Effect of quick lime and superphosphate additives on emergence and survival of *Rumex obtusifolius* seedlings in acid and alkaline soils contaminated by As, Cd, Pb, and Zn

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ABSTRACT

Rumex obtusifolius is a troublesome weed widely spread in temperate grasslands and can be potentially used for detection of soils contaminated by trace elements. We asked how emergence and survival of its seedlings are affected by application of quick lime (Ca) and superphosphate (P) additives in soils contaminated by trace elements. We performed the pot seeding experiment with slightly acid Litavka soil contaminated by arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn) and alkaline Malín soil contaminated by As, Cd, and Zn. We used a control without any additives, Ca and P treatments in both soils. Higher and quicker emergence, together with substantially higher mortality of seedlings, was recorded in Litavka than in Malín. A positive effect of the Ca treatment on seedlings was recorded in Litavka, but a negative in Malín. Small seedlings with narrow and long leaves of reddish colour were recorded in Litavka in the control and in the P treatment both with high availability of Zn, Cd, and Pb. In the Ca treatment, leaves of seedlings were more elliptic and less reddish. In Malín, seedlings were green and substantially more vital in the control and in the P treatment than in Litavka. In the Ca treatment, small and unviable seedlings were recorded. Seedlings of *R. obtusifolius* are sensitive on high availability of Ca, Zn, Cd, and Pb in the soil.

Keywords: broad-leaved dock; calcium; cadmium; lead; zinc; metal toxicity; soil reaction

Rumex obtusifolius subsp. *obtusifolius* (broad-leaved dock) is a highly problematic and widely spread weed species particularly under organic farming in temperate grasslands (Cavers and Harper 1964, Hejcman et al. 2012). Factors that determine *R. obtusifolius* to be a successful weed are high production of long-term viable seeds, regeneration of plants from fragments of underground organs, deep growing taproot with high storage capacity, the perennial character of the species, and quick and large biomass production (Zaller 2004, Gilgen et al. 2010, Strnad et al. 2010, Hrdličková et al. 2011).

Rumex obtusifolius can be negatively related to a high Ca content in the soil and a high pH value, which was shown by Humphreys et al. (1999) and Hann et al. (2012). Bohner (2001) described *R. obtusifolius* as a 'calciphobic' species which precipitates excessive Ca into calcium oxalate crystals. On Ca and Mg rich soils, *R. obtusifolius* can suffer from insufficient K supply because of antagonism between K and Ca/Mg uptake as suggested by Hann et al. (2012).

Growth of *R. obtusifolius* can also be highly affected by the availability of trace elements, but there have not been any studies investigating the effect of As, Cd, Pb, and Zn soil contamination on emergence and survival of *R. obtusifolius* seedlings. As *R. obtusifolius* is a widely spread species, it could potentially be a suitable species for detection of soils contaminated by trace elements by field vegetation mapping.

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Availability of many trace elements to plants, As, Cd, and Zn in particular, is also related to soil P status. In the case of As this is because of competition between phosphates and arsenates for binding sites in the soil (Száková et al. 2009, Liu et al. 2012), and in the case of Cd and Zn, because of Cd and Zn precipitation into phosphates (Dong et al. 2007). Also the soil pH is important as the availability Cd, Fe, Mn, and Zn to plants is high especially in acidic soils (Dong et al. 2007). Addition of Ca (CaO – quick lime) or P ($Ca(H_2PO_4)_2$ – superphosphate) additives can therefore substantially decrease the availability of many trace elements in the soil (Puschenreiter et al. 2005, Kumpiene et al. 2008, Hejcman et al. 2010, Trakal et al. 2011).

Using a pot experiment with Ca and P additives to two soils differing in pH, As, Ca, Cd, Mg, Pb, and Zn status, the aim of this study was to investigate how much emergence and survival of *R. obtusifolius* seedlings are affected by these soil chemical properties.

MATERIAL AND METHODS

Design of the pot experiment. In May 2011 we established the pot experiment in an outdoor weather-controlled vegetation hall in Prague-Suchdol (Czech Republic) with natural temperature and light conditions.

