

# Regulation of macro, micro, and toxic element uptake by *Salix × smithiana* using liming of heavily contaminated soils

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Received: 30 September 2015 / Accepted: 17 November 2015 / Published online: 26 November 2015  
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## Abstract

**Purpose** Willow cultivation in soils heavily contaminated by risk elements is a challenging issue due to phytotoxic effects that restrict plant growth. Liming reduces the mobility of some risk elements in contaminated soils and therefore can be a suitable measure for contaminated soils but can also affect availability of nutrients for planted willows. We investigate how liming affects concentrations of macro, micro, and toxic elements in the organs of willows planted in contaminated soils. **Materials and methods** We established a 3-year pot experiment with *Salix × smithiana* planted in weakly acid and alkaline soils anthropogenically seriously contaminated by As, Cd, Pb, and Zn. Soils were both untreated and treated with two doses of lime and dolomite in the first year before planting. We determined biomass production, mortality, and the concentration of macro- and micronutrients and toxic elements in the willows' aboveground organs. **Results and discussion** Lime application increased biomass production in both soils; dose of lime played an important role for its increase only in alkaline soil. Lime in a higher dose was incompatible with the vitality of just-planted willows in both

soils. Doses of dolomite significantly affected the biomass production and mortality of willows, where lower doses caused a permanent decrease of biomass production and mortality in weakly acid soil. The toxicity of Cd and Zn in leaves was recorded in both untreated soils; the latent deficiency of P and deficiency of Fe in leaves was only recorded in weakly acid untreated soil.

**Conclusions** Lime application irrespective of dose with foliar Fe application seemed to be the most suitable measure for increasing biomass production and decreasing toxic elements, especially Cd and Zn, without decreasing the macro- and micronutrients in the aboveground organs of willows in weakly acid soil. In alkaline soil, only higher doses of lime had a positive effect on the studied parameters. Dolomite application is not a suitable measure for planting willows in both contaminated soils. Dolomite in a lower dose impairs the growth of willows in weakly acid soil.

**Keywords** Biomass production · Dolomite · Plant mortality · Quick lime · Silky-leaf osier

Responsible editor: Maria Luisa Andrade

**Electronic supplementary material** The online version of this article (doi:10.1007/s11368-015-1310-4) contains supplementary material, which is available to authorized users.

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## 1 Introduction

A high concentration of risk elements in soil is potentially toxic for most plants and can lead to their poor and irregular growth and development or even can cause their mortality (Nagajyoti et al. 2010; Solanki and Dhankhar 2011; Leitenmaier and Küpper 2013). Because of the insufficient vegetation cover of extremely contaminated areas, risk elements can be spread to the surroundings by wind or water erosion and can be leached off (Ruttens et al. 2006; Friesl-Hanl et al. 2009; Bolan et al. 2014).

Chemophytostabilisation appears as one of the most promising temporary phytoremediation techniques for heavily

contaminated soils for several risk elements (Alkorta et al. 2010). The application of soil additives to reduce the mobility and bioavailability of risk elements in soil (i.e. in situ chemical immobilisation) is the first step of chemophytostabilisation (Kumpiene et al. 2008; Alkorta et al. 2010). Consequently, it is possible to grow plants that are tolerant to risk elements in stabilised soils and to utilise the site aesthetically and economically (Tlustoš et al. 2007; Friesl-Hanl et al. 2009; Alkorta et al. 2010). Liming of the contaminated soils can reduce the bioavailability of risk elements and is the most widely used remediation treatment (Lee et al. 2004). Quick lime can cause a large increase of soil pH and thus immobilise risk elements in soil (Alkorta et al. 2010). Dolomite can immobilise risk elements by weak pH changes and increased adsorption to the surface of dolomite (Bolan and Duraisamy 2003).

Possible differences in risk element mobility are not only affected by a wide range of soil parameters (e.g. soil pH, the quantity and quality of organic matter, cation exchange capacity, soil texture, or soil type) (Vácha et al. 2002; Kunhikrishnan et al. 2012; Alloway 2013) or by the properties of the risk elements themselves; soil additives, their application doses, and the period under consideration can also play important roles. Soil additives affect not only toxic element mobility but also the macro- and micronutrients availability in the soil. Unsuitable soil additives or improper application doses can reduce the macro- and micronutrient uptake by plants, induce their deficiency, prevent plants from growing in contaminated sites, and thus make the utilisation of soils impossible (Bolan and Duraisamy 2003; Tlustoš et al. 2007; Friesl-Hanl et al. 2009).

There are a variety of plants tolerant to risk elements (Baker 1981; Sheoran et al. 2011). Considerable attention in terms of phytoremediation is focused on fast-growing trees, especially willows (*Salix* spp., *Salicaceae* family) (Pulford and Watson 2003; Meers et al. 2007). This focus is connected with the worldwide spread of willows, with their high biomass production and their ability to tolerate or accumulate risk elements in biomass, and consequently with their possible utilisation for phytoextraction or phytostabilisation (Pulford and Watson 2003; Meers et al. 2007; Tlustoš et al. 2007; Abhilash et al. 2012). Specific willow clones (e.g. *Salix* × *smithiana* Willd.) are able to grow and accumulate substantial concentrations of mobile elements such as Cd and Zn in their aboveground biomass in moderately contaminated soils (Vysloužilová et al. 2003b; Dos Santos Utmazian et al. 2007; Tlustoš et al. 2007). In contrast, the biomass production of willows is limited in heavily contaminated soils because of Cd and Zn phytotoxicity (Vysloužilová et al. 2006; Jensen et al. 2009). Therefore, willows can be used for phytoremediation in slightly or moderately contaminated soils (Vysloužilová et al. 2003b; Jensen et al. 2009). In heavily contaminated soils, it is necessary to reduce the mobility of risk elements in the soil before planting

willows (Vysloužilová et al. 2003a; Lee et al. 2004; Puschenreiter et al. 2005; Tlustoš et al. 2006).

In our previous study (Vondráčková et al. 2013), the effect of lime and dolomite application on the Cd, Zn, Pb, As, Fe, and Mn mobility was studied as the first step of chemophytostabilisation in a system with heavily contaminated soils differing in the soil parameters. Dolomite application was less effective than lime at decreasing element mobility, and only at higher doses. Liming only reduced Cd and Zn mobility in weakly acid soil and was ineffective at reducing Pb and As mobility in slightly acidic and alkaline soils. In the present study, we deal with the long-term effects of liming on the concentrations of a wide range of elements, including macro- and micronutrients in willows as a main step of chemophytostabilisation. According to our knowledge, there is a gap in the literature on the long-term effect of liming on concentrations of macro- and micronutrients in the organs of willows. Using the same long-term anthropogenically contaminated soils as in our previous study, in this study, we asked how (1) biomass production of *S. smithiana*, (2) its mortality, and (3) the concentration of macro (Ca, Mg, K, P), micro (Cu, Fe, Mn, Ni), and toxic elements (Al, Cd, Cr, Pb, Zn) in the aboveground organs of willows were affected by the application of two doses of lime and dolomite in the three following years. We classified zinc as a toxic element, depending upon its total content and availability in the soil and its concentration in plant biomass. Using multivariate analysis, we also investigated relationship among the studied parameters.

