RESEARCH

Open Access

Biomass yield and metal phytoextraction efficiency of *Salix* and *Populus* clones harvested at different rotation lengths in the field experiment

Nikola Prouzová¹, Pavla Kubátová¹, Filip Mercl¹, Jiřina Száková¹, Jana Najmanová¹ and Pavel Tlustoš^{1*}

Abstract

Background Phytoextraction belongs to environmentally well-accepted remediation technologies to remove metals from contaminated soils. Due to long-time requirement, sufficient data for proper phytoextraction evaluation are missing. Four clones of fast-growing trees: two willow species (S1), *Salix viminalis* L. (*Salix schwerinii* E.L.Wolf × S. *viminalis*) × S. *viminalis*) and (S2)—*Salix smithiana* (*Salix* × *smithiana* Willd.), and two poplar clones (P1), *Populus* Max-4 (*Populus nigra* L.×*Populus maximowiczii* A. Henry) and (P2) Wolterson (*P. nigra* L.) were cultivated under field conditions at medium-to-high Cd and Pb, and low Zn soil contamination to assess trees' long-term ability of biomass production and removal of potentially toxic elements (PTEs). The biomass yield and PTE uptake were measured during 8 years of regular growth under three rotation lengths: four harvests following 2-year periods (4 × 2y), two harvests in 4-year periods (2 × 4y), and one harvest representing 8 years of growth (1 × 8y).

Results In most cases, the highest annual dry biomass yield was achieved with a $2 \times 4y$ rotation (P1 = 20.9 t ha⁻¹ y⁻¹, S2 = 18.4 t ha⁻¹y⁻¹), and the yield decreased in order $2 \times 4y > 1 \times 8y > 4 \times 2y$ of harvesting periods. Only clone S1 showed a different pattern. The differences in biomass yield substantially affected the PTE phytoextraction. The greatest amount of Cd and Zn was removed by willow S2, with the highest biomass yield, and the strongest ability to accumulate PTEs. With $2 \times 4y$ rotation, S2 removed a substantial amount of Cd (9.07%) and Zn (3.43%) from the topsoil horizon (0–20 cm) and 5.62% Cd and 2.04% Zn from horizon 20–40 cm; phytoextraction rate was slightly lower for $1 \times 8y$ rotation. The poplar P1 removed the most Pb in the $1 \times 8y$ rotation, but the overall Pb phytoextraction was negligible. The results indicated that lignin and cellulose contents increased, and hemicellulose content decreased with increased concentrations of Cd, Pb and Zn in poplars wood.

Conclusions The data confirmed that phytoextraction over longer harvest periods offered promising results for removing Cd from medium- to high-level contaminated soils; however, the ability of Pb removal was extremely low. The longer harvest period should be more economically feasible.

Keywords Phytoextraction, Cadmium, Lead, Zinc, Willow, Poplar, Short rotation coppice

*Correspondence: Pavel Tlustoš tlustos@af.czu.cz Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/ficenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.



Background

Phytoextraction is an eco-friendly, low-cost alternative for restoring the quality of soils contaminated by potentially toxic elements (PTEs) with the aid of welldeveloped plants [1]. Numerous plant species have been extensively tested as potential candidates for successful phytoremediation of soils contaminated by PTEs from anthropogenic sources. The required criteria for plant species to be successful in phytoextraction are good tolerance to high concentrations of available PTEs in soil, efficient metal uptake, translocation of the accumulated PTEs to aboveground biomass, fast growth, and high production of aboveground biomass [2, 3]. Short rotation coppices (SRCs) of fast-growing trees planted on arable land can fulfill the majority of requirements and be effective from both the economic and ecological points of view [4]. These plants do not accumulate extremely high concentrations of PTEs in biomass like hyperaccumulators, but this shortcoming is compensated by their large annual biomass production, which could be utilized for wood products or as a renewable energy source [1, 5].

The SRCs cultivated on arable land contaminated by PTEs (especially Cd and Zn) can act as bioaccumulators [6], and because of their ability to produce large amounts of biomass [7], they can significantly reduce the content of these pollutants in the soil [2, 6, 8, 9]. The most promising and commonly cultivated SRC trees are the willows (*Salix*) and poplars (*Populus*); many cultivars have been bred and widely distributed as selected clones [10]. An important factor affecting biomass production is the length of time between coppice rotations [11]. The most

common coppice rotations are from every 2 to every 4 years. Shorter rotations can adversely affect the following growth rate, especially for poplars, which are negatively affected by short rotation coppicing; longer periods of growth can be more suitable for them, depending on the clone [11, 12]. Rotation length affects the total cultivation period, which is an important economic parameter [13]. The vitality of a tree can also correlate with its vulnerability as poor health increases susceptibility to infestation by insects or fungi, and possibly greater browsing damage [14].

Most studies [15–18] have focused their research on clones with high biomass production findings, and the ability to take up and store a high amount of PTEs in the aboveground parts, which is crucial for successful phytoextraction. A large number of willow and poplar clones were tested and a high variability in PTE accumulations was found [19-21], not only between individual clones [19, 21], but also for single clones at different sites, due to differences in soil properties [22] and contamination level [19]. High differences in PTEs accumulation were found also between plants grown in pot experiments and in the field, because the roots of plants in pots densely penetrate a limited volume of soil, but in the field, they can easily develop in the large soil volume avoiding contaminated topsoil layer [23]. The PTEs distribution in plant tissues is not uniform, for instance, Cd and Zn are accumulated more in the aboveground biomass, especially in leaves while Pb is often accumulated more in branches [17] and in roots [24]. The results of a study by Kubátová et al. [25] confirmed twofold higher Cd accumulated by

willow leaves (62 mg kg⁻¹) than by branches (30 mg kg⁻¹) and fivefold higher accumulation of Zn in leaves $(1700 \text{ mg kg}^{-1})$ than by branches (300 mg kg^{-1}) . Leaf biomass accounted for only 16-28% of the harvested aboveground biomass of 9-year-old clones. The phytoextraction efficiency of the PTEs was influenced by factors, such as moisture, organic matter, redox potential, and especially soil pH [26]; however, the efficiency of phytoextraction can be increased by agronomic practices, such as soil fertilization and conditioning, enhancing metal bioavailability, plant selection and length of rotation [27]. The length of rotation of willow and poplar clones can affect biomass yield [28], which is an important parameter for phytoextraction [27], as well for wood quality and composition [29]. The harvested wood can be utilized for lumber or as a renewable energy source. The content of the primary wood compounds, cellulose, lignin, hemicellulose and water affects its postharvest treatment [30, 31]. The poplar clones in the study of Guidi et al. [30] and the willow clones in the study of Stolarski et al. [31], harvested in 4-year rotations, achieved higher biomass vield and cellulose content and lower lignin content than clones harvested in 2-year rotations. A high cellulose content is suitable for converting the biomass into second generation biofuels (e.g. bioethanol). According DeBell et al. [32] poplar wood from trees harvested at older ages had higher wood density and fiber length, and was suitable for manufacturing secondary products [29]. Lignin surfaces contain carboxylic- and phenolic-type groups with high affinity for PTE ions to bind them in the order: Pb>Cd>Zn [33]. Juvenile shoots had a higher ratio of bark to wood, and bark contains more lignin than wood [29]; therefore, the juvenile shoots contain higher metal concentrations than older ones [34].

As summarized above, several important research questions have to be answered to optimize the effectiveness of the phytoextraction process. How do short-rotation harvests affect the long-term stability/instability of biomass yield and phytoextraction efficiency? What rotation length of harvest gives the highest PTE removal? How does the harvest rotation period affect wood quality from polluted areas? Therefore, the main objectives of the research presented in this paper were: (i) to assess the effect of rotation length on biomass production and on accumulation of PTEs in selected clones, (ii) to compare the biomass yield and potential of two willow and two poplar clones for accumulating and extracting Cd, Pb and Zn within individual harvests with different rotation lengths, and (iii) to measure the effect of rotation length and PTE content on the composition of the main wood compounds in the biomass.