We used two contaminated soils: (1) slightly acidic Fluvisol termed 'Litavka' from alluvium of the Litavka river collected in the village Trhové Dušníky. The Litavka soil was contaminated with As, Cd, Zn, and Pb by wastes from smelter setting pits (Šichorová et al. 2004, Trakal et al. 2011). (2) The alkaline Luvisol termed Malín collected from a bank of the streamlet Beránka near Malín village. The Malín soil was contaminated by As, Cd, and Zn due to tailings of silver mining in the 13–16[th] centuries (Száková et al. 2009). Chemical properties of the soils used analysed before establishment of the experiment are given in Table 1. In addition to the effect of soil, an effect of Ca (CaO – quick lime) and P ($Ca(H_2PO_4)_2$ – superphosphate) additives

Table 1. Basic characteristics of soil collection sites and chemical properties of investigated soils (in dry matter)

Site and soil properties	Soil	
	Litavka (49°43'N, 14°0'E)	Malín (49°58'N, 15°17'E)
Altitude (m a.s.l.)	450	230
Mean annual temperature (°C)	7.3	8.5
Mean annual precipitation (mm)	623	575
Soil texture	Clay loamy sand	Loam
Soil type	Fluvisol	Luvisol
pH_{CaCl_2}	5.8	7.2
CEC ($mmol_{(+)}$/kg)	55	346
C_{org} (g/kg)	36	27
Ca (mg/kg)	1856	8914
Mg (mg/kg)	160	354
K (mg/kg)	192	234
P (mg/kg)	9	56
Cd (mg/kg)	53.8	11.3
Zn (mg/kg)	6172	1022
Pb (mg/kg)	3305	98
As (mg/kg)	354	688
Fe (mg/kg)	21193	17379
Mn (mg/kg)	2688	371

CEC – cation exchange capacity; C_{org} – content of organic carbon. Values for P, K, Ca, and Mg are plant available concentrations of nutrients determined by Mehlich III extraction procedure. Values for As, Cd, Fe, Mn, and Zn are total concentrations of elements extracted by *aqua regia*. Czech legislation limits for *aqua regia* (pseudo-total) concentrations of elements in light-textured and other soils, respectively (in mg/kg) are 0.4 and 1.0 for Cd, 130 and 200 for Zn, 100 and 140 for Pb and 30 and 30 for As

Table 2. Effect of soils and applied treatments on plant available concentrations of elements extracted by $CaCl_2$ on 28th day after application of additives (according to Vondráčková et al. 2012)

Element (mg/kg)	LC	LCa	LP	MC	MCa	MP
As	0.06	0.2	0.2	0.5	0.3	2.2
Cd	0.8	0.05	0.7	0.01	0.007	0.02
Pb	0.06	2.1	0.1	0.005	0.004	0.01
Zn	33	5.9	30	0.1	0.03	0.3

L – Litavka; M – Malín; C – control; Ca – application of Ca additive; P – application of P additive

on emergence of *R. obtusifolius* was investigated. Based on previous experiments with application of Ca and P additives into investigated soils (Trakal et al. 2011, Vondráčková et al. 2013), we applied to pots 7.3 g CaO per 1 kg of soil and 1.3 g $Ca(H_2PO_4)_2$ per 1 kg of soil. The pot experiment was composed of six treatments replicated five times (30 pots altogether): LC – Litavka soil without any additive as the control; LCa – Litavka soil with Ca additive; LP – Litavka soil with P additive; MC – Malín soil without any additive as the control; MCa – Malín soil with Ca additive and MP – Malín soil with P additive. Plant available concentrations in investigated treatments 28th day after establishment of the experiment are given in Table 2.

We used 5 L pots with 20 cm diameter filled with 5 kg of air dried soil sieved through a 10 mm sieve. We then applied the following fertilisers: 0.5 g N (in the form of NH_4NO_3), 0.16 g P and 0.4 g K (in the form of K_2HPO_4) into each pot. Application of N, P, and K fertilisers was performed in order to make N, P, and K availability non-limiting for growth of *R. obtusifolius* in all treatments. According to our previous experience, amount of applied P fertilizer in the form of K_2HPO_4 was not high enough to change mobility of metals, but was high enough to alleviate P deficiency for *R. obtusifolius* growth. The additives and fertilizers were mixed with the soil and then all pots were watered. The fertilisers and additives were applied on the morning of the 3rd May 2011. In the evening of the same day, we sowed 100 seeds, 1–2 cm deep, into each pot.

Seeds of *R. obtusifolius* were collected during spring 2011 from a region near Prague in central Czech Republic. The collection sites were mainly roadside ditches or abandoned fields with neutral soil pH, good P and K and low As, Cd, Pb, and Zn availability. The seeds from different mother plants were mixed to avoid any maternal effect and stored at room temperature in paper bags and in the dark. Germination of used seeds was 86% tested under laboratory conditions in a 12 h day/night regime at 25°C directly before establishment of the experiment. Pots were regularly watered if necessary to maintain optimal growth conditions during the course of the experiment.