## 2 Materials and methods

### 2.1 Soils

The specific characteristics of the two long-term heavily As-, Cd-, Pb-, and Zn-contaminated soils are given in previous studies (Hejzman et al. 2012, 2014; Vondráčková et al. 2014). The main chemical properties of the soils are as follows: (1) ‘Litavka Fluvisol’—354 mg As/kg (in all elements pseudo-total concentrations were extracted with aqua regia), 54 mg Cd/kg, 3305 mg Pb/kg, and 6172 mg Zn/kg,  $\text{pH}_{\text{CaCl}_2}$  6.5, cation exchange capacity (CEC) 109  $\text{mmol}_{(+)}\text{/kg}$ , and  $C_{\text{org}}$  3.6 %, and (2) ‘Malín Luvisol’—688 mg As/kg, 11 mg Cd/kg, 98 mg Pb/kg, and 1022 mg Zn/kg,  $\text{pH}_{\text{CaCl}_2}$  7.3, CEC 333  $\text{mmol}_{(+)}\text{/kg}$ , and  $C_{\text{org}}$  2.7 %. All the above-mentioned chemical properties represent soils before the establishment of the pot experiment, i.e. without the influence of plants, soil additives, and irrigation with deionised water.

### 2.2 Pot experiment

The 3-year experiment was established in May 2010. The whole soil profile of 5 kg of air-dried soil in each 5-L pot was mixed with nutrient solution, consisting of 0.1 g N as

NH<sub>4</sub>NO<sub>3</sub> per 1 kg of soil, and 0.032 g P and 0.08 g K as K<sub>2</sub>HPO<sub>4</sub> per 1 kg of soil. Application of quick lime (hereafter abbreviated as lime) in doses—7.3 g (dose 1) and 21.9 g (dose 2) per 1 kg of soil containing 686 g Ca/kg of material with pH<sub>CaCl2</sub> 12.0—and of dolomite in doses—21.6 g (dose 1) and 68.1 g (dose 2) per 1 kg of soil containing 220 g Ca/kg and 100 g Mg/kg of material with pH<sub>CaCl2</sub> 8.3—was performed after the application of the nutrient solution. Therefore, we set up a pot experiment with ten treatments each with four replications: LC Litavka control soil without any additive, LL1 Litavka soil with lime in dose 1, LL2 Litavka soil with lime in dose 2, LD1 Litavka soil with dolomite in dose 1, LD2 Litavka soil with dolomite in dose 2, MC Malín control soil without any additive, ML1 Malín soil with lime in dose 1, ML2 Malín soil with lime in dose 2, MD1 Malín soil with dolomite in dose 1, and MD2 Malín soil with dolomite in dose 2.

Subsequently after liming, one 20-cm-long cutting of *Salix × smithiana* Willd. (clone no. S-218, the silky-leaf osier, hybrid of *Salix viminalis* L. and *Salix caprea* L.) (Tlustoš et al. 2007; Zárubová et al. 2015) was planted in each pot (cutting was protruded approx. 1–2 cm above the surface of the soil). *Salix × smithiana* plants were grown in the pots for the three following years. The pots were regularly watered with deionised water to maintain the optimal moisture conditions for plant growth during the vegetation. Due to an Fe deficiency in the willows’ leaves (chlorosis) visible during the first growing season, foliar Fe application (6 % solution of Fe in the form of EDDHMPA—i.e. ethylenediamine (*o*-hydroxy-*p*-methylphenylacetic) acid) was regularly performed four times during vegetation (i.e. in July and August) from the second year. Before each growing season, all the dead plants were replaced by new one of the same clone.

At the end of each growing season before the leaves started to fall (i.e. September), plant biomass was harvested, divided into twigs and leaves, weighed for the determination of dry biomass (DM total—dry mass of twigs and leaves together, DM organ—dry mass of twigs and leaves separately), ground, and analysed. Concurrently, soil samples were collected from the whole soil profile of each pot.

### 2.3 Chemical analysis

At the end of each growing season, soil samples were extracted with 0.01 mol/L CaCl<sub>2</sub> (hereafter abbreviated as Ca; pH 5.9; mobile—CaCl<sub>2</sub>-extractable portions of elements) in a 1:10 ratio (*w/v*) (Tlustoš et al. 1994) and with 0.11 mol/L acetic acid (hereafter abbreviated as AA; pH 2.8; mobilisable—acid-extractable portions of elements) in a 1:20 ratio (*w/v*) (Quevauviller 1998). The CaCl<sub>2</sub>-extractable and acid-extractable concentrations of elements in soil extracts (Table S1, Electronic Supplementary Material) were determined using inductively coupled plasma-optical emission

spectrometry (ICP-OES, VARIAN Vista Pro, Varian, Australia; for P, Cu, Fe, Mn, Ni, Al, Cd, Cr, Pb, Zn) and flame atomic absorption spectroscopy (FAAS, VARIAN, SpectrAA-280, Australia; for Ca, Mg, and K). Soil pH was measured in a suspension of soil and 0.01 mol/L CaCl<sub>2</sub> (1:5, *w/v*; Table 1).

The total concentrations of elements in plant organs (twigs and leaves; air-dried at 60 °C and stainless steel milled) were determined with ICP-OES and FAAS after the dry ashing of the sample at 500 °C under atmospheric pressure (Mader et al. 1998). Certified reference material, NCS DC 73348 Bush Branches and Leaves, was applied for quality assurance of the analytical data.

### 2.4 Statistical analysis

All statistical analyses were performed using the Statistica 12.0 ([www.statsoft.com](http://www.statsoft.com)) and Canoco 4.5 (ter Braak and Šmilauer 2002) software. All data were checked for homogeneity of variance and normality (Levene and Shapiro–Wilk tests). Soil and biomass data did not meet assumptions for the use of ANOVA and thus were evaluated with the non-parametric Kruskal–Wallis test. We assessed the effects of (1) treatment and growing season on the soil pH, biomass production, mortality, and the concentration of

**Table 1** The effect of treatment on soil pH (mean±SE) at the end of the three growing seasons of the experiment

Variable	Treatment	Growing season		
		1st**	2nd**	3rd**
pH <sub>CaCl2</sub>	LC <sup>n.s.</sup>	6.0 <sup>bA</sup> ±0.02	6.2 <sup>bA</sup> ±0.04	6.1 <sup>bA</sup> ±0.06
	LL1 <sup>**</sup>	7.7 <sup>abA</sup> ±0.03	7.6 <sup>abAB</sup> ±0.03	7.4 <sup>abB</sup> ±0.03
	LL2 <sup>n.s.</sup>	7.9 <sup>acA</sup> ±0.01	7.8 <sup>acA</sup> ±0.02	7.7 <sup>aA</sup> ±0.07
	LD1 <sup>*</sup>	6.6 <sup>bcA</sup> ±0.02	6.7 <sup>bbB</sup> ±0.03	6.7 <sup>bcAB</sup> ±0.06
	LD2 <sup>*</sup>	6.6 <sup>bcA</sup> ±0.02	6.9 <sup>bcB</sup> ±0.07	6.7 <sup>bcAB</sup> ±0.04
	MC <sup>n.s.</sup>	7.3 <sup>abA</sup> ±0.03	7.2 <sup>abA</sup> ±0.02	7.2 <sup>abA</sup> ±0.01
	ML1 <sup>*</sup>	7.7 <sup>abA</sup> ±0.04	7.6 <sup>abAB</sup> ±0.04	7.4 <sup>abB</sup> ±0.02
	ML2 <sup>**</sup>	8.3 <sup>aA</sup> ±0.04	7.9 <sup>aAB</sup> ±0.02	7.6 <sup>acB</sup> ±0.02
	MD1 <sup>n.s.</sup>	7.4 <sup>abA</sup> ±0.03	7.3 <sup>abA</sup> ±0.02	7.3 <sup>abA</sup> ±0.02
	MD2 <sup>n.s.</sup>	7.3 <sup>abA</sup> ±0.01	7.4 <sup>abA</sup> ±0.03	7.3 <sup>abA</sup> ±0.03

