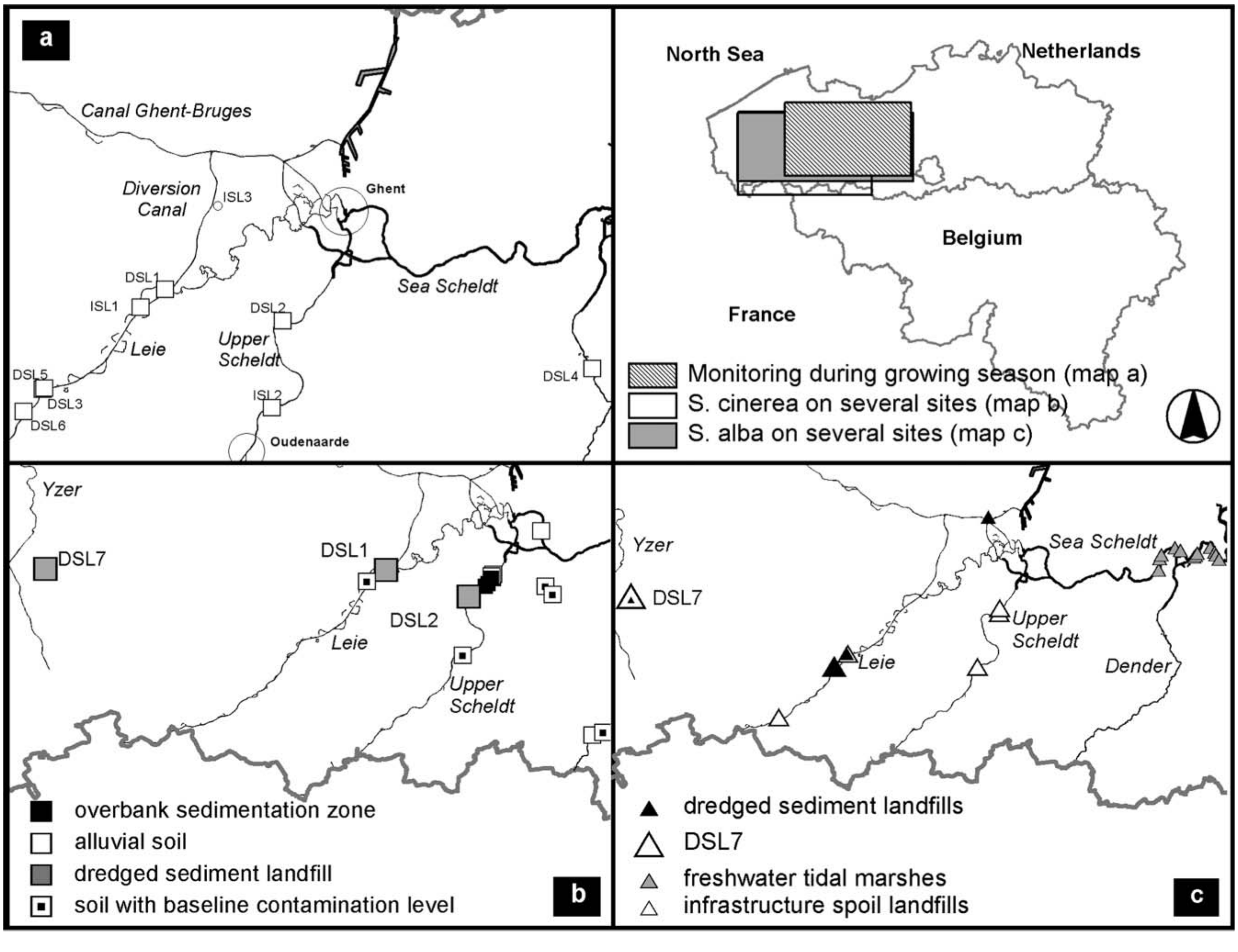

Fig. 1 Study area with the sampled sites for (a) several *Salix* species during the growing season, (b) *Salix cinerea* and (c) *S. alba*.

species and approximately the same age and dimensions were sampled within a circle with a diameter of 15 m. At least four branches from different heights and positions in the crown were sampled. The use of replicates results in a location specific concentration but with an indication of the variability between individual trees within the population. Samples for the evaluation of the temporal influence on foliar concentrations were taken from trees in a fixed sequence with a three week interval between May and November. Only half of the trees had sufficient leaves to be sampled in week 45. Samples for the second part of the study were taken in the second half of August (week 33). Samples were collected by means of a large catapult[19] for higher trees (> 10 m) or an extension crosscut saw for trees smaller than 10 m.[20] Approximately 1000 cm^3 of leaf samples were collected per tree at each sampling location. Leaves were not washed as washing procedures seem to produce misleading results due to incomplete removal of metals on the leaf surface and partial leaching of metals from the leaf tissues.[21] Leaves then were dried for 7 days at 40 °C, mechanically ground (Pulverisette 14, Fritsch, Idar-Oberstein, Germany) and stored in dark glass vials before analysis.

On each plot where *Salix* was sampled, the A horizon was sampled in quadruplicate for physico-chemical characterisation. The minimum observed thickness for the A horizon was 15 cm. For the DSL and FTM, the lower depth of the A horizon was hard to define and therefore samples were taken to a depth of 30 cm. Soil profiles on DSL were previously sampled to a depth of at least 1 m for exploratory goals.

