



# Organic Amendments to Short Rotation Coppice (SRC) Plantation Affect Species Richness and Metal Accumulation of Spontaneously Growing Herbaceous Plants

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## Abstract

Excess potentially toxic metals (PTMs) in soils require ad hoc approaches to salvage. Hence, this study explored the shoot accumulation of cadmium (Cd), lead (Pb), and zinc (Zn) by herbaceous plants growing under previously established *Salix* and *Populus* clones Short Rotation Coppice (SRC) with compost and sewage sludge applications in an abandoned metallurgical site, Podlesí, Czech Republic; PTM decontamination of soils. Soils within the SRC experimental site and outside considered as control were analyzed for their chemical properties by multi-analytical techniques. Shoots of spontaneously growing herbaceous plants under trees in the site and without trees in control were determined for pseudo-total Cd, Pb, and Zn contents. Moderately to slightly acid soils, high cation exchange capacity, and C/N ratio supported mineralization and relative mobility of total Cd (7.7–9.76), Pb (1541–1929), and Zn (245–320 mg kg<sup>-1</sup>) in soils. Although soil amendments improved chemical properties, compost application supported higher species richness than sewage sludge. Over 95% of plants accumulated Cd and Zn above the WHO threshold and green fodder in the Czech Republic, with 36% Pb above the regional limit (40 mg kg<sup>-1</sup>). Approximately 100, 50, and 6% of herbaceous species had Cd, Pb, and Zn accumulation, respectively, higher than published average upper limits in plants (0.2 Cd, 10 Pb, and 150 Zn mg kg<sup>-1</sup>). Dicots recorded higher Cd content, *Tenacetum vulgare* (L.), *Hypericum maculatum* (Crantz), and *Cirsium arvense* (L.); *Stachys palustris* (L.), *Lamium perperum* (L.), and *Campanula patula* (L.) for Pb; *Glechoma hederaceae* (L.), *C. patula*, and *C. arvense* for Zn in all treatments. Appropriate soil amelioration of SRC-supported PTM mobility and excess herbaceous species shoot accumulation, growth, and richness.

**Keywords** Compost · Dicot · Potentially toxic metals · Sewage sludge · Shoot accumulation · Soil chemical properties

## 1 Introduction

Soil contamination by potentially toxic metals (PTMs), typically lead (Pb) and cadmium (Cd), is a widespread problem. For example, Pb is one of the most toxic elements whose extensive use from anthropogenic interferences, ore mining, smelting, and tailings, has caused environmental (Gupta et al. 2019; Kushwaha et al. 2018) and health-related issues (Han et al. 2020; Varrica et al. 2018). Only in the Czech Republic, there are over 800 ore deposits, with a similar number of known metalliferous mineral occurrences (Bufka et al. 2005). Therefore, strict measures are required with the increasing trends in metal production (U.S. Geological Survey 2020; Shahabi-Ghahfarokhi et al. 2021), especially in Europe (Bertrand et al. 2016).

Various biological, chemical, and physical techniques to eliminate PTMs in soils are commonly used (Aparicio et al.

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2021; Ashraf et al. 2018). Conventional strategies for cleaning PTM-contaminated lands, e.g., soil washing (Shen et al. 2019), solidification (Matec Industries 2021), and vitrification (Liu et al. 2018), are often ex situ and require high treatment costs. These approaches can result in the loss of soil fertility and biodiversity (Eissa and Almaroai 2019; FAO 2017; Liu et al. 2019). Meanwhile, using phytoremediation (plant-based approach) to remove PTMs in contaminated soils outscored the demerits of conventional methodologies (Reeves 2006; Tonelli et al. 2020; van der Ent et al. 2003). During the selection process for tolerant plants for effective PTM removal, it is vital to consider species capable of recovering soil health, e.g., perennial herbaceous plants (Chamberlain et al. 2022).

Several factors affect the mobility, immobilization, and uptake of PTM in soils, e.g., pH, element content, root architecture, and organic matter (Borah et al. 2018; Zeng et al. 2011). Although organic amendment, including the application of compost and sewage sludge, improves soil fertility for plant growth, it contributes to microbial count and diversity in soils capable of solubilizing PTMs for plant accessibility. Moreover, the addition of organic matter can influence the number of plant species (Moreno-Peñaranda et al. 2004). As metal-contaminated soils are not suitable for the cultivation of crops, a cleanup campaign becomes pertinent.

According to agricultural and environmental legislation, PTM-contaminated soils are not suitable for the cultivation of food crops and herbage and should undergo extensive remediation actions, a condition that makes the management of polluted sites often not sustainable. In contrast, the use of PTM-polluted soils to grow high biomass woody plants managed as Short Rotation Coppice (SRC) allows for reduced environmental mobility of PTM and produces polluted woody biomass (Zárubová et al. 2015), thus representing an option to reduce both the environmental impact of PTM-polluted soils and produce income from the use produced biomass. Fast-growing species of *Salix* and *Populus* spp. SRC have shown high extraction of PTMs in contaminated soils (Goliński et al. 2015; Hassinen et al. 2009) and produce large amounts of biomass in rotations of 3 to 10 years (Šlapokas and Granhall 1991). It has been widely reported that phytomanagement of PTM-polluted soils with SRC of fast-growing plants (e.g., willow and poplar) allows for restoration of soil physical and chemical fertility (Courchesne et al. 2017) and microbial diversity and activity, especially if aided by the use of soil ameliorants. Meanwhile, the diversity and PTMs accumulation potency of herbaceous species (plants without persistent woody stem above ground) in polluted soils is still not well-understood, especially in the case of perennial species. Studies have been carried out on short-lived herbaceous species or plants characterized by low-biomass (e.g., Chen et al. 2014; Wei et al. 2006), and plant species such as *Lolium perenne* (L.) and

*Poa pratensis* (L.) were used to remove copper (Cu), Cd, and Pb from sewage water (Liu et al. 2019).

Selecting resistant herbaceous plants for PTM removal can offer an even more cost-effective and time-saving approach to improve the phytomanagement of PTM polluted soils and also improve the self-sustenance of SRC phytomanagement interventions (Licinio et al. 2023).

The study focussed on herbaceous plant growth and their ability to accumulate PTMs from contaminated soil under SRC of *Salix* and *Populus* species after different organic treatments. The field trial was established in 2008 (Zárubová et al. 2015).

The study aimed to evaluate the following aspects of herbaceous plant species: (i) the composition of the herbaceous plant species community and the effects of compost and sewage sludge on species count and (ii) the accumulation potential of PTMs of the detected species and the eventual effects of compost and sewage sludge. To our knowledge, this is the first study to focus on the potential of herbaceous plants to accumulate PTMs in the presence of fast-growing woody plants and may help improve the phytomanagement of metal-polluted soils.

## 2 Materials and Methods

### 2.1 Study Site and Field Experiment

The study occurred in Podlesí (49° 42' 24" N, 13° 58' 32" E), near the ancient mining and smelting town Příbram, 58 km south of Prague, Czech Republic. The site represents moderately Cd, Pb, and Zn-contaminated agricultural soil, predominantly from atmospheric deposition due to its proximity to an abandoned smelter (approximately 200 m).

The elevation of the study area is 500 m above sea level (asl), with mean annual precipitation and temperature of 700 mm and 6.5 °C, respectively. The site characterizes modal Cambisols (syn. *modální* Kambizem) with geological substrates formed mainly by schists, sandstones, graywackes, and quartz (Czech Geological Survey 2012; Němeček and Kozák 2005).