According to the multiple comparisons of mean ranks, treatments within the growing season with the same letter (a–c) and growing seasons within the treatment with the same letter (A–B) were not significantly different LC Litavka control soil without any additive, LL1 Litavka soil with lime in dose 1, LL2 Litavka soil with lime in dose 2, LD1 Litavka soil with dolomite in dose 1, LD2 Litavka soil with dolomite in dose 2, MC Malín control soil without any additive, ML1 Malín soil with lime in dose 1, ML2 Malín soil with lime in dose 2, MD1 Malín soil with dolomite in dose 1, MD2 Malín soil with dolomite in dose 2

Calculated with the Kruskal–Wallis test, differences between treatments within the growing season and differences between the growing seasons within the treatment were not statistically significant (<sup>n.s.</sup>) or were significant at 0.05(\*) and 0.01 (\*\*) probability levels

elements in the soil and biomass and (2) organ on the concentration of elements in the biomass. After obtaining significant results from the Kruskal–Wallis test, we used multiple comparisons of mean ranks for the detection of significant differences between different treatments, growing year, and organs. The relationship between selected soil and biomass data was analysed by linear regression. A principal component analysis (PCA), in the Canoco 4.5 program, was applied to all collected data together (soil pH, total and organ biomass, mortality, and concentration of elements in the soil and biomass) in individual soils after three growing periods separately. We used the standardisation of species data because data of a different character were analysed together. The results were visualised in the form of a bi-plot ordination diagram in the CanoDraw program. The PCA is a multivariate method, useful for data presentation, because of overview formation over the correlations between all the analysed data and of showing general trends visible in one ordination diagram.

### 3 Results

#### 3.1 Soil pH

Soil pH was increased in all lime and dolomite treatments (LL1, LL2, LD1, LD2) in Litavka soil but in lime treatments with considerably higher effect (Table 1). On the other hand, soil pH was increased only in lime treatments (ML1 and ML2) in Malín soil.

#### 3.2 Biomass production

Total and organ (total/organ) biomass of *S. × smithiana* was significantly affected by treatment, soil, growing time (time), and additive (Table 2).

The leaf biomass tended to have higher biomass production than twig biomass in most of the treatments in the Litavka soil over time (Table 2). A significantly higher twig biomass than leaf biomass was recorded in the Malín soil (all treatments all together) over time. The twig to leaf biomass ratio was positively related to soil pH in both soils over time ( $r=0.413$ ,  $p<0.01$ ).

Total/organ biomass was significantly increased in the control (MC), lime (LL1, LL2, ML1, and ML2), and dolomite (LD1, LD2, MD1, and MD2) treatments over time (Table 2). A trend towards decreasing twig biomass was recorded in the control treatment (LC) over time.

In the Litavka soil, a tendency towards a decrease in total/organ biomass after the first year and a significant increase of total/organ biomass after the next two growing years were recorded in the lime (LL1 and LL2) treatments compared with the control treatment (LC, Table 2 and Fig. 1a, c, e). After the first year, biomass production was negatively related to soil

pH (total DM:  $r=-0.551$ ,  $p=0.018$ ; organ DM:  $r=-0.521$ ,  $p=0.027$ ) in the LC, LL1, and LL2 treatments all together. A significant decrease of total/organ biomass was recorded in the LD1 treatment in comparison to the LC treatment over time. In the Malín soil, all the dead plants after the first growing season and a significant increase of total/organ biomass after the next 2 years were recorded in the ML2 treatment in comparison to the MC treatment (Table 2 and Fig. 1b, d, f). After the last two growing seasons, biomass production was positively related to soil pH (total DM:  $r=0.666$ ,  $p<0.01$ ; organ DM:  $r=0.569$ ,  $p<0.01$ ) in the MC and ML2 treatments.

The application dose of lime only played an important role in the total/organ biomass in the Malín soil (i.e. an increase of biomass in ML2 treatment only occurred in the last 2 years) and the application dose of dolomite only played an important role in the organ biomass in the Litavka soil (i.e. decrease of biomass in the LD1 treatment).

#### 3.3 Plant mortality

Mean mortality of *S. × smithiana* was significantly affected by treatment, soil, and time (Table 3).

Higher mortality of cuttings was recorded in the Litavka soil than in the Malín soil and after the first season than after the following seasons (Table 3). Higher mortality of cuttings at the harvest of the first year tended to treatments with the highest pH in both soils (LL1, LL2, and ML2; Tables 1 and 3). The mortality of other cuttings was always recorded after winter in the Litavka soil (Table 3). All the dead plants were replaced.

Immediate planting of the cuttings after lime application in the higher dose (LL2 and ML2 treatments) was recorded as incompatible with willow development in both soils (Tables 2 and 3). Regular mortality of cuttings was recorded only in the treatment with dolomite applied in lower doses (LD1).

#### 3.4 Concentration of elements in biomass

The concentrations of Mg, Mn, Ni, Cr, Pb, and Zn were significantly affected by treatments, while the concentrations of P, Cd, Cr, Pb, and Zn were significantly affected by soil. The concentrations of P, Cu, Fe, Mn, Ni, Al, Cd, Cr, and Pb were significantly affected by time, the concentrations of Mg, Mn, Ni, and Zn were significantly affected by additive, and the concentrations of Ca, Mg, K, P, Fe, Mn, Ni, Al, Cd, Cr, and Zn differed between individual organs (Table S2, Electronic Supplementary Material).