We recorded the number of seedlings daily in each pot from the start of the emergence on the 5th day of the experiment up to 28 day of the experiment in 8th June 2011. The field emergence was defined as the maximal number of seedlings recorded during the course of the experiment in each treatment. We used field emergence because the maximal number of seedlings was recorded on different days for each treatment. In addition to field emergence, we analysed mortality defined as the maximal number of seedlings minus the final number of seedlings on the 28th day of the experiment. Mortality was expressed as percentage from used seeds.

Soil pH $CaCl_2$ was measured in suspension of 10 g of soil and 50 mL of solution containing 0.01 mol/L $CaCl_2$ at 20 ± 1°C at the end of the experiment.

Data analysis. Repeated measures and factorial ANOVA followed by comparison using Tukey HSD test were applied to obtained data. The relationship between soil pH and field emergence was evaluated using linear regression. All analyses were performed using the Statistica 8.0 program (Statsoft, Tulsa, USA).

RESULTS

Effect of additives on soil pH. Effects of locality, additive and additive × locality interaction on soil pH were significant at the end of the experiment (Figure 1a). In Litavka soil, the pH value was highly increased after application of Ca additive, but only slightly increased in Malín soil. There was no effect of P addition on the pH value in either soil.

Seedling number during the experiment and field emergence. The number of seedlings in Litavka soil was significantly affected by day and by day × additive interaction, but there was no effect due to soil additives (Figure 2a). In Malín

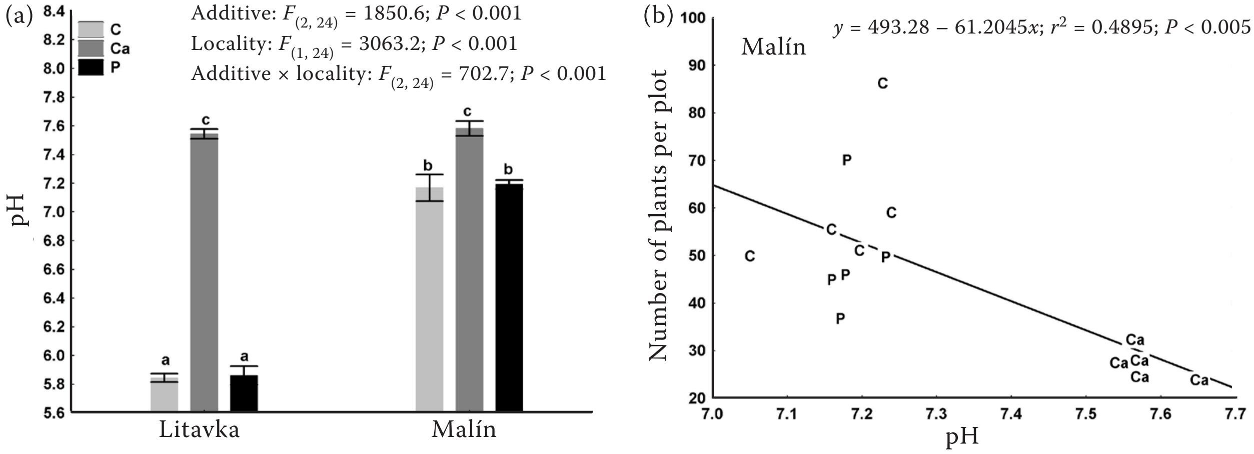

Figure 1. (a) Effect of additive, locality and additive × locality interaction on soil pH ($CaCl_2$) measured at the end of the experiment and (b) effect of soil pH ($CaCl_2$) on emergence of *Rumex obtusifolius* (maximal number of seedlings recorded during the experiment) in Malín soil. C – control treatment without any additive; Ca – application of Ca additive; P – application of P additive. Error bars represent standard errors of the means (SE). *F* and *P* values – results of ANOVA analyses for particular effects (locality, additive and additive × locality interaction) and values in brackets are degrees of freedom. According to the Tukey post-hoc test, treatments with the same letter were not significantly different at the 0.05 probability level