Materials and methods Site description

The study area is located in the Central Bohemian region, Příbram district, Czech Republic (49°42'24''N, 13°58′32′′E) near the former local lead smelter, which is responsible together with ore mining for the historical contamination of the area. Currently, however, no atmospheric deposition occurs from the smelter [35]. The present study did not consider soil characteristics over time, because this continuing experiment occupied less than half of an experimental area ($\sim 5000 \text{ m}^2$) and would have been very labor-intensive. We expect to test other longer term harvest protocols for substantial PTE removal and confirm the results by soil sampling. The experimental plot is 500 m above sea level, with mean annual precipitation of 700 mm and a mean annual temperature of 6.5 °C. The soil is also classified as a weakly acidic modal Cambisol (pH_{H2O} 5.66; pH_{KCl} 5.27) with a CEC of 166 mmol·kg⁻¹, C_{org} of 4.1%, C/N ratio of 9, and a bulk soil density of 1.35 t·m⁻³ in horizon 0–20 cm and 1.39 t \cdot m⁻³ in horizon 20–40 cm. The experiment started in April 2008 in soil contaminated, medium-to-high, with multiple PTEs, mainly Cd, Pb, and Zn. Hundreds soil samples from the top 0-20 cm and from the subsoil (20-40 cm) horizons were taken before the experiment was set up. The average (±standard error) content of aqua regia-extractable (pseudo-total) PTEs were Cd 7.3 \pm 0.22 mg kg⁻¹, Pb 1368 \pm 33 mg kg⁻¹, and Zn 218 \pm 5.9 mg kg⁻¹ in horizon 0–20 cm and Cd 4.4 \pm 0.39 mg kg⁻¹, Pb 672 \pm 37 mg kg⁻¹, and Zn 135 \pm 4.9 mg kg⁻¹ in horizon 20–40 cm. The plantavailable concentrations of Cd, Pb and Zn extracted by 0.01 mol l^{-1} CaCl₂ were in horizon 0–20 cm 2.6, 42 and 21 mg kg^{-1} , respectively and in horizon 20-40 cm 1.5, 28and 12 mg kg^{-1} , respectively.

Experimental design

For the study, two clones of willow and two poplar species were tested. Cuttings (length 20 cm) were planted in plots of four rows (7.5×1.3 m) with each row considered an experimental unit (4 clones×8 repetitions). The cuttings were supplied by the Silva Tarouca Research Institute for Landscape and Ornamental Horticulture, v.v.i (Průhonice, CZ). Experimental units were arranged in a completely randomized design, and plants in the rows were spaced 0.25 m apart (density=30,769 plants ha⁻¹ at the beginning of the experiment). SRCs have been cultivated on this experimental site since 2008. Two willow clones, allochthonous hybrid Tordis (*Salix schwerinii×S. viminalis*)×*S. viminalis* (S1), and autochthonous clone S-218, *Salix×smithiana* (S2), and two poplar clones J-105 (also known as Max-4), *Populus maximowiczii×Populus* nigra (P1), and commonly grown clone Wolterson, Populus nigra, (P2) were chosen as SRC subjects, all the clones were previously verified as suitable for phytoextraction [19, 36–39]. The first uniformly cut harvest was in 2012 (time zero). Some of the units were harvested four times, once after each 2-year growing season, representing the short rotation length $(4 \times 2y)$ condition, while the others were harvested twice, after each 4-year growing season, representing the long rotation period $(2 \times 4y)$. The first 2-year harvest was done in 2014 (2y₂₀₁₄), and further 2-year harvests followed in 2016 (2y₂₀₁₆), 2018 (2y₂₀₁₈) and 2020 $(2y_{2020})$. The 4-year harvests were done in 2016 $(4y_{2016})$ and 2020 $(4y_{2020})$. Another part of the experimental site was maintained and harvested after 8 years $(1 \times 8y)$ of growth for the first time in 2020 (Fig. 1). The biomass yields (DM) for all rotations were defined as the sum of all harvests of one clone during 8 years: the sum of yields from four 2-year harvests, the sum of yields from two 4-year harvests and the yield from one 8-year harvest.

Previous study [25], showed higher leaves accumulation of PTEs (especially Cd and Zn) than branches and stems; however, mature trees account only low amount of leaves from total aboveground biomass; therefore, in longer rotations, leaf biomass does not play an important role. All harvests were made during the winter period (in February), which is the most suitable time for successful re-vegetation of the trees. Trees were planted without any application of nutrients or pesticides.

At harvest, a row and all its plants were cut about 20 cm above the soil surface. Each experimental unit (row) was harvested and weighed for fresh aboveground biomass (branches + stem), and then subsamples of one plant from each unit (8 samples for each clone and each harvest) were collected, weighed in the field immediately after harvest for fresh weight (FW), then dried to constant weight at 60 °C, and dry weight (DW) was recorded. Samples were ground using a stainless-steel mill. The dry matter (DM) of one row (unit) was then calculated from the DW/FW ratio and the fresh matter weight per row. Yield of DM per hectare was calculated by multiplying DM by the number of units per hectare (Additional file 1: Fig. S1).

Analytical methods

Dry biomass was chipped and then ground to particles of ~ 1 mm using a stainless steel Retsch friction mill (Retsch, Haan, Germany) to make homogeneous samples for determination of element contents. Decomposition of the biomass samples (0.5 g) was carried out by the dryashing decomposition method [40]. The total content of Cd, Pb and Zn in aboveground biomass (branches + stem) was determined after dry-ashing decomposition (25 mL

solubilized sample in 65% HNO₃). Detection was done using an inductively coupled plasma source with optical emission spectroscopy (ICP–OES; Agilent 720, Agilent Technologies Inc., USA). Aliquots of the certified reference material (CRM) RM NCS DC 73349, bush branches and leaves, (Analytika, Prague, Czech Republic), were determined under the same conditions for quality assurance of the analytical method. The certified values of the CRM were the following: 0.38 ± 0.08 mg kg⁻¹ for Cd; 47 ± 3 mg kg⁻¹ for Pb, and 55 ± 4 mg kg⁻¹ for Zn. The measured values of this CRM were 0.45, 44.6 and 53.7 mg kg⁻¹ for Cd, Pb and Zn, respectively.

For the contents of specific wood components, cellulose was determined by Seifert's method [41] and holocellulose by Wise's method [42]. Lignin was determined in accordance with the Tappi T 222 om-11 standard [43], ash in accordance with the Tappi T 211 om-02 standard [44], and extractives in accordance with the Tappi T 5 wd-73 [45] and Tappi T 6 wd-73 standards [46].

Indices of the effectiveness of phytoextraction *Total uptake*

Total uptake of PTEs (g ha⁻¹) indicates the amount of PTEs removed by aboveground biomass (without leaves) of SRC clones. It was determined as $C_{\text{plant}} \times \text{DM}_{\text{plant}}$, where C_{plant} is the concentration of PTE in dry biomass (g t⁻¹) and DM_{plant} is the total dry matter plant biomass yield per row converted to per hectare (t ha⁻¹).

Remediation factor

The remediation factor (RF) indicates the total amount of accumulated PTEs in the aboveground biomass (branches+stem) removed from horizon 0–20 cm and from horizon 20–40 cm of the contaminated soil by the investigated SRC clones over a given time period, divided into the total amount of individual element present in each soil horizon, expressed as relative phytoextraction potential in percentage. The RF (%) was determined as follows: $(RF\%) = \frac{C_{plant}DM_{plant}}{C_{soil}W_{soil}} \times 100$, where C_{soil} is the total concentration of PTEs in soil (g t⁻¹), and W_{soil} is the amount of soil (t ha⁻¹) in the topsoil horizon (0–20 cm) and (20–40 cm); modified according to Komárek et al. [38].

To mimic field conditions, and root growth below the topsoil, two layers of soil 0–20 cm and 20–40 cm were taken for the RF calculation. To properly distribute PTE removal between both layers the ratio of plant-available Cd, Pb and Zn concentrations located in horizons 0–20 cm and 20–40 cm was included into the calculation assuming their distribution in both layers. The ratio of the available soil content of each element between both layers was applied as the first assumption for the distribution of total plant uptake from individual layers. Different





February 2012

In February 2012 cutting of all plots (time zero), and 8 plots dividing them in half (other 8 plots remaining whole) - Start of the experiment



At harvest, a row (8-year rotation) or half of row (2- and 4-year rotation) and all its plants was cut about 20 cm above the soil surface. Each experimental unit (row) was harvested and weighed for fresh aboveground biomass, and then subsamples of one plant from each unit (8 samples for each clone and each harvest) were collected, weighed in the field immediately after harvest for fresh weight (FW), then dried to constant weight at 60°C, and dry matter (DM) was recorded. The DM of one row (unit) was then calculated from the DW/FW ratio and the fresh matter weight per row. Yield of DM per hectare was calculated by multiplying DM by the number of units per hectare



Fig. 1 Flow-chart showing the experimental procedure. S1 [(*S. schwerinii* × *S. viminalis*) × *S. viminalis*)], S2 (*S. smithiana*), P1 (*P. maximowiczii* × *P. nigra*), P2 (*P. nigra*), 2y (2-year rotation), 4y (4-year rotation), 8y (8-year rotation), 2y2014 (1. harvest after 2 years in 2014), 2y2016 (2. harvest after 2 years in 2016), 2y2018 (3. harvest after 2 years in 2018), 2y2020 (4. harvest after 2 years in 2020), 4y2016 (1. harvest after 4 years in 2016), 4y2020 (2. harvest after 4 years in 2020), 8y2020 (1. harvest after 8 years in 2020)

root distribution among two horizons was taken into account as the second assumption for the RF calculation. The percentage number of roots in both horizons was interpolated based on the study of Crow and Houston [47]. They found that 75–95% of all willow and poplar SRC roots were within the 0–36 cm-thick ploughed soil layer, mostly roots with diameters of <2 mm, important for the element uptake The top 0–20 cm horizon contained about 60% and the 20–40 cm horizon about 40% these roots, and this ratio was applied as the second parameter for the distribution of total uptake between the two layers, assuming that all roots had the same capacity for taking up elements.

