https://doi.org/10.17221/755/2017-PSE

Effect of tree harvest intervals on the removal of heavy metals from a contaminated soil in a field experiment

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ABSTRACT

Kubátová P., Száková J., Břendová K., Kroulíková-Vondráčková S., Drešlová M., Tlustoš P. (2018): Effect of tree harvest intervals on the removal of heavy metals from a contaminated soil in a field experiment. Plant Soil Environ., 64: 132–137.

Four clones of short rotation coppice (SRCs) were investigated for phytoextraction of soil contaminated by risk elements (REs), especially Cd, Pb and Zn. As a main experimental factor, the influence of rotation length on the removal of REs was assessed. The field experiment with two *Salix* clones (S1 – (*Salix schwerinii* × *Salix viminalis*) × *S. viminalis*; S2 – *S.* × *smithiana*) and two *Populus* clones (P1 – *Populus maximowiczii* × *Populus nigra*; P2 – *P. nigra*) was established in April 2008 on moderately contaminated soil. For the first time, all clones were harvested in February 2012 (2012_{4y}) after 4 years. Subsequently each plot was equally split into halves. The first half of the SRC clones was harvested in February 2014 after 2 years (2014_{2y}) and again it was harvested in February 2016 after further 2 years (2016_{2y}). The second half was harvested in February 2016 after 4 years (2016_{4y}). The results showed that the biomass production for the second 4-year harvest period was significantly higher for all clones but the metal concentration was lower in the mentioned period. 4-year rotation seems to be more advantageous for the phytoextraction than two 2-year rotations. The highest metal removal presented by remediation factors (RFs) per 4 years for Cd (6.39%) and for Zn (2.55%) were found for S2 in the harvest 2016_{4y} treatment. Removal of Pb was the highest by P1 clone with very low RF per 4 years (0.04%). Longer rotation is also economically superior.

Keywords: willow; poplar; cadmium; lead; zinc; contamination

Phytoextraction is an environmentally friendly method, where the risk elements (REs) are removed from soil by their accumulation in plant tissues (Favas et al. 2014). Suitable plants for phytoextraction must accumulate REs in their tissues and produce large amount of biomass. The biomass production can be supported by correct selection of short rotation (SRCs) clones and habitats (Mrnka et al. 2011) or improving soil conditions by control of weeds (Larsen et al. 2014) or fertilization (Sevel et al. 2014) but also the length of rotation (Schweier et al. 2017).

In an experiment with ten *Salix* clones, most reached a maximum mean annual increment (8 to 14 t/ha/year) after a rotation period of 4–5 years, while extremely short rotations (1–2 years) were unsuitable (Willebrand et al. 1993). Conversely, Bullard et al. (2002) reported that two *Salix* clones (*S.* × *dasyclados* and *S. viminalis*) had higher annual yields after 2 years than after 3 years. Sevel et al. (2014) reported that the *Salix* clone Tordis (S1) after 2 years achieved yields of 8.7–11.9 t/ha/year; in the study of Larsen et al. (2014) after 3 years, the yields of 5.2–10.2 t/ha/year were

Supported by the Czech University of Life Sciences Prague, Project No. CIGA 20172023.

https://doi.org/10.17221/755/2017-PSE

achieved, as dependent on the fertilization rate. In a trial with three harvests of three-year rotations, the *Salix* clones showed better biomass yield (S2 clone – 14 t/ha/year) than the *Populus* clones; for them, the 3-year rotations were too short (Weger 2008). In the study of Weger (2009), clone P1 harvested after 6 years in one rotation yielded 11.7 t/ha/year, 9.2 t/ha/year after 3 years in two rotations and only 5.7 t/ha/year when the harvest was done annually. Schweier et al. (2017) reported the yield of P1 clone was on average 10.4% higher in a 7-year rotation than in a 3-year rotation. Nielsen et al. (2014) estimated the biomass production after 5 and 13 years for 36 *Populus* clones (include P1 and P2 clones). Generally, average biomass yield for all clones together was higher after 13 years (3.6 t/ha/year; specifically P1 = 5.1 t/ha/year; P2 = 1 t/ha/year) than after 5 years (2.3 t/ha/year; data for P1 and P2 were not shown). According to Liu et al. (2016), clones cultivated under a 3-year rotation achieved higher mean annual yields, but lower tissue concentrations of elements, including REs, than clones cultivated under 1 and 2-year rotations.