##### 3.4.1 Macronutrients

No significant differences in Ca, Mg, or K concentrations in organs (leaves and twigs together) were recorded between

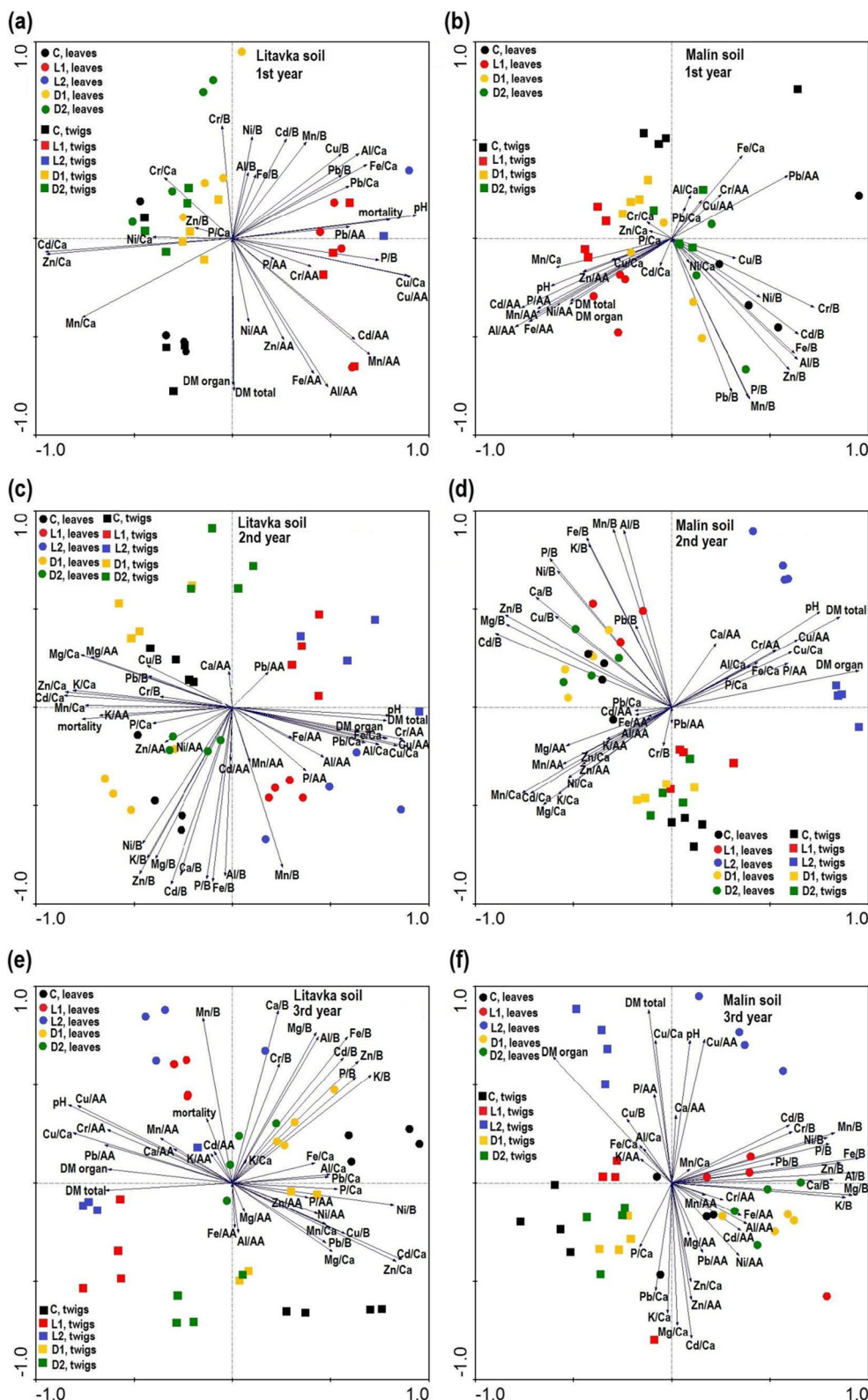
**Table 2** The effect of treatment on dry mass of organ biomass (DM organ; twig and leaf separately) and on dry mass of total biomass (DM total; twig and leaf together) of *S. smithiana* (g/plant; mean±SE) at the end of three growing seasons in the Litavka and Malin soils

Treatment	Growing season												
	1st			2nd			3rd			1st–3rd			
	DM organ**	Leaf**	Twig**	DM organ**	Leaf**	Twig**	DM total**	DM organ**	Leaf**	Twig**	DM total**	Mean DM organ**	Mean DM total**
LC	10.0 <sup>abA</sup> ±1.3	10.2 <sup>abA</sup> ±0.8	20.2 <sup>abA</sup> ±1.8	7.6 <sup>abA</sup> ±2.3	13.0 <sup>abA</sup> ±3.5	20.6 <sup>abA</sup> ±5.8	5.5 <sup>ba</sup> ±2.3	10.3 <sup>bcA</sup> ±3.2	15.8 <sup>abA</sup> ±5.5	7.7 <sup>b</sup> ±1.2	11.2 <sup>ac</sup> ±1.5	18.9 <sup>b</sup> ±2.6	
t <sup>n.s.</sup> , l <sup>n.s.</sup> , tot <sup>n.s.</sup>													
LL1	5.8 <sup>abB</sup> ±3.0	6.8 <sup>abB</sup> ±2.7	12.6 <sup>abB</sup> ±5.6	14.6 <sup>abAB</sup> ±3.0	19.8 <sup>abAB</sup> ±3.5	34.4 <sup>abAB</sup> ±6.4	32.2 <sup>abA</sup> ±1.3	33.6 <sup>ba</sup> ±2.2	65.8 <sup>bcA</sup> ±3.1	17.5 <sup>a</sup> ±3.6	20.1 <sup>a</sup> ±3.6	37.6 <sup>a</sup> ±7.1	
t*, l**, tot**													
LL2	2.6 <sup>abA</sup>	5.7 <sup>abA</sup>	8.3 <sup>abA</sup>	26.1 <sup>abA</sup> ±7.8	28.5 <sup>abA</sup> ±7.3	54.6 <sup>abA</sup> ±15.0	26.6 <sup>abA</sup> ±8.8	20.1 <sup>abA</sup> ±6.7	46.7 <sup>abA</sup> ±15.4	23.7 <sup>a</sup> ±5.5	22.2 <sup>a</sup> ±4.8	45.9 <sup>a</sup> ±10.0	
t <sup>n.s.</sup> , l <sup>n.s.</sup> , tot <sup>n.s.</sup>													
LD1	2.0 <sup>ba</sup> ±0.5	3.4 <sup>ba</sup> ±0.5	5.4 <sup>ba</sup> ±0.9	2.6 <sup>ba</sup> ±2.3	2.4 <sup>ba</sup> ±2.0	5.0 <sup>ba</sup> ±4.3	3.3 <sup>ba</sup> ±1.5	6.1 <sup>ba</sup> ±2.2	9.4 <sup>ba</sup> ±3.6	2.6 <sup>b</sup> ±0.9	4.0 <sup>c</sup> ±1.0	6.6 <sup>bc</sup> ±1.8	
t <sup>n.s.</sup> , l <sup>n.s.</sup> , tot <sup>n.s.</sup>													
LD2	1.7 <sup>ba</sup> ±0.5	2.2 <sup>bb</sup> ±0.7	3.8 <sup>bb</sup> ±1.1	10.1 <sup>abA</sup> ±2.2	10.7 <sup>abAB</sup> ±2.2	20.8 <sup>abAB</sup> ±4.4	17.7 <sup>abA</sup> ±5.6	18.8 <sup>abA</sup> ±4.5	36.5 <sup>abA</sup> ±9.8	9.8 <sup>b</sup> ±2.7	10.5 <sup>bc</sup> ±2.5	20.3 <sup>b</sup> ±5.2	
t <sup>n.s.</sup> , l*, tot*													
MC	8.1 <sup>abB</sup> ±0.2	6.4 <sup>abAB</sup> ±0.3	14.5 <sup>abB</sup> ±0.4	8.6 <sup>abAB</sup> ±0.6	6.4 <sup>abB</sup> ±0.5	15.0 <sup>abAB</sup> ±0.5	28.3 <sup>abA</sup> ±1.7	16.5 <sup>abA</sup> ±1.2	44.7 <sup>bcA</sup> ±2.5	15.0 <sup>b</sup> ±2.9	9.7 <sup>bc</sup> ±1.5	24.7 <sup>b</sup> ±4.3	
t*, l*, tot**													
ML1	15.0 <sup>abAB</sup> ±1.0	11.7 <sup>abAB</sup> ±0.8	26.7 <sup>abAB</sup> ±1.7	9.6 <sup>abB</sup> ±0.6	6.5 <sup>abB</sup> ±0.5	16.1 <sup>abB</sup> ±0.9	23.3 <sup>abA</sup> ±2.6	17.5 <sup>abA</sup> ±1.7	40.7 <sup>abA</sup> ±3.6	16.0 <sup>a</sup> ±1.9	11.9 <sup>ac</sup> ±1.5	27.8 <sup>a</sup> ±3.3	
t**, l**, tot*													
ML2	died	died	died	32.6 <sup>ab</sup> ±1.6	26.1 <sup>ba</sup> ±1.2	58.7 <sup>ab</sup> ±2.6	49.9 <sup>abA</sup> ±1.2	26.3 <sup>acA</sup> ±0.4	76.2 <sup>abA</sup> ±1.4	41.2 <sup>a</sup> ±3.4	26.2 <sup>a</sup> ±0.6	67.4 <sup>a</sup> ±3.6	
t*, l <sup>n.s.</sup> , tot*													
MD1	8.5 <sup>abB</sup> ±0.4	7.5 <sup>abAB</sup> ±0.5	16.0 <sup>abAB</sup> ±0.7	8.5 <sup>abAB</sup> ±0.6	5.6 <sup>abB</sup> ±0.9	14.1 <sup>abB</sup> ±1.3	23.3 <sup>abA</sup> ±2.6	15.8 <sup>abA</sup> ±2.1	39.1 <sup>abA</sup> ±4.6	13.4 <sup>b</sup> ±2.3	9.6 <sup>bc</sup> ±1.5	23.1 <sup>b</sup> ±3.7	
t*, l*, tot**													
MD2	11.8 <sup>abAB</sup> ±0.2	9.3 <sup>abAB</sup> ±0.2	21.0 <sup>abAB</sup> ±0.3	8.8 <sup>abB</sup> ±0.3	5.2 <sup>abB</sup> ±0.5	13.9 <sup>abB</sup> ±0.2	23.3 <sup>abA</sup> ±2.3	15.2 <sup>abA</sup> ±1.6	38.5 <sup>abA</sup> ±3.8	14.6 <sup>a</sup> ±2.0	9.9 <sup>bc</sup> ±1.3	24.5 <sup>bc</sup> ±3.3	
t**, l**, tot**													