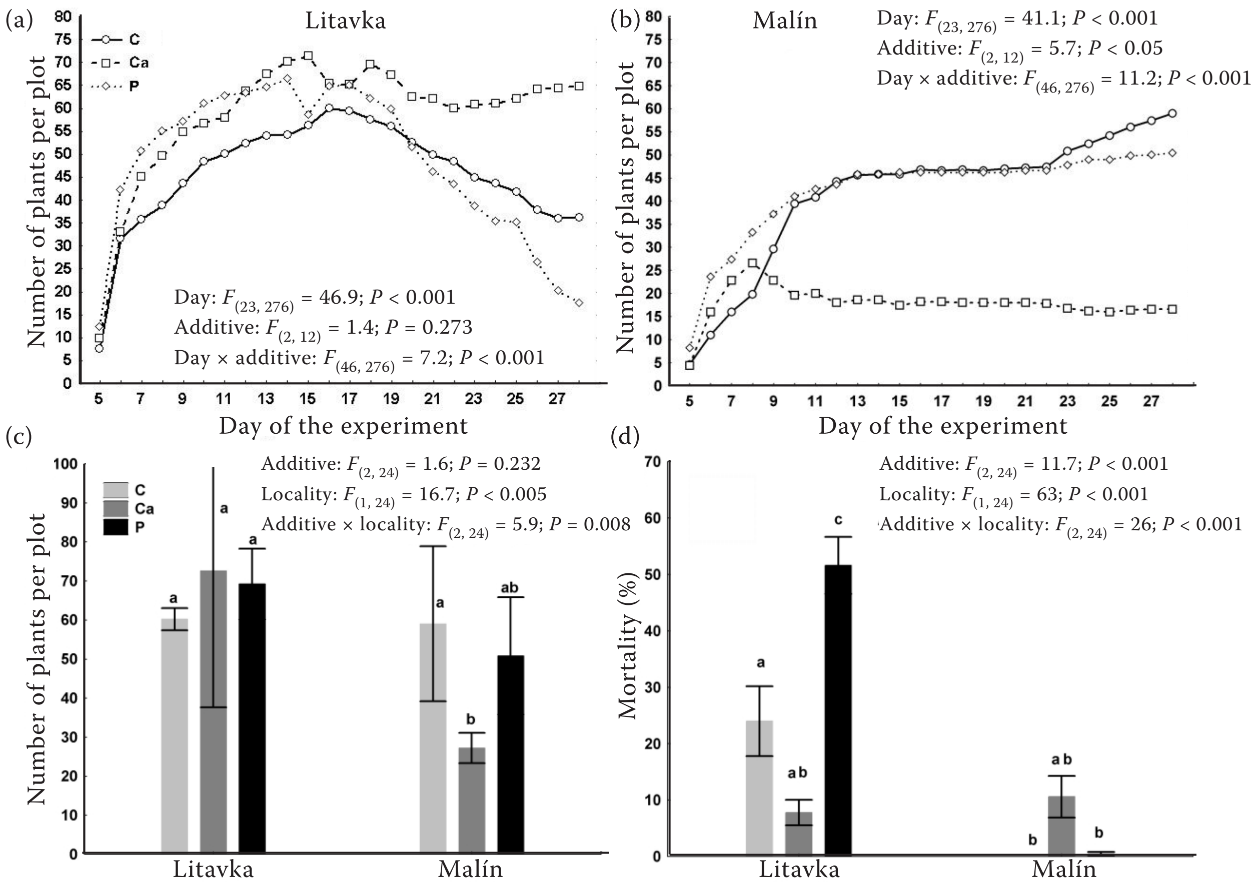

Figure 2. Effect of additives on number of *Rumex obtusifolius* seedlings during the 28 days of the experiment in (a) Litavka soil and (b) Malín soil. (c) Field emergence (maximal number of seedlings) recorded over the study period in Litavka and Malín soils and (d) mortality in Litavka and Malín soils expressed as percentage from used seeds per pot. *F* and *P* values – results of ANOVA analyses for particular effects (day, additive, day × additive interaction, locality, additive × locality interaction) and values in brackets are degrees of freedom. According to the Tukey post-hoc test, treatments with the same letter were not significantly different at the 0.05 probability level. For explanation see Figure 1

soil, the number of seedlings was significantly affected by all factors – day, additive and by day × additive interaction (Figure 2b). In Litavka soil, higher emergence values and quicker emergence was recorded than in Malín soil (Figure 2c), but in Malín soil, substantially lower mortality of seedlings was recorded than in Litavka soil (Figure 2d). In Litavka soil, there was no significant effect of Ca additive on emergence and survival of seedlings in comparison to the control. In Malín soil, there was a large negative effect of Ca additive on the emergence and survival of seedlings in comparison to the control. P addition tended to enhance speed of emergence in both soils but there were differing effects due to P addition on survival of seedlings in both soils. In Litavka soil, P addition increased mortality while there was no effect of P addition on the mortality of seedlings in Malín soil.