MATERIAL AND METHODS

Study site and field experiment. The field experiment was established in April 2008 on multi-RE (mostly Cd, Pb and Zn)-contaminated agricultural soil (49°42'24''N, 13°58'32''E), near the town of Příbram, 58 km south of Prague, Czech Republic. Two previously tested *Salix* clones S1 and S2 and the two *Populus* clones P1 and P2 were grown in contaminated weakly acidic Haplic Cambisol. For planting, homogeneous 20-cm long cuttings of clones were used. Pseudo-total (*aqua regia*-soluble) mean (± standard error) concentrations of REs in the soil (0–20 cm) were as follows: 7.3 ± 0.22 mg Cd/kg, 218 ± 5.9 mg Zn/kg and 1370 ± 33 mg Pb/kg (n = 50). An exact description of this place, soil and clones was published in the study of Zárubová et al. (2015). The experimental area comprised 32 rows (experimental units), 8 plots each with 4 rows and each row containing one clone. Each row covered the area of 7.5 × 1.3 m, and the intra-row original planting distance among plants was 0.25 m. Whole plots were arranged in a completely randomised design with eight replications. Each whole plot contained four sub-plots = rows (experimental units). The first harvest was carried out in February 2012 (2012_{4y}), when the whole site was cut uniformly. Then each row was equally split into two experimental units. The first units of rows were harvested twice after two years, first in February 2014 (2014_{2y}), as published in the study of Kubátová et al. (2016) and second in February 2016 (2016_{2y}). The second part of rows was harvested once after four years in February 2016 (2016_{4y}). The shoots of SRCs clones were cut 20 cm above the soil surface. Each experimental unit (half of row) was harvested and weighed for fresh matter, then subsamples (one whole plant from each unit) were collected, weighed, cutted, dried at 60°C, to determine dry matter and ground using a stainless steel Retsch friction mill (Retsch, Haan, Germany; particle size 0–1 mm). Mortality of clones after each harvest was monitored, so all living plants in each row were counted. The dry matter yield per hectare was estimated as follows: the dry matter weight of unit was multiplied by the number of units per hectare. Dry ashing procedure (Mader et al. 1998) was applied for sample decomposition. The ash was solubilized in HNO_3 (65%) and adjusted by deionised water into 20 mL flasks. The total concentrations of REs in the harvested biomass (stems and twigs without leaves) were determined using the inductively coupled plasma with optical emission spectroscopy (ICP-OES; Agilent 720, Agilent Technologies Inc., Torrance, USA). Certified reference material RM NCS DC 73349 – bush branches and leaves (Analytika, Prague, Czech Republic), was analysed under the same conditions for quality assurance of the analytical method.

Statistical analyses. All statistical analyses were performed using the software packages Statistica 12.0 (www.statsoft.com). All data were checked for homogeneity of variance and normality (Levene and Shapiro-Wilk tests). The collected data did not meet the assumptions for the use of analysis of variance (ANOVA) and were thus evaluated by the non-parametric Kruskal-Wallis test.

Remediation factor (RF). The phytoextraction potential of the examined clones was expressed as relative RF (%), which indicates the proportion of elements removed via the harvested biomass from the pseudototal soil contents of the elements at the site. The RF was calculated as follows (1):

$$\text{RF (\%)} = \frac{C_{plant}DM_{plant}}{C_{soil}W_{soil}} \times 100 \qquad (1)$$

Where: C_{plant} – concentration of REs in the plant dry biomass (g/t); DM_{plant} – dry matter plant biomass yield (t);

https://doi.org/10.17221/755/2017-PSE

C_{soil} – pseudototal concentration of the REs in soil (g/t); W_{soil} – amount of soil (t/ha) in the top horizon (0–20 cm) at soil bulk density of 1.35 t/m^3, modified according to Komárek et al. (2008).