2.3. Chemical analysis

DA : DW was determined after ashing oven-dried material in a muffle oven. Ashing was performed at 550 °C with gradual heating and cooling down over 72 hours. Total foliar and litter N was measured by the Kjeldahl method. Total foliar and forest floor element concentrations were extracted with HNO_3 (p.a. 65%) and H_2O_2 (ultrapur) in a 3 : 1 ratio using microwave digestion and measured with ICP-AES (Varian Liberty Series II, Varian, Palo Alto, CA). Digestion was performed using microwave (Milestone 1200 MS Mega) with the following program: 250 W (5 min.), 0 W (5 min.), 400 W (5 min.), 500 W (5 min.), 600 W (5 min.), ventilation (10 min.). The accuracy of the foliar and forest floor element analysis was checked using BCR 60 (Aquatic plant) for Cd, Cu, Mn and Zn, and CRM 100 (Beech leaves) for Ca, Mg, Na, K, S and P. Values obtained in mg kg^{-1} DW were for Cd 2.21 (certified value: 2.2) , for Cu 54.1 (certified value: 51.2), for Mn 1747 (certified value: 1759), for Zn 325 (certified value: 313), for Ca 5443 (certified value: 5300), for Mg 878 (certified value: 878), for Na 234 (certified value: 255), for K 9814 (certified value: 9600), for S 2683 (certified value: 2690) and for P 1599 (certified value: 1550).

The methods used for soil analysis are described in Vandecasteele *et al.*[22] Soil total concentrations of Cd, Cr, Cu, Ni, Pb, S, P and Zn are actually pseudo-total *aqua regia* extractable concentrations measured with ICP-AES after microwave digestion.

2.4. Statistics

ANOVA was used for comparison of both soil and foliar characteristics for the *S. cinerea* and *S. alba* data set after variables were tested for normality and homoscedasticity. Soil data for Cd, S and electrical conductivity (EC) were log10-transformed for both *S. cinerea* and *S. alba*, and Mn was log10-transformed for *S. cinerea*. Foliar Cd and Mn, and BCF for Cd and Zn were log10-transformed for both *S. cinerea* and *S. alba*. Multiple comparison between the sampled soil types and the soil and foliar reference was performed with the Dunnett test (0.95 confidence level). For *S. cinerea*, foliar data for BAS, ALLUV and OSZ were subsequently applied as reference for DSL2, DSL1 and DSL7. Soil data for *S. cinerea* and soil and foliar data for *S. alba* were compared with BAS and ISL data, respectively. Bioconcentration factors (BCF) for Cd and Zn were defined as the ratio foliar concentration/total soil concentration (*aqua regia*).

3. Results

3.1. Foliar concentrations over the growing season

3.1.1. Trends in uptake patterns. Fig. 2 reveals a generally decreasing trend in foliar concentrations during the growing season for N, P and Cu, and an increasing trend for Ca, Cd, Zn, Mn and dry ash to dry weight ratio (DA : DW). For Na, concentrations strongly declined during the first weeks of the growing season but increased sharply during autumn. Trends were not clearly expressed for S, Mg and K.

Fig. 2 also revealed some trends as a function of the site (DSL or ISL) and willow species. For Zn and Cd, foliar concentrations were mainly determined by soil type, with high foliar concentrations for the dredged sediment landfills (DSL, black symbols). For K, Mn and DA : DW, clear distinctions occurred between *S. cinerea* (square symbols on Fig. 2) and the other willow species, irrespective of the soil type. Differences were less pronounced for Cu and S. Mn concentrations were higher for *S. cinerea*, while K, Cu and S concentrations and DA : DW were lower.

3.1.2. Site effects for Cd and Zn. Site effect was important for Zn and Cd only. Changes in bioconcentration factor (BCF) of these elements during the growing season are shown in Fig. 3. In contrast with the absolute foliar concentrations, BCF values were clearly lower for the willows on DSL, indicating that foliar concentrations did not increase linearly with concentration in the soil. The relative uptake pattern for Zn on dredged sediment landfills was proportional to the uptake of Cd and Mn for all species except *S. cinerea*, where Zn uptake was markedly slower early in the growing season (Fig. 4).

3.2. Comparison of foliar data for *S. cinerea* and *S. alba* grown on different soil types: site effects

Soil-plant relations for Cd and Zn for *S. cinerea* and *S. alba* (Fig. 5) were strongly influenced by other factors than soil concentrations, while for Cu (Fig. 5), S (Fig. 5), Mn and K (Table 4), the species effect on foliar concentrations was more dominant than the effect of soil concentrations. Prior to statistical analysis of foliar data for *S. alba* and *S. cinerea*, soil properties were compared between sites with ANOVA (Table 3).

3.2.1. *S. cinerea*. In general, all sites have a more clayey soil texture and higher soil organic matter (SOM) concentrations than the BAS sites (Table 3). DSL2, DSL1 and OSZ are clearly polluted with metals, and are calcareous soils with high P concentrations (Table 3).

For foliar Mg, K, Na and N, no differences were detected between the sites. For Cd, Ca, Cu, P, S, DA : DW, Zn and Mn, differences from the BAS sites were significant ($p < 0.01$). For foliar Cd and BCF(Cd), differences between sites were highly significant ($p < 0.001$). Multiple comparison of the means confirmed that foliar Cd concentrations were significantly higher for DSL2, DSL1 and OSZ than for BAS. In contrast, ALLUV and DSL7 were not significantly different relative to BAS. BCF(Cd) was significantly lower for all sites except for DSL7. BCF for Zn was significantly lower for all sediment-derived sites (DSL and OSZ) and ALLUV than for the BAS sites.

