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Effect of Rock Phosphate on Zn and Fe Bioavailability and Accumulation by *Salix smithiana* in Heavily Contaminated Soil

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Abstract. High biomass production *Salix smithiana* was proved as the plant able to accumulate substantial amount of Cd and Zn in aboveground biomass. Nevertheless, in heavily contaminated soils (mainly by Zn) willows can suffer from chlorosis because of Fe deficiency induced by excess of Zn amount. Method such as chemophytostabilization seems like very good measure for planting willows in such heavily contaminated soil. In our experiments we evaluated effect of rock phosphate on changes in Zn and Fe bioavailability and accumulation of these elements by willows together with the willows growth on heavily contaminated soil. Addition of rock phosphate reduced plant-available Zn concentrations in soils resulting in significant decrease of Zn content in leaves. In the case of Fe, however, its contents in the leaves significantly decreased as well, although the mobile portion of Fe in soil remained unchanged. Yield of aboveground biomass in rock phosphate treatment was not significantly different in comparison to the control. After the first vegetation period, we can conclude that reduction of Zn contents in willows after rock phosphate application did not lead to suppress of Fe deficiency and improvement of willow growth in heavily contaminated soil.

Key words: Iron, Chemical Immobilization, Chemophytostabilization, Willow, Zinc

Introduction

Fluvisol from the alluvium of the Litavka River in the village Trhové Dušníky (60 km south of Prague, the Czech Republic) is a good example of heavily contaminated soil by several metals like Cd, Pb, and Zn (Borůvka et al., 1996). However, high concentrations of heavy metals are toxic for most of the soil microorganisms and plants, leading to poor and irregular development of vegetation or even total disappearance of plant cover in such sites (Gray et al., 2006).

In this context, chemophytostabilization of metal contaminated soils, or the use of metal-tolerant plants, together with different amendments to reduce metal mobility and bioavailability in soils, are the most promising available measures for sites highly contaminated with metals (Alkorta et al., 2010). Specific willow clones are able to grow and accumulate substantial amounts of Cd and Zn in the aboveground biomass on moderately contaminated soils (Vysloužilová et al., 2003). Nevertheless, willows planted in highly contaminated soils especially by high Zn concentration suffer from chlorosis probably due to Fe deficiency reducing willow production (Alcantara et al., 1994; Vysloužilová et al., 2006).

The aim of our study was to investigate the effect of rock phosphate application on (1) Zn and Fe bioavailability, (2) Zn and Fe accumulation by willows and (3) willow growth in heavily contaminated soil.

Materials and Methods

Slightly acidic soil "Litavka" used in both incubation and pot experiments was characterized as follows: CEC 55 mmol kg^{-1}, C_{org} 3.6 %, 53.8 mg Cd kg^{-1}, 6172 mg Zn kg^{-1}, 3305 mg Pb kg^{-1}. In both experiments, rock phosphate was used (TIMAC AGRO CZECH Ltd, Czech Republic) as the metal stabilization agent.

In the incubation experiment, at constant temperature 25°C was rock phosphate inserted into acid-clean polyethylene 250-ml plastic bottle and mixed with amount of 50 g of air-dried contaminated soil and deionised water at a volume equivalent to 60% maximal water-holding capacity. Each treatment (control, rock phosphate rate 1 representing 1.8 g kg^{-1} soil, rock phosphate rate 2 representing 16.7 g kg^{-1} soil) was performed in five replications. After 42 days soil samples were extracted with 120 ml of 0.01 mol L^{-1} $CaCl_2$ for 6 h.