RESULTS AND DISCUSSION

Biomass yield of SRCs. Generally, the annual biomass production per plant increased non-significantly in the order $2012_{4y} < 2014_{2y} < 2016_{2y} < 2016_{4y}$, with the exception of S1 clone. The S1 clone had higher annual biomass production in harvest 2016_{2y} (884.5 g/plant = 442 g/plant/year) than in harvest 2016_{4y} (1361 g/plant = 340 g/plant/year). However, if the biomass production only after 4 years is compared, the lowest biomass production was in the first harvest in 2012_{4y}, followed by the combined harvests 2014_{2y} + 2016_{2y} and the harvest 2016_{4y} (Table 1). The same trends were also found for dry biomass production per hectare compromising different numbers of trees per plot (Figure 1a). Differences between the yield of harvest 2016_{4y} and yield of harvests 2014_{2y} + 2016_{2y} were small for clone S1, but the yield of harvest 2016_{4y} was 1.5, 2 and 4 times higher than the yield sum of harvests 2014_{2y} + 2016_{2y} for the clones S2, P1 and P2, respectively (Figure 1a). The results confirm that longer harvest periods are advantageous especially for the *Populus* clones. Weih (2004) reported suitable rotation periods of 4–6 years for *Populus* clones while for *Salix* clones suitable rotation periods can be also 3 years. The biomass yield also increased with the increasing number of rotations. According to Havlíčková et al. (2010), the biomass yield of the first harvest

Table 1. The mean (± standard error) dry matter and concentrations of elements (Cd, Pb and Zn) in the biomass of *Salix* (S1 – (*S. schwerinii* × *S. viminalis*) × *S. viminalis*; S2 – *S. smithiana*) and *Populus* (P1 – *P. maximowiczii* × *P. nigra*; P2 – *P. nigra*) clones

Variable	Period (year)	Time	Clone S1	S2	P1	P2
Dry biomass (g/plant)	4	2012	38.77 ± 6.90^{Aa}	132.43 ± 16.03^{Ba}	196.90 ± 43.39^{Ba}	101.97 ± 29.11^{ABa}
	2	2014	403.15 ± 182.59^{ABab}	498.15 ± 137.11^{Bab}	540.93 ± 104.10^{Bab}	71.23 ± 16.55^{Aa}
	2	2016	884.51 ± 106.70^{ABb}	1604.55 ± 318.62^{Bbc}	1126.34 ± 159.64^{Bbc}	173.33 ± 48.81^{Aab}
	2 + 2	total	1287.66 ± 211.75^{ABb}	2102.70 ± 296.84^{Bbc}	1667.27 ± 234.50^{Bbc}	244.56 ± 60.49^{Aab}
	4	2016	1361.23 ± 167.29^{Ab}	3843.20 ± 368.89^{Bc}	4171.81 ± 539.24^{Bc}	792.77 ± 226.02^{Ab}
Cd (mg/kg)	4	2012	54.96 ± 6.83^{Bb}	44.40 ± 7.90^{ABb}	23.60 ± 2.91^{Ab}	25.33 ± 3.36^{Ab}
	2	2014	26.73 ± 1.99^{Cab}	22.35 ± 1.51^{BCa}	10.96 ± 0.68^{Aa}	14.39 ± 1.63^{ABa}
	2	2016	25.84 ± 1.83^{Ba}	29.04 ± 2.07^{Bab}	15.81 ± 1.37^{Aab}	13.27 ± 0.43^{Aa}
	4	2016	27.47 ± 1.28^{Cab}	22.19 ± 1.71^{Ba}	10.65 ± 0.77^{Aa}	14.55 ± 1.00^{ABa}
Pb (mg/kg)	4	2012	28.83 ± 4.02^{Ab}	24.53 ± 3.57^{Ab}	30.09 ± 2.89^{Ac}	19.48 ± 3.20^{Aa}
	2	2014	14.81 ± 0.97^{Aab}	14.36 ± 1.25^{Aab}	27.34 ± 2.74^{Bbc}	16.89 ± 1.74^{Aa}
	2	2016	13.00 ± 1.33^{Aa}	14.10 ± 1.72^{Aa}	15.51 ± 1.44^{Aa}	15.68 ± 1.19^{Aa}
	4	2016	16.39 ± 1.50^{Aab}	16.39 ± 1.34^{Aab}	17.94 ± 1.07^{Aab}	16.47 ± 3.64^{Aa}
Zn (mg/kg)	4	2012	506.26 ± 66.17^{Cb}	342.62 ± 38.87^{BCb}	206.83 ± 23.49^{ABb}	158.29 ± 21.53^{Ab}
	2	2014	251.34 ± 23.95^{Ba}	186.70 ± 15.97^{ABa}	114.66 ± 7.81^{Aa}	120.21 ± 14.28^{Aab}
	2	2016	248.79 ± 14.59^{BCa}	279.14 ± 24.76^{Cab}	168.93 ± 13.94^{ABb}	93.08 ± 5.71^{Aa}
	4	2016	285.64 ± 16.54^{Bab}	267.52 ± 36.19^{Bab}	148.66 ± 8.94^{ABab}	126.95 ± 5.44^{Aab}

