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Phytoremediation of Potential Toxic Elements by Native Tree Species in Mined- Spoiled Soils in Mátraszentimre, Hungary

ABSTRACT

Potential toxic elements, such as Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), and Zinc (Zn) are commonly left behind after mining operation. Being non-biodegradable, these elements serve as source of contamination for the soil and water ecosystems and create hazards to human health. This research work evaluated the phyto extracting ability for potential toxic elements by four endemic tree species, such as Betula pendula (Birch), Carpinus betulus (Hornbean), Fagus sylvatica (Beech), and Salix caprea (Goat willow). These tree species are predominantly growing in an abandoned mining spoil sites in Mátra Mountains, Hungary. Plant and soil samples were collected in the field and analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-OES) method. The soil was highly contaminated with heavy metals, such as Pb, As, and Zn which were 10 to 60 times more than the typical non-contaminated Hungarian soil. Among the trees evaluated, Carpinus betulus showed the highest potential for Pb dendroremediation, with a mean concentration value of 4071.67±45.71 mg kg^{-1} dry weight in roots, 439.05±1.06 mg kg^{-1} dry weight in stems and 92.53±0.73 mg kg^{-1} dry weight in leaves. Betula pendula and Salix caprea bioaccumulated 475.575±2.219 and 395.97±1.43 mg kg^{-1} dry weight of Zn in their leaf biomass. Both trees had a Bio-concentration Factor (BCF) value of greater than 1.0 but less than 10 which classified them as potential phytoextractors of Zn. Salix caprea gave the highest Translocation Factor (Tf) for Cd while Betula pendula gave the highest Tf for Zn.

Keywords: *potential toxic elements, dendroremediation, bioconcentration factor (BCF), translocation factor (Tf)*

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INTRODUCTION

Ecological restoration after an enormous anthropogenic disturbance like mining operation is a difficult task. Mining creates negative impacts in micro-climatic environment and metal waste in the soil ecosystem laden with high amounts of potential toxic elements such as heavy metals (*Gonzales et al. 2008*). Restoration of the ecosystem through soil decontamination from potential toxic elements is the most recalcitrant problem (*Dobson et al. 2007*).

Phytotoxicity problem as a consequence of elevated concentration of heavy metals in soils have both direct and indirect hazards to human health. The indirect effects are through food chain or pollution of ground and surface waters (*Pulford et al. 2002*).

Phytoremediation is an emerging phytotechnology which uses plants in the removal, reduction, immobilization and degradation of heavy metals in the soil (*Lasat 1999*). It is economically, ecologically, socially acceptable and has aesthetic value as phytotechnology (*Stankovic and Devatakovic 2016*). Plant species that are commonly used in the bioremediation are called hyperaccumulators. This group of plants are capable of accumulating and tolerating considerable level of heavy metals. The heavy metals are absorbed from the soil, then translocated and accumulated in plant biomass.

Selection of the most suitable plant species for targeted potential toxic elements is the key for a successful phytoremediation work (*William 2008*). The plant should be able to sustain growth at high concentration of metals while being able to produce large amount of above ground biomass. Among the most studied plant species on phytoremediation are *Pteris vittata*, a fern species found as hyper accumulator of arsenic (*Ma et al. 2001*), *Brassica juncea* for Pb and Zn (*Gisbert et al. 2006*), *Pitygramma calomenalos* for As (*Visoottiviseth et al. 2002*), and *Rinorea niccolifera* for Ni which can accumulate more than 18,000 mg kg^{-1} dry weight of nickel into the leaf tissues (*Fernando et al. 2014*).

Most reported hyperaccumulators are slow growing producing limited biomass. On a per hectare basis and per year growth rate, the total accumulated potential toxic elements are less compared to non-hyperaccumulator tree species. Hyperaccumulator plant species can be trees, shrubs, grasses and ferns but trees are generally preferred as phytoremediator. Trees have many advantages as compared to small plant species like shrubs, grasses and ferns. These are fast growing and produce relatively large volume of harvestable biomass. The use of trees is also cheaper and a more environmentally acceptable technology (*Evangelou et al. 2013*). Although heavy metal uptake of trees is not as high as metallophytes, , the removal of metals by trees from the soil could be more effective due to greater biomass yield (*Greger and Lundberg 2006*). Trees also have long term ecological values because they can live for many years and can even grow in highly contaminated soils (*William 2008*).