Statistical methods

All statistical analyses were performed using the software packages Statistica 12.0 (www.statsoft.com). The relationship between wood components (lignin, cellulose and hemicellulose) and age of shoots of clones (harvested at 2, 4 and 8 years) and the relationship between the concentration of elements (Cd, Pb and Zn) and wood components (lignin, cellulose and hemicellulose) were evaluated using linear regression (LR). All data were checked for homogeneity of variance and normality (Levene and Shapiro–Wilk tests). Collected data did not meet assumptions for the use of analysis of variance (ANOVA) and were thus evaluated by the nonparametric Kruskal– Wallis test.

Results and discussion

Effect of different harvesting period on willow and poplar yield

Due to the low yield of biomass, plots were first uniformly harvested in 2012, 4 years after planting of the cuttings (time zero, Table 1), and yields of time zero were not included in the final evaluation (total DM yields for clones S1, S2, P1, P2 were 0.67, 3.26, 5.14 and 2.58 t ha⁻¹, respectively). For comparison, Scriba et al. [48] showed that S1 achieved a DM yield of 0.66 t ha⁻¹ biomass after the first year of cultivation, an amount of S1 biomass similar to what we found after 4 years of cultivation in our study. However, other researchers reported substantially higher DM yields than were found in our study: Tlustoš et al. [19] measured a yield of 2-5 t ha⁻¹ y⁻¹ in the first rotation for S2, Weger et al. [49] showed 5 t $ha^{-1}y^{-1}$ after the first year, and 10 t $ha^{-1}y^{-1}$ after 3 years for P1, while Laureysens et al. [50] reported 8.2 t ha⁻¹ y⁻¹ DM after the first 4-year rotation for P2. We speculate that the low biomass yield was probably a result of the slow development of roots, the weak growth of young plants due to lack of water, and strong competition of plants with fast growing grasses and other weeds [51]. Thus, yields highly depend not only on the clone but also on the level of soil contamination, nutrient and water availabilities, climate conditions and weed infestation [34].

The subsequent plant growth with already developed roots was much more robust. Succeeding harvests showed increased biomass yield and a large diversity in biomass production was observed (Fig. 2). Throughout the whole 8-year experiment, the lowest biomass production for all the clones and harvest periods was found in the 2-year rotations (ranging from 11 for P2 to 93 t ha^{-1} for S2) and the largest increase in biomass yield in the 4-year rotations ranged from 32 for P2 to 167 t ha^{-1} for P1, with the exception of clone S1 (24 t ha^{-1}). The DM yield in tons per hectare for S2, P1 and P2 clones decreased in the order $2 \times 4y > 1 \times 8y > 4 \times 2y$ (amount of biomass of each clone was evaluated as the sum of the biomass of all rotations during 8 years). For clone S2, however, the differences between all the rotations were not significant. In contrast, clone S1 had the highest total yield in the $1 \times 8y$ rotation (82 t ha⁻¹) and this yield was almost four times higher than the yield in the $4 \times 2y$ (22 t ha^{-1}) and $2 \times 4y$ rotations (24 t ha⁻¹). Poplar clones showed significantly higher yield in $2 \times 4y$ than $4 \times 2y$, and clone P1 had significantly higher yield in the $1 \times 8y$ rotation (126 t ha^{-1}) compared with the 4×2y rotation (73 t ha^{-1} ; Fig. 2). Stolarski et al. [28] reported similar

Table 1 Dry matter yield and Cd, Pb and Zn removal by willow and poplar clones harvested in 2012

Variable	Clones					
	S1	S2	P1	P2		
Dry biomass (t· ha1 ⁻¹)	0.67±0.18 B	3.26±0.57 A	5.14±1.08 A	2.58±0.91 AB		
Cd (g∙ ha ⁻¹)	32.59±9.58 B	132.79±20.49 A	107.82±24.81 AB	62.84±24.71 AB		
Pb (g∙ ha ⁻¹)	15.46±3.94 B	72.84±8.36 AB	141.43±27.97 B	48.71±17.32 AB		
Zn (g∙ ha ⁻¹)	287.71±76.71 B	1086.38±185.17 A	983.57±207.76 A	441.34±190.63 AB		

Dry matter yield of aboveground biomass (branches + stem) and Cd, Pb and Zn removal (mean \pm standard error) by willow, S1 (*S. schwerinii* × *S. viminalis*) × *S. viminalis*) and S2 (*S. smithiana*); and poplar, P1 (*P. maximowiczii* × *P. nigra*) and P2 (*P. nigra*) clones harvested in 2012 (time zero). Clones with the same capital letter for each treatment in each harvest year were not significantly different. Differences between the clones were evaluated by the Kruskal–Wallis test at $p \le 0.05$. Number of replicates, n = 8



Fig. 2 Dry matter yield (mean, t· ha⁻¹) of aboveground biomass (branches + stem) for willow S1 [(*S. schwerinii* × *S. viminalis*) × *S. viminalis*] and S2 (*S. smithiana*); and poplar P1 (*P. maximowiczii* × *P. nigra*) and P2 (*P. nigra*) clones after harvest rotation periods of four 2 years (4 × 2y), two 4 years (2 × 4y) and one 8 years (1 × 8y). For each clone, harvest rotations with the same lowercase letters were not significantly different. Differences between the rotations were evaluated by the Kruskal–Wallis test at $p \le 0.05$. Number of replicates, n = 8

observations: an increase in the yield and the quality of the biomass with extension of the rotation period, on average 3.4% lower in the 2-year and 17.2% lower in the 1-year rotation period compared to the 3-year rotation period. Larsen et al. [52] reported a 39% reduction in willow biomass yield from a 2-year harvest and 17% for a 1-year harvest compared to a 3-year harvest periods. The willow and poplar clones in 2-year rotations not only had a low yield, but also according to Klasnja et al. [53] juvenile shoots were characterized by high ash and moisture content and lower wood density, which is undesirable for fuel wood. According to DeBell et al. [32], the wood density and fiber length that determines paper pulp quality increased with increasing age of clones. The older wood was more suitable for manufacturing secondary products such as pulp, paper, and fiber-board [29].

The lowest total DM yield of our clones was measured in the 2-year harvests (Fig. 2). The yields were especially low in the third harvest $(2y_{2018})$, with the exception of clone S2, and in the fourth 2-year harvest $(2y_{2020})$ for all clones due to growth weakening by too frequent harvesting. Our results suggested that the more often the coppice is harvested, the lower its capacity to regrow from the stool. The yield can also be influenced by climate conditions, mainly the amount of precipitation during the growing period [54]. The low DM yield in the 4th harvest $(2y_{2020})$ could have been caused by the below-average precipitation in 2018 and 2019. The precipitation in February 2018 was only 8.4 mm (Additional file 1: Table S1). Contrarily, the increase of DM yield in the first 4-year and the first 2-year harvest $(2y_{2014})$ could be a result of the above average precipitation in 2012, the total annual precipitation was 707 mm (highest in January) and in 2013 was 751 mm, while the mean total annual precipitation in Příbram-Podlesí since 1961 has been only 630 mm (Additional file 1: Table S1). These weather conditions could stimulate shoot sprouting from stools in the first year after harvest. The DM yield was probably also

affected by the order of harvest. Weger and Bubeník [55] stated that the biomass yield of willow and poplar clones cultivated in 3-year rotation usually increased until the third or fourth harvest. Comparison of individual clone productivity showed substantial differences among them (Fig. 2). The DM yield per hectare decreased in $1 \times 8y$ and $4 \times 2y$ rotations in the order S2 > P1 > S1 > P2. Conversely, in $2 \times 4y$ rotations, the poplar clone P1 (167 t ha⁻¹) achieved higher total yields than the willow clone S2 (147 t ha^{-1}), and P2 (32 t ha^{-1}) was higher than S1 (24 t ha⁻¹). This corresponds to the same order as a harvest performed in time zero, and this trend indicated that the rotation length for poplar clones should be about 4 years, which is supported by the results of Weih [56], who concluded that the optimal rotation period was 4-6 years. Weger et al. [49] even recommended 5-6-year rotations to increase DM yield of P1 clones.