Minerals rich in PTM include galenite (PbS), sphalerite (ZnS), boulangerite (Pb<sub>5</sub>Sb<sub>4</sub>S<sub>11</sub>), and antimonite (Sb<sub>2</sub>S<sub>3</sub>) throughout the study area. The levels of these minerals also cause natural increases in the content of PTMs in the soil (Borůvka and Vacha 2006).

To cover the variability of soil and herbaceous plant samples, we took advantage of the experimental design of the *Salix* and *Populus* clones SRC in the site (see Zárubová et al. 2015 for a detailed description of the experimental design). The SRC consisted of two *Salix* clones, allochthonous (*Salix schwerinii* × *Salix viminalis*) × *S. viminalis* hybrid Tordis and autochthonous *Salix* × *smithiana* clone S-218. Among

*Populus* clones consisted of the most widely planted hybrid clone in the Czech Republic, *Populus maximowiczii* × *Populus nigra* J-105, also known as the Max-4 and *P. nigra* clone Wolterson. Shoots were harvested after four seasons in 2012 and then after two more seasons in 2014.

The *Salix* and *Populus* clones appear in four split-plot randomized blocks with the application of compost (co), sewage sludge (ss), and control (*k*) without any treatment. The ratio of compost and sewage sludge to spoil material was estimated to give an appropriate range of contents as follows: compost composition, 59.9% dry matter, nitrogen (N) 16 kg/t, 20 t/ha dry compost, and 33.4 t/ha with an application of 100 kg/plot with the content of elements given in Table S1 (Kubátová et al. 2016); sewage sludge, 20.4% dry matter, N 46 kg/t dry matter, 6.96 t/ha sludge dry matter, 34.8 t/ha of fresh sludge on a plot size of 29.25 m<sup>2</sup>. The application of sewage sludge was 100 kg/plot applied in 2012. An exact description shows that *Salix* clones accumulated up to 55 Cd, 29 Pb, and 506 Zn mg kg<sup>-1</sup> in dry-weight biomass compared to 25 Cd, 30 Pb, and 206 Zn mg kg<sup>-1</sup> in *Populus* at the phytoremediation experimental site (Kubátová et al. 2016).

## 2.2 Soil Sampling and Preparation

In the experimental site, soil samples were randomly collected from the randomized blocks of *Salix* and *Populus* clones from *k*, co, and ss, according to the intra-row distance of 0.25 m. For each treatment, soil sampling involved random collection around the same tree species on one block (7.5 × 1.3 m) to avoid lateral mixing of adjacent soils. The total size of the experimental site represents approximately 100 m<sup>2</sup>. Each randomized block of different treatments was represented by nine replicates of soil samples.

Additionally, soil samples were randomly collected outside the experimental site, without trees, as control (*c*) with the same soil and parent bedrock. At *c*, the sampling covered nine soil samples taken randomly within an area < 100 m<sup>2</sup>, according to the distribution of the herbaceous plant species. In this case, soil samples are collected around locations with herbaceous plants to detect the direct effect of the physico-chemical properties of the soil.

Each soil sample consisted of five subsamples randomly taken from the arable layers (upper 30 cm) with a soil probe (Purchhauer type, core diameter: 30 mm). In total, 36 mixed soil samples (9 replicates × 4 treatments) were collected as an acceptable method to average out short-range variation, making the results and generalization more reliable (Asare et al. 2020). Samples of each soil were air-dried, disaggregated, and passed through a 2-mm mesh sieved to homogeneity for subsequent chemical analysis. Each representative soil sample was prepared into three replicates.

## 2.3 Determination of Soil Chemical Properties

The pH of all soil samples followed the measurement by a 1/2.5 (w/v) suspension of soil and 0.01 M calcium chloride (CaCl<sub>2</sub>). Carbonates were determined volumetrically following the method proposed by Kalra and Maynard (1991).

The total carbon in soil samples again was determined according to automated thermal analyses in which carbon is converted to carbon dioxide (CO<sub>2</sub>) by flash combustion at 1080 °C (Costech Instruments Elemental Combustion 102 System elemental analyzer, Thermo Scientific 103 Delta V Advantage). Carbonates were previously removed from 10 mg of soil by treatment with hydrogen chloride (HCl) in silver capsules. The total N content was determined from 50 mg of soil using the modified Dumas combustion method at 960 °C with a CNS elemental analyzer (Vario MACRO cube CNS; Elementar Analysensysteme GmbH, Langenselbold, Germany). The cation exchange capacity (CEC) was determined by ISO 11260 (1994).

The total contents of soil PTM (Cd, Pb, and Zn) and sulfur (S) in the soils were extracted using the USEPA 3052 extraction procedure (International Organization for Standardization, USEPA 1996) with an extraction mixture of 65% nitric (HNO<sub>3</sub>), 36% hydrochloric (HCl), and 38% hydrofluoric (HF) acids. The total element content in soil digest was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA), equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material (experimental conditions, power of 1.2 kW, plasma flow of 15.0 Lmin<sup>-1</sup>, auxiliary flow of 0.75 Lmin<sup>-1</sup>, nebulizer flow of 0.9 Lmin<sup>-1</sup>).

## 2.4 Plant Sampling, Classification, and Preparation

A comprehensive identification campaign and sampling of herbaceous plant species were well-performed following a systematic characterization of mature plant species. The geobotanical description of the herbaceous species was carried out in situ following the methodology and traditional techniques adopted in Plant Taxonomy. The nomenclature of the taxa used followed that adopted in Angiosperm Phylogeny Group (APG) IV (<https://www.gbif.org/species/3106>).

Plant materials collected were whole green biomass (shoot above the soil surface) of mature herbaceous plant species in all four sampled locations. The samples were washed with deionized water (H<sub>2</sub>O) to remove ions transported from environmental dust. Next, we dried all the samples in an electric oven (SLW 53 STD, Pol-Eko, Wodzisław Śląski, Poland) at 60 °C for 48 h and then cut them in a stainless steel Retsch friction mill (Retsch, Haan, Germany; particle size 0–1 mm) to obtain homogenized-powdered samples.

## 2.5 Determination of PTMs in Plant Samples

Powdered samples of each dry weigh plant species were further analyzed for PTM following the microwave-assisted acid approach (USEPA 3051 1994), with contents determined by the same ICP-OES (Agilent 720, Agilent Technologies Inc., USA).

### 2.5.1 Analytical Protocol

An aliquot (~ 500 mg of dry matter) of the plant sample was weighed in a digestion vessel for elemental content in the determination of the plant biomass. Concentrated HNO<sub>3</sub> acid of 8.0 mL and 30% peroxide (H<sub>2</sub>O<sub>2</sub>) (2.0 mL) (Analytika Ltd., Czech Republic) were added. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 30 min at 220 °C. After cooling, we quantitatively transferred the plant digests into a 20-mL glass tube, topped with deionized H<sub>2</sub>O and kept them at laboratory temperature until the determination of the contents of PTM.

For quality assurance of the analytical data, a standard reference material (NIST SRM tomato leaves 1573) certified for the determination of total contents of arsenic (As), Cd, Cu, and Zn in plant biomass was used (<http://www.nist.gov/srm>) and contents of PTMs determined by same ICP-OES. ICP-OES has previously been used for the determination of the total Pb content in dried-weight plant samples (Gonçalves et al. 2019; Yener 2019). The results of the control elements (Cd and Zn) revealed a recovery (*R*) of 90% with an obtained SRM value of 0.061 ± 0.01 Cd and 80.4 Zn mg kg<sup>-1</sup> to original values—0.068 ± 0.012 Cd and 82.3 ± 3.9 Zn mg kg<sup>-1</sup>, respectively (Magnusson and Örnemark 2014).

$$R = (\text{mean value of element} \div \text{mean reference value}) \times 100 \quad (1)$$

According to the obtained results, the precision of the analytical method was adequate for this study.

