Influence of Ca/Mg ratio and Cd²⁺ and Pb²⁺ elements on low molecular weight organic acid secretion by *Salix viminalis* L. roots into the rhizosphere

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Abstract A modified hydroponic experiment was focused on the influence of mutual changes in the Ca/Mg ratio and trace elements (Cd and Pb) concentration in solution on the efficiency of low molecular weight organic acid (LMWOA) formation in the *Salix viminalis* rhizosphere. The study showed that changes in the LMWOA contents were caused by the Ca/Mg ratio as well as the kind and concentration of trace element (Cd or Pb) in hydroponic solution. Under 0.5 mM solution of Cd, the total concentration of LMWOAs—depending on the Ca/Mg ratio—decreased according to the formula: 20:1 > 4:1 > 1:10 > 1:1/4, while under 0.5 mM of Pb, it was as follows: 20:1 ≈ 4:1 > 1:1/4 > 1:10. When comparing all the experimental systems, it is important to underline that regardless of the Ca/Mg ratio, the concentration of LMWOAs in the rhizosphere was higher under 0.5 mM Cd than Pb, with the exception of 1:1/4 Ca/Mg ratio, where the opposite observation was made and both of these values were lower than in the control. In addition, accumulation of Cd and Pb in *Salix* roots and young shoots was dependent on Ca/Mg ratio only and decreased according to the

formula 1:10 > 4:1 > 20:1 > 1:1/4, regardless of Cd or Pb supplementation.

Keywords Cadmium · Ca/Mg ratio · Hydroponic · Lead · Low molecular weight organic acids · *Salix*

Introduction

Hundreds of hyperaccumulators have been identified and described in the past three decades (Li et al. 2011). Most hyperaccumulators, such as *Thlaspi* or *Alyssum*, are characterized by a generally slow growth rate and low biomass production, which make these plants impractical for use in phytoextraction in the field (Evangelou et al. 2004; Peer et al. 2005; Puschenreiter et al. 2001). Moreover, many hyperaccumulators have various strategies to actively accumulate metals e.g. *Thlaspi* actively produces roots that go into pools with high metal concentrations.

Recent studies on phytoextraction are focused on plants not recognized as hyperaccumulators, but still very effective in phytoremediation with high biomass effectiveness during the growth season, i.e. maize (*Zea mays*), peas (*Pisum sativum*), oat (*Avena sativa*), Indian mustard (*Brassica juncea*) as well as the trees *Salix* and *Populus* (Chen et al. 2004; Shen et al. 2002). Other important factors include a strongly developed and deep root system, quick establishment, followed by easy adaption to a wide range of environmental conditions (Mleczek et al. 2010), fast growth, elevated rates of photosynthesis and watering demand (Zalesny et al. 2006; Zupančič et al. 2010). *Salix viminalis* L. features all the above-mentioned properties with exceptional abilities to tolerate and accumulate high concentrations of trace elements. All this makes *Salix* an ideal plant to study mechanisms responsible for

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hyperaccumulation as well as to enhance phytoremediation processes.

To date, studies on the mechanism of metal tolerance and accumulation of *Salix* have been mainly focused on the uptake (Lu et al. 2008; Mleczek et al. 2011; Yang et al. 2006) and subcellular distribution characteristics (Li et al. 2006; Tian et al. 2009), with no information on many basic processes in the rhizosphere of the plant (Li et al. 2011; Pilon-Smits 2005). The processes of metal accumulation by the rhizosphere have not been elucidated; nor have the mechanisms of trace element solubilization and absorption by plants from contaminated soils (Gonzaga et al. 2009; Li et al. 2011). Several studies have tried to assess changes in rhizosphere chemistry of hyperaccumulator and non-hyperaccumulator plants with high capacity for metal accumulation for solubilisation of non-labile forms of metals. There was no correlation found between metal uptake and root-induced changes in the rhizosphere such as acidification and release of a wide spectrum of organic compounds (amino acids, low-molecular-weight carboxylic acids, sugars, and simple and flavonoid-type phenolics) (Dessureault-Rompré et al. 2010; Magdziak et al. 2011).

Low molecular weight organic acids (LMWOAs) are common metabolites in many pathways of plants. In soil, the predominant input of them is from root exudates, dead plant material and microbial decomposition products. Organic manures can also contain large amounts of organic acids (Wang and Zhong 2011). These root exudates are implicated in the weathering of soil minerals, mobilisation of soil nutrients (e.g. P and Fe), enhancement of nutrient uptake and stress resistance to acid soils and toxic metals (e.g. Al) (Barceló and Poschenrieder 2002; Hinsinger et al. 1993; Jones 1998; Kidd et al. 2001; Watanabe and Osaki 2002). What is more, root exudates may affect the behaviour/properties of nutrients and trace elements, either enhancing or reducing their availability, by directly affecting acidification, chelation, precipitation and redox reactions, or indirectly, through their effects on microbial activity, physical and chemical properties of the rhizosphere and root growth pattern (Kidd et al. 2009; Tao et al. 2004; Uren and Reisenauer 1988). Organic acid can bind elements such as metals, and their role as detoxification agents has been widely discussed (Jones 1998). They are able to form complexes with metal ions and modify the mobility of the metals in the rhizosphere (Maturi and Reddy 2008; Renella et al. 2004) and their exudation is probably an internal mechanism against toxic effects of trace elements (Gasecka et al. 2012). However, there is little information in the literature concerning the mechanism of how these compounds affect trace element accumulation in plants (Wang and Zhong 2011).

Over the last 20 years, numerous studies have demonstrated that the value of many nutritional elements, as well

as trace elements, may have a considerable effect on growth and the accumulation efficiency of other elements present in the soil. Moreover, the data presented in the literature indicate that the Ca/Mg ratio has a significant effect on the phytoremediation process (Parker et al. 1998; Ösretås and Greger 2006). However, information on their influence on the efficiency of heavy metal accumulation in plant organs in association with LMWOA secretion by plant roots is substantially limited. Following the results of our earlier work (Magdziak et al. 2011; Mleczek et al. 2011) and a small number of works on the role of organic acids in phytoremediation, the aims of this study were (a) to investigate the changes of LMWOA composition and concentration in the rhizosphere of *Salix* under 0.5 mM Cd or Pb supplementation and (b) to assess the role of LMWOAs in the rhizosphere of *Salix* in Cd and Pb accumulation at four different Ca/Mg ratios. Pb and Cd were selected because they are among the most toxic elements for humans and widespread in contaminated ecosystems. The selection of the Ca/Mg ratio was also not accidental as regards the different roles of these nutritional elements in plant growth.

Materials and methods

Plant material

One-year-old cuttings of *S. viminalis* L. cv. 'Cannabina' collected from 3-year-old rootstock without foliage were used in the experiment under fully controlled conditions (phytotron). To induce root formation, standardized rods (25 cm long, 15 mm in diameter) were under complete Knop's medium (10 mL of 10 % $\text{Ca}(\text{NO}_3)_2$, 2.5 mL of 10 % KNO_3 , 1.2 mL of 10 % KCl, 10 mL of 2.5 % KH_2PO_4 , 5 mL of 5 % MgSO_4 and 0.25 mL of 0.25 % FeCl_3 diluted to 1 L with acidified water of pH 5.42 (PN-ISO 10390 1997); electrical conductivity (PN-ISO 1265+AC1: 1997) of nutrient solution was 1.36 mS cm^{-1}). To improve root formation, the solution applied at the beginning of the preliminary incubation period contained 50 % salt contents in the standard Knop medium, which also facilitated easier and faster adaptation of plants to the new conditions.

