Strategies of cadmium and zinc resistance in willow by regulation of net accumulation

T. LANDBERG[1]*, P. JENSÉN[2] and M. GREGER[1]

Department of Botany, Stockholm University, S-10691 Stockholm, Sweden[1]
Department of Horticulture, Division of Root and Substrate Research, Swedish University of Agricultural Sciences, S-23053 Alnarp, Sweden[2]

Abstract

This work was performed to find out if metal resistant clones of *Salix viminalis* L. are capable to achieve high resistance to the metals by regulating their net accumulation. *Salix* clones with low or high resistance in combination with low or high accumulation capacity of either Zn or Cd were cultivated from cuttings in nutrient solution. The investigation included leakage and uptake experiments using ^{65}Zn or ^{109}Cd and analysis of root cation exchange capacity (CEC). Some plants were pre-treated with unlabeled 0.5 μM Cd or 2.5 μM Zn 24 h prior to the experiments to induce possible tolerance mechanisms. To find out if the regulation was a metabolic process, experiments were also performed with 2,4-dinitrophenol (DNP). Clones with high resistance and low Cd accumulation had higher efflux of Cd compared to the other clones, in both untreated and Cd pre-treated plants. This indicates a constitutive property to lower Cd accumulation by high Cd leakage. Pre-treatment with 0.5 μM Cd diminished the Cd net uptake to a level near zero in all clones, likely to be due to decreased the Cd uptake. In contrast, resistant clones with high Cd accumulation had the highest root CEC, which may be used to bind up Cd in the free space. No clear regulation of Zn net uptake was found in Zn-resistant clones. Pre-treatment with Zn decreased the uptake of Zn into the free space in Zn-resistant clones. The resistant high-accumulating clones, however, showed the highest leakage of Zn in both untreated and pre-treated plants, a constitutive process not related to high accumulation. Neither the influx nor the efflux of Cd or Zn was affected by DNP indicating passive transport across the plasma membrane.

Additional key words: cation exchange capacity, cell wall, efflux, heavy metals, influx, tolerance.

Introduction

Resistance to heavy metals varies considerably among different species, cultivars and populations of higher plants (Baker *et al.* 1986, Baker 1989) and numerous resistance strategies have been suggested (reviewed by Ernst *et al.* 1992). Baker (1981) reported that plants found in metal-contaminated areas could be categorised as "accumulators" or "excluders". *Salix* is shown to have a very wide variation of heavy metal resistance and accumulation (Landberg and Greger 1994, Greger and Landberg 1999, Greger *et al* 2001), and different clones can be identified either as "accumulators" or "excluders" and can be found both in contaminated and uncontaminated areas (Landberg and Greger 1996).

It is reasonable to assume that "accumulators" have developed mechanisms to tolerate high contents of heavy metals in their tissues. Such mechanisms may include the formation of different heavy metal complexes or/and compartmentalization (Ernst *et al.* 1992). "Excluders", on the other hand, may use a low net accumulation as regulation mechanism (Strange and MacNair 1991). The net accumulation of the metal is the difference between influx and efflux across the plasma membrane of the root cells. A plant might thus be able to regulate one or both of these processes to achieve low net uptake and thereby strengthen the tolerance.

Cation-exchange capacity (CEC) of the root cell walls may also be an important factor influencing the net uptake of metals. A high CEC implies a high metal adsorption in the cell walls, making the metal ions more available for membrane transport as well as decreasing

Received 16 January 2004 , *accepted* 22 September 2010.
Abbreviation: CEC - cation exchange capacity; DNP - 2,4-dinitrophenol.
* Corresponding author, fax: (+46) 8 165525, e-mail: tommy.landberg@botan.su.se

the leakage back to the external medium. High CEC may also increase the metal content in the cytoplasm and thus affect the tolerance to the metal (Blamey *et al.* 1992). The CEC depends on the quantity and the type of negative charges in the cell wall (Wagatsuma and Akiba 1989, Wang *et al.* 1992). Several authors have demonstrated that CEC has a role in tolerance, and tolerant cultivars in several species have lower CEC than sensitive ones (Wagatsuma and Akiba 1989). On the contrary, Blamey *et al.* (1992) showed no correlations between tolerance and CEC in 12 species.

Uptake and leakage of Cd and Zn in plants and the regulation of these processes is poorly known. The uptake of Cd and Zn has been described either as an active or as a passive process (Cutler and Rains 1974, Cataldo *et al.* 1983, Bowen 1987). Zinc uptake was proportional to the biomass production (Di Baccio *et al.* 2010). Different ion-channels (*e.g.* Ca^{2+}-channels) have been suggested to be important pathways for Cd, and kinetic studies indeed support this view (Hooda and Alloway 1993, Perfus-Barbeoch *et al.* 2002). Exudation of metals *via* certain transport proteins, which enhance heavy metal tolerance, is known in bacteria (Silver and Misra 1988), and Clarkson and Lüttge (1991) and Meharg (1993) have suggested similar processes in plants. This assumption has not, however, been confirmed.