The overall field emergence was significantly affected by locality and by additive × locality interaction, but not by additive (Figure 2c). The field emergence 73% in Litavka soil with Ca additive was not significantly different from the other treatments except the Ca treatment in Malin soil with field emergence 27%. The emergence was not affected by the pH value in Litavka soil (P = 0.33, r^2 = 0.055), but was significantly negatively related to pH value in Malín soil (Figure 1b).

The overall mortality was significantly affected by the locality, additive and by locality × additive interaction, indicating a soil specific effect of additives (Figure 2d). No mortality of seedlings was recorded in Malín soil in C treatment and the highest mortality was in Litavka soil in P treatment (52%).

The effect of all treatments on number, vitality and size of seedlings on the 28th day of the experiment is obvious in photographs of individual pots shown in Figure 3. Very small seedlings with narrow and long leaves with a characteristic reddish colour of leaf tips were recorded in Litavka soil in the control and P treatment. In Ca treatment, leaves of seedlings were more elliptic and less reddish. In Malín soil, lower number of seedlings was recorded than in Litavka soil, but seedlings were green and substantially more vital in the control and P additive treatment than in Litavka soil. In Ca treatment, low numbers of small and unviable seedlings was recorded.

DISCUSSION

Field emergence and consequent survival of *R. obtusifolius* seedlings were highly affected by soil chemical properties. Most detrimental for emergence of *R. obtusifolius* seedlings was high pH, related to high Ca availability in the soil. This was shown clearly in the Ca and Mg rich Malín soil where addition of Ca resulted in very low emergence of *R. obtusifolius* seedlings although there was comparatively low availability of As, Cd, Pb, and Zn. Humphreys et al. (1999) reported weak negative correlation between soil Mg status and pH and abundance of *R. obtusifolius* in grasslands in UK. Hann et al. (2012) found negative correlations between plant available Ca and Mg contents in soil and the density of *R. obtusifolius*, but they did not test effects of Ca application. Bohner (2001) classified *R. obtusifolius* as a 'calciphobic' species which precipitate excessive Ca into insoluble calcium oxalate crystals. As the enhanced synthesis of oxalic acid in Ca rich soils can result in extra energy costs (Kinzel 1983), this might, together with low K supply for this highly K demanding species, decrease competitiveness and increase mortality of *R. obtusifolius* on Ca rich soils (Hann et al. 2012). We demonstrated that negative effects of high Ca availability on *R. obtusifolius* can already be recorded during early development of seedlings.

In the first days of the experiment we observed the highest emergence in P treatments in both soils. This is in accordance with results by Křišťálová et al. (2011), recording the positive effect of high P availability in the soil on early development of *R. obtusifolius* seedlings. A relatively low effect due to P addition on the emergence of seedlings in our study can be explained by P fertiliser application in all pots before the start of the experiment which removed the strong P limitation on early seedlings growth in all treatments. The consequent high mortality of seedlings in P treatment in Litavka soil was probably caused by toxic effects of too high P status.

In contrast to Malín soil, application of Ca increased the survival of *R. obtusifolius* seedlings in Litavka soil. This was due to substantially lower initial availability of Ca in this soil, and a substantial decrease in the availability of Zn and Cd and hence their toxicity on seedlings after application of the Ca additive (Vondráčková et al. 2013). In Malín soil no, or only slight, decrease in Cd and Zn availability was recorded and initial concentrations of Cd and Zn available to plants were about three fold lower than in Litavka soil. Therefore there was no Cd or Zn toxicity even in untreated Malín soil. In Litavka soil, toxicity of Cd and Zn for *R. obtusifolius* seedlings was indicated also by