After 10 days, plants were selected according to the size of the root system to obtain a uniform group (mean initial lengths of main roots for particular tested plants were in the range between 4.5 and 5.0 cm and the diameter of them was 2.0–2.5 mm) and transferred to Knop's medium (0.5 L) containing the appropriate amount of Cd or Pb nitrate (V) stabilized with steamed ultra-pure quartz sand (1.15 kg per pot: pH = 7.2, SiO_2 content >97 %, moisture content 0.061 %) in hydroponic pots (one *Salix* cutting per

pot and seven plants per Cd and Pb concentration). In every pot (13 × 15 cm, diameter × height), part of the rhizosphere was separated from the rest of the sand using inert material to obtain better opportunities to analyse composition and amount of LMWOAs. Plants were cultivated in 0.5 L of 0.1 mM solution of each of the two tested trace elements (control: Cd_{0.1} + Pb_{0.1}) and in four experimental combinations where one of the metals was at 0.5 mM concentration, while the second was at 0.1 mM (Cd_{0.5} or Pb_{0.5}). In these experiments, we have also analysed plants growing in 0 mM concentration, but the use of control in Cd_{0.1} + Pb_{0.1} form was expedient and necessary because this experiment is an extension of our other experimental studies carried out in the environment (the results will be compared and presented in a subsequent work). In addition, the use of control in Cd_{0.1} + Pb_{0.1} form was possible as regards the insignificant differences in analysed plant growth (plant biomass, LMWOA exudation as well as trace element accumulation) between Cd_{0.1} + Pb_{0.1} and Cd and Pb free medium. The concentrations 0.1 and 0.5 mM were selected based on growth of the tested plants in soil with Cd and Pb in the concentration range usually present in Polish soils (Kabata-Pendias and Pendias 1999). In addition, the difference between the two concentrations is high enough to observe its influence on composition and concentration of LMWOAs as well as antagonistic/synergic effects between trace elements and the differences in plant development with no toxic influence. Four (previously tested) Ca/Mg ratios were also chosen to indicate the differences in LMWOA secretion into the rhizosphere, Cd and Pb accumulation and plant growth in physiological ratio (4:1—C_{Ca} = 4.23 mM and C_{Mg} = 1.05 mM), in excess of calcium (20:1—C_{Ca} = 21.16 mM and C_{Mg} = 1.05 mM), in excess of magnesium (1:10—C_{Ca} = 1.06 mM and C_{Mg} = 10.5 mM) and in deficiency of both macroelements (1:1/4—C_{Ca} = 1.06 mM and C_{Mg} = 0.26 mM).

The 21-day experiment was performed in a climate chamber under controlled conditions (air temperature 22 ± 1 °C, relative humidity 79 ± 1 %), equipped with a fluorescent lamp (MASTER TL-D Secura 58 W/830 1SL) providing a radiation (photon) flux of 235 μE s⁻¹ m⁻² (μmol s⁻¹ m⁻²) at the top of the plant for 16 h a day. Plant roots were aerated by a hose system (1 h periods, three times per day). Solution was not replaced during the experiment with metals and no bactericide was used. Plants were harvested 21 days after the beginning of the photoperiod, and then the seedlings were gently removed from the pots. Rhizosphere quartz sand samples were collected with a sample collector in a volume of 100 mL from the separated area, then mixed and dried at room temperature (20 ± 2 °C). Roots and young shoots of *Salix* plants were first washed with tap water, then rinsed with ultrapure, deionized water, dried in an electric drier at 105 ± 5 °C

for 72 h, and then ground for 3 min in a laboratory Cutting Boll Mill 200 by RETSCH. The heavy metal contents were analysed in the milled fraction after passing through sieves of diameter 0.5–1 mm.

Biometric analysis

The cumulative (total) leaf area, and mean length of shoots and roots were measured for biometric analysis. The shoot and root lengths were measured by tape. The cumulative leaf area was measured with a DocuPENTM RC 800 portable scanner (ABBYY) with ABBYY FineReader 6.0 Sprint (ABBYY) and Adobe Photoshop 10.0 (Adobe) software.

Chemical analysis

Chemicals and standards

The LMWOA standards were purchased from SUPELCO with the certified standard grade. Organic solvents were of HPLC grade, purchased from Sigma. For pH adjustment concentrated acid (H₃PO₄, HCl) and other chemicals (methanol, KH₂PO₄, ethyl acetate and acetonitrile) of analytical grade (Sigma) were used. For Cd and Pb analysis, analytical grade HNO₃ (65 %), H₂O₂ (30 %) and a standard Cd²⁺ (Cd(NO₃)₂) and Pb²⁺ (Pb(NO₃)₂) solution (1 g L⁻¹) were purchased from FLUKA. All aqueous solutions were prepared on the basis of Milli-Q water (Millipore Corporation).

LMWOA analysis

The LMWOAs were extracted from 20 g of the samples with 100 mL of water (pH 2 acidified with concentrated HCl) in an orbital shaker at room temperature for 12 h. Extracts were filtered through Whatman No. 42 filters, and organic acids were extracted from the water solution three times with ethyl acetate (20 mL, 5 min) (Baziramakenga et al. 1995). The volume of the solvent was reduced to 5 mL with a rotary evaporator at 40 °C and transferred into an amber glass vial. The residue was rinsed from the flask with 1 mL of distilled water and added to the vial. The solvent was evaporated at room temperature under a stream of nitrogen to obtain 1 mL of water solution, and a 0.5 mL water aliquot was analysed. LMWOAs were determined with a Waters Alliance 2695 Chromatograph coupled with a Waters 2996 Photodiode Array Detector at λ = 220 nm. Separations were performed on a Waters Atlantis C₁₈ column (250 mm × 4.6 mm, 5 μm) at room temperature (25 ± 2 °C) at a flow rate of 0.8 mL min⁻¹. The mobile phase consisted of 25 mM KH₂PO₄ adjusted to pH 2.5 with concentrated H₃PO₄ and methanol (95:5, v/v). For the

analysis of root exudate samples, gradient elution was employed every fifth sample, to obtain 95 % methanol in 15 min to fully flush the column of hydrophobic compounds from previous injections (Cawthray 2003). A reversed-phase liquid chromatography column (RPLC) was used for the separation and quantification of ten LMWOA (malic, malonic, lactic, acetic, maleic, citric, succinic, fumaric, formic and oxalic) in plant root exudates. Compounds were identified by retention times of their peaks in a chromatogram and quantified by comparing peak area with standards at a known compound concentration according to the appropriate standard curve. Retention times (T_R) and limits of detection (LOD) for ten analysed low molecular weight organic acids (LMWOAs) are presented in Table 1.

The content and composition of ten organic acids analysed individually as well as total content of LMWOAs were established based on both the concentration of trace elements (Cd^{2+} , Pb^{2+}) and at four Ca/Mg ratios (4:1, 1:10, 20:1 and 1:1/4) in solution. Data were normalized for the dry mass of the quartz sand used in each experiment.