See Table 1 for more details about the treatments. According to the multiple comparisons of mean ranks, treatments between the same organ within the growing season with the same letter (a–c) and growing seasons within the treatment between the same organ with the same letter (A–B) were not significantly different

Calculated with the Kruskal–Wallis test, differences between treatments of the same organ (i.e. twig, leaf, and total DM separately) within the growing season and differences between the growing seasons within the treatment of the same organ were not statistically significant (l<sup>n.s.</sup>) or were significant at 0.05(\*) and 0.01 (\*\*\*) probability levels

**Fig. 1** Ordination diagrams showing the results of the PCA analysis with element concentrations in organs of *S. smithiana* grown in contaminated soils at the end of growing season—1st (a, b); 2nd (c, d); 3rd (e, f). Treatment abbreviations: control C; lime application in doses (g/kg soil) L1—7.3, and L2—21.9, dolomite application in doses (g/kg soil) D1—21.6, and D2—68.1. Element abbreviations: /B total concentrations of elements in plant biomass, /Ca CaCl<sub>2</sub>-extractable concentrations of elements in soil (extracted with 0.01 mol/L CaCl<sub>2</sub>), /AA acid-extractable concentrations of elements in soil (extracted with 0.11 mol/L CH<sub>3</sub>COOH). Other abbreviations: DM total dry mass of twigs and leaves biomass together, DM organ dry mass of organ biomass (twigs and leaves biomass separately) and pH mean soil pH



soils (all treatments together); the concentration of P in organs was significantly higher in the Malin soil than in the Litavka soil. The concentration of P in leaves was positively related to acid-extractable concentrations of P in both soils over time

( $r=0.458, p<0.01$ ). The concentration of P in leaves was also negatively related to CaCl<sub>2</sub>-extractable and acid-extractable concentrations of Cd, Pb, and Zn (Ca: Cd  $r=-0.380, p<0.01$ ; Pb  $r=-0.268, p<0.01$ ; Zn  $r=-0.336, p<0.01$ ; AA:

**Table 3** The effect of treatment on mortality (%) of *S. smithiana* after winter in the Litavka and Malín soils

Treatment	Mortality				
	Growing season				
	1st	2nd	3rd	1st–3rd <sup>n.s.</sup>	2nd–3rd <sup>n.s.</sup>
LC	0	25	0	8*±8	12.5*±12.5
LL1	50	0	0	17*±17	0*±0
LL2	75 <sup>a</sup>	0	25	33*±22	12.5*±12.5
LD1	25	50	25	33*±8	37.5*±12.5
LD2	100	0	0	33*±33	0*±0
MC	0	0	0	0*±0	0*±0
ML1	0	0	0	0*±0	0*±0
ML2	100 <sup>a</sup>	0	0	33*±33	0*±0
MD1	0	0	0	0*±0	0*±0
MD2	0	0	0	0*±0	0*±0

See Table 1 for more details about the treatments. According to the multiple comparisons of mean ranks, treatments between growing seasons were not significantly different

Calculated with the Kruskal–Wallis test, differences between treatments between growing seasons were not statistically significant (<sup>n.s.</sup>)

<sup>a</sup> Mortality (%) at harvest

Cd  $r = -0.553$ ,  $p < 0.01$ ; Pb  $r = -0.578$ ,  $p < 0.01$ ; Zn  $r = -0.600$ ,  $p < 0.01$  in both soils over time.

There was no significant effect of time on concentrations of Ca, Mg, or K in organs (leaves and twigs together and all treatments in both soils together); the concentration of P in organs was significantly higher in the first year than in the second year.

The application of lime (LL1, LL2, ML1, and ML2 treatments) and of dolomite (LD1, LD2, MD1, and MD2 treatments) did not significantly affect the distribution of Ca, Mg, K, or P concentrations between organs (twig < leaf) in comparison to the control treatment in both soils (LC and MC).

The application dose of lime and of dolomite played no significant role in the potential change of the concentration of all macronutrients in the organs of the willows in both contaminated soils.