The most productive clones in our study were willow S2 and poplar P1, depending on the length of rotation. P1 with a $2 \times 4y$ rotation achieved the highest mean total DM yield (20.9 t ha⁻¹ y⁻¹) while S2 was next highest with 18.4 t ha⁻¹ y⁻¹ for a $2 \times 4y$ rotation. Our findings confirmed that S2 generally achieved the highest yield of all studied willow clones under Czech climatic conditions [57]. The yield of P1 strongly depended on the length of rotation. The DM yields of S2 and P1 in our study were

Composition of wood (%)

slightly higher than in other studies on sites with uncontaminated soil, because we did not include the low yield of harvest in time zero. In the field experiments of Weger [58], average yields of 14.6 t $ha^{-1}y^{-1}$ for clone P1 and 14 t $ha^{-1}y^{-1}$ for clone S2 were found in the third harvest in a 3-year rotation on different sites, but Weger and Bubník [55] reported that S2 yielded 27.6 t $ha^{-1} y^{-1}$ of DM when cultivated under optimum conditions. Clone S1 showed a good DM yield of 10.3 t ha⁻¹ y⁻¹ only in $1 \times 8y$ rotation, but in other rotations, the DM yield was low (3 t ha⁻¹ y^{-1}). The P2 clone exhibited the lowest yields in the 1×8y $(2 \text{ t ha}^{-1} \text{ y}^{-1})$ and $4 \times 2y$ (1.4 t ha⁻¹ y⁻¹) rotations among all observed clones, with only the $2 \times 4y$ rotation showing higher yields than S1 (4 t $ha^{-1} y^{-1}$). Biomass yield is strongly associated, not only with rotation length, but also with environmental condition [59]; therefore, in our study, the locally bred clones, S2 and P1, had substantially better yields than the internationally recognized clones, S1 and P2.

Composition of wood

In the final harvest performed in 2020, we measured the content of the main wood components from willow and poplar shoots harvested after 2, 4 and 8 years (Table 2). The contents of ash and extractives were significantly higher for poplar than willow clones, primarily for clone

Table 2
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of willow and poplar clones from harvest in 2020
Composition of wood of woo

Period

Clones

S1 S2 **P1** P2 2y 2020 2.62±0.13 aA 2.22 ± 0.03 aAB Ash 2.06±0.16 aAB 2.14 ± 0.06 aB 4y 2020 1.90±0.12 aB 1.97±0.08 aAB 2.44 ± 0.04 aA 2.49±0.12 aA 8y 2020 2.03 ± 0.09 aB 2.10±0.13 aAB 2.86±0.16 aA 1.90±0.39 aAB Extractives 2y 2020 6.32±0.45 aAB 4.12±0.47 aB $8.94 \pm 0.10 \text{ aA}$ 8.20±0.12 aAB 4y 2020 3.03 ± 0.51 bB 5.37±0.99 aAB 8.24±0.42 aA 7.26±0.22 abAB 3.85±0.82 bA 8y 2020 4.72 ± 0.31 abA 4.96±0.38 aA 6.36±1.06 aA Lignin 2y 2020 28.19±0.98 aA 26.90±0,77 aA 26.12±1.11 aA 24.27 ± 1.71 aA 4y 2020 24.24 ± 2.42 aA 24.07 ± 1.87 aA 24.72±1.13 aA 25.38±0.39 aA 8y 2020 26.22±0.98 aA 28.47 ± 2.20 aA 26.28±0.24 aA 26.33+0.80 aA Cellulose 2y 2020 35.48±0.35 aA 34.96±1.04 aA 33.89±1.69 aA 31.93±1.70 bA 4y 2020 36.87±0.93 aAB 44.09 ± 4.79 aA 32.36 ± 1.63 aB 32.28 ± 0.69 abA 8y 2020 35.88±0.28 aA 39.72 ± 2.33 aA 33.52±0.74 aA 37.72±0.85 aA Hemicellulose 2y 2020 27.95 ± 1.84 aA 31.88 ± 2.29 aA 28.43±0.25 aA 33.38 ± 2.54 aA 4y 2020 33.96±1.96 aA 24.50 ± 5.47 aA 32.24 ± 2.99 aA 32.59±0.17 aA 8y 2020 31.15±0.71 aA 24.75 ± 5.40 aA 30.98±0.47 aA 30.20±0.46 aA Holocellulose (cellulose + hemicellulose) 2y 2020 63.43 ± 1.75 aA 66.84 + 1.28 aA 62.32 ± 1.54 aA 65.31 + 1.09 aA 4y 2020 70.83 ± 2.87 aA 68.59±0.68 aA 64.60±1.45 aA 64.87±0.85 aA 8y 2020 67.03 ± 0.98 aA 64.47 ± 3.21 aA 64.50±1.21 aA 67.92±0.78 aA

Composition of wood (mean ± standard error) of willow S1 (*S. schwerinii* × *S. viminalis*) × *S. viminalis*) and S2 (*S. smithiana*); and poplar, P1 (*P. maximowiczii* × *P. nigra*) and P2 (*P. nigra*) clones, harvested in 2020 in 2-year, 4-year and 8-year periods. For each clone, periods with the same lowercase letters were not significantly different, and in each period, clones with the same upper-case letters were not significantly different. Differences between the periods and between the clones were evaluated by the Kruskal–Wallis test at $p \le 0.05$. Number of replicates, n = 4

P1. In poplar, the ash content ranged from 1.90% to 2.86% and the extractives from 3.85% to 8.94%. In willow clones, both the ash and the extractives were lower, with no significant differences in the content of ash and extractives among the different harvest periods. Higher extractive contents were generally found in young shoots, and decreased with increasing rotation length similar to the study of Guidi et al. [30] (Table 2).

The main wood components lignin, cellulose and hemicellulose, did not show any significant differences among clones, or length of harvest rotations, with the exception of cellulose content in S2 with 4-year harvest. According to Borukanlu et al. [29], this could be caused by a faster growth rate, which increases tree-ring widths and cellulose content. For all clones, the lignin content varied from 24.1% to 28.5%, hemicellulose from 24.5% to 34.0%, and cellulose from 31.9% to 44.1%. In general, the highest content of holocellulose (cellulose + hemicellulose) and consequently the lowest content of lignin (except for P2) was found in 4-year wood (Table 2). Guidi et al. [30] observed decreased lignin content and increased cellulose content with increasing length of harvest period of poplar clones (Populus deltoides). This is probably due to the higher proportion of bark with high lignin content in young shoots [53]. Conversely, the higher lignin content in 8-year shoots could be a result of the greater stem lignification of the older trees [60].

In our study, no close relationship between harvest period length and lignin, cellulose and hemicellulose content in the harvested biomass was found (Additional file 1: Fig. S2a–c). However, the results indicated, especially for poplar clones, that Cd, Pb and Zn concentrations increased with increasing lignin content (Additional file 1: Fig. S2d, g and j). This could be related to the high lignin affinity for Pb, Cd and Zn ions, binding them to its carboxylic and phenolic surface groups [33]. Lignin is also a cell wall material that acts as a mechanical barrier against external stressors such as metals [61, 62]. PTEs like Cd are known to induce oxidative stress [61], while H_2O_2 elicits secondary reactions, such as enhanced peroxidase activity, which can increase the degree of lignification.

A similar trend, was found for cellulose, where the poplar clones again showed increased concentrations of Cd, Pb and Zn with increasing cellulose content (Additional file 1: Fig. S2e, h and k). Conversely, for the poplar clones and for S1 (only with Cd and Zn), the PTE concentrations tended to decrease with increasing hemicellulose content. This trend for hemicellulose was opposite to the lignin trend, but was statistically significant for Cd and Zn (Additional file 1: Fig. S2f and l). The decrease in Cd, Pb and Zn concentrations with increasing hemicellulose content may be related to the relationship between lignin and hemicellulose. Increased hemicellulose content led to a decrease in lignin and also Cd, Pb and Zn. All the investigated clones showed significant decrease in lignin content with increasing hemicellulose content (Additional file 1: Fig. S2o). However, the relationship between Cd, Pb and Zn concentration and wood components could be affected not only by the increased lignification due to PTEs, and the high affinity of lignin for Pb, Cd and Zn, but also the type of clone, antagonistic and synergistic relationships among PTEs, their mobility and relative content in the soil.