### 2.6 Estimation of Bioaccumulation Factor (BAF)

The *BAF* (soil-shoot quotient) explains the ability of a plant to translocate the metal from the soil to shoot. The use of *BAF* is a vital parameter when associated with the permissible limits of PTMs in plants. The estimation of *BAF* follows the equation of Baker (1981) and Vondráčková et al. (2015).

$$BAF = \frac{\text{Content of PTM in shoot}}{\text{content of PTM in soil}} \quad (2)$$

The accumulation of PTMs in the studied plant species was evaluated against the regulatory limits of plant-origin animal feed raw materials, following the Czech Republic Regulation (Commission Directive 2010/6/EU), WHO

(1996), and the average values expected in plant biomass by Kabata-Pendias (2011).

## 2.7 Statistical Analysis

For data generated from field and laboratory, we checked for homogeneity of variance and normality with the Levene and Shapiro–Wilk tests, respectively. Data for soil chemical properties met assumptions for the use of parametric tests. In that case, the analysis of variance (ANOVA) model with Tukey’s honestly significant difference (HSD) post hoc test for significant ANOVA results was applied to determine significant differences in soil properties in all treatments. Differences between treatments were considered significant at  $p < 0.05$ . Principal component analysis was used to show the variability among herbaceous plants according to monocot/dicot and botanical families.

In the case of PTM data of plants for each treatment, the Kruskal–Wallis analysis of variance model was applied to determine the significant differences since there was no normality. We evaluated the relationships between soil chemical properties using Pearson’s correlation (*r*). Data for soil and plant samples were statistically analyzed using R software version 4.1.3 with “data.table,” “ggplot2,” and “factoextra” (R Development Core Team 2010).

## 3 Results

### 3.1 Soil Chemical Properties

There was a significant effect of the content of Cd, Pb, and Zn between treatments (Table 1). The PTM content in the soil ranged from 7.7 to 9.8 mg Cd kg<sup>-1</sup>, 1541–1929 mg Pb kg<sup>-1</sup>, and 245–320 mg Zn kg<sup>-1</sup> in all treatments (Table 1) compared to global (0.3 Cd, 100 Pb, and 200 Zn mg kg<sup>-1</sup>) and regional regulatory limits (0.5 Cd, 60 Pb, and 150 Zn mg kg<sup>-1</sup>) (Decree of the Ministry of Environment No. 153/2016 Coll., Czech Republic) limits for agricultural soils. Soil pH, contents of total C, N, S, CEC, and C/N ratio corresponded to the range of values characteristic of soils of the temperate climate zones (Antisari et al. 2021) with notable heterogeneity (Fig. 1). A pH<sub>[CaCl2]</sub> range of 5.2–5.8 (moderate to slight acidity) can support relative solubility of Cd, Pb, and Zn (Fig. 1a). Compared to co (pH 5.8) in all treatments, the effect of compost application resulted in a reduction of soil acidity.

Treatments with ameliorants increased the amount of organic matter, indicated by the significantly high contents of total C, N, and S (Fig. 1b, c, e) and a suitable C/N ratio (Fig. 1d) for mineralization of nutrients.

The application of organic matter also relates to the significant increase in CEC (Fig. 1f). The release of positive

**Table 1** Mean content ( $\pm$ SD) of total Cd, Pb, and Zn under different treatment of soils in the SRC experimental site and control without trees

Treatment	Cd	Pb	Zn
$\text{mg kg}^{-1}$			
<i>c</i>	$8.04 \pm 2.12\text{ab}$	$1872 \pm 346\text{b}$	$278 \pm 35.6\text{c}$
<i>co</i>	$9.76 \pm 0.71\text{a}$	$1929 \pm 210\text{a}$	$320 \pm 23.7\text{a}$
<i>k</i>	$7.7 \pm 1.7\text{b}$	$1541 \pm 255.3\text{d}$	$245 \pm 28.8\text{d}$
<i>ss</i>	$7.73 \pm 1.91\text{b}$	$1695 \pm 413\text{c}$	$290 \pm 66.6\text{b}$
<i>p</i> -value	0.002	0.001	0.001

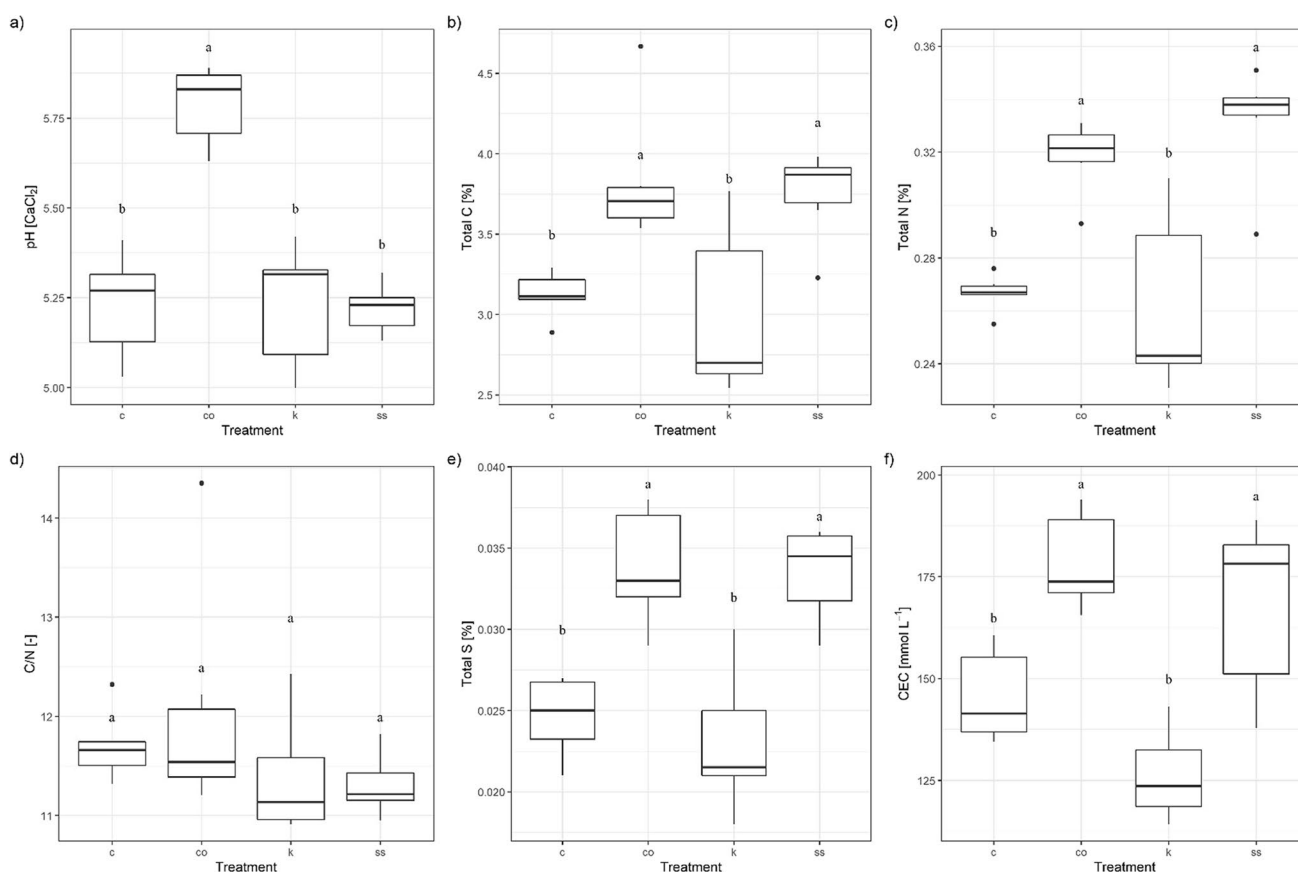
The *p*-values were obtained by one-way ANOVA Test. Using the Tukey post hoc test, the mean contents of each potentially toxic metal (PTM—Cd, Pb, and Zn) in each treatment with different letters are significantly different.