In addition, foliar data for ALLUV and OSZ were used as reference for multiple comparison with data for DSL2, DSL1

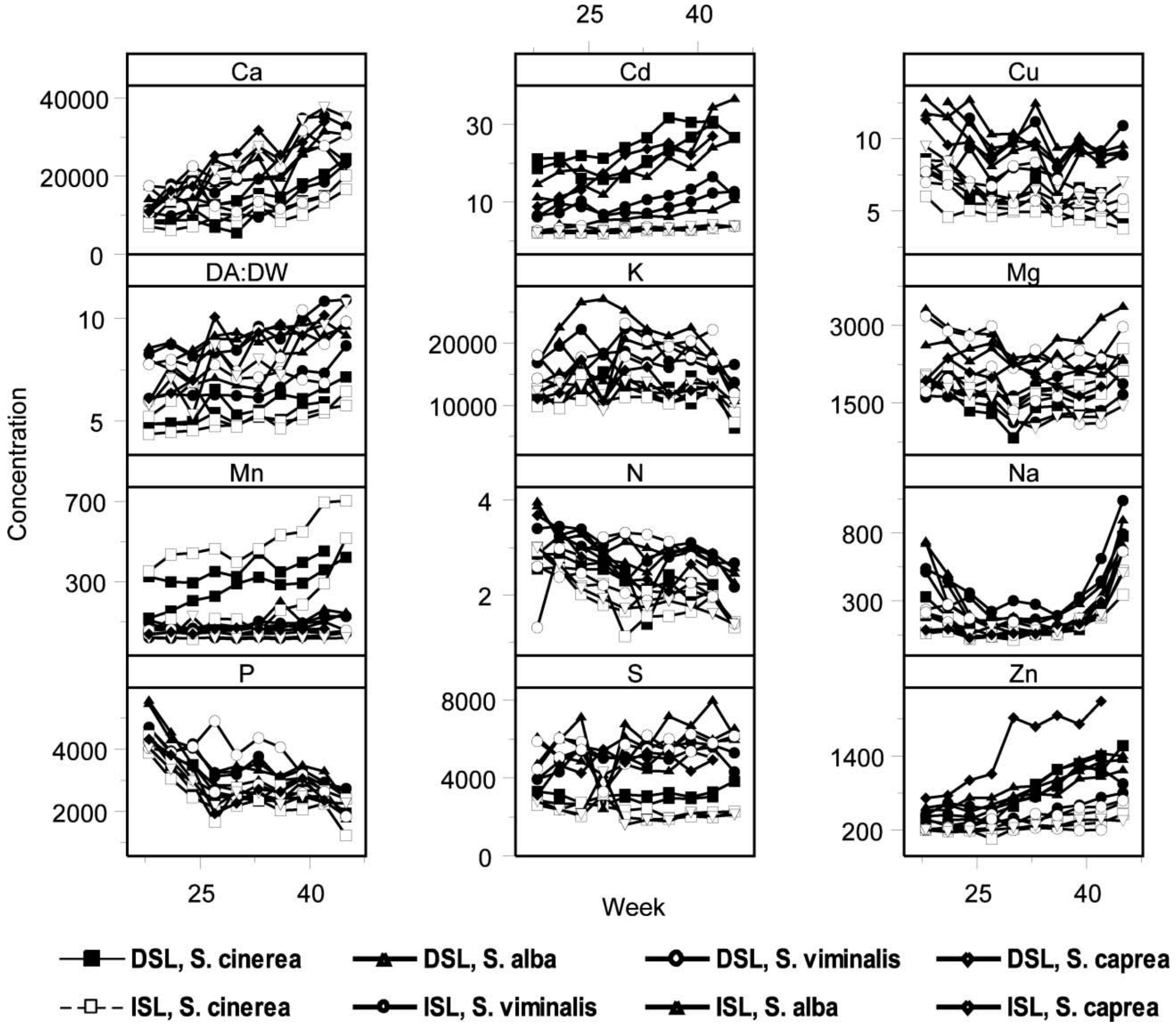

Fig. 2 Foliar uptake patterns for Cd, Zn, Cu, Mn, K, Ca, DA : DW, N, S, P, Mg and N during a growing season for willows on dredged sediment landfills (black symbols) and infrastructure spoil landfills (open symbols) for *S. cinerea* (squares), *S. alba* (triangles), *S. viminalis* (circles) and *S. caprea* (diamonds). N and DA : DW are expressed as %, other elements are expressed as mg kg^{-1} DW.

and DSL7. The choice of the reference largely influenced the evaluation of Cd and Zn foliar concentrations on DSL (Table 4).

3.2.2 *S. alba*. Distinct differences in soil properties between sampled sites were observed (Table 3). SOM, clay content and N_{soil} were comparable on all sites with the exception of the low contents on the infrastructure spoil landfills. The pH_{H_2O} on all sites was higher than 7. $CaCO_3$ content was lower for the DSL7 site, while EC was particularly high for all DSL. Soil P concentration was high for DSL and FTM, S was high at