The present experiments were performed to investigate if, and how, heavy-metal resistant clones of *Salix viminalis* regulate their net accumulation of Zn and Cd. The Cd and Zn uptake, leakage and CEC in "accumulators" and "excluders" were compared.

Materials and methods

Plants: Clones of *Salix viminalis* L. were used in this investigation. The clones were chosen among more than 130 clones, which earlier were screened for resistance, uptake, accumulation and translocation to the shoot of Cd, Zn and Cu after 20-d treatment (Landberg and Greger 1994, Greger and Landberg 1999, Greger *et al* 2001). A total of 16 clones were chosen; 8 clones for Cd studies and 8 clones for Zn studies, in this work called "Cd-clones" and "Zn-clones". The clones had low or high metal tolerance in combination with low or high metal accumulation in the roots of either Cd or Zn (Table 1). All clones originated from non-polluted areas in Skåne, southern part of Sweden, except one of the clones (RH1) which was a breeding of *S. viminalis* and *S. schwerinii* from eastern Siberia.

Twenty-six cuttings of each clone, 10 cm in length, were cultivated hydroponically. During the first 14 d the cuttings were cultivated in 100 µM $Ca(NO_3)_2$ to develop roots, and thereafter in a half strength nutrient solution according to Gussarsson and Jensén (1992) adjusted to pH 6 with KOH. The nutrient medium contained 12.5 nM Zn. Water was added whenever needed. The growth medium was continuously aerated during the whole growth period and renewed once a week. The plants were grown in a climate chamber equipped with light tubes (*Sylvania* F96T12/CW/VHO providing a photon flux density of 180 µmol m^{-2} s^{-1} during 16-h photoperiod). The relative humidity was 60 - 70 % and the temperature 20 - 22 °C.

Uptake experiments: The uptake experiment was performed on day 27 - 29 after cutting planting for Cd and day 33 - 35 for Zn. Plants were transferred to pots containing 300 cm^3 and 850 cm^3 of experimental medium in the case of Cd and Zn, respectively. Only the roots were allowed to touch the solution. The experimental medium contained 1/4 strength nutrient solution adjusted to pH 6 and 0.185 MBq dm^{-3} ^{109}Cd (= 40 nM) or 0.555 MBq dm^{-3} ^{65}Zn (= 83 nM). Prior to each experiment, some plants were pre-treated for 24 h in 1/4 strength nutrient solution supplemented with 0.540 µM Cd or 2.583 µM Zn. The solutions were continuously stirred with aeration. Two samples from the solution, 2 cm^3 each, were taken after 0, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min. In order to measure involvement of possible active uptake, the experiment was also performed in the presence of 100 µM 2,4-dinitrophenol (DNP) for the time periods 60, 120 and 240 min on plants not pre-treated with Cd or Zn.

Leakage experiments: In different set of cuttings, 200 nM ^{109}Cd or 278 nM ^{65}Zn, corresponding to 9.25 MBq dm^{-3} of each isotope, were added to the growth medium at day 4 of cultivation, in order to load plants with the isotopes before the experiments. Nutrients were added to the radiolabelled medium once a week corresponding to 50 % nutrient strength. The leakage experiments were performed after 22 - 24 and 26 - 28 d of cultivation for Cd and Zn, respectively. The plants and their growth medium of each container were divided into 3 groups, 8 clones in each. To one of the groups, 0.5 µM Cd or 2.5 µM Zn were added. To the second group 100 µM DNP was added, and to the third group nothing was added. The experiment was performed after 24 h pre-treatment in Cd or Zn, in DNP, or solely in the cultivation medium.

The leakage solution contained unlabelled 1/4 strength nutrient solution, adjusted to pH 6 with KOH and aerated. The radiolabelled Cd or Zn was replaced by unlabelled ions in corresponding concentrations (200 nM Cd or 278 nM Zn). There were 3 different treatments in line with the pre-treatments: *a*) no extra Cd or Zn, *b*) 0.5 µM Cd or 2.5 µM Zn or *c*) 100 µM DNP. Plants were moved from beaker to beaker and the roots were allowed to successively leak in a series of solutions (250 cm^3 per beaker; 5, 5, 5, 10, 15, 20, 30, 30, 60, 60, 90, 90 and

90 min for Cd and 5, 5, 5, 10, 15, 20, 30, 30, 60, 60, 60, 60 and 60 min for Zn). Two solution samples of 2 cm^3 were taken from each of the beakers for analysis.