The most commonly studied tree in Europe for phytoremediation are willows (*Salix viminalis*) and poplar trees (*Populus alba*), primarily because these trees are fast growing, produce large amount of biomass, and can survive in broad range of climatic and soil conditions (*Greger and Lundberg 2006*). In Sweden, willows are largely cultivated for the phytoextraction of Cd and Zn and bio-energy production (*Greger and Lundberg 2006*). A tree species, *Betula alnoides*, is highly recommended for the reforestation of mining sites with high levels of Pb and Zn like in China and New Zealand (*Wang et al. 2015*).

Trees vary in their ability to grow in highly contaminated soil. The most ideal tree species are those naturally growing in mine tailing areas as these have evolved sophisticated adaption mechanisms to tolerate potential toxic levels of metals in the soil (*Mendez and Maier 2008*). Local or endemic tree species are desirable since these are already adapted to local conditions and just need the ability to survive in harsh environment of potential toxic elements. Sources for mass propagation are also accessible and readily available. The search for a nobel tree species for phytoremediation of potential toxic elements is the need of time.

The mining dump site serves as source of potential toxic elements and poses danger to the downstream ecosystems like rivers, farms and resident communities. Phytoremediation activity is needed in the area to lessen the downward mobility of heavy metals and to protect the soil ecosystems and water sources.

The study was conducted to determine the phytoextracting ability of endemic tree species growing in heavy metal contaminated soils in an abandoned mining site in Mátraszentimre, Hungary.

MATERIALS AND METHODS

Study Area.

This research work was conducted in the abandoned mined spoil dumping site located in Mátraszentimre, Heves county, Hungary (**Figure 1**). The mining operation, a closed type of mining, ceased 52 years ago due to falling prices of metals in the world market.

The mean annual temperature is 5.9 °C and mean annual rainfall is 670-750 mm per year in which the highest rainfall was observed at the end of October and at the beginning of November (*Odor et al. 1998*). The natural vegetation surrounding the dump sites are mixed beech and evergreen forest with oak trees inter sparse with bushy and grassy spaces. Pine plantations can also be normally observed in plantations (*Odor et al. 1998*).

Soil Sampling and Analysis

Soil samples were collected in eight sampling points along the main dump sites (**Figure 1**) last June 17, 2019. Sampling points in forest adjacent to the main dump site served as control and considered to be not contaminated with heavy metals from mining operation. In each sampling point, surface soils were collected using soil auger from 0-25 cm depth. Soil samples were mixed thoroughly in each sampling point to make composite samples. Around 1 kg composite sample from each sampling point was taken and brought for laboratory analysis in the Hungarian University of Agriculture and Life Sciences, Institute of Environmental Sciences, Gödöllö, Hungary. The samples were air dried, ground and sieved in 2 mm pore opening sieve. Around 50 g soil sample was used for chemical analysis. Soil samples were digested with HNO_3 and H_2O_2, and concentrations of potential toxic elements in soils were analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-OES) method paralleled with Hungarian Standards (MSZ-21470-50-2006) *(Caic et al. 2019*). This is the standard protocol for environmental soil test for determination of total and soluble toxic elements, heavy metals, and chromium (VI).