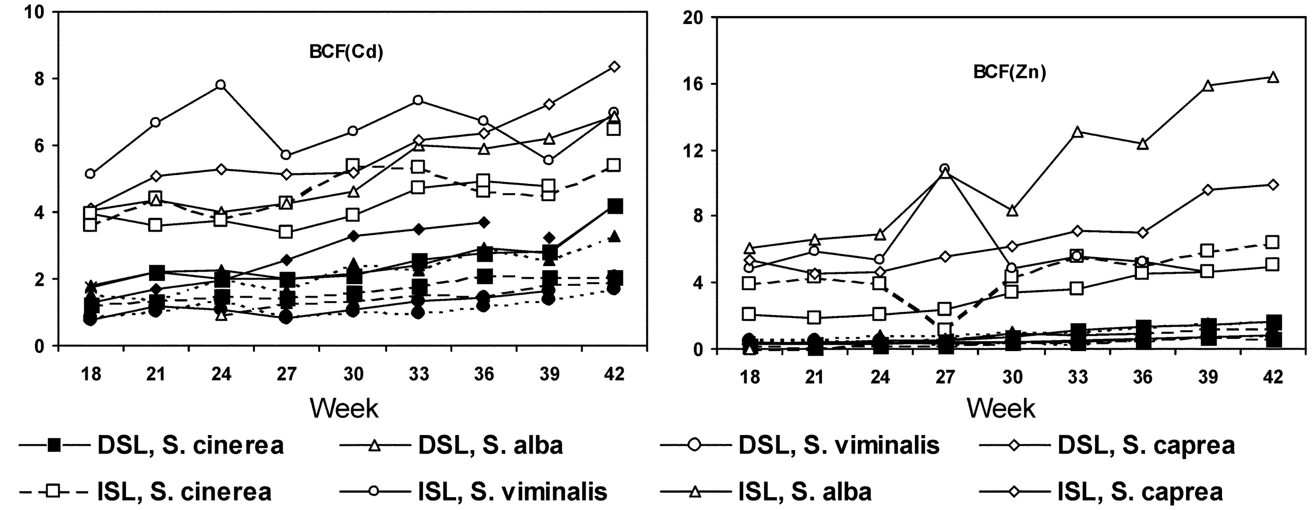

Fig. 3 Evolution of the bioconcentration factor (BCF) during a growing season for willows on dredged sediment landfills (black symbols) and infrastructure spoil landfills (open symbols) for *S. cinerea* (squares), *S. alba* (triangles), *S. viminalis* (circles) and *S. caprea* (diamonds).

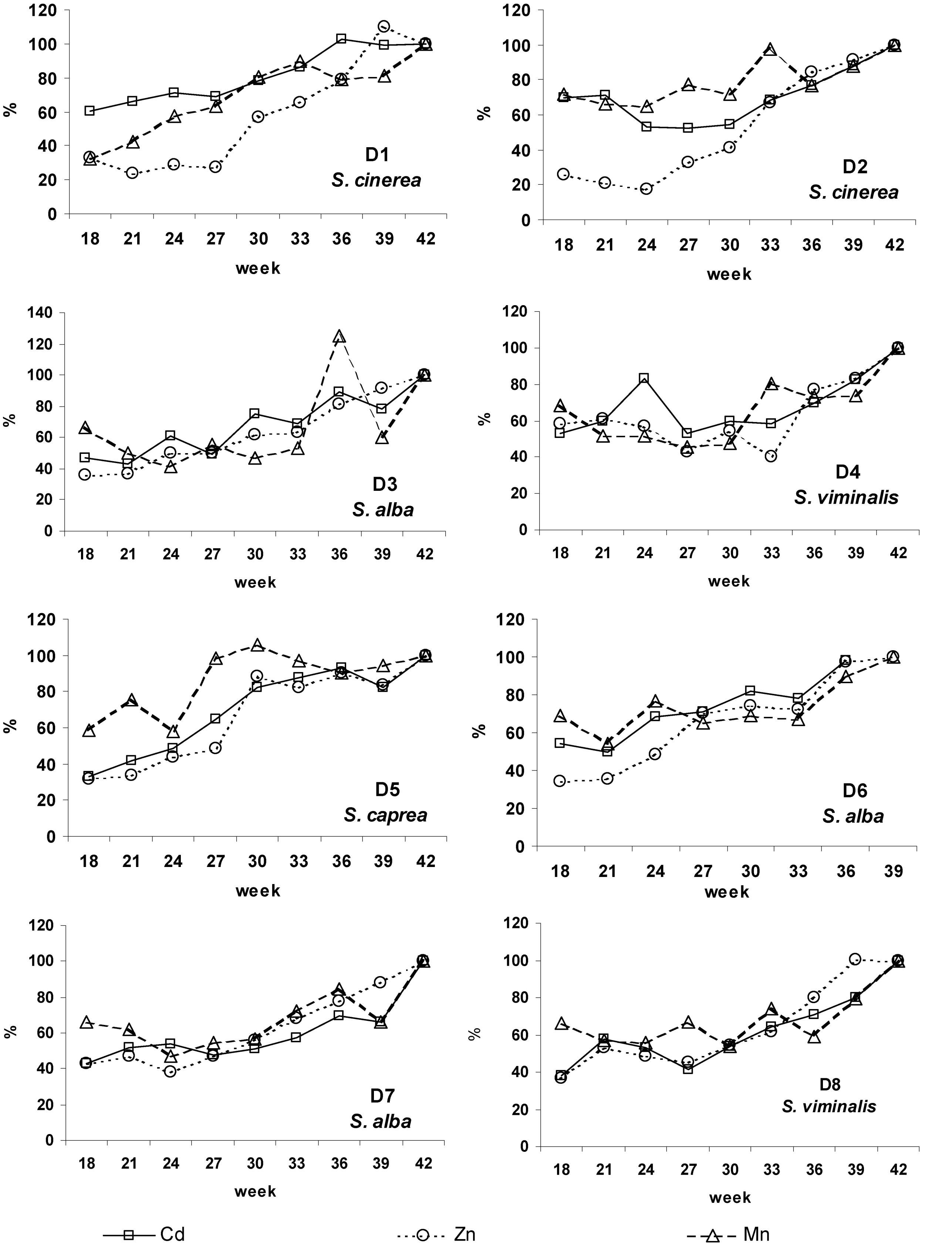

Fig. 4 Relative uptake of Cd (squares), Mn (triangles) and Zn (circles) during the growing season for willows on 8 plots (D1–D8, see Fig. 1 and Table 2) on polluted dredged sediment landfills. Values are averages for 4 individually sampled trees and are expressed relative to the concentration measured in week 42.

DSL7, intermediate at DSL and FTM and lowest for ISL. Metal concentrations were higher for DSL and FTM, and lowest for DSL7 and ISL. Only for Zn, soil concentrations were higher for DSL than for FTM.