Cd^{2+} and Pb^{2+} phytoextraction

The material was mineralized with a CEM Mars 5 Xpress (CEM, Matthews, NC) microwave mineralization system (55 mL vessels) using 8 mL HNO_3 and 2 mL H_2O_2 . Digestion of the plant materials was performed according to a microwave programme composed of three stages: first stage: power 800 W, time 10 min, temperature 120 °C; second stage: power 1,600 W, time 10 min, temperature 160 °C; third stage: power 1,600 W, time 10 min, temperature 200 °C. Materials after digestion were filtered through 45 mm filters (Qualitative Filter Papers Whatman, Grade 595: 4–7 μm), and then whole contents were com-

plement to a final volume bulbs of 50 mL by deionized water.

Cd and Pb concentration was determined by flame atomic absorption spectrometry (FAAS) using an AA Duo-AA280FS/AA280Z spectrometer (Agilent Technologies, Mulgrave, Victoria, Australia). Calibration curves were prepared in advance, with five replicates per Cd or Pb concentration. The limits of detection were 0.94 and 10.89 $\mu\text{g dm}^{-3}$ for Cd and Pb, respectively. Results were validated on the basis of certified reference materials, i.e. NIST 1575a (Pine Needles) and NCS DC 73350 (Leaves of poplar) analysed in every tenth measuring set, and simultaneous analyses of randomly selected samples using inductively coupled plasma optical emission spectrometry (ICP-OES) with Vista MPX apparatus by Varian.

The bioaccumulation factors (BAFs) were calculated as the ratio of Cd or Pb concentration in *Salix* organs (roots and shoots) to the initial metal concentration in the cultivation medium (Eq. 1). Depending on the BAF value, the efficiency of Cd or Pb accumulation was described on a four-grade scale as follows: $\text{BAF} > 1$ (I—intensive), 1–0.1 (M—medium), 0.1–0.01 (W—weak) and 0.01–0.001 (L—no accumulation) (Kabata-Pendias and Pendias 1999).

$$\text{BAF} = \frac{\text{Me}^{n+} \text{ content in } \text{Salix} \text{ organ (mg kg}^{-1} \text{ DW)}}{\text{Me}^{n+} \text{ content in the cultivation medium (mg kg}^{-1} \text{ DW)}} \quad (1)$$

Statistical analyses

The experimental data were analysed statistically using STATISTICA 9 software (StatSoft Inc.). In particular, 2-factor analyses of variance for efficiency of Cd and Pb accumulation in roots and shoots under different Ca/Mg ratios in *S. viminalis* and the content of LMWOAs observed in *Salix* rhizosphere under Cd and Pb supplementation were performed. The appropriate *F* statistics for tests of significance factor effects and for the interaction effect between two factors were applied. The differences between means for the combination of factor levels were tested using Duncan's multiple range test (Gomez and Gomez 1984).

Results

Biomass analysis

Simultaneously with the total amount of cadmium and lead in tested parts of *Salix*, morphometric analyses were performed. The length of roots and shoots, leaf surface area and total plant biomass are presented in Table 2.

Table 1 Retention times (T_R) and limits of detection (LOD) for ten analysed low molecular weight organic acids (LMWOAs)

LMWOAs	T_R (min)	LOD (μM)
Oxalic acid	4.46	0.011
Formic acid	4.60	0.002
Malic acid	5.30	0.007
Malonic acid	5.64	0.009
Lactic acid	6.52	0.014
Acetic acid	7.08	0.008
Maleic acid	7.81	8.6×10^{-7}
Citric acid	8.96	4.95×10^{-3}
Fumaric acid	10.65	4.3×10^{-6}
Succinic acid	11.18	8.475×10^{-3}

Table 2 The responses of biometric parameters to the different levels of Pb^{2+} and Cd^{2+} and Ca/Mg ratios in *S. viminalis* L

Parameter	System	20:1	4:1	1 : $1/4$	1:10
Root length (cm)	Cd _{0.5}	11.49 ± 1.14 ^c	17.09 ± 0.68 ^a	16.62 ± 1.02 ^a	13.27 ± 0.53 ^b
	Pb _{0.5}	15.07 ± 0.65 ^a	13.41 ± 1.18 ^b	15.20 ± 0.76 ^a	12.01 ± 0.53 ^b
	Control	10.32 ± 0.74 ^c	14.91 ± 0.38 ^b	18.17 ± 1.13 ^a	10.52 ± 0.60 ^c
Shoot length (cm)	Cd _{0.5}	9.02 ± 1.02 ^b	13.85 ± 0.65 ^a	16.02 ± 1.97 ^a	6.91 ± 0.61 ^b
	Pb _{0.5}	13.86 ± 1.15 ^a	14.36 ± 0.47 ^a	12.15 ± 0.33 ^b	8.07 ± 0.42 ^c
	Control	5.11 ± 1.08 ^c	9.85 ± 1.28 ^b	17.43 ± 0.53 ^a	6.28 ± 1.20 ^c
Leaf area (cm ²)	Cd _{0.5}	12.09 ± 0.71 ^c	17.20 ± 1.12 ^b	23.15 ± 1.19 ^a	10.96 ± 0.32 ^c
	Pb _{0.5}	18.55 ± 0.75 ^a	16.07 ± 0.43 ^b	12.67 ± 1.92 ^c	10.29 ± 1.46 ^c
	Control	10.02 ± 0.71 ^c	24.35 ± 2.00 ^b	29.89 ± 1.99 ^a	13.12 ± 1.55 ^c
Total plant biomass (g)	Cd _{0.5}	21.94 ± 2.45 ^a	25.78 ± 3.84 ^a	24.84 ± 4.05 ^a	22.57 ± 3.24 ^a
	Pb _{0.5}	24.16 ± 3.77 ^a	23.28 ± 4.62 ^a	23.69 ± 2.61 ^a	22.09 ± 2.58 ^a
	Control	20.34 ± 2.96 ^b	24.69 ± 2.15 ^{ab}	27.55 ± 2.86 ^a	19.76 ± 3.40 ^b

Mean values ($n = 7$) ± SDa, b, c In particular rows for Cd (Pb) under various Ca/Mg ratios are significantly different at $P = 0.05$ **Table 3** Low molecular weight organic acid contents [$\mu\text{M kg}^{-1}$ DW] in *S. viminalis* L. rhizosphere according to trace element concentration and Ca/Mg ratio in the medium