Plant Sampling and Analysis

Samples of tree species were collected around the eight

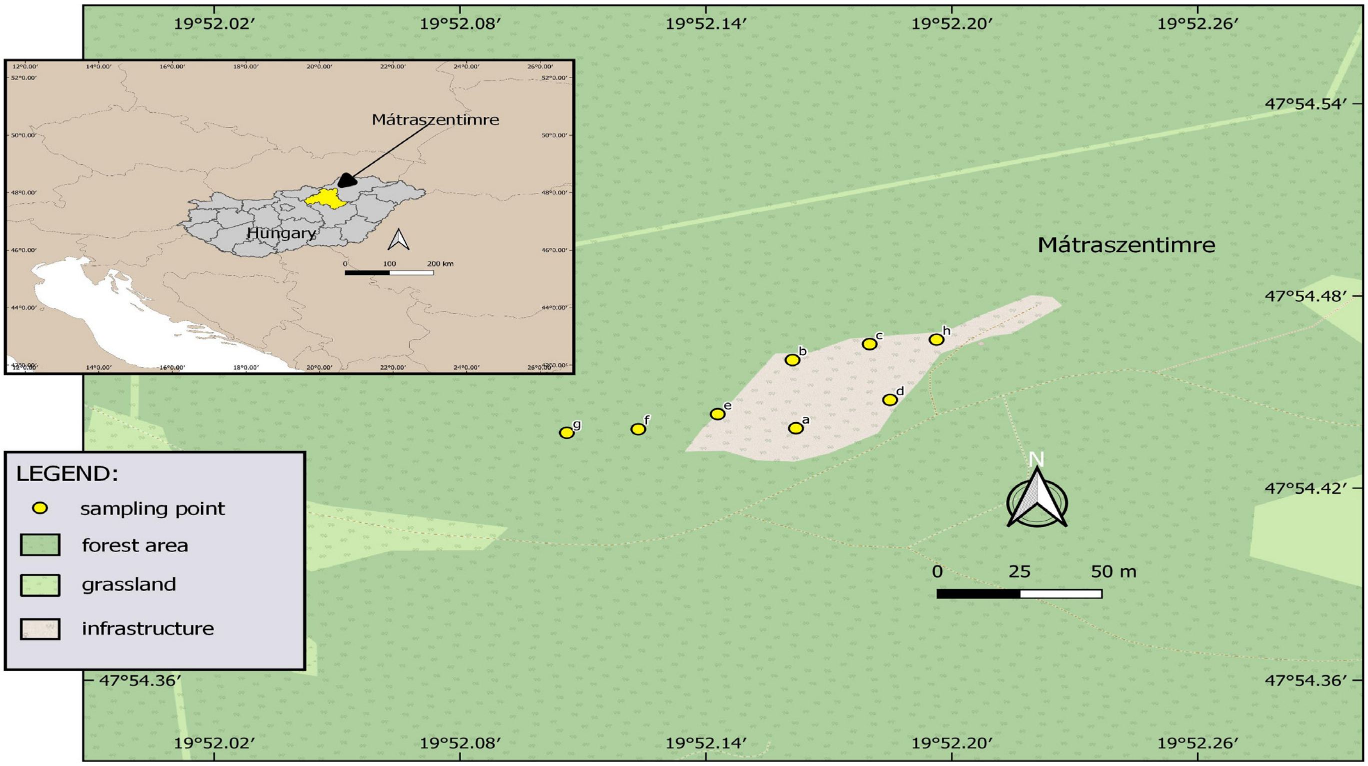

Figure 1. Sampling Area (Mátraszentimre, Mátra Mountains, Hungary), maps taken from Google Earth.

sampling points in the main dump site. Four endemic tree species identified in the area were assessed. The native tree species were *Betula pendula* (Birch), *Carpinus betulus* (Hornbean), *Fagus sylvatica* (Beech), and *Salix caprea* (Goat willow). The number of replications for each tree species was unequal because tree samples were not observed on all sampling sites. Unequal number of plant samples for each species was observed due to inherit heterogeneity of the mining site. *Betula pendula* (Birch) were observed in six sampling points, *Carpinus betulus* (Hornbean) in seven sampling points, *Fagus sylvatica* (Beech) in four samping points, and *Salix caprea* (Goat willow) in three sampling points. Seven to ten10) plant samples for each species were taken from each sampling point. Whole plant samples of trees with height 0.61 m and below growing within one meter diameter distance around the soil sampling point were collected. Plant samples were washed initially with tap water and later with distilled water to remove soil contaminants. The samples were air dried for two weeks and cut into 1 cm pieces. Root, stem and leaf samples for each tree species were prepared separately. Plant samples were ground and ash. Extraction to determine the levels of potential toxic elements were carefully followed using Hungarian Standard through microwave-assisted digestion with HNO_3 and H_2O_2. Concentrations of potential toxic elements were analyzed using ICP-OES based on Hungarian Standard (MSZ-21470-50-2006) (*Caic et al. 2019*). This is the standard protocol for environmental soil test for determination of total and soluble toxic elements, heavy metals, and chromium (VI).