In contrast to the soil Cd and Zn concentrations for both FTM and DSL, which were significantly higher than the reference, foliar Cd and Zn concentrations were significantly higher only for DSL (Table 4). Both BCF(Cd) and BCF(Zn)

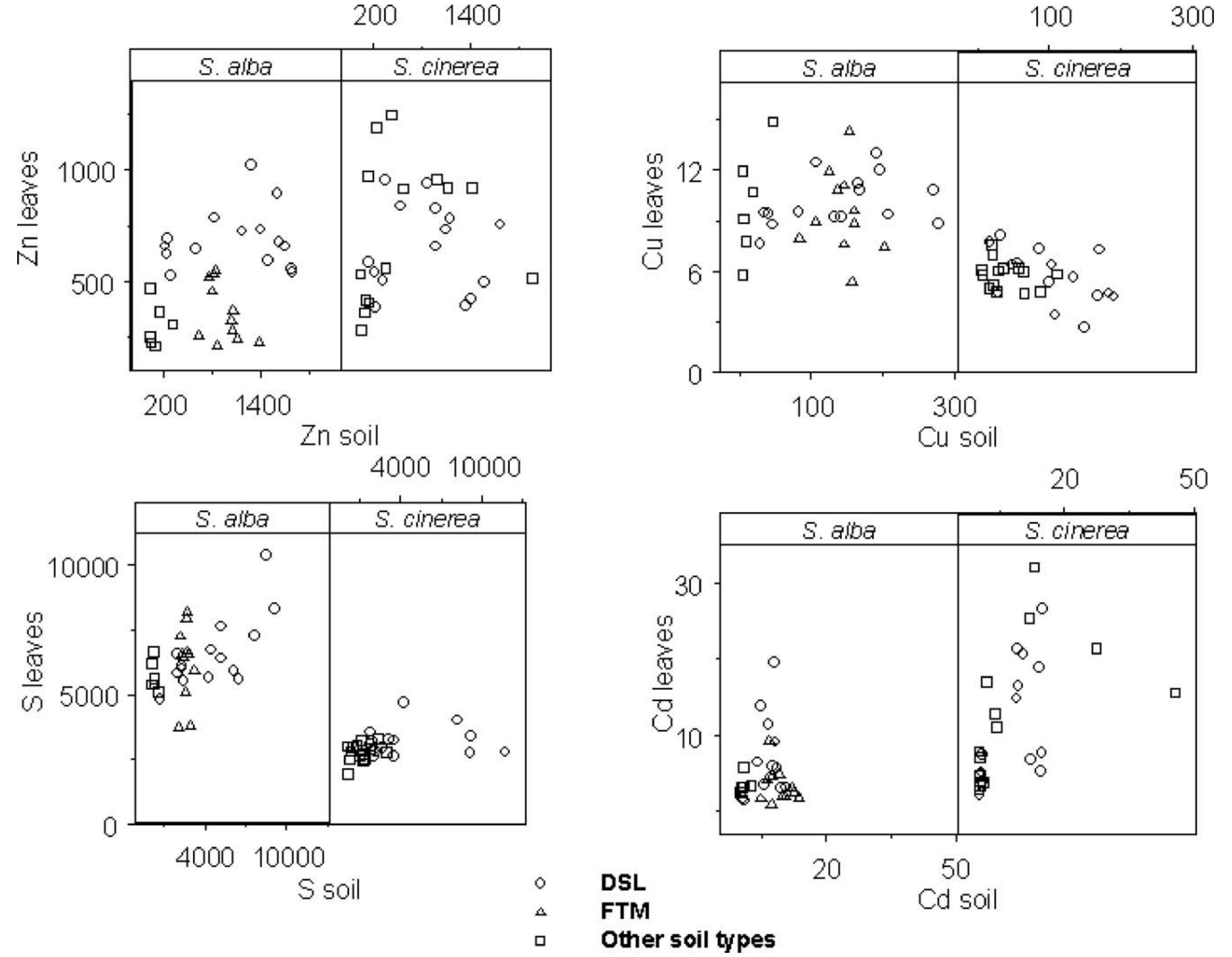

Fig. 5 Foliar Cd, Cu, Zn and S concentrations (mg kg^{-1} DW) in relation to soil concentrations (mg kg^{-1} dry soil) for dredged sediment landfills (circles), freshwater tidal marshes (triangles) and other soils (squares). Values are averaged per plot.

were lower for FTM and DSL in comparison with ISL (Table 4). There was a clear difference in tree age between the sites, demonstrated by the average tree diameters at breast height (Dbh). These were 20, 25, 44 and 104 cm for DSL7, DSL, ISL and FTM, respectively.

4. Discussion

4.1. Evolution of foliar concentration over a growing season

4.1.1. Trends in uptake patterns. The observed trends over the growing season for Cd, Mn, Na, Mg, N, P, Ca, and S

Table 4 Average foliar characteristics for the sampled *S. cinerea* and *S. alba*. Values indicated in **bold** are significantly different (Dunnett test with a 0.95 confidence level, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) from the foliar reference (BAS resp. ISL). Additionally, *S. cinerea* data for DSL2, DSL1 and DSL7 were compared with ALLUV and OSZ, and significantly different results are in *italics* resp. (bracketed) (*p*-values not shown). Number of replicates (*n*) is indicated on the first row (ALLUV: alluvial soils, OSZ: contaminated overbank sedimentation zones, DSL: dredged sediment landfills, FTM: freshwater tidal marshes)