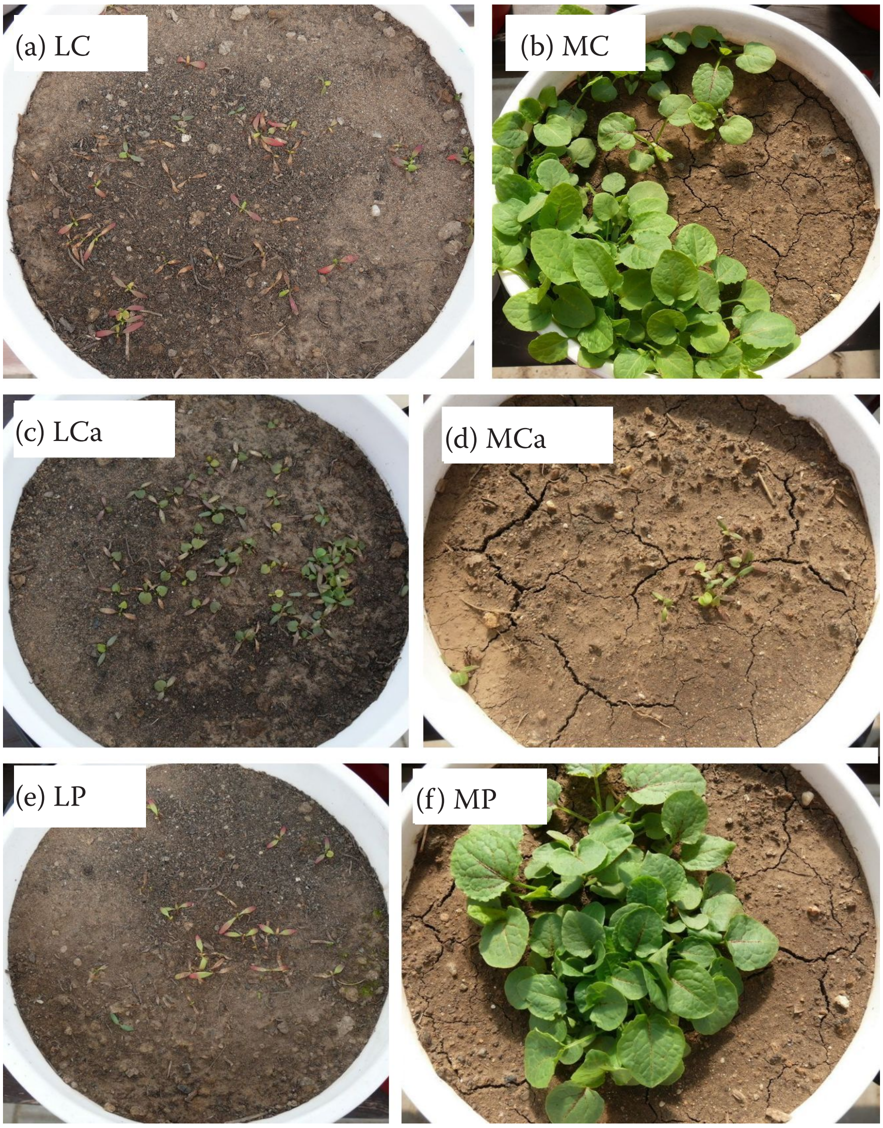

Figure 3. Photographs of *Rumex obtusifolius* plants in investigated treatments on the 28th day of the experiment. (a) LC – Litavka soil without any additive as the control; (b) MC – Malín soil without any additive as the control; (c) LCa – Litavka soil with Ca additive; (d) MCa – Malín soil with Ca additive; (e) LP – Litavka soil with P additive; (f) MP – Malín soil with P additive

substantially higher concentrations of both elements in leaves than in Malín soil (unpublished data). In Litavka soil, the concentration of Zn in leaves exceeded the critical limit for Zn toxicity, estimated by Broadley et al. (2007) for a wide range of species to be 300 mg Zn/kg. Although the concentration of Zn was lower in Ca treatment in comparison to P treatment and the control, the concentration of Zn in leaves still exceeded 500 mg/kg. In addition, toxicity of Zn was clearly visible by the reduction in biomass growth and the yellow, brown and reddish colour of leaves (Figure 3). These symptoms are connected with Zn toxicity resulting from Fe deficiency in leaves, as has been described in studies on other plant species (Sagardoy et al. 2009, Cui and Zhao 2011, Song et al. 2011).

In addition to Zn toxicity in Litavka soil, Cd and Pb toxicities were probably also highly important, as concentrations of Cd in leaves of *R. obtusifolius* ranged from 5–14 mg/kg and concentrations of Pb were around 100 mg/kg. These concentrations were approximately one order higher than in Malín soil (unpublished data).

Finally we can conclude that *R. obtusifolius* suffers from high Ca availability in the soil since emergence of seedlings. As *R. obtusifolius* is a widely spread weed species with high seed production, restricted growth and conspicuous changes in leaf shape and colour (yellow, brown and reddish rather than green) of its seedlings can indicate soils with toxic Zn, Cd and Pb contents.

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