Bio-concentration Factor (BCF)

The BCF (Bio-concentration Factor) value of all four tree species were calculated. BCF is a measure of ratio between the levels of heavy metals accumulated inside the whole plant or plant parts over the level of heavy metals in the contaminated soil. It is an indicator of the ability of the plant to accumulate potential toxic elements with respect to the level of potential toxic elements in the soil.

The BCF for each tree species was calculated using the following formula (*Zayed et al. 1998*):

BCF= Concentration of heavy metals in plant tissue/Concentration of heavy metals in soil (1)

It is a good indicator to easily determine if a given tree under observation can be classified as hyper accumulator, accumulators, and phyto excluder in a given contaminant in soil. Plants are classified based on its BCF: hyperaccumulators if BCF >10; accumulators if 1<BCF<10; and excluders if BCF<1 (*Zayed et al. 1998*). It will also measure the phyto-remediating capacity of plant species even at lower concentrations of potential toxic elements in soil environment.

Translocation Factor (TF)

Translocation factor is a measure of plant ability to translocate the heavy metals from the roots to upper harvestable parts of the plant (stems and leaves). Translocation factor is the ratio between concentration of heavy metals between the shoot (leaves + stems) and the root (*Zayed et al. 1998*). It measures the mobility of potential toxic elements from roots to harvestable organs.

TF= Concentration of heavy metals in stem + leaves/Concentration of heavy metals in roots (2)

Statistical Analysis

All statistical analysis were carried out by means using SAS. Treatment effects were determined by analysis of variance (ANOVA). Differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Concentrations of Heavy Metals in Soil Samples from the Mining Site

The mined spoil soil located in Matraszentimre, Northern Hungary had elevated concentrations of As, Cd, Cu, Pb and Zn (**Table 1**). The contamination of the mine spoiled soils can be attributed to mining activities conducted in the area 52 years ago. Concentration of Cd is 4.77±0.28 times higher than the normal soil observed in the forest and above the acceptable limit in Hungarian soil. The mean concentration levels of Cu in mined spoil soils is 32.81 ±0.28 mg kg^{-1} dry weight of soil which is 4.25 times higher than the forest soil but lower than the acceptable limit of Hungarian soil.

The concentration levels of Pb had a mean value of 1929.71±6.4 mg kg^{-1} dry weight of soil, more than 92 times of Pb in the forest soil and 19 times higher than the acceptable level. The concentration levels of Zn at the site were nearly 2 times of Zn in a typical limit of Hungarian soil. It had a mean value of 421.26 ±6.4mg kg^{-1} dry weight of soil. Likewise, As concentration levels at the site were very high. The As mean concentration value of 885.57±9.56 mg kg^{-1} dry weight of soil was 39 times of As in normal forest soil in the research area.

Concentrations of Heavy Metals in Plant Samples in the Mining Site

Arsenic. Total As in different tree species were not significantly different (**Table 2**). The levels of As varied significantly among tree species in roots and stem but not in leaves. The As in roots of *Carpinus betula*, *Betula pendula*, and *Salix caprea*, with mean values of 17.247±2.294 mg kg^{-1}, 16.274±0.831 mg kg^{-1}, and 14.755±0.953 mg kg^{-1}, respectively, were significantly higher than *Fagus sylvatica* (3.187±0.560 mg kg^{-1}). Stem values were noted to have the same significant differences but at a much lower value than the roots. *Carpinus betulus* had the highest mean values for both root and stem. The As levels in the leaves of the four tree species were statistically the same but the highest numeric value was obtained in *Betula pendula* (1.464±1.67 mg kg^{-1}). The levels of As were below the detected value compared to other plant species considered phyto accumulator of As.