SD standard deviation, SRC Short Rotation Coppice, *c* control without trees, *co* treatment with compost application, *k* no organic matter treatment in the experimental site, and *ss* treatment with sewage sludge

ions ( $\text{H}^+$ ) in the acid soil reaction in the case of compost application can contribute to the comparatively high CEC. The correlations between soil chemical properties for each treatment are shown in Table 2. There was a negative correlation between pH and all soil chemical properties in *k* ( $r = -0.84$  to  $-0.98$ ) and vice versa for the other treatments except for pH and N (in *c*) and S (in *co*). The possibility of sorption is well-indicated by the relationship between C and N in both *co* and *ss* treatments, while the pH and S in the application of compost were negative ( $r = -0.3$ ).

### 3.2 Description of Herbaceous Species Richness and Diversity

Different treatments resulted in varied counts of perennial herbaceous species, botanical families, and monocot/dicot (Tables S2 – S4). Exactly 53 herbaceous plants were recorded, with 58% and 51% for the *c* and *co* treatments,



**Fig. 1** Effect of different soil treatments on **a** pH and content of **b** total C, **c** total N, **d** C/N ratio, **e** total S, and **f** CEC. Mean values with different letters significantly differ using the Tukey HSD post hoc test. *p*-value (one-way ANOVA) was significant at  $<0.05$ . Error bars

indicate standard deviation. Abbreviations: *c*, control without trees; *co*, treatment with compost application; *k*, no organic matter application in the experimental site; and *ss*, treatment with sewage sludge application

**Table 2** Correlations between chemical properties of soils at different treatments

	pH	CEC	Tot C	Tot N	Tot S	C/N ratio
<i>c</i>						
pH		0.51*	0.3	-0.01	0.63*	0.47
CEC			0.38	-0.07	0.65*	0.68*
Tot C				0.80*	0.86*	0.85*
Tot N					0.62*	0.36
Total S						0.80*
C/N ratio						
<i>co</i>						
pH		0.68*	0.34	0.40	-0.3	0.01
CEC			-0.17	0.41	-0.39	-0.36
Tot C				0.4	0.42	0.92*
Tot N					0.1	0.01
S						0.41
C/N ratio						
<i>k</i>						
pH		-0.86*	-0.98*	-0.96*	-0.84*	-0.91*
CEC			0.88*	0.93*	0.93*	0.65*
Tot C				0.99*	0.89*	0.93*
Tot N					0.95*	0.86*
S						0.69*
C/N ratio						
<i>ss</i>						
pH		0.78*	0.06	0.34	0.80*	-0.65*
CEC			0.50*	0.70*	0.97*	-0.33
Tot C				0.93*	0.57*	0.51*
Tot N					0.74*	0.17
S						-0.22
C/N ratio						

Tot total, *c* control without trees, *co* treatment with compost application, *k* no organic matter treatment in the experimental site, and *ss* treatment with sewage sludge application. Locations with an asterisk (\*) indicate a moderate ( $r = -0.5/0.5$  to  $-0.7/0.7$ ) to strong significant correlation ( $r = -0.8/0.8$  to  $-0.98/0.99$ ) at  $p < 0.05$

**Table 3** Characteristics of biomass under different treatments

Treatment	No. of species	No. of monocot	No. of dicot	No. of Families
<i>c</i>	31	21	10	13
<i>co</i>	27	23	8	15
<i>k</i>	24	15	10	14
<i>ss</i>	24	15	9	9

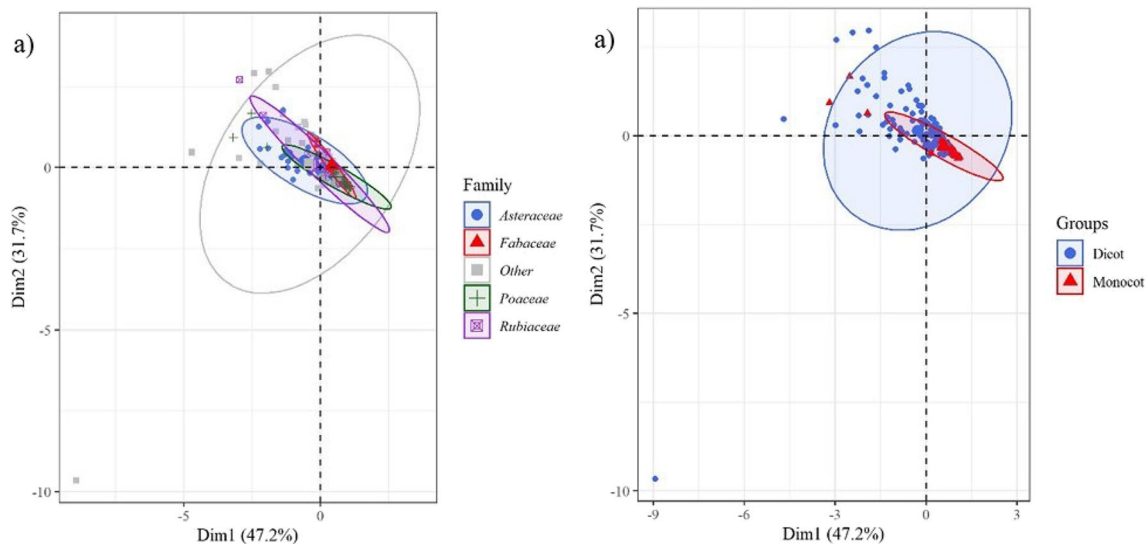
No. number, *c* control without trees, *co* treatment with compost application, *k* no organic matter application in the experimental site, and *ss* treatment with sewage sludge application

respectively, and 45% each for *k* and *ss* (Table 3). Although organic matter application plays a significant role in species richness, the quality of the materials and conditions of preparation is vital (Weisser et al. 2017). The application of

sewage sludge supported the nutrient status of the soil and plant species but not the richness. The botanical families and monocot/dicot showed 47.2 and 31.7% variability in the first and second axes of the ordination diagrams (Fig. 2a, b). In all fields, we recorded 20 botanical families with a high prevalence of species in Poaceae, Asteraceae, Fabaceae, and Rubiaceae (Fig. 2a).

### 3.3 PTM Accumulation by Herbaceous Plants and Bioaccumulation Factor (BAF)

The contents of PTMs accumulated by each herbaceous plant species and monocots/dicots under different treatments are shown in Figs. 3, 4, 5 and 6. The contents of Cd in *c*, *co*, *k*, and *ss* treatments ranged from 0.51 to 16.5 mg kg<sup>-1</sup> in *Lathyrus pratensis* (L.) and *Tanacetum vulgare* (L.), 0.22–29.6 mg kg<sup>-1</sup> in *Urtica dioica* (L.)



**Fig. 2** Ordination diagram of the first and second axis of the distribution and variability of **a** botanical families and **b** taxa (monocot and dicot) of studied herbaceous plant species

and *Hypericum maculatum* (Crantz), 0.23–43.7 mg kg<sup>-1</sup> in *Holcus lanatus* (L.) and *H. maculatum*, and finally 0.23–19 mg kg<sup>-1</sup> in *Elymus repens* (L.) and *Cirsium arvense* (L.), respectively (Fig. 3a–d).