Differences between the clones and harvest periods were evaluated by the Kruskal-Wallis test at $P \le 0.05$. Clones with the same capital letter for each harvest period were not significantly different. In each harvest period, clones with the same lowercase letter were not significantly different

https://doi.org/10.17221/755/2017-PSE

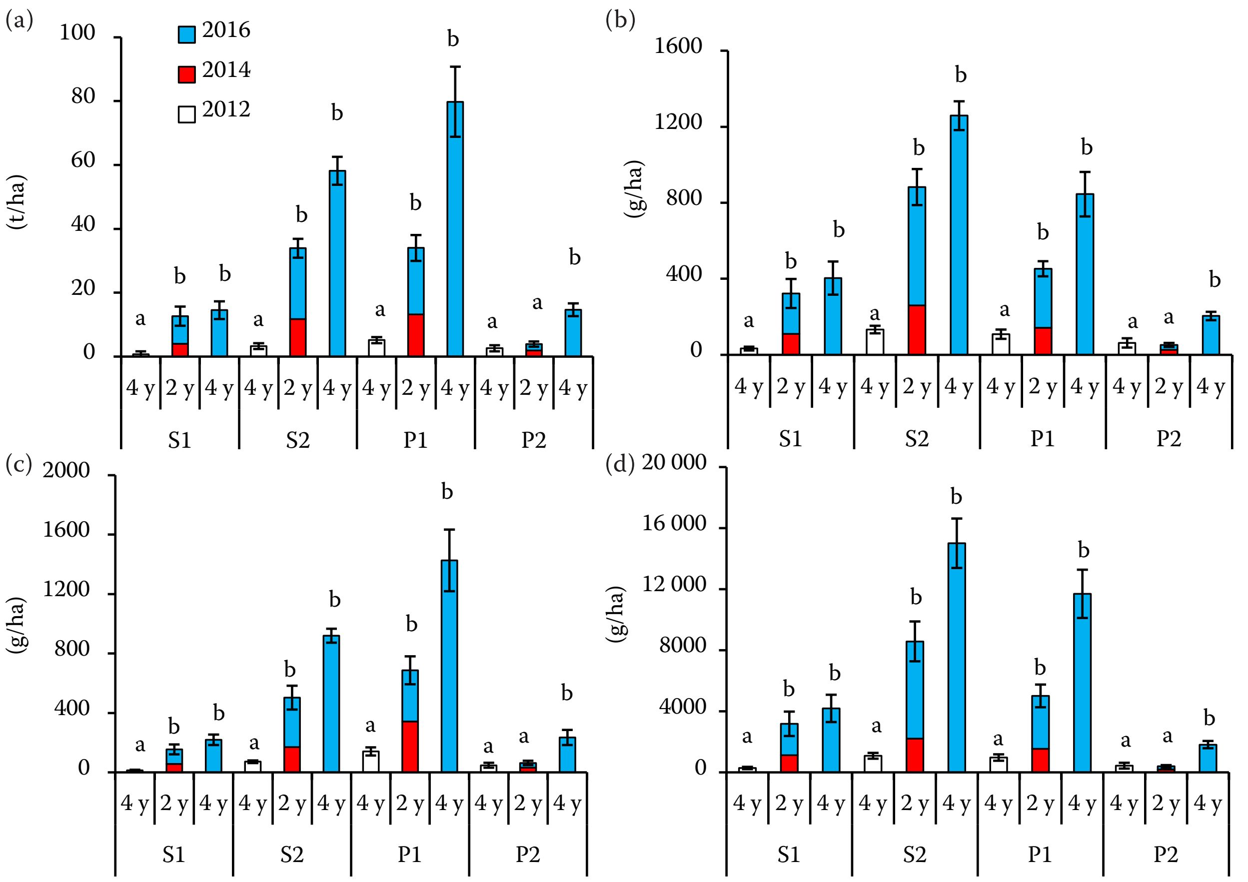

Figure 1. The mean (± standard error) dry matter yield (a) and the mean amount of Cd (b), Pb (c) and Zn (d) removed by the harvested biomass of *Salix* (S1 – (*S. schwerinii* × *S. viminalis*) × *S. viminalis*; S2 – *S. smithiana*) and *Populus* (P1 – *P. maximowiczii* × *P. nigra*; P2 – *P. nigra*) clones (30 769 plants/ha minus mortality). In each clone, periods of harvest with the same lowercase letter were not significantly different. Differences between the harvest periods were evaluated by the Kruskal-Wallis test at $P \leq 0.05$; 2 y – 2 years period; 4 y – 4 years period

represented only ~30% of the yield from the following harvests. Weger (2008) reported that biomass yield of SRC clones in the third rotation was about 20% higher than in the second one.

Clone P1 had the highest yields in all harvests, only with the exception of harvest 2016_{2y}. Yields obtained with the P1 clone ranged from 1.29 t/ha/year (5.1 t/ha) in harvest 2012_{4y} to 19.95 t/ha/year (79.8 t/ha) in harvest 2016_{4y}. Similarly, clone S2 achieved a yield as high as 14.55 t/ha/year (58.2 t/ha) in harvest 2016_{4y} (Figure 1a). Mrnka et al. (2011) reported yields ranging