doi: 10.17221/181/2015-PSE

Biochar immobilizes cadmium and zinc and improves phytoextraction potential of willow plants on extremely contaminated soil

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ABSTRACT

The availability of risk elements in soil can be possibly reduced by various soil additives. Among them, the attention has been recently focused on the research of unconventional soil additive – biochar. The aim of this study was (i) to observe the effect of biochar application on risk elements transport through the soil profile and (ii) to assess the availability of risk elements in biochar amended soil to willow growth. The experiment was established at greenhouse conditions and extremely contaminated soil, reaching 43 mg/kg cadmium (Cd) and 4340 mg/kg zinc (Zn), was used. To observe risk element content in leachate, the lysimeter cylinders were tested. The rates of biochar were 0 (control); 5, 10, and 15% per mass of soil. The results showed that biochar significantly increased biomass production whereas the plant Cd and Zn contents remained unchanged in most cases. In leachate, Cd and Zn content decreased by 99% at all the biochar treatments. We can summarize that biochar appears to be a very effective regulator of availability of observed risk elements and improver agent for biomass production of plants and remediation efficiency.

Keywords: heavy metals; soil contamination; *Salix* × *smithiana*; phytoremediation; stabilization

Under the European Union (EU) Thematic Strategy for Soil Protection, the European Commission identified soil contamination; occurrence of 342 000 polluted sites was reported, most commonly polluted with heavy metals and mineral oil (Panagos et al. 2013). The soil contamination is presented as the most important problem of soil protection nowadays, while the so-called old load is the most significant health threat (Němeček et al. 2010). Risk elements soil contamination in the region of Příbram in the central part of the Czech Republic was described and pollution by cadmium (Cd), zinc (Zn) was determined as extreme at specific parts of this location (Vondráčková et al. 2013).

For metal-polluted soil, phytoremediation appears to be an economically and aesthetically attractive *in situ* technology (Pulford and Watson 2003). Willow potential for phytoextraction technologies was observed on heavily and moderate polluted calcareous, sandy soils or Cambisol (Meers et al. 2007, Tlustoš et al. 2007, Jensen et al. 2009).

The phytoextraction was introduced in Příbram Fluvisol in pot experiments and the willow biomass reduction due to extremely high content of zinc was observed (Vysloužilová et al. 2003). However, for reasonable efficiency of phytoextraction the biomass production in field conditions will mainly determine metal removal (Meers et al. 2007). Thus, in specific cases it should be considered to combine phytoextraction and stabilization technologies to improve plant growth and support the phytoextraction potential.

Among a wide scale of available soil stabilization materials, inorganic and organic substances based on coal-like materials or combustion by-products have been investigated, e.g. coal or bio-fuel fly

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Supported by the Czech University of Life Sciences Prague, Project No. CIGA 20132007.

doi: 10.17221/181/2015-PSE

ashes (Clark et al. 2001), wood fly ash (Ochecová et al. 2014) or lignite (Uzinger and Anton 2008).

In recent years the investigation was focused on biochar, stable carbon-rich charred biomass and its utilization as a soil additive (Qayyum et al. 2014). The biochar sorption ability of organic pollutants (Zhang et al. 2011) and heavy metals (Beesley et al. 2010) was observed. The specificity of heavy metal has a high impact on biochar sorption ability of these contaminants. While biochar application increases soil pH, the mobility of arsenic (As) increased and due to increased dissolved carbon also copper (Cu) mobility increased; opposite pattern was observed for Cd and Zn (Beesley et al. 2010, Jiang et al. 2012).

Element transport through the soil profile can be observed with lysimeter (Jordan 1968). The utilization of cylinder pots placed in laboratory, greenhouse or into field conditions were described as a suitable way for this type of investigation (Trakal et al. 2011).

As a general conclusion from the review paper, the potential of combination of biochar amendment and phytoremediation technologies have been suggested (Paz-Ferreiro et al. 2014). However, relevant study supporting this statement with experimental data is still missing.

The aim of our study was (i) to evaluate the potential effect of elevated rates of biochar application on risk elements transport through the soil profile, and (ii) to assess the effect of biochar amendment on plant growth as well contaminant accumulation in willow tissues was evaluated.