Ca/Mg ratio	Metal content (mM)	LMWOAs contents [$\mu\text{M kg}^{-1}$ DW]						
		Formic CH ₂ O ₂	Malonic C ₃ H ₃ O ₄	Acetic C ₂ H ₄ O ₂	Citric C ₆ H ₈ O ₇	Oxalic C ₂ H ₂ O ₂	Lactic C ₃ H ₆ O ₃	Total of ten LMWOAs
20:1	Cd _{0.5}	0.429 ± 0.042 ^a	0.084 ± 0.024 ^b	49.756 ± 8.092 ^a	0.578 ± 0.090 ^b	n.d.	1.358 ± 0.445 ^a	52.722 ± 7.840 ^a
	Pb _{0.5}	0.081 ± 0.012 ^b	n.d.	23.829 ± 2.632 ^b	3.682 ± 0.819 ^a	0.327 ± 0.015	1.221 ± 0.019 ^b	29.825 ± 2.399 ^b
	Control	0.115 ± 0.007 ^b	9.738 ± 0.847 ^a	1.461 ± 0.033 ^c	0.032 ± 0.002 ^b	n.d.	0.896 ± 0.050 ^c	12.496 ± 0.867 ^c
4:1	Cd _{0.5}	0.076 ± 0.009 ^b	2.019 ± 0.409 ^b	41.686 ± 3.821 ^a	0.249 ± 0.002 ^a	n.d.	0.819 ± 0.024 ^c	45.208 ± 3.708 ^a
	Pb _{0.5}	0.061 ± 0.011 ^b	6.097 ± 0.357 ^a	20.956 ± 3.932 ^b	0.082 ± 0.011 ^a	0.276 ± 0.016	1.287 ± 0.043 ^a	29.243 ± 3.636 ^b
	Control	0.357 ± 0.009 ^a	0.144 ± 0.066 ^c	2.159 ± 0.052 ^c	0.415 ± 0.025 ^a	n.d.	1.114 ± 0.040 ^b	4.445 ± 0.078 ^c
1: $1/4$	Cd _{0.5}	0.054 ± 0.004 ^a	4.582 ± 0.274 ^a	4.145 ± 0.151 ^b	5.235 ± 0.274 ^c	n.d.	1.227 ± 0.033 ^a	15.733 ± 0.241 ^b
	Pb _{0.5}	0.072 ± 0.003 ^a	4.083 ± 0.313 ^a	11.720 ± 0.163 ^{ab}	9.773 ± 0.373 ^a	n.d.	0.703 ± 0.037 ^b	26.901 ± 0.745 ^a
	Control	0.054 ± 0.006 ^a	1.714 ± 0.224 ^b	16.417 ± 1.606 ^a	8.499 ± 0.375 ^b	n.d.	0.801 ± 0.035 ^b	27.656 ± 1.489 ^a
1:10	Cd _{0.5}	0.038 ± 0.004 ^{ab}	27.240 ± 1.799 ^a	6.495 ± 0.483 ^a	3.771 ± 0.207 ^a	n.d.	0.683 ± 0.038 ^b	39.130 ± 2.052 ^a
	Pb _{0.5}	0.069 ± 0.004 ^a	0.573 ± 0.188 ^b	6.927 ± 0.071 ^a	3.687 ± 0.999 ^a	n.d.	1.242 ± 0.030 ^a	13.478 ± 1.217 ^b
	Control	0.018 ± 0.001 ^b	n.d.	8.235 ± 0.579 ^a	2.258 ± 0.452 ^b	n.d.	0.609 ± 0.035 ^b	11.427 ± 0.176 ^b

Mean values ($n = 7$) ± SD

nd not detected

a, b, c In particular columns for various Ca/Mg ratios are significantly different at $P = 0.05$

Results of biometric analyses present significant differences in growth of tested parts of *Salix* cuttings depending on trace element (Cd^{2+} or Pb^{2+}) supplementation and the mutual Ca/Mg ratio. Depending on these two factors, different *S. viminalis* L. responses to added trace elements were observed, indicating inhibition or stimulation of plant growth. However, it should be noted that greater changes in tested *Salix* parts depend mainly on the Ca/Mg ratio.

LMWOA contents

The efficiency of acid exudation to the rhizosphere (mean values from two independent experiments, each in three replications), depending on the dominant metal under 0.5 mM, is shown in Table 3.

Among the ten analysed LMWOAs in the *S. viminalis* L. rhizosphere, the dominant acids were acetic, citric, malonic, lactic and formic acids, while oxalic, maleic, fumaric

Table 4 Trace elements' accumulation [mg kg^{-1} D.W.] in *S. viminalis* L. organs under different Pb^{2+} and Cd^{2+} concentrations and Ca/Mg ratio in the medium

Salix organ/ system	Cd				Pb			
	20:1	4:1	1 : $\frac{1}{4}$	1:10	20:1	4:1	1 : $\frac{1}{4}$	1:10
Root								
Cd _{0.5}	2.22 ± 0.16 ^c	3.51 ± 0.31 ^b	1.58 ± 0.12 ^d	3.76 ± 0.41 ^a	5.71 ± 0.42 ^c	8.06 ± 0.51 ^b	4.90 ± 0.41 ^d	8.92 ± 0.76 ^a
Pb _{0.5}	3.27 ± 0.28 ^c	3.63 ± 0.29 ^b	3.01 ± 0.28 ^d	3.96 ± 0.34 ^a	12.13 ± 0.86 ^c	14.52 ± 1.03 ^b	11.23 ± 0.98 ^d	16.81 ± 1.42 ^a
Control	1.69 ± 0.14 ^c	2.26 ± 0.17 ^b	1.14 ± 0.11 ^d	2.63 ± 0.21 ^a	3.71 ± 0.28 ^c	4.34 ± 0.37 ^b	2.78 ± 0.24 ^d	4.79 ± 0.39 ^a
Shoot								
Cd _{0.5}	2.42 ± 0.22 ^c	2.91 ± 0.19 ^b	1.85 ± 0.14 ^d	3.64 ± 0.29 ^a	4.21 ± 0.33 ^c	4.64 ± 0.29 ^b	3.86 ± 0.33 ^d	5.37 ± 0.58 ^a
Pb _{0.5}	2.01 ± 0.19 ^c	2.78 ± 0.24 ^a	1.84 ± 0.15 ^d	2.19 ± 0.21 ^b	6.65 ± 0.55 ^c	7.33 ± 0.64 ^b	6.21 ± 0.59 ^d	8.63 ± 0.37 ^a
Control	1.86 ± 1.13 ^c	2.04 ± 0.16 ^b	1.78 ± 0.14 ^d	2.72 ± 0.22 ^a	3.61 ± 0.29 ^c	4.38 ± 0.35 ^b	3.42 ± 0.30 ^c	5.02 ± 0.42 ^a

Mean values ($n = 7$) ± SD^a, ^b, ^c In particular rows for Cd (Pb) under various Ca/Mg ratios are significantly different at $P = 0.05$

and succinic acids were present at much lower levels. Malic acid was below the detection limit in any studied system.

The changes in the content of LMWOAs were caused by the Ca/Mg ratio as well as the trace element in solution at 0.5 mM concentration. Under 0.5 mM Cd supplementation (Cd_{0.5}), the total concentration of LMWOAs, depending on the Ca/Mg ratio, decreased according to the formula $20:1 > 4:1 > 1:10 > 1:\frac{1}{4}$ and was 52.7, 45.2, 39.1 and 15.7 $\mu\text{M kg}^{-1}$ D.W., respectively. For the system under 0.5 mM Pb supplementation (Pb_{0.5}), the total concentration of LMWOAs decreased according to the formula $20:1 \approx 4:1 > 1:\frac{1}{4} > 1:10$ and was 29.8, 29.2, 26.9 and 13.5 $\mu\text{M kg}^{-1}$ D.W. Comparing all the experimental systems, it is important to underline that regardless of Ca/Mg ratio, the total concentration of LMWOAs in the rhizosphere was higher under Cd_{0.5} than Pb_{0.5}, and for systems under 1:10, 20:1 and 4:1 Ca/Mg ratio, it was 2.9, 1.8 and 1.5 times higher, respectively. The exception was the

experimental systems at $1:\frac{1}{4}$ Ca/Mg ratio. In this system, the total amount of LMWOAs was higher under 0.5 mM of Pb (26.9 $\mu\text{M kg}^{-1}$ DW) when compared to Cd supplementation (15.7 $\mu\text{M kg}^{-1}$ DW), and both of these values were lower than in the control system (27.7 $\mu\text{M kg}^{-1}$ DW). At $1:\frac{1}{4}$ Ca/Mg ratio, the total concentration of LMWOAs for the 0.5 mM Pb system was almost 1.7 times higher than for 0.5 mM Cd.