Concentrations of PTEs in wood

The ability of willows and poplars to accumulate Cd (11-29 mg kg⁻¹), Zn (93-279 mg kg⁻¹) and Pb (13-47 mg kg⁻¹) in the wood biomass was confirmed under field conditions (Fig. 3). Considering the total content of PTEs in the soil (Cd 7.3 mg kg⁻¹, Zn 218 mg kg⁻¹, and Pb 1368 mg kg⁻¹) the clones showed higher accumulation capacity for Cd and Zn, while the Pb accumulation was low relative to its soil content due to low soil Pb availability. The Cd and Zn concentrations in trees showed similar trends (Fig. 3), consistent with the findings of Tőzsér et al. [63], who reported a positive correlation between Cd and Zn accumulation. We observed the highest Cd concentrations in aboveground biomass (branches+stem) of both willow clones: S2 harvested in 2y₂₀₁₆ (29 mg kg⁻¹) and S1 harvested in $4y_{2016}$ (28 mg kg⁻¹). The same clones also showed the highest Zn concentrations, S2 harvested in $2y_{2016}$ (279 mg kg⁻¹) and S1 harvested in $4y_{2016}$ (259 mg kg^{-1}) . The ability of willow clones to accumulate higher Cd and Zn concentrations than poplar clones had already been shown by other authors [34, 64]. High Cd and Zn concentrations were found in both willow clones, especially in 2-year rotations (Fig. 3), where the DM yield was low (Fig. 2). This could be caused by the limited internal dilution effect of PTEs under low biomass yield [65, 66]. Michels et al. [67] reported poplar Cd concentrations varying from 13.0 to 26.5 mg kg⁻¹, similar to our results, with the range of Cd content in wood from 10 to 15.8 mg kg^{-1} (Fig. 3). These authors also found higher Zn concentrations in wood biomass $(304-524 \text{ mg kg}^{-1})$ compared to our study (93–169 mg kg⁻¹), with lower Cd (3.0 mg kg^{-1}) and higher Zn (378 mg kg^{-1}) soil content compared to our site.

The highest Pb concentrations accumulated in P1 (47.4 mg kg⁻¹) and P2 (42.4 mg kg⁻¹) in the $1 \times 8y$ rotation. In the shorter rotations, Pb concentrations in poplar clones were lower, ranging from 15.5 to 31.7 mg kg⁻¹; however, the P1 clone had higher Pb concentrations in all the rotations. The increased capacity of P1 to accumulate Pb was also documented in previous studies [25, 34]. Fischerová et al. [68] compared various species of



Fig. 3 Concentrations of Cd, Pb and Zn (mean ± standard error, mg·kg⁻¹) in aboveground biomass (branches + stem) of willow S1 [(*S. schwerinii*×*S. viminalis*) × *S. viminalis*)] and S2 (*S. smithiana*); and poplar, P1 (*P. maximowiczii*×*P. nigra*) and P2 (*P. nigra*) clones in individual harvests. Numbers on bars (2–8) indicate rotation length. For each clone, harvests with the same lowercase letters were not significantly different. Differences between harvests were evaluated by the Kruskal–Wallis test at $p \le 0.05$. Number of replicates, n = 8

fast-growing trees and hyperaccumulators and confirmed the high capacity of the clone 'Henry' (Populus maximow*iczii*×*P. nigra*), the same hybrid as P1, to accumulate Pb. They explained it by the released poplar root exudates, which can mobilize additional soil Pb. Clone P2 showed high Pb concentrations only in the $1 \times 8y$ rotation. The experiments of other authors [25, 34, 39] also confirmed low Pb content in wood by this clone compared to other tested clones. Lead concentrations in branches and stems of the willow clones ranged from 13 mg kg⁻¹ in 2-year rotations to 33.8 mg kg⁻¹ in the $1 \times 8y$ rotation (Fig. 3), which was significantly higher compared to shorter $2 \times 4y$ and $4 \times 2y$ rotations for all clones with no significant differences in Pb concentration between the two rotations. The significantly higher Pb content found in the $1 \times 8y$ rotation was probably related to the longer exposure time of the trees. In a 3-year-long experiment, Tőzsér et al. [63] found a significantly increased accumulation of Cd and Zn that correlated with exposure time of trees, whereas the Pb concentration was not affected, probably, due to low Pb mobility and limited transport from roots to branches [69, 70].

Removal of PTEs by trees, and their remediation efficiency

The total removal of PTEs by willow and poplar SRCs followed the order, Cd < Pb < Zn (Fig. 4), which differed from the order of total soil PTEs contents. Cd and Zn removal decreased in the harvest order $2 \times 4y > 1 \times 8y > 4 \times 2y$ for the most investigated clones, except for S1, which corresponded to biomass yield. The clones, S2, P1 and P2 showed higher Cd and Zn removal in 2×4y rotations than in $1 \times 8y$ rotation, but only P2 showed the significant differences. Comparing the $2 \times 4y$ and $4 \times 2y$ rotations, the differences were significant for both poplar clones. The removal of Cd and Zn by the $4 \times 2y$ rotations was significantly lower than the $1 \times 8y$ rotation, only for P1. In contrast, Cd and Zn removal by S1 was significantly higher in the $1 \times 8y$ rotation than in $4 \times 2y$ or $2 \times 4y$ rotations (Fig. 4). In general, the trend for Cd and Zn removal paralleled the biomass yield. Our findings also confirmed that the biomass yield was the crucial parameter determining the phytoextraction efficiency of SRCs clones [34, 38]. By defining the removal of PTEs as concentration of PTEs in biomass × DM yield of biomass, it is obvious that clone ability to accumulate high PTEs concentration also influence the efficiency of phytoextraction, as shown by clone S1 for Cd and Zn removal in 8-year rotation. Clones suitable for phytoextraction of PTEs must optimally combine high biomass productivity with high metal uptake and translocations [71]. The removal of Cd and Zn by individual clones in $2 \times 4y$ and $4 \times 2y$ rotations decreased in the order, S2>P1>S1>P2. In these rotations, S1 with high concentrations of Cd and Zn showed



Fig. 4 Mean amounts of Cd, Pb and Zn (g·ha⁻¹) removed by the harvested biomass of willow S1 [(*S. schwerinii*×*S. viminalis*)×*S. viminalis*] and S2 (*S. smithiana*); and poplar, P1 (*P. maximowiczii*×*P. nigra*) and P2 (*P. nigra*) clones after different harvest rotation periods: four harvests at 2-year intervals $4 \times 2y$ ($2y_{2014}$, $2y_{2016}$, $2y_{2018}$, $2y_{2020}$), two harvests at 4-year intervals $2 \times 4y$ ($4y_{2016}$, $4y_{2020}$), and one harvest at 8 years $1 \times 8y$ ($8y_{2020}$) are shown in individual columns. For each clone, harvest rotations with the same lowercase letters were not significantly different and for each harvest rotation, clones with the same upper-case letters were not significantly different. Differences between the rotations were evaluated by the Kruskal– Wallis test at $p \le 0.05$. Number of replicates, n = 8

higher removal than P2. In $1 \times 8y$ rotations, S1 exceeded both poplar clones in Cd and Zn removal, although P1 in a $1 \times 8y$ rotation achieved a substantially higher DM yield than S1 in the order S2>S1>P1>P2 (Fig. 4). S2 was the most promising clone for removal of Cd and Zn in every rotation period. For example, with a 2×4y rotation, S2 removed 2474 g Cd ha⁻¹ and 27.865 g Zn ha⁻¹. The next most efficient clone for PTE removal in $2 \times 4y$ and $4 \times 2y$ rotations was P1; but in the $1 \times 8y$ rotation, S1 was slightly better.

Pb removal per hectare decreased in the order $1 \times 8y > 2 \times 4y > 4 \times 2$ for all the investigated clones (Fig. 4) and decreased in the order P1 > S2 > S1 > P2 in all rotations. The most promising clone for Pb removal was P1, similar to our previous studies [25, 34]. Interestingly, despite the fact that the $1 \times 8y$ rotation showed reduced biomass production compared to the $2 \times 4y$ rotation, the significantly higher Pb concentrations in aboveground biomass confirmed the increased Pb removal compared with the other rotations. This finding suggests that longer rotations can lead to higher efficiency of Pb phytoextraction and this is likely to be related to the increasing content of cellulose and lignin in the wood of poplar clones (Additional file 1: Fig. S2).

The relative removal of contaminants represented by RF decreased in both horizons as follows: Cd > Zn > Pb (Table 3). The significant differences in RFs of Cd, Pb and Zn among the rotations ($4 \times 2y$, $2 \times 4y$, $1 \times 8y$) and clones (S1, S2, P1, P2) corresponded with the data of PTE removal because of mean element soil metal content was applied for the calculation. According to Crow and

Table 3 Mean reme	ediation	tactors
-------------------	----------	---------

Houston [47], 75–95% of willow and poplar SRC roots occur within the plough soil about 30–36 cm deep with the root distribution about 60% in upper (0–18 cm) layer, and 40% in bottom (19–36 cm) layer. Therefore, RFs in our study were calculated for a 40-cm depth profile representing the majority of roots in our shallow soil. The Pb RF achieved by the best P1 clone was below 0.11% in horizon 0–20 cm and 0.1% in horizon 20–40 cm. Very low values were caused by extremely high Pb concentration in both soil layers (1368 mg kg⁻¹; 672 mg kg⁻¹) and the low mobility and plant accumulation capacity of this element.