between 6–18 t/ha/year for P1 clone. Weger (2008) found yields of 14.63 t/ha/year for clone P1 and of 14 t/ha/year for clone S2 in the third harvest in a 3-year rotation. Above average biomass yields of both P1 and S2 clones in our study can be explained by a very high density of planting (30 769 plants/ha) at the beginning of the experiment (real densities in 2016 for clones S1, P1, S2 and P2 were 15 100, 19 400, 23 700 and 21 800 plant/ha, respectively) as well as by suitability for climate conditions. Typical SRC planting densities for production of energy biomass range from 6000 to 12 000 plants for *Populus* clones and 10 000–20 000 for *Salix* clones (Havlíčková et al. 2010). Clone S1 had a yield from 0.17 t/ha/year (0.67 t/ha) in harvest 2012_{4y} to 4.3 t/ha/year (8.6 t/ha) in harvest 2016_{2y}. Clone P2 had a yield from 0.65 t/ha/year (2.58 t/ha) in harvest 2012_{4y} to 3.65 t/ha/year (14.6 t/ha) in harvest 2016_{4y} (Figure 1a). These S1 and P2 clones achieved lower or comparable results to the findings of other studies. Larsen et al. (2014) reported yields of 5.2–10.2 t/ha/year for clone S1 in the first rotation after 3 years; Sevel et al. (2014) in a 2-year rotation reported yields of 8.7 to 11.9 t/ha/year. In the study by Verlinder et al. (2013) biomass yield of the P2 clone was 1.27 t/ha/year after one year, and in the study by Verlinder et al.

https://doi.org/10.17221/755/2017-PSE

(2015) it was 3.75 t/ha/year after 4 years. Nielsen et al. (2014) reported yields of only 1 t/ha/year for clone P2 after 13 years.