The analysis of the results clearly indicates that systems with one of the trace elements at a higher concentration show an influence on the level of analysed acids. Compared to the control system, these values were increased from 1.2 (Pb_{0.5}—control in 1:10 Ca/Mg) to 10.2 (Cd_{0.5}—control in 4:1 Ca/Mg) times. As mentioned earlier, the exception was the system under $1:\frac{1}{4}$ Ca/Mg. The total amount of LMWOAs under 0.5 mM Pb supplementation was similar to the control group, while under Cd supplementation, this value was 1.8 times lower in comparison to the control.

Table 5 The bioaccumulation factor (BAF) values in *S. viminalis* L. organs under different Pb^{2+} and Cd^{2+} concentrations and Ca/Mg ratio in the medium

Salix organ/system	Cd				Pb			
	20:1	4:1	1 : $\frac{1}{4}$	1:10	20:1	4:1	1 : $\frac{1}{4}$	1:10
Root								
Cd _{0.5}	0.040 ^(W)	0.063 ^(W)	0.028 ^(W)	0.067 ^(W)	0.276 ^(M)	0.389 ^(M)	0.237 ^(M)	0.431 ^(M)
Pb _{0.5}	0.292 ^(M)	0.324 ^(M)	0.269 ^(M)	0.354 ^(M)	0.117 ^(W)	0.140 ^(W)	0.109 ^(W)	0.162 ^(W)
Control	0.151 ^(M)	0.202 ^(M)	0.102 ^(M)	0.235 ^(M)	0.179 ^(M)	0.210 ^(M)	0.134 ^(M)	0.231 ^(M)
Shoot								
Cd _{0.5}	0.043 ^(W)	0.052 ^(W)	0.033 ^(W)	0.065 ^(W)	0.203 ^(M)	0.224 ^(M)	0.186 ^(M)	0.259 ^(M)
Pb _{0.5}	0.179 ^(M)	0.248 ^(M)	0.164 ^(M)	0.196 ^(M)	0.064 ^(W)	0.071 ^(W)	0.060 ^(W)	0.083 ^(W)
Control	0.166 ^(M)	0.182 ^(M)	0.159 ^(M)	0.243 ^(M)	0.174 ^(M)	0.212 ^(M)	0.165 ^(M)	0.243 ^(M)

Mean values ($n = 7$) ± SDM medium ($1 > \text{BAFs} > 0.1$) and W weak ($0.1 > \text{BAFs} > 0.01$) cadmium or lead ion accumulation

Efficiency of trace elements accumulation under different Ca/Mg ratios

In *S. viminalis* L., similar efficiency of Cd accumulation in roots and shoots was observed (Table 4). In the case of Pb, significantly higher efficiency of sorption was observed in the roots than in the shoots. However, the efficiency of Cd and Pb accumulation, regardless of the type of tested willow part, decreased according to the formula $1:10 > 4:1 > 20:1 > 1:1/4$ Ca/Mg ratio.

These results for both trace elements and both tested parts of *Salix* (roots and shoots), higher effectiveness of accumulation was observed in comparison to the control system. It can be assumed that the relationship observed in this study is affected by the higher concentration of the trace elements in the Knop medium. In the case of Cd sorption by the roots in Cd_{0.5} system, accumulation of this metal increased depending on the Ca/Mg ratio in the systems. This trend was not observed for the shoots in Pb_{0.5} system. Probably, the differences in the capacity for Cd sorption between the two parts of the willow were due to differences in the mobility of both metals in the plant with plant physiological fluids. In the case of Pb sorption, significant differences in accumulation for roots and shoots was observed under supplementation with 0.5 mM Pb solution.

Calculated values of bioaccumulation factors, presented in Table 5, indicated medium or weak accumulation of Cd and Pb ions depending on metals' concentration in Knop medium. BAFs values were from 0.354 to 0.028 (roots) and 0.248 to 0.033 (shoots) for Cd, and from 0.431 to 0.117 (roots) and 0.259 to 0.060 (shoots) for Pb. Weak accumulation of Cd by *Salix* roots and shoots only under the Cd_{0.5} system was observed. For the other systems (Pb_{0.5}, control), medium accumulation was observed. In the case of Pb sorption by roots and shoots, medium accumulation was observed with the exception of weak Pb sorption by tested *Salix* shoots growing under this element supplementation (Pb_{0.5}).

Statistical analysis

To compare the composition and the content of ten LMWOAs observed in the *Salix* rhizosphere under both Cd and Pb supplementation, multivariate analysis of variance was performed. Statistically significant differences for Cd_{0.5}–Pb_{0.5} contrast in concentration of all identified LMWOAs formed in the *Salix* root zone were observed, except for fumaric acid. In the case of Pb_{0.5}–c contrast, the same situation was observed, with the exception of malonic acid. For Cd_{0.5}–c contrast, a statistically significant difference was also observed, but in this contrast exceptions were formic, maleic and citric acids. Simultaneously, for

Table 6 Contrast analysis (Cd_{0.5}–c, Pb_{0.5}–c) for changes in concentration of LMWOAs in rhizosphere ($\mu\text{M kg}^{-1}$ D.W.) under 0.5 mM Pb or Cd and phytoaccumulation efficiency in roots and shoots

Parameter	Cd _{0.5} –c	Pb _{0.5} –c	Cd _{0.5} –Pb _{0.5}
Succinic acid	0.072*	0.143*	–0.071*
Citric acid	–0.343	1.505*	–1.848*
Fumaric acid	0.228*	0.228*	–0.001
Maleic acid	0.023	0.057*	–0.034*
Malonic acid	5.582*	–0.211	5.793*
Lactic acid	0.167*	0.257*	–0.090*
Formic acid	0.008	–0.074*	0.082*
Acetic acid	18.461*	8.799*	9.663*
Root	0.838*	1.538*	–0.700*
Shoot	0.605*	0.105*	0.500*

* Significant contrasts at $P = 0.05$

all analysed Ca/Mg ratio and presented contrasts (Cd_{0.5}–c, Pb_{0.5}–c), statistically significant differences were observed in Cd and Pb accumulation by roots and shoots. The contrast analysis, showing the effectiveness of acid exudation under supplementation of trace elements (0.5 mM) and metal accumulation in relation to the control system (c), is presented in Table 6.

These results indicate statistically significant differences under 20:1 and 4:1 Ca/Mg ratio for succinic, acetic, formic, lactic and malonic acids for both analysed contrasts (Pb_{0.5}–c and Cd_{0.5}–c). The opposite situation was observed for fumaric and citric acids, detected in the plant root zone under 1:1/4 and 1:10 Ca/Mg ratio. In other cases, statistically significant differences were observed sporadically, showing no particular trends (Supporting information). The presented statistical analyses showed that at four analysed Ca/Mg ratios and for analysed contrasts (Cd_{0.5}–c, Pb_{0.5}–c), significant differences were observed in accumulation of trace elements in roots and shoots.