Cadmium. Significant levels of Cd were observed in stems, leaves and total biomass of *Salix caprea* with a mean concentration value of 4.702±1.579 mg kg^{-1} and 4.302±0.017 mg^{-1} kg dry weight in leaves and stems, respectively (**Table 3**). This plant species yielded highest Cd level in roots (1.577±0.024 mg kg^{-1}) with no significant difference to other three species evaluated. Cadmium was not detected in stems and leaves of *Fagus sylvatica* and it had the lowest Cd level in the roots.

Copper. No significant differences were observed on the concentration levels of Cu among the four tree species (**Table 4**). *Carpinus betulus* contained highest level of

Table 1. Concentrations of potential toxic (mg kg^{-1}) elements in mined –spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Potential Toxic Elements	Forest Soil	Mine Spoiled Soil	p-value	Acceptable Limit in Hungarian Soil[a8]
Cadmium (Cd)	0.01±0.02	4.77 ±0.28	0.038*	1.0
Copper (Cu)	7.71±0.04	32.81 ±0.07	0.025*	75.0
Lead (Pb)	20.9±1.5	1929.71±6.4	0.023*	100
Zinc (Zn)	74.6±0.5	421.26±6.4	0.022*	200
Arsenic (As)	22.48±0.88	885.57±9.56	0.023*	15

Mann-Whitney U test was used due to non-homogeneity of variance
*significant
** Hungarian Joint Decree No 10/2000 (*Gazdag and Sipter 2008*)

Cu in the roots and leaves biomass, having concentration mean values of 11.490±0.240 and 7.226±0.24 mg kg^{-1} dry wt., respectively. The highest Cu level in the stem (mg kg^{-1} dry weight) and the lowest Cu level in the leaves (mg kg^{-1} dry wt) were obtained in B*etula pendula*. *Fagus sylvatica* had the lowest concentration value of Cu in the root and stem. It contained a mean value of 2.578±0.018 mg kg^{-1} dry weight in roots, 2.73±0.098 mg kg^{-1} dry weight in stems and 6.801±0.057 mg kg^{-1} dry wt. in leaves.

Lead. The concentration levels of Pb on trees were significantly different among tree species (**Table 5**). It was also significantly different in roots, stems and leaves. The tree species that contains highest level of Pb in the biomass was *Carpinus betulus*. It has a concentration mean value of 4071.67±45.713 mg kg^{-1} dry weight in roots, 439.05±1.061 mg kg^{-1} dry weight in stems and 92.532±0.730 mg kg^{-1} dry wt. in leaves. It was followed by Pb concentrations observed in *Betula pendula* with a mean value of 1227.120±4.917 mg kg^{-1} dry wt in roots, 260.630±1.857 mg kg^{-1} dry wt in stems, and 8.276±0.267 mg kg^{-1} dry wt in leaves of Pb. The mean concentration levels of Pb in *Salix caprea* were 216.14±0.966 mg kg^{-1} dry wt in roots, 46.166±0.720 mg kg^{-1} dry wt in stems, and 9.006±0.054 mg kg^{-1} dry wt in leaves. Lowest concentration levels of Pb were observed in *Fagus sylvatica* with a mean value of 75.834±1.101 mg kg^{-1} dry wt. in roots, 2.805± 0.024 mg kg^{-1} dry wt. in stems, and 2.5000±0.133 mg kg^{-1} dry wt. in leaves.

Table 2. Concentration levels (mg kg^{-1}) of As in roots, stem and leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Tree Species	Roots	Stems	Leaves	Total
Betula pendula	16.274±0.831 a	0.283±0.141 b	1.464±1.67 a	18.59±5.97 a
Carpinus betulus	17.247±2.294 a	5.833±2.025 a	0.627±0.24 a	18.00±9.71 a
Fagus sylvatica	3.187±0.560 b	0.633±0.612 b	0.843±0.018 a	5.32±4.89 a
Salix caprea	14.755±0.953 a	0.749±0.109 b	0.285±0.070 a	15.52±9.72 a
p-value	0.044*	<0.001***	0.760ns	0.220ns

Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

Table 3. Concentration levels (mg kg^{-1}) of Cd in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Tree Species	Roots	Stems	Leaves	Total
Betula pendula	0.591±0.06 a	0.360±0.09 b	0.475±0.76 b	1.420±1.34 b
Carpinus betulus	0.942±0.06 a	0.613±0.01 b	0.050±0.015 b	5.80 ±6.98 ab
Fagus sylvatica	0.158±0.011 a	bdl	bdl	0.17±0.25b
Salix caprea	1.577±0.024 a	4.302±0.017 a	4.702±1.579 a	10.58±3.14 a
p-value	0.120ns	<0.010**	<0.001***	0.040*

Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

Table 4. Concentration levels (mg kg^{-1}) of Cu in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Tree Species	Roots	Stems	Leaves	Total
Betula pendula	6.209±0.176 a	8.684±0.061 a	4.145±0.040 a	16.12±3.33 a
Carpinus betulus	11.490±0.240 a	3.658±0.143 a	7.226±0.24 a	16.21±9.17 a
Fagus sylvatica	2.578±0.018.a	2.737±0.098 a	6.801±0.057 a	11.19±6.27 a
Salix caprea	9.861±0.070 a	4.398±0.018 a	5.708±0.229 a	19.97±4.57 a
p-value	0.272ns	0.310ns	0.718ns	0.44ns

Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

Zinc. The tree species that contained the highest level of Zn in the total biomass of *Betula pendula* (Birch tree) (**Table 6**). It was followed by level of Zn in *Salix caprea*. The mean observed value of Zn were 243.975±1.504 mg kg^{-1} dry wt. in roots, 583.180±1.504 mg kg^{-1} dry wt. in stems, 475.575±2.219 mg kg^{-1} dry wt. in leaves. It was followed by Zn concentration levels found in *Carpinus betulus* with an observed mean value of 335.320±4.439 mg kg^{-1} dry wt. in roots, 351.660±2.216 mg kg^{-1} dry wt. in stems, and 111.200±0.561 mg kg^{-1} dry wt. in leaves of Zn. High Zn values were also observed on the leaves of *Salix caprea* with concentration levels of 395. 970±1.43 mg kg^{-1} Zn in leaves, 285. 825±1.217 mg kg^{-1} dry wt. in stems, and 216.055±0.292 mg kg^{-1} dry wt. in roots. Lowest value was observed in *Fagus sylvatica* with mean levels of 133.947±1.362 mg kg^{-1} dry wt. in roots, 171. 427±1.332 mg kg^{-1} dry wt. in stems, and 71.285±0.709 mg kg^{-1} dry wt. in leaves.

Bioconcentration Factor (BCF)

Bioconcentration factor measures the potential toxic elements accumulated by each tree species based on equation 1, the concentration of potential toxic elements present in plant tissues over the concentration values potential toxic elements in the contaminated soil where it is growing. BCF value >1.0 means the plant can bio-accumulate potential toxic elements higher than the concentration levels of heavy metals present in the soil. BCF value <1.0 means the plant species has lower concentration of potential toxic elements in their plant tissues compared to the concentration levels of potential toxic elements present in the mined spoil soils.

The root, stem and leaves of all four tree species had less than 1.0 BCF for As, Cu and Pb but not for Cd and Zn. For Cd, the roots, stems and leaves of S*alix caprea* had BCF values of 1.577±0.346, 4.299±2.516 and 4.387±1.134, respectively while the roots of *Carpinus betulus* had BCF value of 1.131±1.009. For Zn, the root BCF of all tree species ranged from 1.111±0.056 to 1.368±1.259 while the stem of all except *Fagus sylvatica* were also above 1.0, ranging from 1.447±0.244 to 4.396±5.459. The Zn levels in the leaves of two tree species (*Betula pendula* and *Salix caprea*) were likewise above 1.0, at 3.030±1.284 and 2.071±0.205, respectively. Based on the analyses of significant differences in BCF values among tree species for each potential toxic element, as expected, the BCF of roots, stems and leaves for As, Cu and Pb were not significantly different. For Cd, however, significant differences among BCF of tree species were not for stem but not for stem and leaves. The stems and leaves BCF of *Salix caprea* were significantly higher than those of the other tree species. Although all