Risk element concentrations in wood (twigs and stem together) of SRCs. For all clones the highest concentrations of all REs were found in the first harvest 2012_{4y}. In this harvest biomass yield was the lowest (Table 1). According to Tinker et al. (1981) a high growth rate of the plant may cause internal 'dilution' of REs. Also concentrations of REs are generally higher in the bark than in the wood and wood/bark ratio increases with the age of the shoots (Zárubová et al. 2015). Concentrations of REs in other harvests did not show any uniform trend. Generally, in all harvests *Salix* clones S1 and S2 accumulated considerably higher concentrations of Cd and Zn than *Populus* clones. *Populus* clone P1 accumulated higher (in harvest 2014 significantly, $P \leq 0.05$) concentrations of Pb than other clones (Table 1). These findings are in line with our previous research (Fischerová et al. 2006, Zárubová et al. 2015).

Removal of elements from the soil. Removal of elements per hectare and per year from the soil by shoots of all clones closely corresponded to the biomass yield per hectare. Generally, annual removal of Cd, Pb and Zn for S2, P1 and P2 clones increased non-significantly in the order $2012_{4y} < 2014_{2y} < 2016_{2y} < 2016_{4y}$, with the exception of Cd and Zn for P2 clone, because the Cd and Zn removal in harvest 2012_{4y} was higher than the removal in harvests 2014_{2y} and 2016_{2y} due to low biomass production. Also, annual removal of Cd by clone S1 had a similar trend, as the lowest biomass production per hectare was found in the first harvest and increased non-significantly in the order $2012_{4y} < 2014_{2y} < 2016_{4y} < 2016_{2y}$. However, annual Pb and Zn removal was slightly higher in 2016_{4y} than in 2016_{2y} (Figure 1). The comparison of harvests after only 4 years as to the removal of metals showed a very close relationship for all clones and for all REs, with the lowest amount of REs being removed in harvest 2012_{4y}, followed by the combined harvests 2014_{2y} + 2016_{2y} and harvest 2016_{4y}, with the exception of P2 clone. Removal of Cd and Zn for P2 clone was higher in the first harvest 2012_{4y} than in the combined harvests 2014_{2y} + 2016_{2y}. This finding confirms that the biomass production is very important for the phytoextraction potential proposed by Komárek et al. (2008). The P1 clone had the highest biomass yield in all harvests, with the exception of harvest 2016_{2y}, but this clone removed only the highest amount of Pb in all harvests (Figure 1c). The S2 clone removed the highest amount of Cd and Zn in all harvests (Figure 1b, d), which is in line with the results of Zárubová et al. (2015).

Remediation factor (RF). The RFs (considering top 20 cm of the soil) after 4 years increased in the order $2012_{4y} < 2014_{2y} + 2016_{2y} < 2016_{4y}$ for all clones and for all REs, with the exception of RFs of Cd and Zn for clone P2 that were higher in the first harvest 2012_{4y} than in the combined harvests 2014_{2y} + 2016_{2y}. The highest RFs for Cd and for Zn in all harvest periods were found for clone S2 (RFs for Cd: 0.67% in 2012_{4y}, 4.53% in 2014_{2y} + 2016_{2y} and 6.39% in 2016_{4y}; RFs for Zn: 0.19% in 2012_{4y}, 1.46% in 2014_{2y} + 2016_{2y} and 2.55% in 2016_{4y}). The RF for Cd was 9.5 times higher in harvest 2016_{4y} than the RF in harvest 2012_{4y}; the RF for Zn was 13 times higher in harvest 2016_{4y} as compared to the RF in the harvest 2012_{4y}. The RFs for Pb were extremely low in all harvests and for all clones, ranging from 0.0004% (S1 in 2012_{4y}) to 0.04% (P1 in 2016_{4y}). The highest RFs for Cd and Zn in our study were slightly higher than in other field experiments (Jensen et al. 2009, Zárubová et al. 2015).

Rotation lasting 4 years seems to be the most promising approach as compared to two 2-year harvest rotations because of the higher biomass production, associated with higher removal of REs as well as higher RFs.

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Received on November 27, 2017
Accepted on March 7, 2018
Published online on March 15, 2018