The RFs for Cd and Zn made a different more promising story. In $2 \times 4y$ rotations, S2 showed 9.07% Cd extracted from horizon 0–20 cm and 5.62% from horizon 20–40 cm, while the extracting Zn levels were 3.43% from horizon 0–20 cm and 2.04% from horizon 20–40 cm after removal by S2 from the soil content during 8 years. The calculated Cd, Pb and Zn RFs in our field experiment were higher than in other field experiments. For instance, Jensen et al. [72] reported after 1 year an RF of Cd=0.13% and RF of Zn=0.029% and Zárubová et al. [34] after 4 years an RF of Cd=0.85% and RF of Zn=0.15%. In mentioned field experiments RFs only horizon 0–20 cm

	Rotation period	Clones				
		S1	S2	P1	P2	
	Horizon 0–20					
Cd (%)	4×2y	1.81±0.37 bBC	6.49±0.73 aAB	3.09±0.25 bB	0.39±0.08 bC	
	2×4y	2.17±0.28 abB	9.07±0.89 aA	5.66±0.45 aA	1.35±0.28 aB	
	1×8y	5.46±1.02 aA	8.20±0.99 aA	4.99±0.50 aAB	0.66±0.26 bB	
Pb (%)	4×2y	0.006±0.001 bB	0.023±0.003 bA	0.032±0.003 bA	0.003±0.001 bB	
	2×4y	0.008±0.001 bB	0.048±0.005 abA	0.064±0.007 abA	0.009±0.002 aB	
	1×8y	0.047±0.007 aAB	0.089±0.013 aA	0.109±0.012 aA	0.012±0.004 aB	
Zn (%)	4×2y	0.63±0.15 bBC	2.19±0.31 aAB	1.06±0.11 bB	0.12±0.03 bC	
	2×4y	0.76±0.10 abB	3.43±0.39 aA	2.28±0.25 aA	0.41 ± 0.09 aB	
	1×8 y	1.96±0.40 aA	2.64±0.38 aA	1.90±0.22 aA	0.21±0.09 bB	
	Horizon 20–40					
Cd (%)	4×2y	1.13±0.23 bBC	4.03±0.46 aAB	1.92±0.15 bB	0.24±0.05 bC	
	2×4y	1.35±0.17 abBC	5.62±0.55 aAB	3.51±0.28 aB	0.83±0.18 aC	
	1×8y	3.38±0.63 aA	5.08±0.61 aA	3.09±0.31 aAB	0.41±0.16 bB	
Pb (%)	4×2y	0.005±0.001 bB	0.020±0.002 bA	0.028±0.005 bA	0.003±0.001 bB	
	2×4y	0.007±0.001 bB	0.042±0.004 abA	0.056±0.006 abA	0.008±0.001 aB	
	1×8y	0.041±0.007 aAB	0.077±0.012 aA	0.096±0.010 aA	0.010±0.003 aB	
Zn (%)	4×2y	0.38±0.09 bBC	1.31±0.19 aAB	0.63±0.07 bB	0.07±0.02 bC	
	2×4y	0.45±0.06 abBC	2.04±0.23 aAB	1.36±0.15 aB	0.25±0.05 aC	
	1 × 8 y	1.17±0.24 aA	1.58±0.23 aA	1.14±0.13 aA	0.13±0.05 bB	

Mean $(\pm SE)$ remediation factors (%) expressed per 8 years for willow and poplar clones in 0–20 cm horizon and in 20–40 cm horizon. Differences between clones S1 (*S. schwerinii*×*S. viminalis*) × *S. viminalis*) and S2 (*S. smithiana*); and poplar, P1 (*P. maximowiczii*×*P. nigra*) and P2 (*P. nigra*) and harvest rotations period: four 2 years (4×2y), two 4 years (2×4y) and one 8 years (1×8y), were evaluated by Kruskal–Wallis tests. Clones with the same uppercase letters for each harvest rotation were not significantly different. Individual harvest rotations for each clone with the same lowercase letters were not significantly different. Number of replicates, n = 8

was observed. Results of our field experiments calculated per annum were always lower than the values reported by Vysloužilová et al. [17] after 3 years: RF of Cd=22.3% and RF of Zn=4.3% for willow clones. Komárek et al. [38] reported an annual RF of Cd=1.27%, RF of Pb=0.4% and RF of Zn=0.33% for poplar clones, while Fischerová et al. [68] reported an annual RF for Cd=8.1%, RF of Pb=0.025% and RF of Zn=2.92% for willow (Cd, Zn) and poplar clones (Pb). Higher RF values are always found in pot experiments because of the greater removal capability of trees with limited root space. These findings were in agreement with the conclusions of Dickinson and Pulford [6] that a substantial reduction in soil Cd contamination could be achieved through phytoextraction by selected *Salix* clones even under field conditions.

Our experiments confirmed the efficient extraction of PTEs from the soil. Using our best clone, S2, with 4-year rotations, the removal of 1 mg Cd· kg⁻¹ of soil from horizon 0–20 cm would require 12 years, while the extraction of 1 mg of Zn· kg⁻¹ from horizon 0–20 cm would only take 1 year. From horizon 20–40 cm, it would take 32 years for 1 mg Cd and 3 years for 1 mg of Zn. Removal of 1 mg Pb· kg⁻¹ of soil using clone P1 with 8-year rotations would require 5.5 years from horizon 0–20 cm and 12.5 years from horizon 20–40 cm. It should be noted that the phytoextraction potential depends not only on the SRC clone and rotation period, but also on the specific area, bioavailability of the metal to plants, the soil properties, and the soil fertility [73].

Conclusions

In summary, we found large differences between clones of willow and poplar species, and harvest intervals in biomass production and the removal of individual PTEs from the medium-high contaminated soil. The 4-year growing period resulted in higher yields of the tested clones, with the exception of S1. The yield of tree biomass did not adversely affect the accumulation of the PTEs, and the dilution of PTEs concentration by dry matter production was not significant; therefore, PTE removal was closely correlated with biomass production. The best phytoextraction potential for Cd and Zn removal was found for clone S2 in $2 \times 4y$ rotations and for Pb removal for clone P1 in $1 \times 8y$ rotations. The phytoextraction potential presented by remediation factor (RF) showed very promising results for removal of mobile Cd (9.07%, representing 0.7 mg $Cd \cdot kg^{-1}$ of soil) in the top horizon 0–20 cm with $2 \times 4y$ rotations from seriously contaminated soil. The RF of Zn reached 3.43% in the top horizon 0-20 cm, and these values corresponded to the removal of 7.0 mg $\text{Zn} \cdot \text{kg}^{-1}$ of soil from horizon 0–20 cm. Pb remediation efficiency was negligible regardless of the clone. Plant accumulation of PTEs in poplar clones correlated well with contents of lignin and cellulose and negatively with hemicellulose, but this trend was not exhibited for willows.

Abbreviations

- S1 Salix viminalis L. (Salix schwerinii E.L.Wolf × S. viminalis) × S. viminalis)
- S2 Salix smithiana (Salix × smithiana Willd.)
- P1 Populus (Populus nigra L.× Populus maximowiczii A. Henry)
- P2 Wolterson (P. nigra L.)
- PTE Potentially toxic element
- SRC Short rotation coppices
- FW Fresh weight
- DW Dry weight
- DM Dry matter
- CRM Certified reference material
- LR Linear regression
- RF Remediation factor

Supplementary Information

The online version contains supplementary material available at https://doi. org/10.1186/s40538-024-00600-1.

Additional file1

Acknowledgements

Not applicable

Author contributions

PT: methodology, validation, writing—review and editing, supervision, project administration. NP: methodology, writing—original draft preparation, investigation. PK: writing—original draft preparation, data curation, visualization. JS: formal analysis, writing—review and editing. FM: writing—review and editing. JN: resources, investigation. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by the European Regional Development Fund (project NUTRISK no. CZ.02.1.01/0.0/0.0/16_019/0000845).

Availability of data and materials

The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 00 Prague 6 - Suchdol, Czech Republic.