	S. cinerea							*S. alba*				
	BAS	ALLUV	OSZ	DSL1	DSL2	DSL7	*p*	ISL	FTM	DSL7	DSL	*p*
Foliar samples (*n*)	20	16	20	16	20	22		24	44	10	43	
Plots	5	4	5	4	5	6		6	11	3	11	
Cd/mg kg^{-1} DW	5.1	9.0	***21.3***	***15.5***	***15.2***	(*4.7*)	***	3.3	3.3	2.2	**7.8**	***
Zn/mg kg^{-1} DW	508	765	**909**	(578)	700	(638)	**	300	361	**626**	**732**	***
Cu/mg kg^{-1} DW	6.2	5.5	5.4	**4.7**	5.1	(*6.7*)	***	10	9.4	9.3	10.6	
Mn/mg kg^{-1} DW	536	429	262	267	(389)	(815)	**	33	64	**82**	**122**	***
Ca/mg kg^{-1} DW	16123	**12635**	**12459**	13116	**11700**	**12483**	**	25438	**20589**	22795	**19143**	***
P/mg kg^{-1} DW	3774	4222	3968	*3135*	(*3081*)	*3220*	**	2852	2973	**3570**	3064	*
S/mg kg^{-1} DW	2596	2676	2896	**3114**	2966	***3235***	**	5692	6188	**7289**	6073	*
N (%)	2.16	2.39	2.40	2.25	(2.10)	2.33		2.39	**2.67**	2.3	**3.07**	**
DA : DW (%)	6.1	**5.3**	5.6	**5.1**	(**4.9**)	**5.2**	***	9.4	8.9	8.6	**8.3**	**
Na/mg kg^{-1} DW	79	77	93	66	81	106		107	179	195	80	**
K/mg kg^{-1} DW	11659	11005	12881	12005	(10370)	12396		21514	**17456**	19543	19490	**
Mg/mg kg^{-1} DW	2146	1989	**1675**	**1704**	1851	1777	*	2370	2624	**3016**	2138	***
BCFCd	9.18	**4.53**	***1.73***	***1.29***	***1.40***	(5.41)	***	3.92	**0.41**	3.35	**1.09**	***
BCFZn	6.48	**2.66**	***1.11***	(***0.44***)	***0.68***	(**2.28**)	***	4.85	**0.42**	2.46	**0.56**	***

(Fig. 2) are comparable with data from Ernst[23] for several deciduous tree species, from Piczak *et al.*[6] for trees in urban areas and data from Vervaeke and Lust[24] for young *S. triandra* and *S. fragilis* on a dredged sediment landfill. Increasing Zn concentrations over the growing season were also reported by Vervaeke and Lust[24] and by Piczak *et al.*[6]

Foliar S, K, Mn and Cu concentrations in *S. cinerea* were clearly different to other sampled *Salix* species (Fig. 2, Table 4). *S. cinerea* is a characteristic species in willow brushwood on submerged sites, while the other sampled species are typically found in riparian willow forest that is periodically flooded.[1] Even in summer *S. cinerea* can survive in stagnant water,[17] and is thus characterised as a wetland plant species. The observed differences in uptake patterns may be related to a different biogeochemical behaviour of the elements between the soils. In reduced soils, Cu and S may present as insoluble sulfides, while Mn may be more available when oxidation–reduction potential decreases.[25] Zn seems to be less available than Cd and Mn in the first half of the growing season for *S. cinerea* (Fig. 4). The differences in foliar concentrations between *S. cinerea* and *S. alba* for Cu, Mn, S and K are also clearly illustrated by the data in Table 4 and Fig. 5. In contrast to observations on cereals on a polluted dredged sediment landfill,[26] no Mn deficiency was observed or expected based on the foliar concentrations.

4.1.2. Site effects for Cd and Zn. Both on DSL and sites with baseline contamination levels, foliar Cd concentrations are higher for the wetland plant species *S. cinerea* (Table 4), while one would expect a lower availability based on the lower oxidation–reduction potential in wetlands.[25] Most sampled plots for *S. cinerea* on sediment-derived sites were characterised by stagnant water until mid May. Soil drying and oxidation in the beginning of June may have caused an increased bioavailability of Cd on these particular plots. Subsequent soil drying and oxidation is expected to result in a higher bioavailability of metals in the soil substrate.[4] Van den berg *et al.*[27] concluded that fluctuating hydrological conditions in a polluted wetland soil resulted in higher metal concentrations in pore water during summer as a result of both organic matter decomposition and sulfide dissolution.

4.2. Site effects on foliar uptake for *S. cinerea* and *S. alba*

For both *S. cinerea* and *S. alba*, foliar Cu concentrations (Table 4) were hardly influenced by Cu concentrations in the soil (Fig. 5, Table 3). However, a large difference in foliar concentration between both species was observed as a consequence of site characteristics. Nevertheless, values were substantially lower than foliar concentrations between 183 and 681 mg Cu kg^{-1} DW measured for *Salix* species near a Cu refinery site.[28]

Otte and Wijte[29] concluded that besides soil composition and trace element speciation, other habitat or site characteristics determine plant Cu and Zn concentration for *Urtica dioica*. In our study, we also observed some site effects for Cd and Zn bioavailability. Foliar Cd concentrations were significantly higher for the polluted DSL and the OSZ relative to the reference situations (Table 4). However, BCF for Cd was highest for the reference situation (Fig. 3). Foliar baseline values for Cd and Zn in willow leaves are characterised by high concentration factors.[2,30,31] No difference in BCF for Cd was observed between the polluted DSL and the OSZ (Table 4). The soil pollution status of the upper horizon was comparable for OSZ, DSL1 and DSL2 (Table 3). However, both substrate types have a different morphology, history and genesis. Due to sedimentation, the pathway of pollution for OSZ results in pollution concentrated in the upper cm of the soil profile. The developed soil profile is gradually polluted from the top layer. In the DSL studied here, the new soil profile is established at once over a larger thickness (> 100 cm in the studied sites), with the soil-forming processes starting from a reduced sediment layer. DSL are thus considered to be new soils at the time of disposal. The undetectable influence of the thickness of the polluted sediment layer on foliar concentrations, observed through comparison of OSZ and DSL, was remarkable. For *S. cinerea*, foliar Cd concentrations and bioavailability were found to be independent from the thickness of the polluted soil horizon, which is an important conclusion for ecological risk assessment. Foliar Zn concentrations were only significantly higher for OSZ compared to BAS. BCF(Zn) for OSZ was higher than for DSL2 and DSL1, while soil Zn concentrations for the three sites were comparable (Table 4).