MATERIAL AND METHODS

Biochar and soil characterization. Biochar was purchased from Erspol., Ltd. (Czech Republic). Biochar derived from coconut shells was characterized by ash content: 12%, pH_{CaCl_2}: 8.9, cation exchange capacity (CEC): 73 $mmol_+/kg$, specific surface area (SSA_{BET}): 486 m^2/g (activated by water steam), particle fraction: 4 × 2 × 2 mm. Soil was sampled from top layer (0–30 cm) of grassland in Trhové Dušníky (Czech Republic) 49°71'8.8742N, 14°0'12.8814E. The type of soil was Fluvisol, pH_{CaCl_2}: 6.6, CEC: 157 $mmol_+/kg$. Soil was air-dried and homogenized.

For determination of the total content of risk elements in soils 0.5 g of soil sample was decomposed in a closed system with microwave heating in the device Ethos 1 (MLS GmbH, Leutkirch, Germany) in a mixture of 8 mL HNO_3, 5 mL HCl and 2 mL HF. Plant-available risk elements in soil were determined according to Ure et al. (1993). The element contents in the soil digests and extracts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., Santa Clara, USA).

The risk element content in biochar was determined by the neutron activation analysis (INAA) (Kubešová and Kučera 2010).

Experimental design. Each pot was filled with 8 kg of contaminated soil. *Salix* × *smithiana* was chosen as an experimental plant. At each treatment two willow cuttings were planted. The experiment consists of 4 treatments: control (no applied biochar), and rates of 5, 10, and 15% of biochar from total mass of soil. Pots were uniquely fertilized with 0.1 g N; 0.16 g P; 0.4 g K per 1 kg of soil. Trees were harvested twice, firstly in July, secondly in early October to test the maximum accumulation potential of plants. The twigs and leaves were analysed separately. Total element contents in plant biomass were determined in the digests obtained by dry ashing decomposition (Street et al. 2006) and Cd and Zn contents were determined by ICP-OES. The experiment was established at greenhouse controlled conditions. To observe risk element content in leachate, the lysimeter cylinders were used. The pots were 40 cm tall. At the bottom end each pot was drained with gravel and placed onto funnel. The leachate was collected into polyethylene laboratory bottle pitched on the funnel and analysed (ICP-OES) each 5 weeks during vegetation.

Statistics. All statistical analyses were performed using the Statistica12 software (Tulsa, USA).

RESULTS AND DISCUSSION

Soil and biochar element content. Nutrient and risk element content of experimental biochar and soil are given in Table 1. There are no legislative limits of risk elements for biochar use in the Czech Republic. If the content of risk elements in used biochar is compared to limits for field application of ash (according to public notice No. 131/2014), the content of Cd is under limit (the limits are: Cd: 5 mg/kg, for Zn no limit was established). Within Europe, plant-available Cd and Zn concentrations in ordinary uncontaminated arable land are up

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Table 1. Element content of biochar and soil

		K	Ca	Mg	Fe	Cd	Zn	C
		(g/kg)				(mg/kg)		(% w/w)
Biochar	total*	0.5	2.9	2.2	4.1	< 0.1	8.3	C_{total}: 93
Soil	total**	10 ± 2	2 ± 0.07	1.9 ± 1.6	6.3 ± 0.8	42.7 ± 0.4	4341 ± 1	C_{org}: 3.6 ± 0.01
	available***	0.1 ± 10^{-3}	1.6 ± 0.06	0.089 ± 6.10^{-3}	0.005 ± 10^{-3}	24.74 ± 1.4	2236 ± 187	

*determined by INAA; ***Aqua regia* and hydrofluoric acid decomposition, *** 0.11 mol/L CH_3COOH extraction

to 0.05 and 0.2 mg/kg, respectively (Uprety et al. 2009). In our experimental soil, these forms are three and four orders higher, respectively. The high content of risk elements in light soil can indicate a risk of groundwater contamination, moreover the contamination can be spread by near river.

Biomass yield. Figure 1a compares the willow biomass yield of leaves and twigs separately and evidently, the aboveground biomass yield was significantly higher at summer harvest compared to the autumn one. However, differences among individual treatments were more balanced at autumn harvest. The yield of leaves was higher than the yield of twigs at all observed treatments. Significantly lower biomass yield of aboveground biomass was observed at control at both harvests in comparison to other treatments. With elevating rates of biochar the yield of aboveground biomass increased. The strong phytotoxic symptoms were observed at control. Yellow leaves indicated Fe deficiency due to competition between Zn and Fe uptake. Amended treatments generally did not show phytotoxic symptoms. Meers et al. (2007) planted willows on contaminated soil in pot experiment (5 mg/kg Cd, 276 mg/kg Zn; *aqua regia* extraction) and achieved yield (leaves and twigs) of 4 g per one plant. Soil in our experiment was more contaminated and at amendments treat-