### 3.4.2 Micronutrients

No significant differences in Cu, Fe, Mn, or Ni concentrations in organs (leaves and twigs together) were recorded between soils (all treatments together).

The Cu concentration in organs (leaves and twigs together) was significantly lower in the first year than in the second year (all treatments in both soils together), the concentration of Fe in organs was significantly higher in the last growing season than in the first season, the concentration of Mn in organs was

significantly higher in the last 2 years than in the first year, and the concentration of Ni in organs was significantly lower in the last growing season than in the first 2 years.

The application of lime (the LL1, LL2, ML1, and ML2 treatments) and of dolomite (the LD1, LD2, MD1, and MD2 treatments) did not significantly affect the distribution of Cu, Fe, Mn, or Ni concentrations between organs (Cu: twig = leaf; Fe, Mn, and Ni: twig < leaf) in comparison to the control treatment in both soils (LC and MC).

The application dose of lime and of dolomite played no significant role in changing in Fe, Mn, and Ni concentrations in the organs of willows in any of the contaminated soils. The application dose of dolomite played an important role in changing in Cu concentration in the organs of the willows in Litavka soil.

### 3.4.3 Toxic elements

No significant differences in Al concentration in organs (leaves and twigs together) were recorded between soils (all treatments together); the concentrations of Cd, Pb, and Zn in organs were significantly higher in the Litavka soil than in the Malín soil; and the concentration of Cr in organs was significantly higher in the Malín soil than in the Litavka soil.

The concentrations of Al, Cd, and Cr in organs (leaves and twigs together) were significantly higher in the last growing season than in the first season (all treatments in both soils together); the concentrations of Cr and Pb in organs were significantly higher in the first year than in the second year; no significant differences in Zn concentration in organs were recorded between seasons.

In the Litavka soil, the application of lime (the LL1 and LL2 treatments) and of dolomite (the LD1 and LD2 treatments) did not significantly affect the distribution of Al, Cd, Cr, or Zn concentrations between organs (twig < leaf) in comparison to the LC treatment. The application of lime (the LL1 and LL2 treatments) significantly affected the distribution of Pb concentration between organs (twig = leaf) in comparison to the LC treatment (twig > leaf), and the application of dolomite (the LD1 and LD2 treatments) did not significantly affect the distribution of Pb concentration between organs (twig > leaf) in comparison to the LC treatment. In the Malín soil, the application of lime (the ML1 and ML2 treatments) and of dolomite (the MD1 and MD2 treatments) did not significantly affect the distribution of Al, Cd, Cr, or Zn concentrations between organs (Al, Cd, and Zn: twig < leaf; Cr: twig = leaf) in comparison to the MC treatment. The application of lime (the ML1 and ML2 treatments) and of dolomite (the MD1 and MD2 treatments) significantly affected the distribution of Pb concentration between organs (twig < leaf) in comparison to the MC treatment (twig = leaf).

The application dose of lime and of dolomite played no significant role in changing in Al, Cd, Cr, and Zn concentrations in the

organs of the willows in any of the contaminated soils. The application dose of dolomite played an important role in changing in Pb concentration in the organs of the willows in Litavka soil.

### 3.5 Results of the PCA analysis

In the Litavka soil, the first axis of the PCA analysis explained 31 % (Fig. 1a, c) and 26 % (Fig. 1e) of the variability of the analysed data; the first two axes explained 48 % (Fig. 1a), 50 % (Fig. 1c), and 42 % (Fig. 1e) of the variability of the analysed data; and the first four axes together explained 71 % (Fig. 1a), 74 % (Fig. 1c), and 63 % (Fig. 1e) of the variability of all the analysed data. In the Malín soil, the first axis of the PCA analysis explained 24 % (Fig. 1b), 28 % (Fig. 1d), and 22 % (Fig. 1f) of the variability of the analysed data; the first two axes explained 40 % (Fig. 1b), 47 % (Fig. 1d), and 38 % (Fig. 1f) of the variability of the analysed data; and the first four axes together explained 64 % (Fig. 1b), 63 % (Fig. 1d), and 60 % (Fig. 1f) of the variability of the analysed data.

The length and direction of the vectors of the parameters (soil pH, DM total/organ, mortality, and concentration of elements in the soil and biomass) indicate links among themselves with respect to the treatment. For example, the mortality of cuttings was positively correlated with soil pH at the first growing year (Fig. 1a; the angle between vectors was less than 90°) and was negatively correlated with soil pH at the second year in the Litavka soil (Fig. 1c). A long vector for a particular variable indicates a large effect on the results of the analysis. For example, there was low effect of mortality on all the analysed data after the third season in the Litavka soil (Fig. 1e), as the vector for mortality was very short.

In the Litavka soil, the first ordination axis divided marks for individual pots into the lime group on the right side and dolomite/control group on the left side of the diagram for the first two growing seasons (Fig. 1a, c). For the last growing year (Fig. 1e), the first ordination axis divided marks for individual pots into positive lime groups on the left side, dolomite in dose 2 in the middle, and negative dolomite in dose 1/control group on the right side of the diagram. This indicates a high effect of lime addition over time and the increasing efficiency of dolomite in dose 2 from the last season on the analysed data in the Litavka soil. In the Malín soil, the first ordination axis divided marks for individual pots into lime in the dose 2 group on the right (2nd year) or the upper side (3rd year) and lime in the dose 1/dolomite/control group on the left (2nd year) or the bottom side (3rd year) of the diagram (Fig. 1d, f). This indicates a high effect of lime in dose 2 on the analysed data in the Malín soil in the last two growing seasons. In the last two growing years in both soils (Fig. 1c–f), in contrast with the first year (Fig. 1a, b), the data for leaves (circles) were clearly separated from all marks for twigs (squares). This indicates a large effect of organs on all

recorded data in the last two growing seasons and a minimal effect in the first year in both soils.

## 4 Discussion

### 4.1 Biomass production

Higher leaf biomass in the slightly acidic Litavka soil and higher twig biomass in the alkaline Malín soil was connected with different soil pH and the original level of risk elements in both soils. Similar results (i.e. twig biomass > leaf biomass of willows in soil with higher pH and a lower level of risk elements) have been recorded by Tlustoš et al. (2007). A reduced twig biomass and stable low leaf biomass with visible symptoms of Zn toxicity inducing Fe chlorosis in the Litavka control soil over time was probably caused by extremely high concentrations of Cd and Zn in the leaves (36.5–73 mg Cd/kg; 2074–3488 mg Zn/kg) exceeding their normal levels in plants (0.05–2 mg Cd/kg, 10–150 mg Zn/kg) (Pugh et al. 2002). A decrease of leaf biomass over time recorded in other studies with heavily acidic contaminated soils (Vysloužilová et al. 2003b; Tlustoš et al. 2007) were not observed, which is probably because of foliar Fe application from the second growing season in our experiment. Moreover, foliar Fe application in combination with lime application seems to be an appropriate measure for increasing the biomass production of willows in slightly acidic heavily contaminated soils. In heavily acidic contaminated soils, it is necessary to immobilise the risk elements before planting willow cuttings, which has also been recorded by other authors (Vysloužilová et al. 2003a; Lee et al. 2004; Puschenreiter et al. 2005; Tlustoš et al. 2006).