Received: 24 February 2024 Accepted: 21 May 2024 Published online: 03 June 2024

References

- 1. Pulford ID, Dickinson NM. Phytoremediation technologies using trees. In: Prasad MNV, Sajwan KS, Naidu R (eds) Trace elements in the environment: biochemistry, biotechnology and bioremediation. CRC Press. 2005
- Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals—concepts and applications. Chemosphere. 2013;91(7):869–81. https://doi.org/10. 1016/j.chemosphere.2013.01.075.
- Sheoran V, Sheoran AS, Poonia P. Factors affecting phytoextraction: a review. Pedosphere. 2016;26(2):148–66. https://doi.org/10.1016/S1002-0160(15)60032-7.
- Maxted AP, Black CR, West HM, Crout NMJ, Mcgrath SP, Young SD. Phytoextraction of cadmium and zinc by *Salix* from soil historically amended with sewage sludge. Plant Soil. 2007;290:157–72. https://doi.org/10.1007/ s11104-006-9149-5.
- Dimitriou I, Mola-Yudego B. Poplar and willow plantations on agricultural land in Sweden: area, yield, groundwater quality and soil organic carbon. For Ecol Manage. 2017;383:99–107. https://doi.org/10.1016/j.foreco.2016. 08.022.
- Dickinson NM, Pulford ID. Cadmium phytoextraction using short-rotation coppice Salix: The evidence trail. Environ Int. 2005;31(4):609–13. https:// doi.org/10.1016/j.envint.2004.10.013.
- Kuzovkina YA, Volk TA. The characterization of willow (*Salix* L.) varieties for use in ecological engineering applications: co-ordination of structure, function and autecology. Ecol Eng. 2009;35:1178–89. https://doi.org/10. 1016/j.ecoleng.2009.03.010.
- Greger M, Landberg T. Use of willow in phytoextraction. Int J Phytoremediat. 1999;1(2):115–23. https://doi.org/10.1080/15226519908500010.
- Pilipović A, Zalesny RS Jr, Rončević S, Nikolić N, Orlović S, Beljin J, Katanić M. Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments. J Environ Manag. 2019;239:352–65. https://doi. org/10.1016/j.jenvman.2019.03.072.
- Clifton-Brown J, Harfouche A, Casler MD, Dylan Jones H, Macalpine WJ, Murphy-Bokern D, et al. Breeding progress and preparedness for massscale deployment of perennial lignocellulosic biomass crops switchgrass, miscanthus, willow and poplar. GCB Bioenergy. 2019;11(1):118–51. https://doi.org/10.1111/gcbb.12566.
- Schweier J, Molina S, Ghirardo A, Grote R, Díaz-Pinés E, Kreuzwieser J, et al. Environmental impacts of bioenergy wood production from poplar short rotation coppice grown at a marginal agricultural site in Germany. GCB Bioenergy. 2017;9:1207–21. https://doi.org/10.1111/gcbb.12423.
- Karp A, Shield I. Bioenergy from plants and the sustainable yield challenge. New Phytol. 2008;179(1):15–32. https://doi.org/10.1111/j.1469-8137.2008.02432.x.
- Hauk S, Knoke T, Wittkopf S. Economic evaluation of short rotation coppice systems for energy from biomass—a review. Renew Sust Energ Rev. 2014;29:435–48. https://doi.org/10.1016/j.rser.2013.08.103.
- Labrecque M, Teodorescu T. Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). Biomass Bioenerg. 2005;29:1–9. https://doi.org/10. 1016/j.biombioe.2004.12.004.
- 15 Pajević S, Borišev M, Nikolić N, Arsenov DD, Orlović S, Župunski M. Phytoextraction of heavy metals by fast-growing trees: a review. In: Ansari A, Gill S, Gill R, Lanza G, Newman L, editors. Phytoremediation. Cham: Springer; 2016.
- Ruttens A, Boulet J, Weyens N, Smeets K, Adriaensen K, Meers E, et al. Short rotation coppice culture of willows and poplars as energy crops on metal contaminated agricultural soils. Int J Phytoremediat. 2011;13(S1):194–207. https://doi.org/10.1080/15226514.2011.568543.
- Vysloužilová M, Tlustoš P, Száková J. Cadmium and zinc phytoextraction potential of seven clones of *Salix* Spp. planted on heavy metal contaminated soils. Plant Soil Environ. 2003;49(542):547. https://doi.org/10.17221/ 4191-PSE.
- Zalesny RS Jr, Zhu JY, Headlee WL, Gleisner R, Pilipovi´c A, Acker JV, et al. Ecosystem services, physiology, and biofuels recalcitrance of poplars grown for landfill phytoremediation. Plants-Basel. 2020;9:1357. https:// doi.org/10.3390/plants9101357.
- Tlustoš P, Száková J, Vysloužilová M, Pavlíková D, Weger J, Javorská H. Variation in the uptake of arsenic, cadmium, lead, and zinc by different species of willows *Salix* Spp grown in contaminated soils. Cent Eur J Biol. 2007;2:254–75. https://doi.org/10.2478/s11535-007-0012-3.

- 20 Mleczek M, Gasecka M, Waliszewska B, Magdziak Z, Szostek M, Rutkowski P, et al. Salix viminalis L—a highly effective plant in phytoextraction of elements. Chemosphere. 2018;212:67–78. https://doi.org/10.1016/j. chemosphere.2018.08.055.
- He J, Ma C, Ma Y, Li H, Kang J, Liu T, et al. Cadmium tolerance in six poplar species. Environ Sci Pollut Res. 2013;20(1):163–74. https://doi.org/10. 1007/s11356-012-1008-8.
- Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustoš P, Zupan M, Wenzel WW. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut. 2007;148(1):107–14. https://doi.org/10.1016/j.envpol.2006.10.035.
- 23. Zhivotovsky OP, Kuzovkina YA, Schulthess CP, Morris T, Pettinelli D. Lead uptake and translocation by willows in pot and field experiments. Int J Phytoremediat. 2011;13(8):731–49. https://doi.org/10.1080/15226514. 2010.525555.
- Połeć-Pawlak K, Ruzik R, Lipiec E, Ciurzyńska M, Gawrońska H. Investigation of Pb (II) binding to pectin in *Arabidopsis thaliana*. J Anal At Spectrom. 2007;22(8):968–72. https://doi.org/10.1039/b704157h.
- Kubátová P, Žilinčíková N, Száková J, Zemanová V, Tlustoš P. Is the harvest of Salix and Populus clones in the growing season truly advantageous for the phytoextraction of metals from a long-term perspective? Sci Total Environ. 2022;838: 156630. https://doi.org/10.1016/j.scitotenv. 2022.156630.
- Shen X, Dai M, Yang J, Sun L, Tan X, Peng Ch, et al. A critical review on the phytoremediation of heavy metals from environment: performance and challenges. Chemosphere. 2022;291: 132979. https://doi. org/10.1016/j.chemosphere.2021.132979.
- Lasat MM. Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res. 1999;2:1–25. https://doi.org/10.4148/1090-7025.1015.
- Stolarski MJ, Szczukowski S, Tworkowski J, Wróblewska H, Krzyżaniak M. Short rotation willow coppice biomass as an industrial and energy feedstock. Ind Crop Prod. 2011;33(1):217–23. https://doi.org/10.1016/j. indcrop.2010.10.013.
- Borukanlu MR, Zadeh OH, Moradpour P, Khedive E. Effects of growth rate of eastern poplar trees on the chemical and morphological characteristics of wood fibers. Eur J Wood Wood Prod. 2021;79:1479–94. https://doi.org/10.1007/s00107-021-01711-4.
- Guidi W, Tozzini C, Bonari E. Estimation of chemical traits in poplar short-rotation coppice at stand level. Biomass Bioenerg. 2009;33:1703– 9. https://doi.org/10.1016/j.biombioe.2009.09.004.
- Stolarski MJ, Szczukowski S, Tworkowski J, Klasa A. Yield, energy parameters and chemical composition of short-rotation willow biomass. Ind Crop Prod. 2013;46:60–5. https://doi.org/10.1016/j.indcrop.2013.01.012.
- DeBell DS, Singleton R, Harrington CA, Gartner BL. Wood density and fiber length in young *Populus* stems: relation to clone, age, growth rate, and pruning. Wood Fiber Sci. 2002;14(4):529–34.
- Guo X, Zhang S, Shan X. Adsorption of metal ions on lignin. J Hazard Mater. 2008;151:134–42. https://doi.org/10.1016/j.jhazmat.2007.05.065.
- Zárubová P, Hejcman M, Vondráčková Š, Mrnka L, Száková J, Tlustoš P. Distribution of P K, Ca, Mg, Cd, Cu, Fe, Mn, Pb and Zn in wood and bark age classes of willows and poplars used for phytoextraction on soils contaminated by risk elements. Environ Sci Pollut Res. 2015;22(18801):18813. https://doi.org/10.1007/s11356-015-5043-0.
- Ministry of the Environment of the Czech Republic. Air quality https:// www.mzp.cz/cz/kvalita_ovzdusi [in Czech]. Accessed 20 November 2023.
- Kidd P, Mench M, Álvarez-López V, Bert V, Dimitriou I, Friesl-Hanl W, et al. Agronomic practices for improving gentle remediation of trace element contaminated soils. Int J Phytoremediat. 2015;17:1005–37. https://doi.org/10.1080/15226514.2014.1003788.
- Vysloužilová M, Tlustoš P, Száková J, Pavlíková D. As, Cd, Pb, and Zn uptake by *Salix* spp clones grown in soils enriched by high loads of these elements. Plant Soil Environ. 2003;49:191–6. https://doi.org/10. 17221/4112-PSE.
- 38 Komárek M, Tlustoš P, Száková J, Chrastný V. The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. Environ Pollut. 2008;151(27):38. https://doi.org/10.1016/j. envpol.2007.03.010.