Discussion

Salix, used in the experimental hydroponic system, is a plant/tree not recognized as a hyperaccumulator, but with a high ability to absorb and accumulate relatively high amounts of trace elements, such as Zn, Cd, Cu, Pb and Ni (Magdziak et al. 2011; Mleczek et al. 2009, 2011; Tlustoš et al. 2007). Exudation of LMWOAs has been put forward as one of the major mechanisms by which fungi (Johansson et al. 2008a, 2009) and plants (Gasecka et al. 2012; Magdziak et al. 2011; Mucha et al. 2010) resist metal toxicity. The individually determined as well as total concentration of LMWOAs in the *Salix* rhizosphere under 0.5 mM Cd or Pb supplementation explains the high accumulation ability

of tested *Salix* taxa in relation to other plants (unpublished data). Under 0.5 mM Cd supplementation, a higher level of total concentration of LMWOAs was observed than under 0.5 mM Pb. This difference may be explained in two ways.

LMWOAs exuded by *Salix* roots into the rhizosphere play an important role in the mobilization and bioavailability of Cd and Pb to plants due to pH decreasing and metal–ligand complexation in the root zone (Gidarakos and Giannis 2006). The soluble form of Cd^{2+} used in every experimental system is highly mobile and becomes more mobile at pH below 7 (because of excellent solubility) (Johansson et al. 2008a; Kabata-Pendias and Pendias 1999), which makes it more prone (than Pb) to toxicity. In the described hydroponic experiments, pH of the quartz sand solution was 5.7. The exudates (LMWOAs) from the root, which were detected in rhizosphere of *Salix*, were found to transform Cd and Pb species in rhizosphere into bioavailable forms, similar to lettuce seedlings under higher concentration of Pb (Liao et al. 2006) or Cd for *Kandelia candel* (L.) (Haoliang et al. 2007; Lin et al. 2001; Wang et al. 2009). What is more, LMWOAs found in the *Salix* root zone environment comprise mono-, di- and tri-carboxylic acids and they function as ligands increasing trace element desorption from quartz sand, thus enhancing its mobility by complexing of metal cations (Strobel 2001). Therefore, Cd and Pb from the experimental environment by LMWOAs can reasonably be indicative of its bioavailability to *Salix* seedlings. LMWOAs may form complexes with ions of Cd and Pb through their carboxyl groups ($-\text{COO}$). Acetate can only form monodentate complexes with metals, while malonate, oxalate and citrate carrying two or three $-\text{COO}$ groups can form chelates with five- or six-membered ring structure which are more stable than monodentate complexes, hence leading to enhanced Cd desorption from the quartz sand (Wang et al. 2009).

On the other hand, efficiency of LMWOAs exudation by *Salix* roots depends as well as on the physico-chemical properties and higher concentration of studied metals. Cd and Pb, used in our studies, have some diverse properties as specific transporters (Cosio et al. 2006; Sharma and Dietz 2009; Sharma and Dubey 2005), which need to be considered to define the effect of these metals on concentrations of analysed acids (Johansson et al. 2008b). For this reason, the theory is that higher concentration of total LMWOAs under Cd 0.5 mM supplementation was due to the fact that free Cd^{2+} could have destroyed the physiological barriers in roots by removing Fe^{2+} , Ca^{2+} and other divalent cations from the plasma membrane, which is thought to be important for the root selectivity properties (Luo et al. 2005), more than free Pb^{2+} ions. It seems that our results confirm the above theory.

It is also worth underlining that similar increased efficiency of Cd and Pb accumulation in shoots was observed

for LMWOAs as well as EDTA addition. This may indicate that EDTA not only increases Pb mobility of soluble complexes but also makes easier transport of the complexes via membranes to roots and later to shoots. The above-mentioned improved mobility of the metal in the plant (Vassil et al. 1998) is the result of damage of the physiological barriers in roots (controlling uptake and translocation of solutes) by chelating EDTA to active sites in the membranes (Williams et al. 2006). The results obtained in our experiments indicate that LMWOAs secreted by *Salix* roots at relatively high concentration may have a similar effect. In addition, metals themselves can damage the membrane (Kaszuba and Hunt 1990; Pasternak 1987) and may confer random metal complexes of quartz sand–solution access to root xylem and to the shoot via the transpiration stream (Vassil et al. 1998), resulting in plant necrosis. This theory could also explain why *S. viminalis* L. seedlings growth was better and higher accumulation was observed under Pb supplementation (Duquène et al. 2009; Evangelou et al. 2006).

Earlier published data show the influence of Ca/Mg levels on alleviation of phytotoxicities of Mn (Robson and Loneragan 1970), Ni (Gabrielli and Pandolfini 1984), Pb (Jowett 1964) and Zn (Simon and Lefebvre 1977), but quantitative studies of the alleviation of Pb and Cd toxicity and exudation of LMWOAs by either Ca or Mg in higher plants are to our knowledge unknown. Petersen and Bottger (1991) reported that natural organic acids secreted into the rhizosphere influenced acidification of the root zone. Higher concentrations of analysed LMWOAs in hydroponic systems decreased the pH of sand, and solubility and mobility of particular elements, which influenced reduction of the trace elements' adsorption by structural elements of the sand and increased the concentrations of soluble forms of metals and bioavailability to the plant (Harter 1983). Such a situation was observed for three analysed systems under 1:10, 4:1 and 20:1 Ca/Mg ratio, when the amount of secreted LMWOAs was always higher under 0.5 mM Cd or Pb supplementation, when compared to the control system. In analyses of root exudates of *Salix* seedlings, growing under hydroponic conditions, roots of willow exuded relatively high amounts of LMWOAs to complexation (chelating) ions of studied trace elements, reducing the pH of quartz sand used in the study as well as increasing their bioavailability to the plants (Hammer and Keller 2002; Zhao et al. 2000). The changes in the root zone, in consequence, resulted in increased Cd and Pb phytoaccumulation efficiencies of studied parts of *S. viminalis* L. (Liao et al. 2006). A different situation was observed for the system with 1:1/4 Ca/Mg ratio, when the concentration of LMWOAs in the rhizosphere under 0.5 mM Cd and Pb supplementation was lower than in the control system, which confirms the above-mentioned Ca versus Cd

competition at higher Ca concentration. In conclusion, the obtained results in the hydroponic system, higher concentrations of various trace elements and Ca/Mg ratio suggest that both factors have a significant impact on the LMWOAs exuded by roots.

The above results confirm previous reports on the relationship between the amount of LMWOAs secreted by *Salix* under higher supplementation of trace elements (Drzewiecka et al. 2012; Gasecka et al. 2012) as well as the influence of the Ca/Mg ratio on the increased acid biosynthesis (Magdziak et al. 2011). Also, significant differences in composition of analysed LMWOAs revealed an essential influence of Ca and Mg on acid secretion. According to the literature data, the relationship between both macroelements (Cieslinski et al. 1998) indicates that dependence in composition between these compounds results in effectiveness of the complexation reaction and then in formation of the metal complexed form.