Table 5. Concentration levels (mg kg^{-1}) of Pb in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Tree Species	Roots	Stems	Leaves	Total
Betula pendula	1227.120±4.917 b	260.630±1.857 b	8.276±0.267 b	1496±185 a
Carpinus betulus	4071.67±45.713 a	439.05±1.061 a	92.532±0.730 a	4603±357 a
Fagus sylvatica	75.834±1.101 d	2.805± 0.024 d	2.5000±0.133 d	78.63±5.6 c
Salix caprea	216.14±0.966 c	46.166±0.720 c	9.006±0.054 c	271.31±33.85 b
p-value	0.004**	0.014*	0.014*	0.03*

Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

Table 6. Concentration levels (mg kg^{-1}) of Zn in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Tree Species	Roots	Stems	Leaves	Total
Betula pendula	243.975±1.504 a	583.180±1.504 a	475.575±2.219 a	1283.91±185.13 a
Carpinus betulus	335.320±4.439 a	351.660±2.216 a	111.200±0.561 b	645.16±290.44 bc
Fagus sylvatica	133.947±1.362 a	171. 427±1.332 a	71.285±0.709 b	357.09±163.46 c
Salix caprea	216.055±0.292 a	285. 825±1.217 a	395. 970±1.43 a	897.85±156.21 ab
p-value	0.634ns	0.128ns	<.001***	0.003**

Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

of the BCFs for Zn in the roots and stems of the four tree species were all above 1.0, none was found significantly different. The BCF of Zn in the leaves of *Betula pendula* and *Salix caprea* were significantly higher than those in *Carpinus betulus* and *Fagus sylvatica*.

Translocation Factor (TF)

TF values of less than 1.0 for As and Pb were noted in all four tree species. However, Cu and Zn had TF values greater than 1.0, in all four trees, with high values ranging from 1.593±0.910 to 4.682±1.402. For Cd, TF value of more than 1.0 was obtained only in *Salix caprea*. Based on the analyses of TF values among trees, significant translocation factor was observed on concentration levels of Cd in *Salix caprea* with an observed value of 3.174±0.229. Significant TF values for Zn was also observed on the harvestable tissues of *Betula pendula* and *Salix caprea* with a value of 4.682±1.402 and 3.171±0.005, respectively. Translocation factor for As, Cu, and Pb were found to be not significant.

Heavy Metals in the Soil

The mined spoil soils under study had elevated concentrations of As, Cd, Cu, Pb and Zn. The As mean concentration level of 885.57 mg kg^{-1} dry weight was higher than the 15 mg kg^{-1} As per dry weight of soil considered toxic to plants and animals. The mean concentration of Cd of 4.77 mg kg^{-1} dry weight of soil was generally toxic. The accepted level of Cd in Hungary is 1.0 mg kg^{-1} Cd per unit dry wt. of soil. The concentration levels of Pb were also above the normal soils in Hungary. The mean concentration level of Pb was 1929.71 mg kg^{-1} dry weight of soil. These values, so with As and Cd concentration levels, were much higher than the acceptable

Table 7. Bioconcentration Factor of different tree species for the uptake of As, Cd, Cu. Pb, and Zn.

Potential Toxic Elements	Betula pendula	Tree Carpinus betulus	Species Fagus sylvatica	Salix caprea	p-value	Remarks
As in Roots	0.067±0.051 a	0.049±0.029 a	0.066±0.105 a	0.055±0.047 a	0.985	ns
As in Stem	0.017±0.006 a	0.019±0.002 a	0.014±0.010 a	0.013±0.010 a	0.624	ns
As in leaves	0.066±0.092 a	0.033±0.022 a	0.017±0.006 a	0.015±0.007 a	0.780	ns
Cd in roots	0.183±0.173 a	1.131±1.009 a	0.158±0.242 a	1.577±0.346 a	0.067	ns
Cd in Stem	0.359±0.590 b	0.332±0.140 b	bdl	4.299±2.516 a	0.008	**
Cd in Leaves	0.475±0.792 b	0.050±0.055 b	bdl	4.387±1.134 a	< 0.001	***
Cu in Roots	0.169±0.034 a	0.457±0.142 a	0.077±0.035 a	0.420±0.352 a	0.058	ns
Cu in Stem	0.254±0.370 a	0.273±0.158 a	0.203±0.219 a	0.188±0.158 a	0.975	ns
Cu in leaves	0.301±0.328 a	0.370±0.376 a	0.117±0.025 a	0.222±0.120 a	0.707	ns
Pb in roots	0.425±0.285 a	0.698±0.595 a	0.393±0.431 a	0.173±0.161 a	0.612	ns
Pb in Stem	0.216±0.227 a	0.148±0.035 a	0.003±0.001 a	0.067±0.005 a	0.257	ns
Pb in Leaves	0.202±0.335 a	0.069±0.059 a	0.037±0.063 a	0.013±0.006 a	0.651	ns
Zn in roots	1.368±1.259 a	1.208±0.510 a	1.172±0.558 a	1.111±0.056 a	0.983	ns
Zn in Stem	4.396±5.459 a	2.056±1.775 a	0.835±0.112 a	1.447±0.244 a	0.556	ns
Zn in Leaves	3.030±1.284 a	0.568±0.574 b	0.648±0.274 b	2.071±0.205 a	0.016	ns