Analysis of ^{109}Cd and ^{65}Zn: The plants were divided into root, old stem and shoot. The plant material was dried for 24 h at 105 °C, weighed and then wet digested in 20 cm^3 14 M HNO_3 using a microwave oven (*CEM MDS 81 D*). The isotopes were analysed with a liquid scintillator (*Philips PW - 4700*, The Netherlands). The radioactivity of the wet digested samples and solution samples was measured after mixing with 5 cm^3 scintillation cocktail (*Quicksafe A*, *Zinsser Analytic*, Germany).

Determination of cation exchange capacity: To prepare cell walls according to Fry (1988), fresh root material from untreated roots was stirred in 70 % ethanol with an *Ultramix PT2000* (*Polytron*, *Kinematica,* Switzerland) and filtrated to wash out low molecular mass compounds as sugars and aminoacids. Then, the material was stirred in 90 % dimethylsulfoxide (DMSO) and filtrated to wash out starch. Thereafter, the material was stirred in phenol/acetic acid/water (5:2:1) and filtrated to wash out proteins. The material was then washed with 70 % alcohol. Finally, the material was stirred in 0.01 M HCl for 300 s and, thereafter, washed five times with distilled water. The cation exchange capacity was measured according to Crooke (1964). The cell wall material was added to 1 M KCl solution adjusted to pH 7.00. After 30 min, 0.01 M KOH was titrated to the solution to re-establish pH 7.00 and the CEC was calculated.

Calculations and statistics: The uptake of metal in the roots was calculated from the decrease in labelled metal in the solution during a certain time period and related to the root mass. A curve on the time course of uptake could then be drawn (Fig. 1). Uptake into free space was estimated to be in the first 30 min (*i.e*. 0 - 30 min), the cellular uptake occurred between next 30 and 90 min and during the later phase the leakage started and thereby the net uptake could be seen. The leakage of metal from the roots was calculated from the content of metal left in the roots after various elution times. Thereafter, a curve on the time course of leakage could be drawn (Jensén and Kylin 1980; Fig. 2). The total leakage [%] = $[(Me_{T0} - Me_{TX})/Me_{T0}] \times 100$, where Me_{T0} and Me_{TX} are the contents of labelled metal in the root at start and at time 510 min (Cd) or 430 min (Zn).

Mean values were calculated from 5 - 7 replicates and each replicate was based on two samples. Ten replicates were used in the CEC experiment. The least significant differences in correlation tests and Student's *t*-tests were calculated at $P = 0.05$ level.

Results

The uptake of Zn showed a typical hyperbolic curve while that of Cd did not (Fig. 1). Plants pre-treated with Zn and untreated plants showed differences, the uptake pattern of ^{65}Zn was similar but the pre-treated plants had, on average, 40 % lower uptake of ^{65}Zn compared with the untreated plants (Fig. 1). There was a higher uptake of Zn into the free space (*i.e*. the initial uptake) in untreated plants compared to pre-treated plants of resistant clones (Table 2). The cellular uptake rate of Zn was lower in untreated plants of sensitive high accumulating and resistant low accumulating clones then the other categories.

The uptake pattern of Cd differed between untreated and pre-treated plants (Fig. 1). The Cd uptake of untreated plants showed a fast initial uptake-rate (*i.e*. uptake into the free space), peaking after about 30 min. Then, for about 1.5 - 3 h, the uptake rate dropped to about 1/50 of the peak. This pattern was less pronounced

Table 1. Properties of *Salix* clones to tolerate and accumulate Cd and Zn. Clones were chosen from 130 clones with various tolerance and accumulation properties (Landberg and Greger 1994, Landberg and Greger 1996, Greger and Landberg 1999 and Greger *et al*. 2001). Tolerance index (TI) is calculated as root or shoot dry mass accumulation at treatment with 7 µM Cd or 70 µM Zn for 21 d divided by that of controls. Metal accumulation was analysed after 21 d treatments with 1µM Cd or 10 µM Zn, respectively. RH - resistant high accumulators, RL - resistant low accumulators, SH - sensitive high accumulators, SL - sensitive low accumulators.

	Clone	Tolerance, TI		Accumulation [µg g^{-1}(d.m.)]	
		root	shoot	root	shoot
Cd	RH1	0.70	0.87	83.4	5.19
	RH2	0.96	0.83	39.0	5.31
	RL1	0.75	0.78	16.3	1.25
	RL2	0.85	0.77	17.5	1.10
	SH1	0.00	0.38	76.2	4.12
	SH2	0.00	0.31	39.3	3.06
	SL1	0.00	0.35	17.3	1.34
	SL2	0.00	0.19	16.0	0.96
Zn	RH1	0.64	0.75	1002	750
	RH2	0.76	0.80	1643	958
	RL1	0.80	0.78	236	125
	RL2	0.64	0.79	349	12
	SH1	0.12	0.35	698	1297
	SH2	0.05	0.33	1320	1326
	SL1	0.09	0.18	96	26
	SL2	0.07	0.27	263	66