A second effect, previously defined as species effect, is also a site effect, as volunteer *Salix* species occur when typical soil conditions prevail. These soil conditions (*e.g.* oxidation–reduction potential) are reflected in the foliar composition. A third site effect was found for *S. alba* with a distinct difference in Cd uptake for DSL and FTM (Table 4) while both soil types had a comparable soil pollution status for Cd. The differences observed in the tree diameter and hydrological regime, and the Zn concentrations in the soil and subsequently the difference in soil Cd : Zn ratio, might be the reason for the difference in foliar Cd concentration between DSL and FTM.

Otte[32] argued that wetland plants are strongly adapted to flooded and anaerobic substrate situations, and that they are only stressed when conditions outside the usual range prevail *e.g.* conditions of environmental pollution. We found no evidence of acute toxicity for willows on the sampled landfills. Neither did foliar fluctuating asymmetry (ESI1, Table 1S†) indicate any potential environmental stress for *S. cinerea*. Deviations from symmetry might be masked due to small sample size,[33] genetic or tree age differences, or different competitive regimes between trees.[34,35]

Future research should focus on the relation between hydrology (and oxidation–reduction potential) of polluted sediment-derived soils and metal uptake by willows, as one might expect a lower bioavailability in reduced soils.

4.3. Ecological risk assessment

Selecting adequate indicators for ecological risk assessment of metal contamination is not easy since potential responses or indicators are acute toxicity, growth reduction, reduced reproduction or elevated concentrations in willows, leaf-feeding insects or litter-dwelling organisms.

Punshon and Dickinson[8] for willow cuttings grown in solution cultures with high Cd, Zn and/or Cu concentrations and Vervaeke and Lust[24] for *S. fragilis* on a dredged sediment landfill found foliar concentrations over 80 mg Cd kg^{-1} DW. These concentrations did not cause any visible signs of phytotoxicity. The highest foliar Cd concentration in this study was 72 mg kg^{-1} DW measured for a *S. alba* on the D7 site. Lunácková *et al.*[36] reported a negative effect of Cd on leaf expansion of several *Salix* and *Populus* spp. In our data, reduced leaf area for *S. cinerea* was not observed as an effect of pollution (ESI1, Table 1S†). Morphological toxicity symptoms (reduced shoot length and leaf yield) for *Zea mays* were observed at foliar concentrations of 123 and 73 mg Cd kg^{-1} DW in the third and fourth leaf.[37] No reference data about toxic foliar concentrations for willow species were found, but most authors refer to general toxic plant concentration ranges given by Kabata-Pendias,[38] who states that critical ranges in plants are between 100 and 400 mg kg^{-1} DW for Zn and between 5 and 30 mg kg^{-1} DW for Cd. Eriksson and Ledin[30] reported baseline Cd concentrations in leaf samples between 0.31 and 1.96 mg kg^{-1} DW for *S. viminalis* on different non-polluted soils in Sweden. Nissen and Lepp[31] found Zn concentrations in several willow species in the UK ranging between 82 and 296 mg Zn kg^{-1} DW, while a baseline range of 0.5–2.9 mg Cd kg^{-1} DW and 128–338 mg Zn kg^{-1} DW was reported by Vandecasteele *et al.*[2] Severson *et al.*[39] reported baseline concentrations for *Salix repens* on the remote Frisian islands in Germany of <0.9–3.8 mg Cd kg^{-1} DW and 130–480 mg Zn kg^{-1} DW. Foliar concentrations are important indicators for Cd, Zn and Cu in site-specific ecological risk assessment, since these data are also indicative for food web transfer of metals. Cd and Zn concentrations remained relatively constant during litter decomposition (ESI2, Table 2S†). Hence, no Cd and Zn accumulation in the forest floor was observed. On average, forest floor Cd and Zn concentrations were five times higher than in willow forests with baseline soil contamination levels (ESI2, Table 2S†).

The large differences in baseline concentrations and uptake patterns between plant species complicate a straightforward definition of general normal and toxic plant concentrations. Data in Table 4 illustrate the subjectivity of choosing reference values for evaluation of foliar concentrations. We propose that foliar data for uncontaminated dredged sediment landfills (DSL7 in this study) can be used for site-specific ecological risk assessment, as these sites are showing the background contamination of a highly urbanised area and have similar site characteristics as the polluted DSL. By choosing these sites as a reference, we avoid the application of unrealistically strict baseline data.

Conclusions

Foliar concentrations for several volunteer willow species were compared for sediment-derived soils and uncontaminated soils and a distinction was made between species effects and site effects on element concentrations. The site effect was important for Zn and Cd only, while other elements were mainly species-specific. Over a range of soil types, distinct differences in foliar Mn, Cu, S and K concentrations were observed between *S. alba* and *S. cinerea*.

Relative uptake patterns throughout the growing season were similar for Cd, Zn and Mn for all species except *S. cinerea*, for which Zn uptake was markedly slower early in the growing season. On polluted dredged sediment landfills, Cd and Zn foliar concentrations increased during the growing season until autumn.

Potential effects of pollution on *S. cinerea* were estimated based on foliar fluctuating asymmetry as a sensitive early indicator. No increase in fluctuating asymmetry was determined for any of the sites studied. Results illustrated that the selection of appropriate reference values for evaluation of foliar concentrations on polluted sites is species-specific. Foliar concentrations on polluted dredged sediment landfills were elevated compared with an uncontaminated dredged sediment landfill. On the other hand, foliar Cd and Zn concentrations measured on "man-made" polluted dredged sediment landfills were as high as values measured for "naturally" polluted overbank sedimentation zones. Soil genesis and thickness of the polluted horizon were not found to affect foliar concentrations for Cd and Zn.