Lime application irrespective of dose was the most suitable measure for increasing biomass production in the slightly acidic Litavka soil from the second season (i.e. after the reduction of the negative effect of the high soil pH induced by the lime application in the first year). Similar results (i.e. increased biomass production of willows and poplars after liming) were recorded by other authors (Tlustoš et al. 2006; Vamerali et al. 2009; Trakal et al. 2011). Dolomite application in the lower dose was recorded as the least suitable measure for increasing the biomass production in the slightly acidic Litavka soil. In the alkaline Malín soil, lime application in the higher dose was recorded as the most suitable measure for increasing biomass production because of the adequately high soil pH and the reduction of Ni, Cd, and Zn concentrations in organs, especially in the second growing year.

### 4.2 Plant mortality

Lower mortality of willows over time and tripled biomass production in the third season were recorded in the alkaline Malín control soil compared with the slightly acidic Litavka



control soil. This is in contrary to the results of Tahvanainen and Rytönen (1999), in which the optimum soil pH for cultivation of willows ranges from 5.5 to 6.5. This inconsistency is connected with the high concentration of risk elements in our soils (i.e. high soil pH helps to decrease risk element mobility and the mortality of plants and to increase biomass production) (Trakal et al. 2011).

Lime application in the higher dose in the slightly acidic Litavka soil and the alkaline Malín soil was incompatible with the vitality of just-planted cuttings due to the high soil pH. Therefore, an artificially increased soil pH ranging from 7.9 up to 8.3 is not appropriate for willows in the early stages of their growth. This finding is in contrary to the results of Hytönen and Kaunisto (1999), in which it was shown that willows require high soil pH for their good root development. This inconsistency is probably connected with the formation of free hydroxides released from lime that can burn the roots and the presence of high loads of risk elements in the soil which can be released into the soil due to the mineralisation of organic matter induced by the high dose of lime (Mühlbachová and Tlustoš 2006).

The dose of dolomite played an important role in the mortality of willows and in their growth. Dolomite application in the lower dose is not a suitable measure for increasing the growth of willows in slightly acidic contaminated soils because of their high mortality. The dolomite application in the higher dose is possibly a measure for better growth of willows in slightly acidic contaminated soils, but only from the third year because of its poor and gradual efficiency (Mayfield et al. 2004).

### 4.3 Concentration of elements in biomass

#### 4.3.1 Macronutrients

Concentrations of Ca, Mg, and K in leaves in both the contaminated control soils were in the range or higher than their foliar level for the optimal growth of willows in non-contaminated soils (4.5 g Ca/kg, 2–2.5 g Mg/kg, 8–18 g K/kg) (Jug et al. 1999). The concentration of P in leaves in the slightly acidic contaminated control soil was usually below or at foliar P content for the optimal growth of willows in non-contaminated soils (2.1 g P/kg) (Jug et al. 1999), which was probably caused by an insufficient supply of P in the soil (the value of 9 mg P/kg, determined with the Mehlich III extraction procedure, belongs to the category of low available concentration of P in soil, i.e. <50 mg P/kg in arable land). In heavily contaminated control soils differing in soil pH, there is no limit to the cultivation of willows due to Ca, Mg, and K deficiency in leaves. Latent deficiency of P can be a problem for willows grown in slightly acidic contaminated soils.

The higher concentration of P in leaves of willows grown in the Malín soil compared with the Litavka soil is connected

with the higher mobilisable concentration of P (i.e. acid-extractable) in the Malín soil and thus with the higher P availability for plants and due to negative interferences of P in leaves and high concentrations of Cd, Pb, and Zn in soil.

The concentration of P in twigs was higher after the first growing year than after the second year, which is probably due to the growth limitation in the first growing season and due to increased P fixation rate by Ca with time. Concentrations of other macronutrients in organs were stable throughout the experiment.

In both contaminated control soils, a tendency for higher transport of all macronutrients from twigs into leaves (i.e. into the most metabolically active organ with high nutrient requirements) (López-Lefebvre et al. 2001) was recorded. Lime and dolomite applications do not restrict transport from twigs into leaves of willows grown in any of the contaminated soils.

Concentrations of Ca, Mg, and K in leaves in most cases in both lime- and dolomite-treated soils were in the range or higher than their foliar level for the optimal growth of willows. The ML2 treatment with 0.9 g Mg/kg in leaves was the exception. This was probably due to a dilution effect because biomass production was quadrupled due to the lime application in the higher dose. The concentration of P in leaves in lime- and dolomite-treated slightly acidic soil was lower than the foliar P level for the optimal growth of willows because liming is not an appropriate measure for increasing the concentration of P in leaves in slightly acidic contaminated soil with an insufficient supply of P in the soil. The concentration of P in leaves in most of the cases in lime- and dolomite-treated alkaline soil was higher than the foliar P level for the optimal growth of willows. The ML2 treatment with 1.7 g P/kg in leaves was the exception and was probably because of the dilution effect. In heavily contaminated lime- and dolomite-treated soils differing in soil pH, there is no limit for the cultivation of willows due to Ca, Mg, and K deficiency in leaves. Latent deficiency of P also remains a problem for willows in lime- and dolomite-treated slightly acidic contaminated soils.

#### 4.3.2 Micronutrients

Concentrations of Cu and Fe in leaves in most of the cases in all of the contaminated control soils were in the range or slightly higher than their common foliar concentrations in willows grown in acidic non-contaminated soils (3.5–9.2 mg Cu/kg; 50–1524 mg Fe/kg) (Syso et al. 2014) with the exception of the LC treatment after the first growing year with 38 mg Fe/kg in leaves. The visible deficiency of Fe for willows (also confirmed by the deficiency level <40 mg Fe/kg) (Levy et al. 1999) was probably caused by the phytotoxicity of Zn (phytotoxicity level >100–500 mg Zn/kg) (Kabata-Pendias 2011). Similar results were recorded by other authors (Vysloužilová et al. 2003b; Tlustoš et al. 2007). Concentrations of Mn and Ni in leaves in both contaminated control soils were lower than their common

foliar concentrations in willows grown in acidic non-contaminated soils (168–779 mg Mn/kg, 5.3–13 mg Ni/kg) (Syso et al. 2014). The deficiency of Fe in leaves is a serious problem for the cultivation of willows in slightly acidic heavily contaminated soils and can be somewhat solved by foliar Fe application during vegetation.

Concentrations of Cu, Fe, and Mn in organs were lower after the first season than after the following seasons because of their precipitation by the high soil pH caused by liming in the first growing season. The higher Fe concentration in leaves after the last 2 years was also caused by foliar Fe application from the second growing season.