The mean accumulation of Cd by plants in all treatments was 4.29 in *c*, 4.52 in *co*, 4.77 in *k*, and 3.22 mg kg<sup>-1</sup> for *ss*. *H. lanatus* recorded the overall highest accumulation of Cd in all treatments. The accumulation of Cd by plants in all treatments was above published average upper limits in plants (0.2 mg Cd kg<sup>-1</sup>) given by Kabata-Pendias (2011).

The lowest Pb content in the *c* treatment was 4 mg kg<sup>-1</sup> in *Alopecurus pratensis* L. and the highest was 70 mg kg<sup>-1</sup> in *Stachys palustris* (L.) (Fig. 4a). Furthermore, the content of Pb in *co*, *k*, and *ss* treatments ranged from 5.7 to 61 mg kg<sup>-1</sup> in *Crepis biennis* (L.) and *Lamium purpureum* (L.), 2.33–33 mg kg<sup>-1</sup> in *Poa* sp. and *C. patula*, and 4.9–30 mg kg<sup>-1</sup> in *Trifolium* sp. and *S. palustris*, respectively (Fig. 4b–d). The mean Pb accumulation by plants in all treatments was 19.7 in *c*, 18.4 in *co*, 11.8 in *k*, and 13.3 mg kg<sup>-1</sup> for *ss*. Exactly 19 (61%), 21 (78%), 10 (42%), and 12 (50%) herbaceous species from *c*, *co*, *k* and *ss* treatment accumulated a Pb content above the published upper limits in plants—10 mg Pb kg<sup>-1</sup> (Kabata-Pendias 2011).

The content of Zn was above the average upper limits in plants—150 mg kg<sup>-1</sup> Zn—according to Kabata-Pendias (2011), only in *Glochoma hederacea* (L.), *Campanula patula* (L.), and *C. arvense*. The Zn content in *c* treatment ranged from 20.9 to 185 mg kg<sup>-1</sup> in *Calamagrostid epigejos* (L.) and *Glochoma hederacea* L., respectively (Fig. 5a).

Similarly, for *co*, *k* and *ss* treatments, the Zn content ranged from 30 to 162 mg kg<sup>-1</sup> in *C. biennis* and *Campanula*

*patula* (L), 18.9 to 164 mg kg<sup>-1</sup> in *H. lanatus* and *C. patula* and 25 to 167 mg kg<sup>-1</sup> in *Cardus acanthiodes* (L.) and *C. arvense*, respectively (Fig. 5b–d). Mean Zn accumulation in the herbaceous plants ranged from 56 to 71 mg kg<sup>-1</sup> in treatments *k* and *co*, respectively.

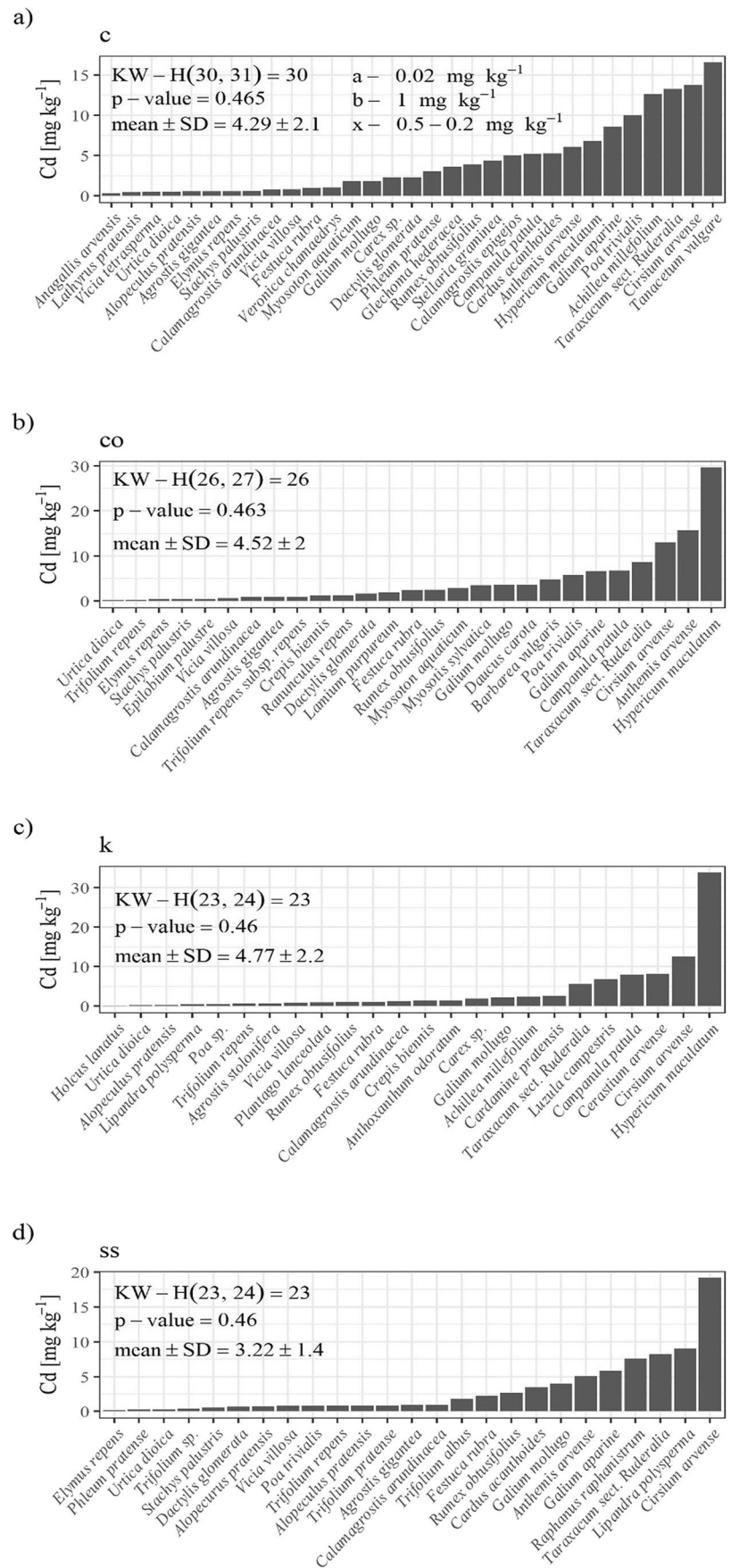
Accumulation of Cd, Pb, and Zn by monocot/dicot under different treatments is in Fig. 6. Generally, there was the highest accumulation of PTMs in dicots compared to monocots (Fig. 6). Compared to 245–320 mg Zn kg<sup>-1</sup> in the soils, all the plants accumulated lesser in each treatment. There were high accumulations of Cd (*BAF* > 1) visible among 11 herbaceous species under different treatments (Table 4). The *BAF* of Pb and Zn for all the plants studied was below 1 (*BAF* < 1) as no plant accumulated PTM above the content of the soil (Table S6).

## 4 Discussion

The current study demonstrated the potential of herbaceous plants growing under phytoremediation scheme of *Salix* and *Populus* SRC plantation to accumulate Cd, Pb, and Zn from contaminated soil in varying contents in their shoots. Some plants possess the ability to persist in PTM-contaminated soils via accumulation (Sun et al. 2006) of excess PTMs (Dalvi and Bhalerao 2013) into the aerial parts. Identification of these herbaceous plants (common weeds) in contaminated soils and accumulation is a prerequisite for the bioindication of soil pollution and the availability of metals to plants and crops (Pietrelli et al. 2022).