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References

1 D. Bal, H. M. Beije, M. Fellinger, R. Haveman, A. J. F. M. van Opstal and F. J. van Zadelhoff, *Handboek Natuurdoeltypen, Tweede, geheel herziene editie*, Expertisecentrum LNV, Wageningen, 2001.
2 B. Vandecasteele, B. De Vos and F. M. G. Tack, *Sci. Total Environ.*, 2002, **299**, 191.
3 S. P. Singh, F. M. Tack and M. G. Verloo, *Water, Air, Soil Pollut.*, 1998, **102**, 313.
4 F. M. G. Tack, S. P. Singh and M. G. Verloo, *Environ. Pollut.*, 1998, **103**, 109.
5 G. M. Pierzynski and A. P. Schwab, *J. Environ. Qual.*, 1993, **22**, 247.
6 K. Piczak, A. Lesniewicz and W. Zyrnicki, *Environ. Monit. Assess.*, 2003, **86**, 273.
7 E. L. Zvereva and M. V. Kozlov, *J. Ecol.*, 2001, **89**, 21.
8 T. Punshon and N. M. Dickinson, *New Phytol.*, 1997, **137**, 303.
9 S. Trapp, K. C. Zambrano, K. O. Kusk and U. Karlson, *Arch. Environ. Contam. Toxicol.*, 2000, **39**, 154.
10 B. Vandecasteele, R. Lauriks, B. De Vos and F. M. G. Tack, *Environ. Monit. Assess.*, 2003, **89**, 263.
11 G. Merrington, D. Miller, M. J. McLaughin and M. A. Keller, *Arch. Environ. Contam. Toxicol.*, 2001, **41**, 151.
12 H. C. Pedersen and M. Saether, *Ecotoxicology*, 1999, **8**, 1–7.
13 J. Mertens, S. Luyssaert, S. Verbeeren, P. Vervaeke and N. Lust, *Environ. Pollut.*, 2001, **115**, 17.
14 M. Lodenius, *Bull. Environ. Contam. Toxicol.*, 2002, **69**, 320.
15 D. Drobne and S. P. Hopkin, *Ecotoxicol. Environ. Saf.*, 1995, **31**, 1.
16 F. M. G. Tack, M. G. Verloo, L. Vanmechelen and E. Van Ranst, *Sci. Total Environ.*, 1997, **201**, 113.
17 E. J. Weeda, R. Westra, C. Westra and T. Westra, *Nederlandse oecologische flora; wilde planten en hun relaties, Deel I*, KNNV uitgeverij, Hilversum, 1999, 304 pp.
18 R. D. Meikle, *Willows and poplars of Great Britain and Ireland, BSBI Handbook n° 4*, Botanical Society of the British Isles, London, 1984, 198 pp.
19 F. Mathias, *Guide pratique du grimpeur-élagueur*, Editions SFA, Châteauneuf-du-Rhône, 2001, 152 pp.
20 D. F. Blair, *Arborist Equipment, A guide to the tools and equipment of tree maintenance and removal*, International Society of Arboriculture, Savoy, IL, 1995, 291 pp.
21 M. V. Kozlov, E. Haukioja, A. V. Bakhtiarov, D. N. Stroganov and S. N. Zimina, *Environ. Pollut.*, 2000, **107**, 413.
22 B. Vandecasteele, B. De Vos and F. M. G. Tack, *Environ. Pollut.*, 2003, **122**, 7.
23 W. Ernst, in *Element concentration Cadaster in Ecosystems*, ed. H. Lieth and B. Markert, VCH, Weinheim, New York, Basel, Cambridge, 1990, pp. 17–40.
24 P. Vervaeke and N. Lust, *Eindverslag IWT-project: Ecotechnologische behandeling en inrichting van baggerspeciedepots op basis van bebossingstechnieken, IWT nr. 960201*, Laboratorium voor Bosbouw, Universiteit Gent, 2001, 373 p.
25 R. P. Gambrell, *J. Environ. Qual.*, 1994, **23**, 883–891.
26 K. W. Smilde, W. van Driel and B. van Luit, *Sci. Total Environ.*, 1982, **25**, 225.
27 G. A. Van den berg, J. P. G. Loch and H. J. Winkels, *Water, Air, Soil Pollut.*, 1998, **102**, 377.
28 N. M. Dickinson, *Chemosphere*, 2000, **41**, 259.
29 M. L. Otte and A. H. B. M. Wijte, *Environ. Monit. Assess.*, 1993, **28**, 263.
30 J. Eriksson and S. Ledin, *Water, Air, Soil Pollut.*, 1999, **114**, 171.
31 L. R. Nissen and N. W. Lepp, *Biomass Bioenergy*, 1997, **12**, 115.
32 M. L. Otte, *Environ. Exp. Bot.*, 2001, **46**, 195.
33 M. Mogie and M. Cousins, *J. Theor. Biol.*, 2001, **211**, 181.
34 J. E. Rettig, R. C. Fuller, A. L. Corbett and T. Getty, *Oikos*, 1997, **80**, 123.
35 C. G. Hochwender and R. S. Fritz, *Evolution*, 1999, **53**, 408.
36 L. Lunácková, E. Masarovicová, K. Kráová and V. Stresko, *Bull. Environ. Contam. Toxicol.*, 2003, **70**, 576.
37 A. Lagriffoul, B. Mocquot, M. Mench and J. Vangronsveld, *Plant Soil*, 1998, **200**, 241.
38 A. Kabata-Pendias, *Trace elements in soils and plants, Third Edition*, CRC Press, Boca Raton, 2000, pp. 413.
39 R. C. Severson, L. P. Gough and G. Van den Boom, *Water, Air, Soil Pollut.*, 1992, **61**, 169.