Our study indicates that the contents of LMWOAs in the *S. viminalis* L. rhizosphere is influenced by both 0.5 mM trace elements (Cd^{2+} , Pb^{2+}) supplementation and four Ca/Mg ratios (4:1, 20:1, 1:10 and 1:1/4) in experimental hydroponic systems, which strongly supports our idea that the above factors (LMWOA concentration levels, presence of Cd, Pb and Ca/Mg ratio) are significantly related. Both of these factors have a significant influence on the composition and content of analysed organic acids present in the root zone of *Salix*. A higher concentration of LMWOAs was always observed for systems, where the concentration of Cd or Pb was higher in comparison to the control system (with the exception of the 1:1/4 Ca/Mg ratio system). It also indicates that the addition of Pb or Cd at higher concentration stimulates exudation of LMWOAs into the rhizosphere.

Moreover, the results show that *S. viminalis* L. was able to accumulate high amounts of Cd and Pb, which suggests that probably due to very strong adsorption to roots and other plant tissues, the element is not transported to ribosomes and in consequence is not able to denature macromolecules responsible for genetic information, which works in *Salix* as an internal, particular mechanism for metal detoxification (Mucha et al. 2010). It has to be underlined that the above changes of LMWOA concentration levels caused by the Cd and Pb presence and Ca/Mg ratio was always accompanied by significant morphological changes, i.e. decreased length of roots, shoots and leaf surface area but with no evidence of necrotic symptoms, which may confirm the above conclusion about the mechanism.

It is the first time, to our knowledge, that data are presented about LMWOA secretion as a response to Cd and Pb contamination and depending on Ca/Mg ratio.

Conclusions

The effectiveness of LMWOA secretion significantly depends on both which trace element is present in the medium and what its concentration levels are. For each metal, the total amount of LMWOAs decreased according to a similar formula, which suggests a significant influence of both Ca/Mg ratio and trace element supplementation on the accumulation efficiency. The efficiency of Cd and Pb accumulation by roots or shoots was generally higher in $\text{Cd}_{0.5}$ and $\text{Pb}_{0.5}$ systems than in the control, which may suggest synergistic effects of both elements on their phytoextraction by the plant from the soil.

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References

- Barceló J, Poschenrieder C (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium resistance and tolerance: a review. *Environ Exp Bot* 48:75–92
- Baziramakenga R, Simard RR, Leroux GD (1995) Determination of organic acids in soil extracts by ion chromatography. *Soil Biol Biochem* 27:349–356
- Cawthray GR (2003) An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. *J Chromatogr A* 1011:233–240
- Chen YH, Li XD, Shen ZG (2004) Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. *Chemosphere* 57:187–196
- Cieslinski G, Van Rees KCJ, Szmigielska AM, Krishnamurti GSR, Huang PM (1998) Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on Cd bioaccumulation. *Plant Soil* 203:109–117
- Cosio C, Vollenweider P, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.) I. Macrolocalization and phytotoxic effects of cadmium. *Environ Exp Bot* 58:64–74
- Dessureault-Rompré J, Luster J, Schulin R, Tercier-Waeber ML, Nowack B (2010) Decrease of labile Zn and Cd in the rhizosphere of hyperaccumulating *Thlaspi caerulescens* with time. *Environ Pollut* 158:1955–1962
- Drzewiecka K, Mleczek M, Gasecka M, Magdziak Z, Goliński P (2012) Changes in *Salix viminalis* L. cv. ‘Cannabina’ morphology and physiology in response to nickel ions—hydroponic investigations. *J Hazard Mater* 217–218:429–438
- Duquène L, Vandenhove H, Tack F, Meers E, Baeten J, Wannijn J (2009) Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments. *Sci Total Environ* 407:1496–1505