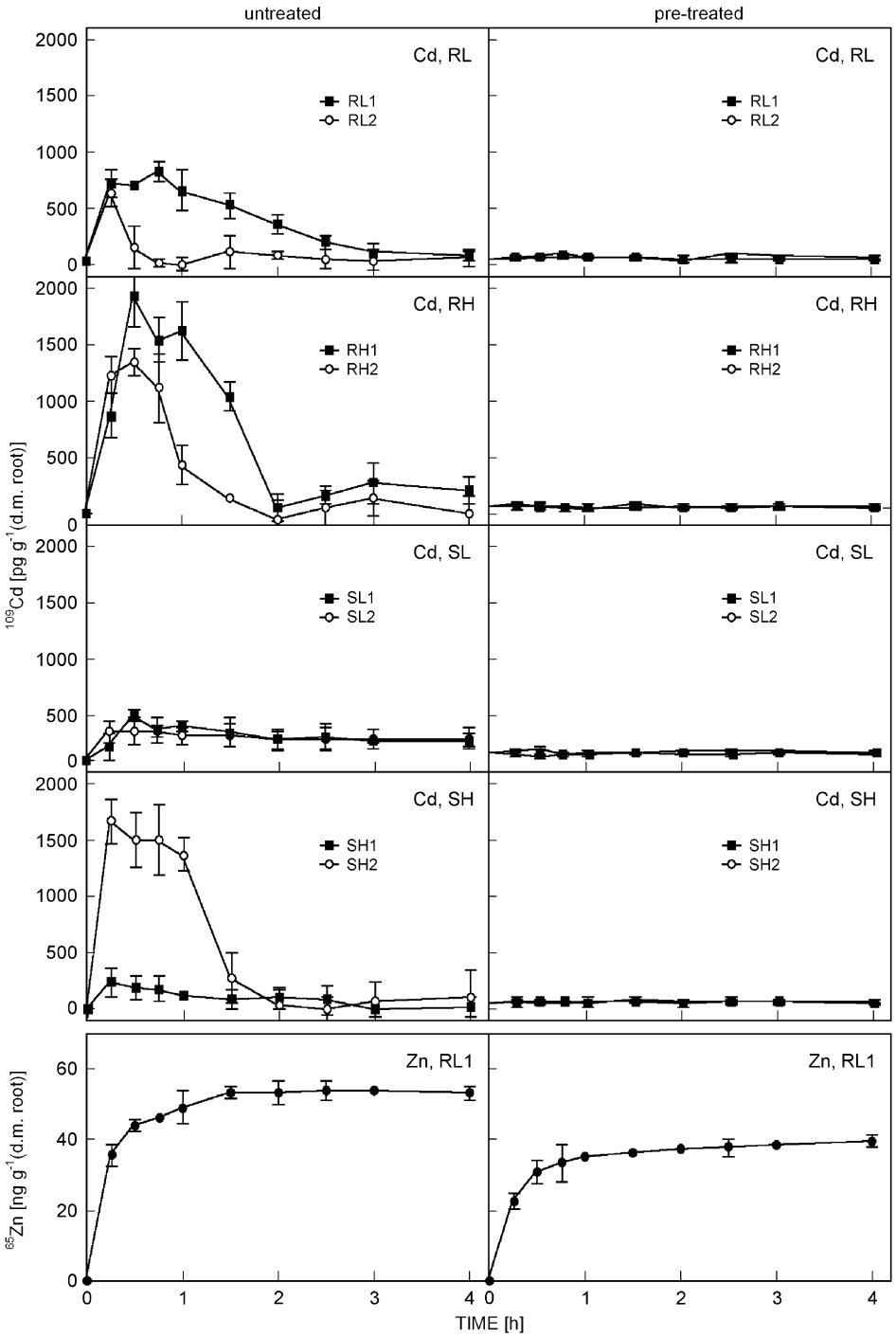

Fig. 1. Time course of uptake of Cd and Zn in different clones of *Salix viminalis*. Uptake of Zn illustrates representative uptake patterns of Zn in all clones. In the case of Cd all different categories of clones are shown. Some plants were pre-treated 24 h prior to the experiment with unlabelled 0.5 μM Cd or 2.5 μM Zn (*on the right*). Means ± SE, n = 6. RL - resistant low accumulators, RH - resistant high accumulators, SL - sensitive low accumulators, SH - sensitive high accumulators.

in sensitive clones, with exception of clone SH2. After the high initial uptake the uptake stabilised at a low level, similar to that found in pre-treated plants, which completely lacks the high initial Cd uptake rate.