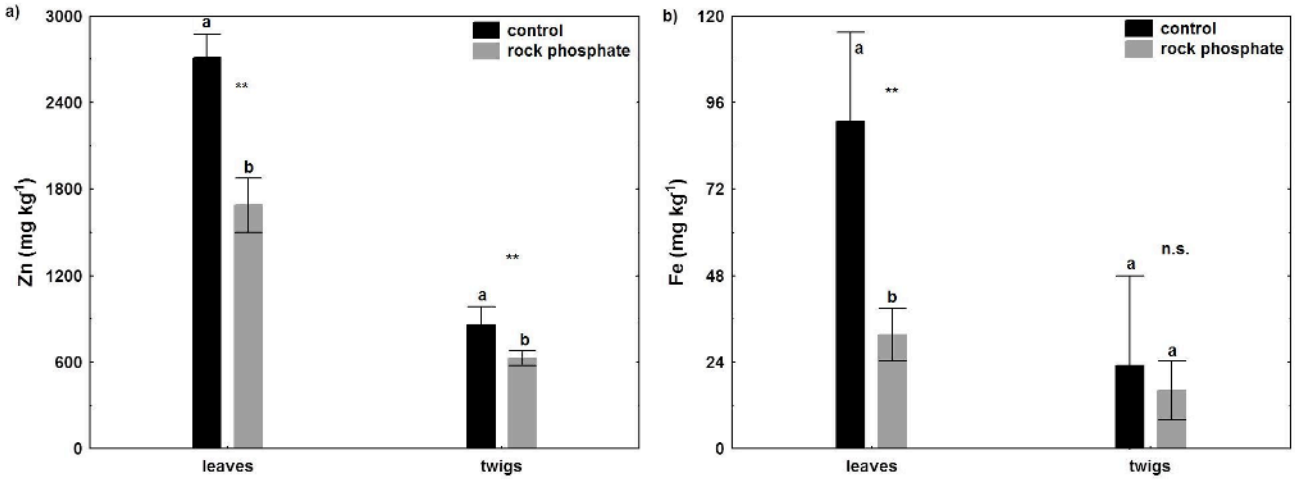

Fig. 1. Zn (a) and Fe (b) contents in leaves and twigs of willow in heavily contaminated soil treated with different treatments (control, rock phosphate). Treatments with the same letter are not significantly different

Plant-available Zn and Fe in soil extracts were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian VistaPro, Varian, Australia).

For pot experiment was selected clone of high biomass production willow (*S. smithiana* Willd., S-smithF-218). Five kg of air dried soil was thoroughly mixed with homogenized rock phosphate (26.5 g kg^{-1} soil) and inserted to the pot. One 20 cm willow cutting was planted in each pot and the pots were kept in an outdoor weather-controlled vegetation hall. Four replications were used for each treatment (control, rock phosphate). After five months of planting, aboveground biomass (leaves and twigs) were harvested, checked for fresh and dry biomass, ground and analyzed as well as soil samples were collected and analyzed. Soil samples (3 g) were extracted with 30 ml 0.01 mol L^{-1} $CaCl_2$ for 6 h. Plant-available Zn and Fe concentrations in soil extracts and total plant Zn and Fe contents were determined by ICP-OES.