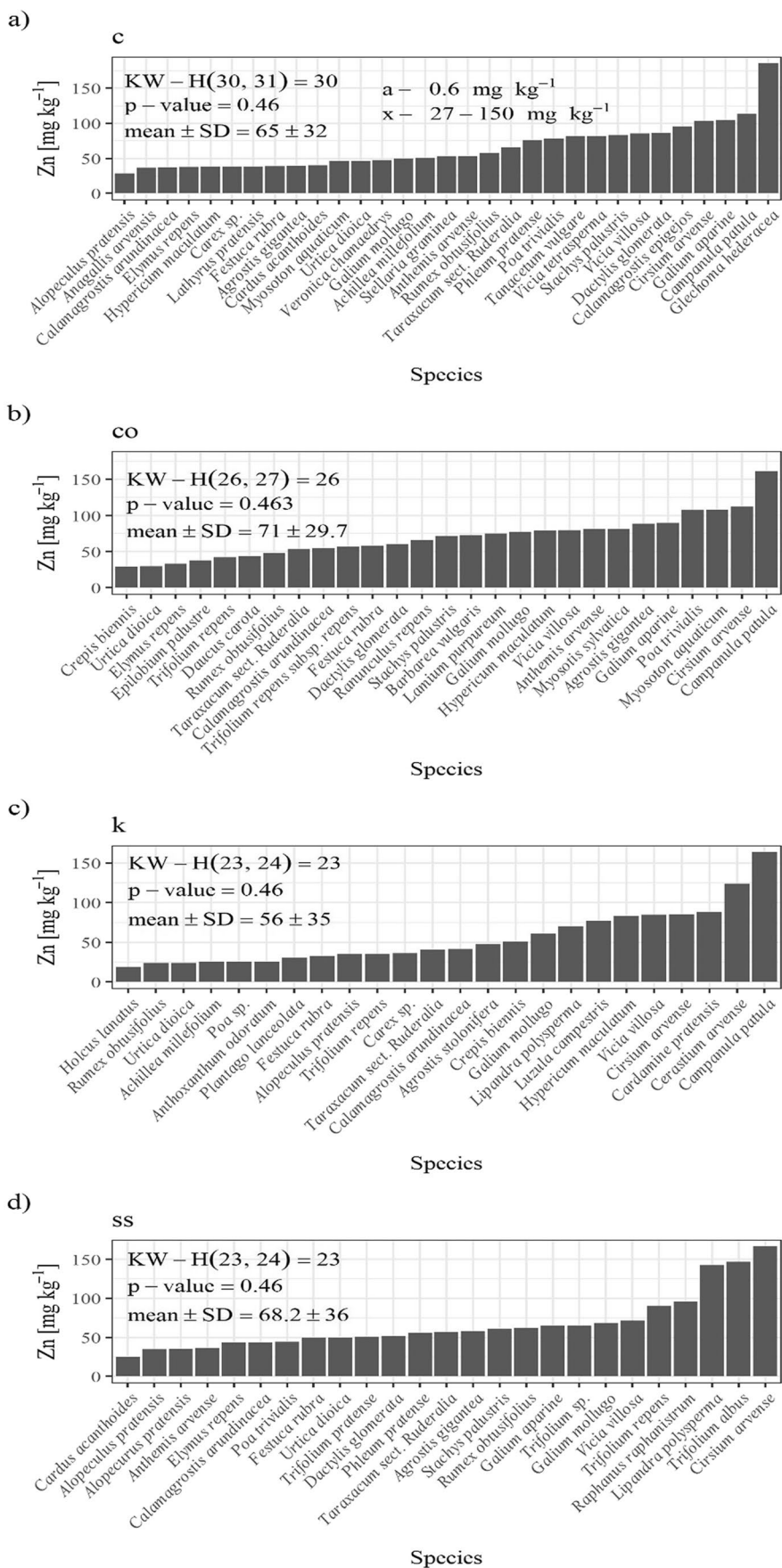
Studies have shown that herbaceous species including *Artemisia vulgaris* L., *Heracleum sphondylium* L., and

**Fig. 3** The mean content of Cd in shoots of herbaceous plant species under different soil treatments **a c**, **b co**, **c k**, and **d ss**. The *p*-value was obtained by the Kruskal–Wallis analysis of variance (ANOVA). Abbreviations: *c*, control without trees outside the Short Rotation Coppice (SRC) experimental site; *co*, treatment with compost application; *k*, no organic matter application in the experimental site; and *ss*, treatment with sewage sludge application. *a*, WHO limit; *b*, green fodder (Czech legislation); *x*, normal level according to Kabata-Pendias (2011). SD, standard deviation