The leakage pattern was similar for ^{109}Cd and ^{65}Zn (Fig. 2). The leakage rate was high at the beginning of the experimental period, showing the leakage from the free space compartment, while it was considerably lower at the end of the period. The leakage was similar for ^{109}Cd and ^{65}Zn, probably due to treatment with the same loading concentrations of the two isotopes. Resistant low accumulating clones showed significantly higher leakage

of ^{109}Cd, 50 - 100 % higher than the other categories of clones, both in untreated and pre-treated plants (Fig. 2, Table 3). This pattern was not recognised for ^{65}Zn, where the resistant high accumulating clones showed the highest total leakage and the lowest total leakage was found in sensitive high accumulating clones not pre-treated with Zn. There was no significant difference in total leakage of ^{65}Zn between untreated and pre-treated Zn-clones.

Neither the uptake nor the leakage of ^{109}Cd and ^{65}Zn was affected by DNP (data not shown), and neither was changes due to pre-treatment in uptake or leakage influenced by DNP.

The CEC was similar in the Cd-clones and the Zn-clones (Table 4). For Cd, resistant clones with high accumulation had a higher CEC. This also held for Zn-clones but differences were not significant (Table 4). The CEC did not correlate with the uptake in the free space ($r \leq 0.023$), the uptake rate ($r \leq 0.009$), or the total leakage ($r \leq 0.006$) for both metals.

Table 2. Uptake of ^{65}Zn shown as uptake in the free space [ng g^{-1}(d.m. root)], uptake rate into the cells and net uptake rate [pg g^{-1}(d.m. root) s^{-1}] (for detail see Materials and methods). Some plants were pre-treated 24 h prior to the experiment with unlabelled 2.5 µM Zn. Means ± SE, n = 6. SL - sensitive low accumulators, SH - sensitive high accumulators, RL - resistant low accumulators, RH - resistant high accumulators. * Significant differences between untreated and pre-treated plants. Different letters show significant differences within each column.

Clone	Uptake in the free space		Uptake rate into the cells		Net uptake rate	
	untreated	pre-treated	untreated	pre-treated	untreated	pre-treated
SL1	8.6 ± 0.93^{B}	4.6 ± 0.75^{C}	4.31 ± 0.27^{A}	3.44 ± 0.18^{A}	0.33 ± 0.07^{AB}	0.28 ± 0.02^{A}
SL2	25.3 ± 2.97^{A}	18.6 ± 2.55^{AB}	4.25 ± 0.62^{A}	0.78 ± 0.09^{C}*	0.30 ± 0.05^{A}	0.24 ± 0.04^{A}
SH1	25.6 ± 3.49^{A}	23.6 ± 1.26^{A}	1.14 ± 0.19^{C}	4.61 ± 0.59^{A}*	0.37 ± 0.02^{AB}	0.29 ± 0.04^{A}
SH2	4.4 ± 0.59^{C}	2.4 ± 0.19^{D}	1.92 ± 0.26^{B}	0.75 ± 0.04^{C}*	0.25 ± 0.09^{A}	0.29 ± 0.06^{A}
RL1	29.6 ± 1.77^{A}	18.6 ± 1.61^{A}*	2.11 ± 0.11^{B}	2.94 ± 0.51^{A}	0.13 ± 0.02^{C}	0.42 ± 0.03^{B}*
RL2	27.5 ± 3.16^{A}	14.4 ± 0.94^{B}*	2.67 ± 0.32^{B}	1.50 ± 0.14^{B}	0.27 ± 0.03^{A}	0.32 ± 0.02^{A}
RH1	24.9 ± 4.08^{A}	9.9 ± 1.94^{B}	5.36 ± 0.32^{A}	2.00 ± 0.06^{B}*	0.29 ± 0.00^{A}	0.24 ± 0.03^{A}
RH2	31.6 ± 4.11^{A}	14.6 ± 0.85^{B}*	5.25 ± 0.63^{A}	3.11 ± 0.39^{A}	0.31 ± 0.06^{A}	0.36 ± 0.07^{AB}

Discussion

The aim of this study was to find out how Cd and Zn resistant clones of *Salix* regulate the net uptake of Cd and Zn. The results indicate that resistant clones with low Cd accumulation may regulate the Cd net uptake by a high leakage of Cd by roots to achieve high resistance. No such conclusion could be drawn for Zn.

The large difference in Cd uptake pattern between untreated and pre-treated plants was that the pre-treated plants showed a very low Cd uptake (Fig. 1). It indicates induction of a mechanism that decreased the Cd uptake. This mechanism seems to be initialised within 30 - 60 min after the start of the ^{109}Cd uptake in untreated plants, and thus it had already been initialised in the Cd pre-treated plants prior to the start of the experiment. After about 2 - 3 h, the net uptake rate in untreated plants dropped down to a very low level, similar to that in pre-treated plants. In the nature, the Cd uptake is therefore likely kept low, as suggested by Baker (1981). The low net uptake of Cd was due to decreased uptake of Cd since the Cd leakage was not changed by the pre-treatment with Cd (Table 3, Fig. 2). The nature of this mechanism cannot be resolved with the present data, but it is not active uptake since DNP had no effect on it (data not shown).