- 39 Laureysens I, Blust R, De Temmermanc L, Lemmensa C, Ceulemans R. Clonal variation in heavy metal accumulation and biomass production in a poplar coppice cul-ture: I. Seasonal variation in leaf, wood, and bark concentrations. Environ Pollut. 2004;131(485):494. https://doi.org/ 10.1016/j.envpol.2004.02.009.
- 40 Mader P, Száková J, Miholová D. Classical dry ashing of biological and agricultural materials: Part II. Losses of analytes due to their retention in an insoluble residue. Analysis. 1998;26:121–9. https://doi.org/10.1051/ analusis:1998121.
- Seifert K. Über ein neues Verfahren zur Schnellbestimmung Der Rein-Cellulose. Das Pap. 1956;10:301–6 (in Germany).
- Wise LE, Murphy M, D'Addieco AA. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Pap Trade J. 1946;122:35–43.
- 43. Tappi Test Methods. Tappi T 222 om-11. Acid-insoluble lignin in wood and pulp. Georgia: Tappi Press Atlanta. 2006.
- 44. Tappi Test Methods. Tappi T 211 om-02. Ash in wood, pulp, paper and paperboard: combustion at 525 °C Georgia: Tappi Press Atlanta 2007.
- 45. Tappi Test Methods. Tappi T 5 wd-73. Dichlormethane soluble in wood, alcohol-benzen solubles in wood. Georgia: Tappi Press Atlanta 2015.
- Tappi Test Methods. Tappi T 6 wd-73. Alcohol-benzene solubility of wood Georgia.: Tappi Press Atlanta; 2015.
- Crow P, Houston TJ. The influence of soil and coppice cycle on the rooting habit of short rotation poplar and willow coppice. Biomass Bioenerg. 2004;26:497–505. https://doi.org/10.1016/j.biombioe.2003.09.002.
- Scriba C, Lunguleasa A, Spirchez C, Ciobanu V. Influence of INGER and TORDIS energetic willow clones planted on contaminated soil on the survival rates, yields and calorific value. Forests. 2021;12:826. https://doi. org/10.3390/f12070826.
- Weger J, Vávrová K, Kašparová L, Bubeník J, Komárek A. The influence of rotation length on the biomass production and diversity of ground beetles (Carabidae) in poplar short rotation coppice. Biomass Bioenerg. 2013;54:284–92. https://doi.org/10.1016/j.biombioe.2013.02.012.
- Laureysens I, Bogaert J, Blust R, Ceulemans R. Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. Forest Ecol Manag. 2004;187:295– 309. https://doi.org/10.1016/j.foreco.2003.07.005.
- Kubátová P, Hejcman M, Száková J, Vondráčková S, Tlustoš P. Effects of sewage sludge application on biomass production and concentrations of Cd, Pb and Zn in shoots of *Salix* and *Populus* clones: improvement of phytoremediation efficiency in contaminated soils. BioEnergy Res. 2016;9:809–19. https://doi.org/10.1007/s12155-016-9727-1.
- Larsen SU, Jørgensen U, Lærke PE. Harvest interval and row spacing of SRC willow influence yield and nutrient content. Biomass Bioenerg. 2019;126:181–9. https://doi.org/10.1016/j.biombioe.2019.05.012.
- Klasnja B, Kopitovic S, Orlovic S. Wood and bark of some poplar and willow clones as fuelwood. Biomass Bioenerg. 2002;23:427–32. https://doi. org/10.1016/S0961-9534(02)00069-7.
- Larsen S, Jørgensen U, Lærke P. Willow yield is highly dependent on clone and site. BioEnergy Res. 2014;7(4):1280–92. https://doi.org/10.1007/ s12155-014-9463-3.
- Weger J, Bubeník J. The evaluation of yield and growth of native willows after 14 years of short rotation coppice. Acta Pruhoniciana. 2011;97:39–46 (in Czech).
- Weih M. Intensive short rotation forestry in boreal climates: present and future perspectives. Can J For Res. 2004;34:1369–78. https://doi.org/10. 1139/X04-090.
- 57 Štícha V, Nuhlíček O, Bubeník J, Weger J, Macků J, Vanická J, et al. Evapotranspiration—tool for seasoning wood of hybrid poplar (*Populus maximowiczii* A. Henry × *Populus nigra* L. 'Max 4-5') and *Salix* × *smithiana* Willd. (*Salix caprea* L. and S. *viminalis* L.). Ind Crop Prod. 2021;162: 113265. https://doi.org/10.1016/j.indcrop.2021.113265.
- Weger J. Yield of selected poplar and willow clones after 9 years of coppicing. Acta Pruhoniciana. 2008;89:5–10 (in Czech).
- 59. Mrnka L, Doubková P, Habart J, Sudová R, Tlustoš P, Vohník M, Vosátka M Willow and poplar short rotation coppice plantations in soils contaminated by risk elements: review and handbook for growers in the Czech Republic Institute of Botany of the ASCR Průhonice. 2011.
- Kenney W, Sennerby-Forsse L, Layton E. A review of biomass quality research relevant to the use of poplar and willow for energy conversion. Biomass. 1990;21:163–88. https://doi.org/10.1016/0144-4565(90)90063-P.

- 61. Abeed AHA, Salama FM. Attenuating effect of an extract of Cd-hyperaccumulator Solanum nigrum on the growth and physio-chemical changes of Datura innoxia under Cd stress. J Soil Sci Plant Nutr. 2022;22:4868–82. https://doi.org/10.1007/s42729-022-00966-x.
- Kováčik J, Klejdus B, Hedbavny J, Zoń J. Significance of phenols in cadmium and nickel uptake. J Plant Physiol. 2011;168:576–84. https://doi. org/10.1016/j.jplph.2010.09.011.
- Tőzsér D, Magura T, Simon E. Heavy metal uptake by plant parts of willow species: a meta-analysis. J Hazard Mater. 2017;336:101–9. https://doi.org/ 10.1016/j.jhazmat.2017.03.068.
- Kacálková L, Tlustoš P, Száková J. Phytoextraction of risk elements by willow and poplar trees. Int J Phytoremediat. 2015;17:414–21. https://doi. org/10.1080/15226514.2014.910171.
- 65. Tinker PB, MacPherson A, West TS. Levels, distribution and chemical forms of trace elements in food plants. Philos Trans R Soc B. 1981;294:41–55.
- Kubátová P, Száková J, Břendová K, Kroulíková-Vondráčková S, Mercl F, Tlustoš P. Effects of summer and winter harvesting on element phytoextraction efficiency of *Salix* and *Populus* clones planted on contaminated soil. Int J Phytoremediat. 2018;5:499–506. https://doi.org/10.1080/15226 514.2017.1393393.
- Michels E, Annicaerta B, De Moor S, Van Nevel L, De Fraeye M, Meiresonne L, et al. Limitations for phytoextraction management on metal-polluted soils with poplar short rotation coppice—evidence from a 6-year field trial. Int J Phytoremediat. 2018;20(1):8–15. https://doi.org/10.1080/15226 514.2016.1207595.
- Fischerová Z, Tlustoš P, Száková J, Šichorová K. A comparison of phytoremediation capability of selected plant species for given trace elements. Environ Pollut. 2006;144:93–100. https://doi.org/10.1016/j.envpol.2006.01. 005.
- Seregin IV, Kozhevnikova AD. Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium. Russ J Plant Physiol. 2008;55(1):1–22. https://doi.org/10.1134/S1021443708010019.
- Petráš R, Jamnická G, Mecko J, Neuschlová E. State of mineral nutrition and heavy metals distribution in aboveground biomass of poplar clones. Pol J Environ Stud. 2012;21(2):447–53.
- Thijs S, Witters N, Janssen J, Ruttens A, Weyens N, Herzig R, et al. Tobacco, sunflower and high biomass SRC clones show potential for trace metal phytoextraction on a moderately contaminated field site in Belgium. Front Plant Sci. 2018;9:1879. https://doi.org/10.3389/fpls.2018.01879.
- Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK. The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environ Pollut. 2009;157:931–7. https://doi.org/10.1016/j.envpol.2008.10. 024.
- Hernández-Allica J, Becerril J, Garbisu C. Assessment of the phytoextraction potential of high biomass crop plants. Environ Pollut. 2008;152(1):32– 40. https://doi.org/10.1016/j.envpol.2007.06.002.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.