Results and Discussion

Rock phosphate application significantly reduced plant-available (0.01 mol L^{-1} $CaCl_2$ extractable) Zn concentration (Tables 1 and 2) in heavily contaminated soil compared with the control. This was probably because of Zn adsorption on or co-precipitated with pyromorphite (Ma et al., 1994). Higher application rate increased rock phosphate efficiency. This conclusion is in agreement with Thawornchaisit and Polpraselt (2009). On the other hand, there was no effect of rock phosphate application on the concentration of plant-available Fe (Tables 1 and 2). This was probably connected with Fe bounds into less soluble iron phosphate (strengite) in acid soils (Hsu, 1975). Plant-available Zn and Fe concentrations were higher in the contaminated soil in the presence of willows according the results from incubation and pot experiments (Tables 1 and 2). It is known that plant roots exudate substances playing an important role in bioavailability of elements in soil (Kabata-Pendias, 2004). It is obvious from the results in Figure 1a that rock phosphate application significantly reduced Zn content in leaves and twigs of willows in comparison to the control. It corresponds with the results of plant-available Zn concentration from incubation and pot experiments. However, significant decrease of Fe content in leaves (Figure 1b) was recorded after rock phosphate application. Nevertheless, there was no change in Fe content in twigs after rock phosphate application. Therefore, we can conclude that Fe content in leaves not correlated with plant-available Fe concentrations in soil treated with rock phosphate. Decrease in Fe content in leaves of willows is probably induced by competition between Zn and Fe in soil solution (Alcantara et al., 1994) regardless of rock phosphate application. Therefore, rock phosphate application was not effective measure to reduce Zn under phytotoxicity value (500 mg Zn kg^{-1}) for general plants (Sauerbeck, 1989) and prevent Fe deficiency. It is probably in connection with unsuccessful reduction of Zn/Fe ratio in aboveground biomass of willows, especially in leaves (Table 3). Rock phosphate application reduced Zn content in leaves and twigs. Nevertheless, Zn/Fe ratio was increased pushing the Zn phytotoxicity up at treated trees. From Figure 2 is obvious that rock phosphate application showed no effect on the production of dry aboveground biomass of willow. We can speculate that it was connected with the Fe deficiency as discussed above. After the first vegetation period rock phosphate application was evaluated that as unsuitable measure to prevent decrease in willow production causing by early leaf fall because of Fe deficiency. Rock phosphate application is suitable measure to reduce Zn bioavailability in heavily contaminated soil but in connection with changes in Fe content and dry matter willow production is insufficient. So we can recommend for prevent Fe deficiency foliar application of Fe during the vegetation.

Table 1. Plant-available Zn and Fe concentrations in heavily contaminated soil treated with different treatments (control, rock phosphate rates 1 and 2) after 42nd days (results from incubation experiment). Treatments with the same letter are not significantly different.

treatment	element (mg kg^{-1})	
	Zn	Fe
control	30.2±0.4^{a}	0.50±0.03^{a}
rock phosphate (1)	27.4±0.6^{b}	0.48±0.03^{a}
rock phosphate (2)	11.6±0.2^{c}	0.41±0.03^{a}

Table 2. Plant-available Zn and Fe concentrations in heavily contaminated soil treated with different treatments (control, rock phosphate) after five months of planting (results from pot experiment). Treatments with the same letter are not significantly different.

treatment	element (mg kg^{-1})	
	Zn	Fe
control	128±13^{a}	1.6±0.10^{a}
rock phosphate	52±3^{b}	0.77±0.35^{a}

Table 3. Changes in Zn/Fe ratio in aboveground biomass of willows planted in heavily contaminated soil treated with different treatments.

treatment	Zn/Fe	
	leaves	twigs
control	30	37
rock phosphate	53	39

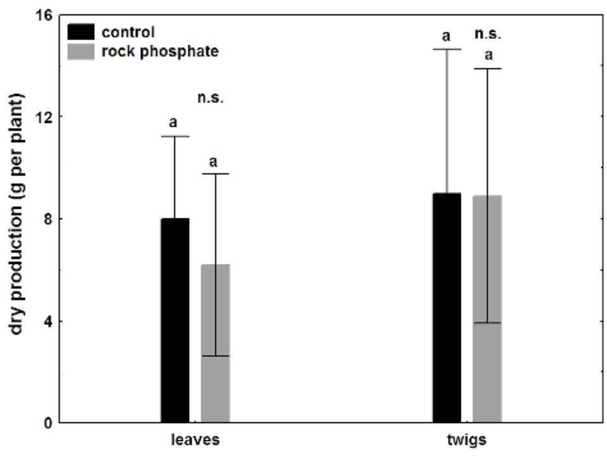

Fig. 2. Dry production of willow (leaves, twigs) in heavily contaminated soil treated with different treatments (control, rock phosphate). Treatments with the same letter are not significantly different.

Conclusion

Rock phosphate is suitable measure to decrease Zn bioavailability in heavily contaminated soil and to reduce Zn content in aboveground biomass of willow. After rock phosphate application, there was no effect on Fe bioavailability. Reduction in Fe content in willow after rock phosphate application is probably caused by competition between Zn and Fe due to unsuccessful reduction of Zn/Fe ratio especially in willow leaves. Rock phosphate application was no effect on willow growth.

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