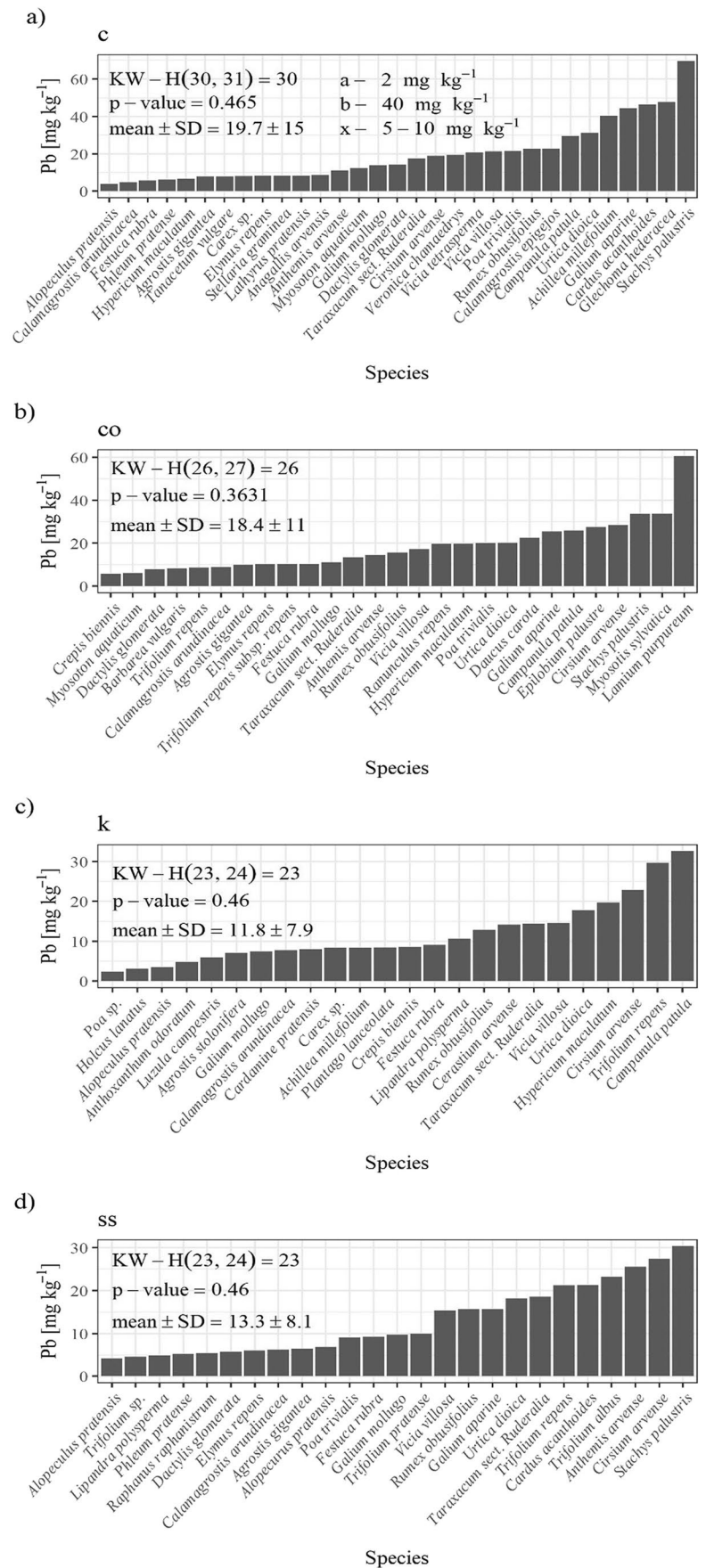


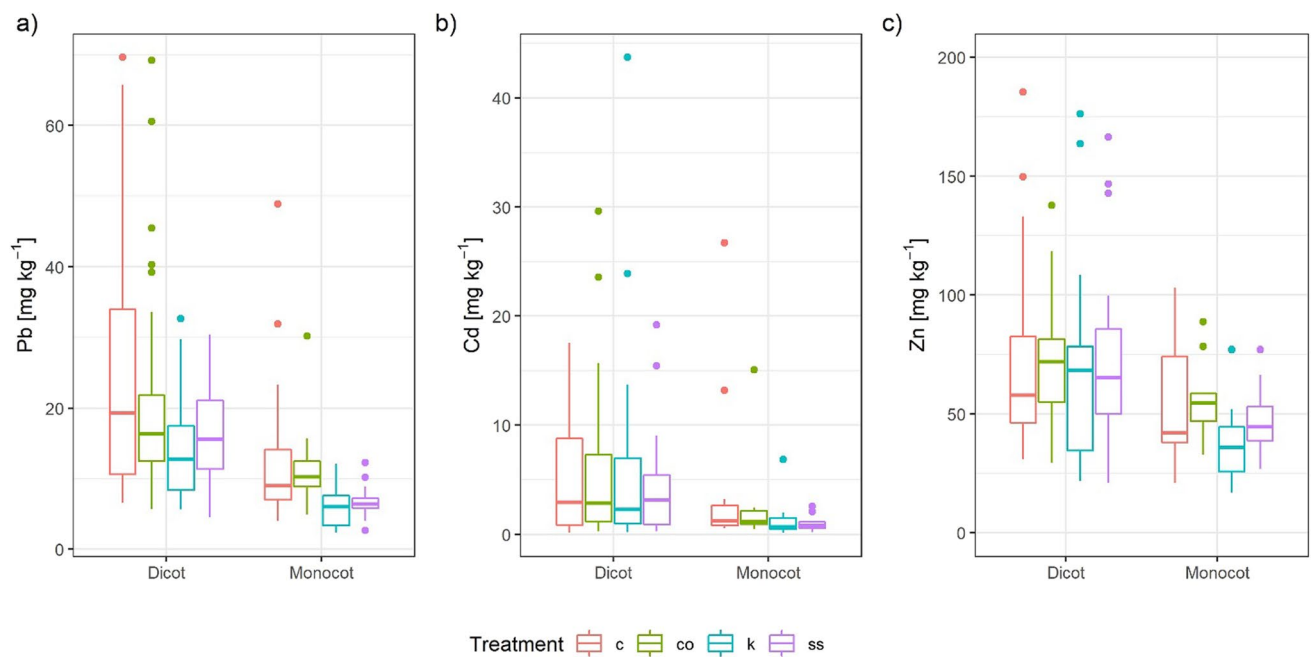


**Fig. 4** The mean content of Pb in shoots of herbaceous plant species under different soil treatments **a c**, **b co**, **c k**, and **d ss**. The *p*-value was obtained by the Kruskal–Wallis analysis of variance (ANOVA). Abbreviations: *c*, control without trees outside the Short Rotation Coppice (SRC) experimental site; *co*, treatment with compost application; *k*, no organic matter application in the experimental site; and *ss*, treatment with sewage sludge application. *a*, WHO limit; *b*, green fodder (Czech legislation); *x*, normal level according to Kabata-Pendias (2011). SD, standard deviation



**Fig. 5** The mean content of Pb in shoots of herbaceous plant species under different soil treatments **a c**, **b co**, **c k**, and **d ss**. The *p*-value was obtained by the Kruskal–Wallis analysis of variance (ANOVA). Abbreviations: *c*, control without trees outside the Short Rotation Coppice (SRC) experimental site; *co*, treatment with compost application; *k*, no organic matter application in the experimental site; and *ss*, treatment with sewage sludge application. *a*, WHO limit; *b*, green fodder (Czech legislation); *x*, normal level according to Kabata-Pendias (2011). SD, standard deviation





**Fig. 6** Effect of different soil treatments on the mean content of **a** Cd, **b** Pb, and **c** Zn in monocots and dicots. Error bars and dots indicate  $\pm$ SD (standard deviation) and outliers, respectively. Abbreviations: *c*, control without trees outside the Short Rotation Coppice

(SRC) experimental site; *co*, treatment with compost application, *k*, no organic matter application in the experimental site; and *ss*, treatment with sewage sludge application

*Bistorta officinalis* L. exhibit marked accumulation of Zn, Cu, Mn, Ni, As, Pb, and Cr in their tissues (Nworie et al. 2019). Thus, such sites remain unsuitable for agricultural production as metals will pose a risk to food crops. Moreover, spontaneous growth of native species *A. vulgaris* and

*Trifolium repens* L. growing on Ni, Cr, Cd, Pb, Zn, and Cu contaminated soils along the Spreča river valley (the north-east region of Bosnia and Herzegovina) were consider more suitable for aerial accumulation of metals than *L. perenne*, *Urtica dioica* L., *Mentha arvensis* L., *Medicago sativa* L., *Urtica urens* L., and *Achillea millefolium* L. (Murtić et al. 2021).

**Table 4** Bioaccumulation factor of herbaceous plants from different treatments

Species (family)	<i>c</i>	<i>co</i>	<i>k</i>	<i>ss</i>
Cd				
<i>Achillea millefolium</i> (Asteraceae)	1.6	-	-	-
<i>Anthemis arvensis</i> (Asteraceae)	-	1.6	-	-
<i>Calamagrostis epigejos</i> (Poaceae)	1.6	-	1.03	-
<i>Campanula patula</i> (Campanulaceae)	-	1.2	-	1.2
<i>Cerastium arvensis</i> (Caryophyllaceae)	-	1.3	1.6	2.5
<i>Cirsium arvensis</i> (Asteraceae)	-	1.3	-	-
<i>Galium aparine</i> (Rubiaceae)	1.1	-	-	-
<i>Hypericum maculatum</i> (Hypericaceae)	-	3	-	-
<i>Lipandra polysperma</i> (Amaranthaceae)	-	-	-	1.1
<i>Poa trivallis</i> (Poaceae)	1.2	-	-	-
<i>Taraxacum sect. Ruderalia</i> (Asteraceae)	1.7	-	-	1.1

*c* control without trees outside the Short Rotation Coppice (SRC) experimental site, *co* treatment with compost application, *k* no organic matter application in the experimental site, and *ss* treatment with sewage sludge application. Only bioaccumulation factor (BAF) > 1 was presented

The current research considered the cutting of aerial parts of plants to allow roots to regenerate shoots for continuous above-ground accumulation—this method is a permanent approach to Cd, Pb, and Zn removal from contaminated soils. The shoot system periodically requires harvesting to avoid continuous drop in biomass.