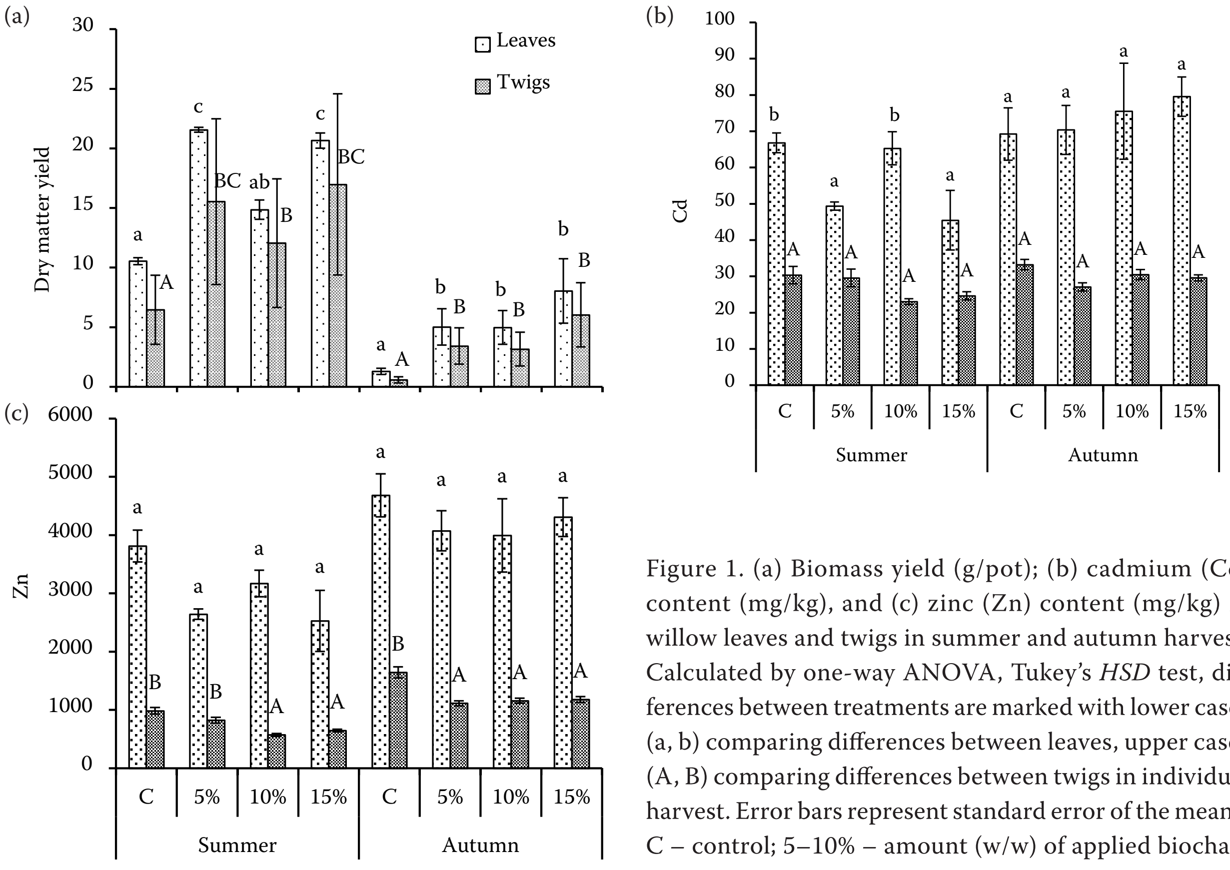

Figure 1. (a) Biomass yield (g/pot); (b) cadmium (Cd) content (mg/kg), and (c) zinc (Zn) content (mg/kg) in willow leaves and twigs in summer and autumn harvest. Calculated by one-way ANOVA, Tukey's *HSD* test, differences between treatments are marked with lower cases (a, b) comparing differences between leaves, upper cases (A, B) comparing differences between twigs in individual harvest. Error bars represent standard error of the means. C – control; 5–10% – amount (w/w) of applied biochar

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ment with 5% of biochar gave almost 11 g/plant in summer. Thus, biochar application was able to overcome the phytotoxicity of the extremely contaminated soil.