The resistant low accumulating clones showed much higher Cd-leakage rate than the other clone categories (Table 3, Fig. 2). Since this difference between the clone categories was similar in untreated and pre-treated plants, it may indicate a constitutional property to have a high leakage rate in resistant-low accumulating clones that

Table 3. Total leakage [% of labelled metal in start] of ^{109}Cd (510 min) and ^{65}Zn (430 min). Some plants were pre-treated 24 h prior to the experiment with unlabelled 0.5 µM Cd or 2.5 µM Zn. Means ± SE, n = 5 - 7, * - significant differences between untreated and pre-treated plants. Different letters show significant differences within each column. For other detail see Table 1.

Clone	Cadmium		Zinc	
	untreated	pre-treated	untreated	pre-treated
SL1	17.9±1.21^{AD}	21.9±2.03^{A}	11.7±1.12^{A}	7.5±1.48^{A}
SL2	19.4±1.14^{A}	23.9±2.45^{A}	13.2±1.51^{A}	9.1±0.74^{A}
SH1	8.8±0.52^{C}	23.5±2.10^{A}*	1.2±1.21^{B}	10.4±1.73^{AB}*
SH2	21.4±1.34^{A}	21.6±0.98^{A}	4.3±3.12^{BC}	5.2±2.43^{A}
RL1	37.6±4.05^{B}	32.2±0.38^{B}	11.0±0.97^{A}	12.8±1.10^{B}
RL2	27.6±2.39^{B}	34.2±2.11^{B}	6.5±0.86^{C}	5.3±0.89^{A}
RH1	15.4±0.12^{D}	23.4±1.30^{A}*	38.5±4.18^{D}	39.8±4.01^{C}
RH2	19.6±0.22^{A}	21.6±0.41^{A}	20.9±2.64^{E}	17.5±4.20^{B}

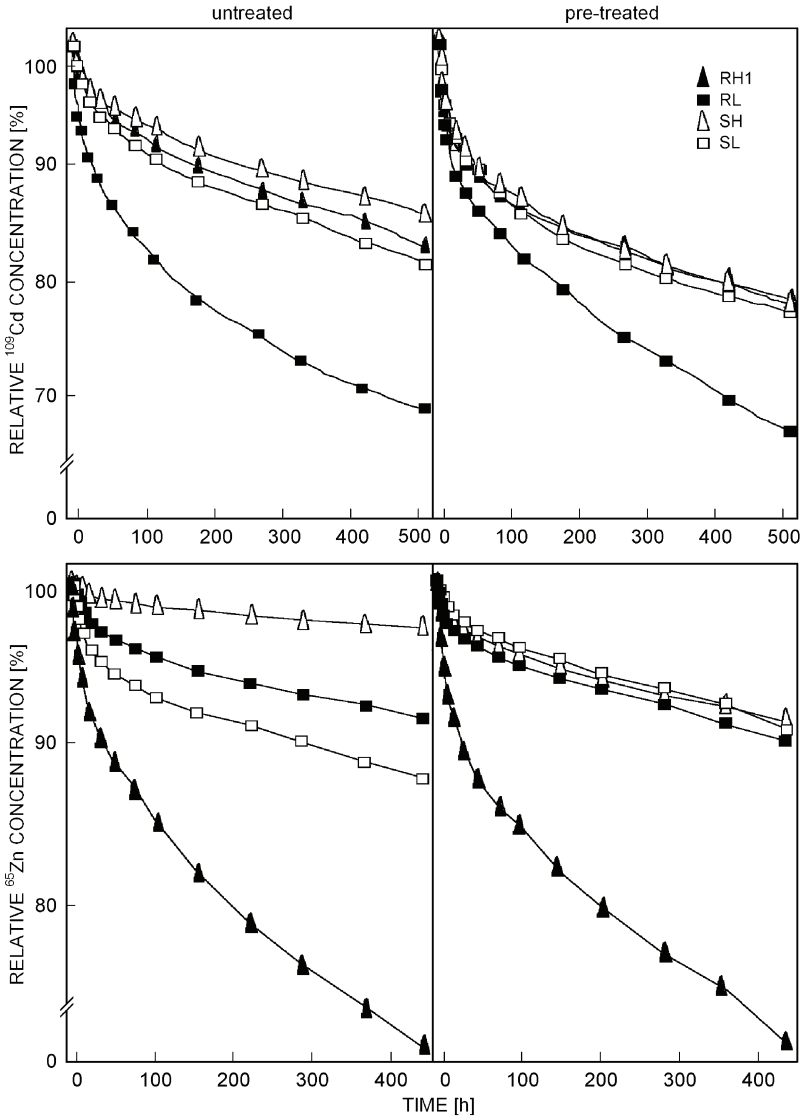

Fig. 2. Time course of leakage of ^{109}Cd and ^{65}Zn from roots of *Salix viminalis*. Some plants were pre-treated 24 h prior to the experiment with unlabelled 0.5 μM Cd or 2.5 μM Zn. Means ± SE, n = 5 - 7. Data are average of clones of the same clone categories. For other details see Fig. 1.