- Evangelou MWH, Daghan H, Schaeffer A (2004) The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere* 57:207–213
- Evangelou MWH, Ebel M, Schaeffer A (2006) Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. *Chemosphere* 63:996–1004
- Gabrielli R, Pandolfini T (1984) Effect of Mg^{2+} and Ca^{2+} on the response to nickel toxicity in a serpentine endemic and nickel-accumulating species. *Physiol Plant* 62:540–544
- Gasecka M, Mleczek M, Drzewiecka K, Magdziak Z, Rissmann I, Chadzinikolau T, Golinski P (2012) Physiological and morphological changes in *Salix viminalis* L. as a result of plant exposure to copper. *J Environ Sci Heal A* 47:33–40
- Gidakos E, Giannis A (2006) Chelate agents enhanced electrokinetic remediation for removal cadmium and zinc by conditioning catholyte pH. *Water Air Soil Pollut* 172:295–312
- Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. Wiley, New York
- Gonzaga MIS, Ma LQ, Santos JAG, Matias MIS (2009) Rhizosphere characteristics of two arsenic hyperaccumulating *Pteris* ferns. *Sci Total Environ* 407:4711–4716
- Hammer D, Keller C (2002) Changes in the rhizosphere of metal-accumulating plants evidenced by chemical extractants. *J Environ Qual* 31:1561–1569
- Haoliang L, Chongling Y, Jingchun L (2007) Low-molecular-weight organic acids exuded by Mangrove (*Kandelia candel* (L.) Druce) roots and their effect on cadmium species change in the rhizosphere. *Environ Exp Bot* 61:159–166
- Harter RD (1983) Effect of soil pH on adsorption of lead, copper, zinc and nickel. *Soil Sci Soc Am J* 47:47–51
- Hinsinger P, Elsass F, Jaillard B, Robert M (1993) Root-induced irreversible transformation of a trioctahedral mica in the rhizosphere of rape. *J Soil Sci* 44:0535–0545
- Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2008a) Quantitative analysis of exudates from soil-living basidiomycetes in pure culture as a response to lead, cadmium and arsenic stress. *Soil Biol Biochem* 40:2225–2236
- Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2008b) Quantitative analysis of root and ectomycorrhizal exudates as a response to Pb, Cd and As stress. *Plant Soil* 313:39–54
- Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2009) Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO_2 . *Soil Biol Biochem* 41:1111–1116
- Jones DL (1998) Organic acids in the rhizosphere—a critical review. *Plant Soil* 205:25–44
- Jowett D (1964) Population studies on lead-tolerant *Agrostis tenuis*. *Evolution* 18:70–81
- Kabata-Pendias A, Pendias H (1999) Biogeochemia pierwiastków śladowych. Biogeochemistry of Trace Elements, 3rd edn. Wyd Naukowe PWN, Warsaw (in Polish)
- Kaszuba M, Hunt GRA (1990) Protection against membrane damage: a H NMR investigation of the effect of Zn^{2+} and Ca^{2+} on the permeability of phospholipid vesicles. *J Inorg Biochem* 40:217–225
- Kidd PS, Llugany M, Poschenrieder C, Gunsé B, Barceló J (2001) The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *J Exp Bot* 52:1339–1352
- Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. *Environ Exp Bot* 67:243–259
- Li TQ, Yang XE, Yang JY, He ZL (2006) Zn accumulation and subcellular distribution in the Zn hyperaccumulator *Sedum alfredii* Hance. *Pedosphere* 16:616–623
- Li T, Di Z, Yang X, Sparks DL (2011) Effects of dissolved organic matter from the rhizosphere of the hyperaccumulator *Sedum alfredii* on sorption of zinc and cadmium by different soils. *J Hazard Mater* 192:1616–1622
- Liao YC, Chien SW, Wang MC, Shen Y, Hung PL, Das B (2006) Effect of transpiration on Pb uptake by lettuce and on water soluble low molecular weight organic acids in rhizosphere. *Chemosphere* 65:343–351
- Lin Q, Chen YX, Chen HM, Yu YL, Luo YM, Wong MH (2001) Chemical behavior of Cd in rice rhizosphere. *Chemosphere* 50:755–761
- Lu LL, Tian SK, Yang XE, Wang XC, Brown P, Li TQ, He ZL (2008) Enhanced root to shoot translocation of cadmium in the hyperaccumulating ecotype of *Sedum alfredii*. *J Exp Bot* 59:3203–3213
- Luo C, Shen Z, Li X (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* 59:1–11
- Magdziak Z, Kozłowska M, Kaczmarek Z, Mleczek M, Chadzinikolau T, Drzewiecka K, Golinski P (2011) Influence of Ca/Mg ratio on phytoextraction properties of *Salix viminalis*. II. Secretion of low molecular weight organic acids to the rhizosphere. *Ecotox Environ Safe* 74:33–40
- Maturi K, Reddy KR (2008) Extractants for the removal of mixed contaminants from soils. *Soil Sediment Contam* 17:586–608
- Mleczek M, Magdziak Z, Rissmann I, Golinski P (2009) Effect of different soil conditions on selected heavy metal accumulation by *Salix viminalis* tissues. *J Environ Sci Heal A* 44:1–8
- Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K, Strażyńska K, Stachowiak A (2010) Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. *Biomass Bioenerg* 34:1410–1418
- Mleczek M, Kozłowska M, Kaczmarek Z, Magdziak Z, Golinski P (2011) Cadmium and lead uptake by *Salix viminalis* under modified Ca/Mg ratio. *Ecotoxicology* 20:158–165
- Mucha AP, Almeida CMR, Bordalo AA, Vasconcelos MTSD (2010) LMWOA (low molecular weight organic acid) exudation by salt marsh plants: natural variation and response to Cu contamination. *Estuar Coast Shelf S* 88:63–70
- Ösretás AH, Greger M (2006) Interactions between calcium and copper or cadmium in Norway spruce. *Biol Plant* 50:647–652
- Parker DR, Pedler JF, Thomason DN, Li H (1998) Alleviation of copper rhizotoxicity by calcium and magnesium at defined free metal-ion activities. *Soil Sci Soc Am J* 62:965–972
- Pasternak CA (1987) A novel form of host defense: membrane protection by Ca^{2+} and Zn^{2+} . *Biosci Rep* 7:81–91
- Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS (2005) Phytoremediation and hyperaccumulator plants. In: Klomp LWJ, Martinoia E, Tamás MJ (eds) Molecular biology of metal homeostasis and detoxification: from microbes to man. Top Curr Gen. Springer-Verlag, New York
- Petersen W, Bottger M (1991) Contribution of organic acids to the acidification of the rhizosphere of maize seedlings. *Plant Soil* 132:159–163
- Pilon-Smits E (2005) Phytoremediation. *Annu Rev Plant Biol* 56:15–39
- PN-ISO 10390 (1997) Soil quality. Determination of pH
- PN-ISO 1265+AC1 (1997) Soil quality. Determination of electrolytic conduction
- Puschenreiter M, Stoger G, Lombi E, Horak O, Wenzel WW (2001) Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: rhizosphere manipulation using EDTA and ammonium sulfate. *J Plant Nutr Soil Sci* 164:615–621
- Renella G, Landi L, Nannipieri P (2004) Degradation of low molecular weight organic acids complexed with heavy metals in soils. *Geoderma* 122:311–315

- Robson AD, Loneragan JF (1970) Sensitivity of annual *Medicago* species to manganese toxicity as affected by calcium and pH. *Aust J Agric Res* 21:223–232
- Sharma SS, Dietz K-J (2009) The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci* 14:43–50
- Sharma P, Dubey RS (2005) Lead toxicity in plants. *Braz J Plant Physiol* 17:35–52
- Shen ZG, Li XD, Wang CC, Chen HM, Chua H (2002) Lead phytoextraction from contaminated soil with high-biomass plant species. *J Environ Qual* 31:1893–1900
- Simon E, Lefèbvre C (1977) Aspects de la tolerance aux metaux lourds chez *Agrostis tenuis* Sibth., *Festuca ovina* L. et *Armeria maritima* (Mill.). *Willd Oecol Plant* 12:95–110
- Strobel BW (2001) Influence of vegetation on low-molecular-weight carboxylic acids in soil solution—a review. *Geoderma* 99:169–198
- Tao S, Liu WX, Chen YJ, Xu FL, Dawson RW, Li BG, Cao J, Wang XJ, Hu JY, Fang JY (2004) Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil. *Environ Pollut* 129:5–12
- Tian SK, Lu LL, Yang XE, Labavitch JM, Huang YY, Brown P (2009) Stem and leaf sequestration of zinc at the cellular level in the hyperaccumulator *Sedum alfredii*. *New Phytol* 182:116–126
- Tlustoš P, Száková J, Vysloužilová M, Pavlíková D, Weger J, Javorská H (2007) Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* spp. grown in contaminated soils. *Cent Eur J Biol* 2:254–275
- Uren NC, Reisenauer HM (1988) The role of root exudates in nutrient acquisition. In: Tinker PB, Lauchli A (eds) *Advances in plant nutrition*, 3. Praeger, New York, pp 79–114
- Vassil AD, Kapulnik Y, Raskin I, Salt DE (1998) The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol* 117:447–453
- Wang H, Zhong G (2011) Effect of organic ligands on accumulation of copper in hyperaccumulator and nonaccumulator *Commelina communis*. *Biol Trace Elem Res* 143:489–499
- Wang H, Jia Y, Wanga S, Zhua H, Wua X (2009) Bioavailability of cadmium adsorbed on various oxides minerals to wetland plant species *Phragmites australis*. *J Hazard Mater* 167:641–646
- Watanabe T, Osaki M (2002) Mechanisms of adaptation to high aluminium conditions in native plant species growing in acid soils: a review. *Commun Soil Sci Plant Anal* 33:1247–1260
- Williams AC, Nascimento W, Amarasiriwardena D, Xing B (2006) Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. *Environ Pollut* 140:114–123
- Yang X, Li T, Yang J, He Z, Lu L, Meng F (2006) Zinc compartmentation in root, transport into xylem, and absorption into leaf cells in the hyperaccumulating species of *Sedum alfredii* Hance. *Planta* 224:185–195
- Zalesny RS Jr, Wiese AH, Bauer EO, Riemenschneider DE (2006) Sapflow of hybrid poplar (*Populus nigra* L. × *P. maximowiczii* A. Henry “NM6”) during phytoremediation of landfill leachate. *Biomass Bioenerg* 30:784–793
- Zhao FJ, Lombi E, Breedon T, McGrath SP (2000) Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant Cell Environ* 23:507–514
- Zupančič MJ, Pajk N, Zupanc V, Zupančič M (2010) Phytoremediation of landfill leachate and compost wastewater by irrigation of *Populus* and *Salix*: biomass and growth response. *Waste Manage* 30:1032–1042