Risk elements content in aboveground biomass. The content of Cd was significantly higher in willows leaves in comparison to twigs at summer and also at autumn harvest (Figure 1b). At summer harvest significantly higher Cd content was determined at control and at 10% treatment. There were no differences in Cd content in twigs at all treatments both in summer and autumn. Similarly to Vysloužilová et al. (2003) and Meers et al. (2007) Cd and Zn were transferred from roots to aboveground tissues and all treatments confirmed higher Cd and Zn accumulation in leaves than in twigs. Higher concentration of risk elements was observed both in leaves and twigs in the autumn, in the end of the vegetation of willows. This was confirmed by Lettens et al. (2011) reporting increased foliar concentrations of Cd, Zn, Cu and Mn towards the end of the season.

Zinc content in plant tissues was hundredfold higher in comparison to the content of Cd (Figure 1c). This trend was also observed in the study of Tlustoš et al. (2007). The content of Zn was higher at autumn harvest. There was a tendency for higher concentration in the control treatment. Differences between elevating rate of biochar were not significant. If we compare mean values of foliar Zn content, the higher Zn concentrations were determined at autumn harvest. In twigs, significantly higher Zn content was at control and 5% treatment at summer harvest and only at control at autumn harvest.

The Zn phytotoxicity threshold was determined at 400 mg/kg (Kabata Pendias and Pendias 2001). Despite of exceeding these values in aboveground biomass of willows, plants at amendment treatments did not have phytotoxic symptoms. Beesley et al. (2010) documented the decrease of phytotoxicity of Zn by biochar amendment into soil by using seed phytotoxicity test. With our results, we can confirm and expand this statement on high plants. High concentration of both Cd and Zn in plant leaves require annual harvesting both to avoid the risk element return by leaf-bound in autumn (Robinson et al. 2000).

Figure 2 shows the Cd and Zn concentration in leachate collected during the whole willow

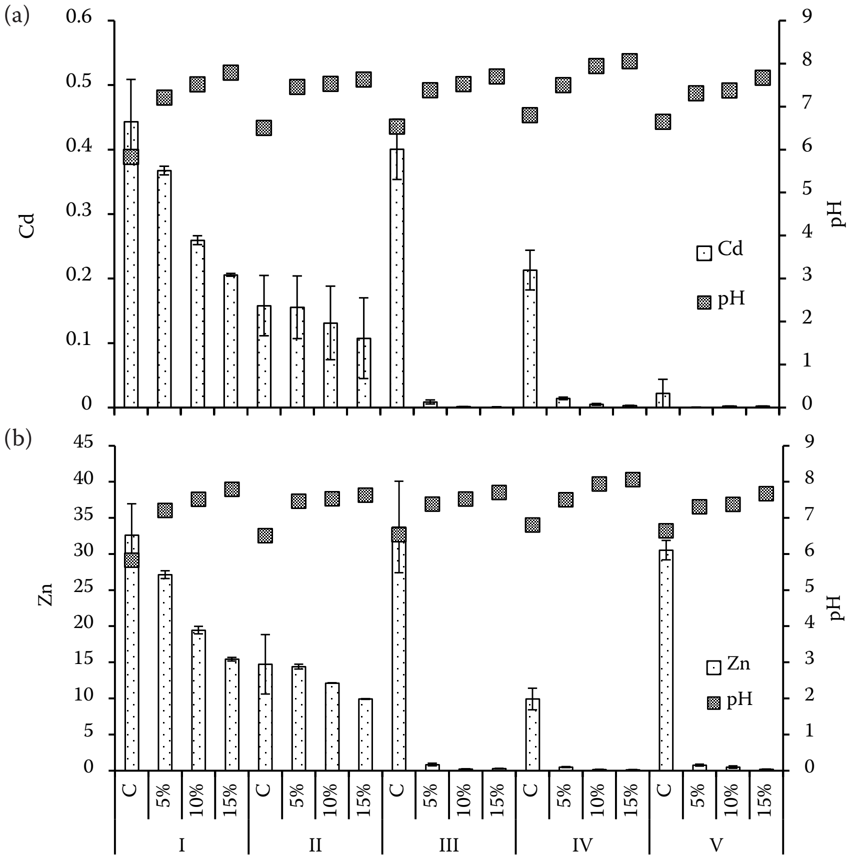

Figure 2. (a) Cadmium (Cd) and (b) zinc (Zn) content in leachate collected during willow vegetation (mg/L). Error bars represent standard error of the means. C – control; 5–10% – amount (w/w) of applied biochar; I, II, III, IV, V – samplings

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vegetation. First sampling was after 1 month of growth. There was a visible decrease of Cd content in leachate with elevating rate of biochar at first and second sampling. After three months the reduction of Cd at biochar amendment treatments was by 97, 99 and 99% at 5, 10 and 15% of biochar amended treatments, respectively in comparison to concentrations determined after one month of willow growth. Similarly Jiang et al. (2012) observed, the acid-soluble Cd decrease by 86% after addition of 3% biochar with no significant difference between 3% and 5% of biochar addition. It can be concluded that lower dose of biochar is a sufficient stabilization agent.