Table 4. Cation exchange capacity [mol kg^{-1}] in different clones of *Salix viminalis*. Means ± SE, n = 10. Different letters show significant differences within each column. For other details see Table 1.

Clone	Cadmium	Zinc
SL1	54.1 ± 3.9^{AB}	51.6 ± 2.8^{A}
SL2	47.1 ± 2.0^{A}	45.0 ± 3.5^{A}
SH1	45.9 ± 2.6^{A}	45.7 ± 4.0^{A}
SH2	49.9 ± 3.0^{A}	51.2 ± 4.2^{A}
RL1	44.8 ± 0.9^{A}	49.6 ± 2.5^{A}
RL2	46.3 ± 1.8^{A}	43.5 ± 4.3^{A}
RH1	59.4 ± 0.3^{B}	55.7 ± 4.6^{A}
RH2	63.7 ± 2.8^{B}	52.9 ± 3.1^{A}

may be important for the high Cd resistance. Strange and MacNair (1991) suggested a low net uptake in metal-excluding plants. The efficiency of compartmentalisation my also be important (Durand *et al.* 2010).

Resistant high Cd-accumulating clones has high root CEC (Table 4) and high initial Cd uptake into the free space (Fig. 1) as shown in untreated plants. High accumulation of Cd in cell walls with high levels of weak electrostatic charges has been found in tolerant populations of the moss *Rhytidiadelphus squarrosus* (Wells *et al.* 1995). In high accumulating clones, but sensitive to Cd, the CEC was not high (Table 4). The mechanism of resistance in high Cd-accumulating clones could therefore be due to the high CEC in the free space of the roots. In resistant high accumulating clones, a larger proportion of total Cd may be distributed to the

free space, where the metal ions become less toxic.

Therefore, resistance to Cd in *Salix* may be reached by three mechanisms: *a*) a decreasing uptake activated in all clones in the presence of Cd; *b*) a constitutional, already existing high leakage in the resistant low accumulating clones and *c*) high CEC in the free space of roots of resistant high accumulating clones.

The uptake pattern of Zn in both untreated and pre-treated plants differed from that of Cd. Zinc showed a hyperbolic pattern, also described by Guillermo and Daniel (1988), a common shape for nutrient elements (Marschner 1995) and probably due to that Zn is an essential for plants while Cd is not (Fig. 1). However, the initial Zn uptake was generally lower in pre-treated compared to untreated plants of resistant clones (Table 2). This is likely the reason for the lower Zn net uptake rate in the latter phase of the uptake curve in the pre-treated plants compared to untreated ones (Fig. 1). In contrast, the uptake pattern of the latter phase did not show difference between pre-treated and untreated resistant plants (Table 2). This may indicate that Zn could have saturated the free space during the pre-treatment of the resistant plants. The lower initial uptake in pre-treated resistant clones may also be due to a smaller free space or a mechanism activated by the Zn pre-treatment in resis-tant compared to sensitive clones. However, such a me-chanism did not affect the net-uptake rate of Zn (Table 2).

The mechanism behind Zn resistance in resistant high Zn-accumulating clones could be due to high cellular uptake (Table 2) and high CEC of the root tissue (Table. 4). The high cellular uptake, however, decreased at Zn pre-treatment (Table 2). The high root CEC, might be constitutive, as, *e.g.*, the properties of pectin substances in cell wall can affect the CEC as suggested by Blamey *et al.* (1992). However, the resistant high accumulating clones also had a high Zn leakage, which was not changed by the Zn pre-treatment and therefore was a constitutive property. An explanation for the high leakage of Zn in high Zn accumulating and resistant clones could not be found.

References

Baker, A.J.M.: Accumulators and excluders - strategies in the response of plants to heavy metals. - J. Plant Nutr. **3**: 643-654, 1981.

Baker, A.J.M.: Terrestial higher plants which hyperaccumulate metalic elements – a review of their distribution, ecology and phytochemistry. - Biorecovery **1**: 81-126, 1989.

Baker, A.J.M., Grant, C.J., Martin, M.H., Shaw, S.C., Whitebrook, J.: Induction and loss of cadmium tolerance in *Holcus lanatus* L. and other grasses. - New Phytol. **102**: 575-587, 1986.