Increasing the time cycles of the SRC allows biodiversity to develop in the coppice. The older the planted crop, the shadier the conditions for the ground vegetation, which is associated with a shift from annual to perennial and from light-demanding to shade-tolerant species (Baum et al. 2009). Studies have shown that SRC willow plantation supports high plant species richness and abundance compared to arable lands. For example, a study found 27% higher cover of plant species richness in SRC willow plantation compared to neighboring arable land (Kwapong 2023). Meanwhile, the condition of organic amelioration can support the mobility and accumulation of PTMs by plant species, enhance diversity, and richness (Niroschika et al. 2020). Under 65 °C thermal conditions, microbes in the compost

during preparation can undergo aerobic respiration, which maintains the decomposition process that produces high quality compost, resulting in spore germination to increase plant species and growth (Chen et al. 2020). Under field conditions, the control environment (*c*) exhibited species richness considering the chemical characteristics of the soil, which partly support the release of elements for relatively easy plant uptake and from minimal or no competition for resources compared to locations with tree canopies (Vetaas et al. 2020). Although the number of species in the treatment *k* was the same in the application of sewage sludge, it could not yet match the species count in the compost application. The differences in the level of plant accumulation of PTMs also relate to plant and other extrinsic characteristics, e.g., microbial activities in the root rhizosphere, which could lead to the solubility of PTMs.

The relationship between soil parameters has implications on the metal content in plants. For example, Adams et al. (2004) predicted the Cd content in wheat grains based on the relationship between the soil Cd content and pH. For example, acidic pH supports the solubility of elements, e.g., PTMs, in soils (Matsumoto et al. 2017). In this study, positive ions from pH, CEC, Cd<sup>2+</sup>, Pb<sup>2+</sup>, and Zn<sup>2+</sup> cannot fully participate in chemical reactions that involve bonding and adsorption on surfaces (Strawn 2021), supported by the relatively weak correlation between pH and C and N (organic matter components). The positive impact of organic ameliorants includes the comparatively high contents of nutrients (N, C, and S) and the improved physical state of plants during the field study. Increased acidity resulting from the application of the sewage sludge contributed to the high release of H<sup>+</sup>, which can reduce the mobility of PTMs and uptake due to adsorption by negative charges of the organic material—evident in the comparatively low mean accumulation of studied PTMs to those in *co*.

Lesser species count can result from competition. For example, the prevalence of *Rumex obtusifolius* L. (Polygonaceae) with broad leaves may obscure the emergence of less competitive species. For example, decreased species diversity resulted from the colonization by *Rumex alpinus* L., in the alps of Austria, Italy, and the Gaint Mountains of Czech Republic (Jungová et al. 2022).

Competition has an additional selection for species that maintain high roots and densities (Craine and Dybzinski 2013; Jungová et al. 2022). Although these dynamics are pertinent, different plants show varying uptake and aerial accumulation mechanisms (Matanzas et al. 2021) due to their genotypes and susceptibility to specific PTMs. For instance, *A. millefolium*, (Asteraceae), *Anthemis arvensis* L. (Asteraceae), *Calamagrostis epigejos* L. (Poaceae), *C. patula* (Campalunaceae), *Cerastium arvensis* L. (Caryophyllaceae), *C. arvensis* (Asteraceae), *Galium aparine* L. (Rubiaceae), *Hypericum maculatum*

(Crantz (Hypericaceae), *Lipandra polysperma* L. (Amaranthaceae), *P. trivialis*, and *Taraxacum sect. Ruderalia* (Asteraceae) exhibited a high shoot accumulation of Cd. Meanwhile, by the conventional standard of classifying plant hyperaccumulators, according to Reeve et al. (2006) and van der Ent et al. (2003), all the plant species were below the expected contents. Although *Salix* and *Populus* sp. are nonhyperaccumulators, these species accumulate 250 mg Cd kg<sup>-1</sup> and 3300 mg Zn kg<sup>-1</sup> with high biomass growth (Wieshammer et al. 2007). Thus, considerable attention to plant shoot accumulation is vital. However, some plants usually exclude (root accumulators—phytostabilization) Pb uptake due to restricted translocation by apoplastic barriers in the root, e.g., Casparian strips (Collin et al. 2022). Hence, plants with an above-ground Pb content greater than regulatory limits are considered suitable for phytomanagement. For example, species—*A. millefolium*, *Galium aparine*, *C. acanthoides*, *G. hederacea*, and *S. Palustris* in treatment *c* and only *L. purpureum* in *co*—recorded Pb above the permissible limits of raw materials of plant origin/animal feed (40 mg Pb kg<sup>-1</sup>), according to Directive 2010/6/EU of the Commission.

Meanwhile, Pb shoot accumulation in all the studied plants was above WHO limit (2 mg Pb kg<sup>-1</sup>). Cai et al. (2020) suggested that *Rudbeckia hirta* L. has restoration potential and its accumulation of Pb in the shoot when the content was up to 1000 mg kg<sup>-1</sup> compared to the lower contents of *Polygonum lapathifolium* L., *Medicago sativa* L., *Talinum paniculatum* (jacq.), *Capsicum annuum* cv., and *Trifolium repens* L.

Herbaceous species usually colonize on contaminated soils with potential use in phytoremediation of metals (Mongkhonsin et al. 2019). The advantage of using these herbaceous plants is associated with their often annual, biennial, or perennial nature, which indicates accumulation can be continual and fast growth of new species. According to WHO limit for Cd, Pb, and Zn many of the studied plants and their potential bioactive substances may lose their medicinal potency (Asare et al. 2022).

During phytoextraction, many plant species are considered according to their high biomass production (Zárubová et al. 2015). In this study, dicots recorded the highest contents of PTMs in all the treatments—for Cd (*C. Tenacetum vulgare*), *H. maculatum* for both *co* and *k*, and again *C. arvensis* for *ss* with *H. maculatum* accumulating > 100% in *co*. Again, dicots with BAF > 1 were greater than monocots in the accumulation of Cd. Furthermore, under different treatments, Pb (*S. palustris*, *L. perperium*, and *C. patula*) and Zn (*G. hederacea*, *C. patula*, and *C. arvensis*) were highest in all dicots. Since most of the studied plants (e.g., *S. palustris* and *C. arvensis*) have consumable parts and products (European Medicine Agency 2016), it is paramount to consider a low threshold, e.g., WHO, to avoid health-related issues.

Phytomanagement of PTM-contaminated soils should consider occupying an entire contaminated surface for all seasons and employ herbaceous plants with high accumulation potential. The impact of soil contamination on PTM uptake by plants requires field studies to select more effective species that can withstand varied environmental conditions.

## 5 Conclusions

The Short Rotation Coppice (SRC) of hybrid *Salix* and *Populus* clones resulted in the growth of multiple understorey herbaceous species. Meanwhile, the application of suitable organic ameliorants increases the number of herbaceous species and shoot accumulation of soil cadmium (Cd), lead (Pb), and zinc (Zn), especially compost treatment.

The study further indicated that herbaceous plant species (common weeds) accumulate potentially toxic metals (PTMs)—Cd, Pb, and Zn in their shoots from contaminated soils and can be considered a more cost-effective approach for the phytomanagement of PTM contaminated sites.

The accumulation of PTM by herbaceous plants is an indication of their availability to crops that make such fields not suitable for arable purposes. The conditions of the chemical properties of natural soils partly influence the accumulation of PTMs by herbaceous plants. Changes in the diversity of vegetation can serve as an indicator of the progress of clean-up process using phytoremediation with SRC.

Furthermore, dicots exhibited supremacy in the accumulation of Cd (*Tanacetum vulgare* L., *Hypericum maculatum* Crantz, and *Cirsium arvense* L.), Pb (*Stachys palustris* L., *Lamium Purpureum* L., and *Campanula patula* L.), and Zn (*Glechoma hederaceae* L., *C. patula*, and *C. arvense*) and best suited in the cleaning Cd/Zn/Pb-contaminated soils due to their biomass comparatively higher than monocots.

SRC plantation resulted in the growth of tolerant herbaceous plants suitable for phyto-management of Cd/Pb/Zn-contaminated sites, according to the level of contamination, while the condition of organic ameliorants can foster nutrient enrichment to soils and improves species richness.

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## Declarations

**Competing interests** The authors declare no competing interests.

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