Questionable and worthy to further observation is the Cd and Zn significant reduction in leachate at all biochar treatments, while the concentration of these elements in aboveground biomass changed little. This phenomenon can be caused by the plant rhizosphere ability to release weakly bound Cd and Zn for plants. The higher concentrations of elements at autumn harvest compared to summer one, can be explained by low autumn biomass yield, which caused lower dilution effect (elements content in the amount of biomass), as Vaněk et al. (2012) described for nutrients.

The pH value of leachate increased (in average by 1 unit) with elevating rate of biochar (Figure 2). Leachate from the control treatment was slightly acidic and the highest pH was 8 at 15% treatment. This effect in our study is comparable with Laird et al. (2010) who observed that the application of biochar (0.5, 1, 2% of biochar was applied in their study) resulted in higher pH values (up to 1 pH unit) relative to the non-amended control.

As was mentioned above, trace elements behaviour is primarily related to soil pH. So the biochar ability to increase pH value is probably one of the crucial factors of reduction of the observed element leachability.

Alkaline materials like coal fly ash and red mud also decreased Zn leaching by 99.7% and 99.6%, respectively (Ciccu et al. 2003); comparatively, our slightly alkaline biochar provides reduction of Zn leaching. The decrease of leached Zn was 97, 99 and 98% at 5, 10 and 15% treatment between first and third sampling, respectively.

Balance of Cd and Zn removal and leachability. An evaluation of the total Cd and Zn removal by the willow plants was calculated by using remediation factor (Rf) derived as a ratio of element removed by harvested biomass to the total content of elements in soil (Figure 3). A percentage removal of Cd by willow plants was higher than Zn removal. The lowest Rf both of Cd and Zn was observed at control: 1 and 0.6%, respectively, of the total soil content. The highest removal of both elements was detected at 15% treatment: 2.7 for Cd and 1.3 for Zn. Tlustoš et al. (2007) investigated willow phytoextraction potential on highly contaminated Fluvisol, resulting in phytoextraction efficiency not exceeding 1% for both Cd and Zn, respectively. Jensen et al. (2009) concluded that phytoextraction by willows on heavily polluted soils is unsuited, because of the poor growth. On moderately contaminated Cambisol, they achieved the extraction of total Cd by 0.13% and of total Zn by 0.29%. Total leaching of Cd and Zn was calculated as a ratio of total element in soil to the amount of leached water during the whole willows vegetation period. The restriction of the risk element transport through soil profile in comparison to control was remarkable. Beesley et al. (2010) observed reduction of water-soluble Zn and Cd in incubation experiment, where they applied 50% of biochar. In our experiment 5% of biochar provided a significant reduction of Cd and Zn leaching. Thus, biochar application was proved as the effective measure for improvement of phytoremediation efficiency at the extremely contaminated soil.

Finally, we can summarize that biochar appears to be a very effective regulator of availability of observed risk elements and it improves biomass growth and thus increases total uptake of Cd and Zn and decreases leaching of these elements.

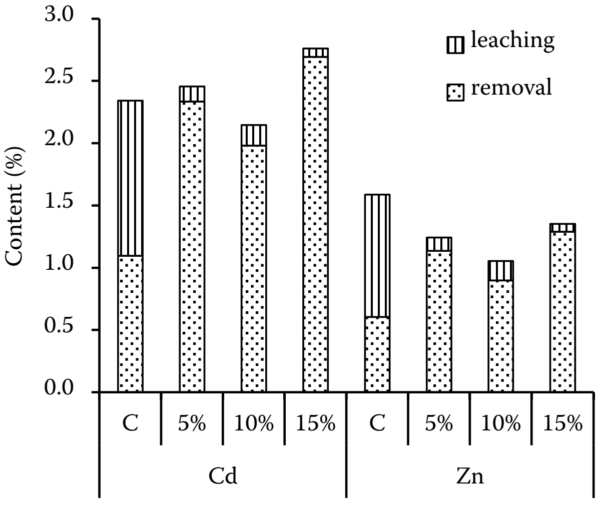

Figure 3. Relative comparison of zinc (Zn) and cadmium (Cd) removal and leaching from contaminated soil (%). C – control; 5–10% – amount (w/w) of applied biochar

doi: 10.17221/181/2015-PSE

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Received on March 18, 2015
Accepted on June 23, 2015

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