Blamey, F.P.C., Robinson, N.J., Asher, C.J.: Interspecific differences in aluminium tolerance in relation to root cation-exchange capacity. - Plant Soil **146**: 77-82, 1992.

Bowen, J.E.: Physiology of genotyping differences in zinc and copper uptake in rice and tomato. - Plant Soil **99**: 115-125, 1987.

Cataldo, D.A., Garland, T.R., Wildung, R.E.: Cadmium uptake kinetics in intact soyabean plants. - Plant Physiol. **73**: 844-848, 1983.

Clarkson, D.T., Lüttge, U.: Inducible and repressible nutrient uptake systems. - Progress Bot. **51**: 93-112, 1991.

Crooke, W.M.: The measurement of the cation-exchange capacity of plant roots. - Plant Soil **21**: 43-49, 1964.

Cutler, J.M., Rains, D.W.: Characterisation of cadmium uptake by plant tissue. - Plant Physiol. **54**: 67-71, 1974.

Di Baccio, D., Minocci, A., Sebastiani, L.: leaf structural modifications in *Populus* × *euramericana* subjected to Zn excess. - Biol. Plant. **50**: 502-508, 2010.

Durand, T.C., Hausman, J.F., Carpin, S., Alberic, P., Baillif, P., Label, P., Morabito, D.: Zinc and cadmium effects on growth and ion distribution in *Populus tremula* × *Populus alba*. - Biol. Plant. **50**: 191-194, 2010.

Ernst, W.H.O., Verkleij, J.A.C., Schat, H.: Metal tolerance in plants. - Acta bot. neerl. **41**: 229-248, 1992.

Fry, S.C.: The Growing Plant Cell Wall: Chemical and Metabolic Analysis. - Longman Scientific & Technical, Essex 1988.

Greger, M., Landberg, T.: Use of willow in phytoextraction. - Int. J. Phytoremed. **1**: 115-123, 1999.

Greger, M., Landberg, T., Berg, B.: *Salix* with Different Properties to Accumulate Heavy Metals for Production of Biomass. - Akademitryck AB, Edsbruk 2001.

Guillermo, E.S.M., Daniel, H.C.: Bidirectional Zn-fluxes and compartmentation in wheat seedling roots. - J. Plant Physiol. **132**: 312-315, 1988.

Gussarson, M., Jensén, P.: Effects of copper and cadmium on uptake and leakage of K^+ in birch (*Betula pendula*) roots. - Tree Physiol. **11**: 305-313, 1992.

Hooda, P.S., Alloway, B.J.: Effects of time and temperature on the bioavailibility of Cd and Pb from sludge-amended soils. - J. Soil Sci. **44**: 97-110, 1993.

Jensén, P., Kylin, A.: Effect of ionic strength and relative humidity on the efflux of K^+ (^{86}Rb) and Ca^{2+} (^{45}Ca) from roots of intact seedlings of cucumber, oat and wheat. - Physiol. Plant. **50**: 199-207, 1980.

Landberg, T., Greger, M.: Can heavy metal tolerant clones of *Salix* be used as vegetation filters on heavy metal contaminated land. - In: Aronsson, P., Perttu, K. (ed.): Willow Vegetation Filters for Municipal Wastewaters and Sludges – a Biological Purification System. Pp. 133-144. SLU Info/Repro, Uppsala 1994.

Landberg, T., Greger, M.: Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. - Appl. Geochem. **11**: 175-180, 1996.

Marschner, H.: Mineral Nutrition of Higher Plants. - Academic Press, London 1995.

Meharg, A.A.: The role of plasmalemma in metal tolerance in angiosperms. - Physiol. Plant. **88**: 191-198, 1993.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A,. Forestier, C.: Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. - Plant J. **32**: 539-548, 2002.

Silver, S., Misra, T.K.: Plasmid-mediated heavy metal resistance. - Annu. Rev. Microbiol. **42**: 717-743, 1988.

Strange, J., MacNair, M.R.: Evidence for a role for the cell membrane in copper tolerance of *Mimulus guttatus* Fisher *ex* DC. - New Phytol. **119**: 383-388, 1991.

Wagatsuma, T., Akiba, R.: Low surface negativity of root protoplasts from aluminium-tolerant plant species. - Soil Sci. Plant Nutr. **35**: 443-452, 1989.

Wang, J., Evangelou, B.P., Nielsen, M.T.: Surface chemical properties of purified root cell walls from two tobacco genotypes exhibiting different tolerance to manganese toxicity. - Plant Physiol. **100**: 496-501, 1992.

Wells, J.M., Brown, D.H., Beckett, R.P.: Kinetic analysis of Cd uptake in Cd-tolerant and intolerant populations of the moss *Rhytidiadephus squarrosus* (Hedw.) Warnst and the lichen *Peltigera membrnacea* (Ach.) Nyl. - New Phytol. **129**: 477-486, 1995.