In both contaminated control soils, a tendency for the higher transport of Fe, Mn, and Ni from twigs into leaves was recorded. Lime and dolomite applications do not restrict their transport from the twigs into the leaves of willows grown in any of the contaminated soils. In the weakly acidic contaminated control soil, a tendency for restricted transport of Cu from twigs into leaves was recorded as well as in the lime and dolomite treatments. In the alkaline contaminated control soil, a tendency for higher transport of Cu from twigs into leaves was recorded as well as in the lime and dolomite treatments. Similar results (i.e. higher concentration of Cu in twigs than in leaves of willows in slightly acidic and soil slightly contaminated by Cd, Cu, and Zn and ambiguous transport of Cu in aboveground organs of willows in alkaline non-contaminated soil) have been recorded by Kacálková et al. (2015). Different agrochemical characteristics of contaminated soils could probably change the distribution of Cu in aboveground biomass.

Concentrations of Cu and Fe in leaves in most cases in the lime- and dolomite-treated soils were in the range or slightly higher than the common foliar concentrations in willows grown in acidic non-contaminated soils. The LL1 treatment with 28 mg Fe/kg in leaves, the LL2 treatment with 24.8 mg Cu/kg in leaves, and the LD1 treatment with 15.0–24.5 mg Cu/kg in the leaves of the willows were the exceptions. It is obvious from previous results that the lower dose of dolomite was connected with lower biomass production and higher mortality of willows in slightly acidic contaminated soil. Concentrations of Mn and Ni in leaves in lime- and dolomite-treated soils were lower than their common foliar concentrations in willows grown in acidic non-contaminated soils. The dolomite application in the lower dose is not an appropriate measure for the cultivation of willows in slightly acidic contaminated soils. The deficiency of Fe in leaves of willows in slightly acidic contaminated soils can be partially solved by lime application in combination with foliar Fe application during vegetation.

#### 4.3.3 Toxic elements

In the present study, we are not concerned with As concentration because willows are not suitable plants for As uptake and accumulation (Tlustoš et al. 2007).

Concentrations of Cd and Zn in leaves in both contaminated control soils were considerably higher (27.7–76.8 mg Cd/kg; 732–3488 mg Zn/kg) than their common foliar concentrations in willows grown in acidic non-contaminated soils (0.5 mg Cd/kg, 175–256 mg Zn/kg) (Syso et al. 2014). Concentrations of Al and Cr in leaves in both contaminated control soils were lower than their common foliar concentrations in willows grown in acidic non-contaminated soils: <100–200 mg Al/kg valid for general plant species (Watanabe and Osaki 2002), 153 mg Al/kg valid for *Salix* ‘Brekaviev’ (Vike 2005), and 1.1–6.4 mg Cr/kg (Syso et al. 2014). The concentration of Pb in leaves was higher in slightly acidic contaminated control soil and was lower in alkaline contaminated control soil than its common foliar concentration in willows grown in acidic non-contaminated soils (1.0–1.1 mg Pb/kg) (López-Lefebvre et al. 2001). In heavily contaminated control soils with different soil pH, cultivation of willows is limited by Cd and Zn toxicity, which induces Fe deficiency in leaves, especially in slightly acidic soil conditions.

The higher concentrations of Cd, Pb, and Zn in willow organs in the Litavka soil are connected with the higher original level of these elements in the soil. The higher concentration of Cr in organs in the Malín soil is connected with possible Cr uptake as anion ( $\text{CrO}_4^{2-}$ ) (higher soil pH is connected with higher Cr availability for plants) (Kabata-Pendias 2011).

Concentrations of Al, Cd, and Cr in organs were higher after the last growing year than after the first year because of the reduced immobilisation effect of liming over time (Lee et al. 2004). Concentrations of Cr and Pb in organs were higher after the first year than after the second year, which is probably because of the reduced effect of liming on the mineralisation of organic matter and subsequently on the reduced release of Cr and Pb bound in the lime over time (Yobouet et al. 2010; Kabata-Pendias 2011).

In both contaminated control soils, a tendency for higher transport of Al, Cd, and Zn from twigs into leaves was recorded. Lime and dolomite applications do not restrict the transport of Al, Cd, or Zn from twigs into leaves of willows grown in any of the contaminated soils. Similar results (i.e. higher Cd and Zn concentrations in leaves than in twigs of willows) were recorded by other authors (Tlustoš et al. 2007; Kacálková et al. 2015). In slightly acidic contaminated control soil, a tendency for higher transport of Cr from twigs into leaves was recorded as well as in the lime and dolomite treatments. In the alkaline contaminated control and treated soils, a comparable concentration of Cr in twigs and in leaves was recorded. In the slightly acidic contaminated control soil, a tendency for restricted transport of Pb from twigs into leaves was recorded. Similar result (i.e. higher concentration of Pb in twigs than in leaves) was recorded by Tlustoš et al. (2007). Lime and dolomite application increased the transport of Pb from twigs into leaves at a comparable level in willows (twigs=leaves)

grown in slightly acidic contaminated soil. In the alkaline contaminated control soil, a comparable concentration of Pb in twigs and in leaves was recorded. Lime and dolomite application increased the transport of Pb from twigs into leaves of willows grown in alkaline contaminated soil.

The comparison of concentrations of all risk elements in leaves in lime- and dolomite-treated contaminated soils with their common foliar concentrations in willows grown in acidic non-contaminated soils was the same as in both contaminated control soils. The dolomite application in the lower dose is not an appropriate measure for the cultivation of willows in slightly acidic contaminated soils because of low biomass and higher mortality of willows. Nevertheless, lime application in combination with foliar Fe application during vegetation caused a reduction of Cd and Zn in leaves (not below the limit of their phytotoxicity) as well as a reduction of the Zn to Fe ratio in leaves of willows, thus indicating that it can partially solve the Zn phytotoxicity that induces Fe deficiency and biomass reduction in slightly acidic contaminated soils.

## 5 Conclusions

Contaminated soils with different soil pH had an effect on the amount of organ biomass production of willows in our 3-year study. Higher twig biomass than leaf biomass was recorded in alkaline soils and vice versa in slightly acidic soils.

Lime application was the most effective measure for increasing biomass production of willows in heavily contaminated soils with slightly acidic to alkaline soil pH. The dose of lime only played a significant role in increasing biomass production in alkaline contaminated soils (i.e. the higher dose). The time that had passed since the lime application affected the biomass production and mortality of willows in both heavily contaminated soils. Immediate planting of cuttings after lime application in higher doses was fatal for them. Dolomite application did not increase the biomass production of willows in heavily contaminated soils with slightly acidic to alkaline soil pH. Lower doses of dolomite caused a decrease in biomass production as well as regular mortality of willows in slightly acidic contaminated soils due to the low immobilisation of risk elements.

Liming is not an appropriate measure for changing the distribution of all macro, all micro, or almost all toxic elements except Pb between aboveground organs of willows in heavily contaminated soils. The latent deficiency of P in leaves can be questionable in untreated as well as in lime- and dolomite-treated slightly acidic contaminated soils. Willow cultivation in slightly acidic contaminated soils treated with dolomite in lower dose is not suitable. The toxicity of Cd as well as the toxicity of Zn, which induces Fe deficiency in leaves and is connected with biomass reduction, can be partially solved by lime application with a combination of foliar

Fe application during vegetation in slightly acidic contaminated soils.

**Acknowledgments** The finalisation of the paper was supported by the National Agency of Agriculture Sciences (NAZV QJ 1210211) and by the Czech University of Life Sciences in Prague (CIGA 20142005).

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