bdl- below detection limits
* Mean ± Standard Division (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

Table 8. Translocation Factor of different potential toxic elements in shoots and leaves of different tree species.

Potential Toxic Elements	Betula pendula	Tree Carpinus betulus	Species Fagus sylvatica	Salix caprea	p-value	Remarks
As	0.153±0.136 a	0.308±0.370 a	0.705±0.751 a	0.070±0.002 a	0.560	ns
Cd	0.838±0.932 b	0.563±0.145 b	bdl	3.174±0.229 a	0.002	**
Cu	2.430±3.00 a	1.593±0.910 a	2.856±1.282 a	1.865±0.555 a	0.839	ns
Pb	0.839±0.932 a	0.576±0.124 a	0.210±0.098 a	0.273±0.1267a	0. 476	ns
Zn	4.682±1.402 a	2.091±1.019 b	1.5690±0.968 b	3.171 ±0.005 a	0.037	*

* Mean ± Standard Division (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

concentration levels based on Hungarian Joint Decree No 10/2000 with a toxicity limit of 15 mg kg^{-1} for As, 1.0 mg kg^{-1} Cd, 100 mg kg^{-1} Pb, and 200 mg kg^{-1} Zn (*Gazdag and Sipter 2008*). The mean concentration level of Zn of 421.89 mg kg^{-1} dry weight of soil was also above the 200 mg kg^{-1} dry weight of soil, which is the acceptable level in soils in Hungary.

The concentration levels of Cu had a mean value of 32.28 mg kg-1 dry weight of soil. This was a little higher than the normal plant requirements of 20-30 mg kg^{-1} (*Marschner 1995*). Unlike the levels of As, Cd, Pb and Zn, the levels of Cu were within the Hungarian acceptable standard with a limit concentration value of not more than 75 mg kg^{-1}.

The results of this research work were mostly above the reported contaminated soils in Hungary such as the road side soils (*Simon 2001*), soils in Gyongyosoroszi (*Simon 2005*) and the sediments in Toka Creek (*Odor et al. 2008*) suggested their high potential toxicity in plants.

Compared to contaminated soils of other countries, soil heaps in smelter plant in Belgium contains very high Zn (*Kopponen et al. 2001*). Similarly, it is suggested that the levels acceptable and not toxic for soils is 1.0 mg kg^{-1} dry wt for Cd, 60-125 mg kg^{-1} dry wt for Cu and 70 to 400 mg kg^{-1}mg kg^{-1} dry wt for Zn. Concentration of Zn between 100 and 900 mg could have phytotoxic effects on plants (*Kabata-Pendias and Pendias 2001*). Based on these criteria, the concentration levels of Cd, Cu and Zn under study were still within acceptable concentration levels.

Concentration of Potential Toxic Elements in Trees

Screening and evaluation of trees growing in soils polluted with potential toxic elements is very important in selecting the most suitable species for any successful revegetation program (*Gonzales et al. 2008*). Plants growing in metalliferous soils cannot prevent metal uptake. The strategy of survival is base on tolerance rather than avoidance of metal toxicity. Among the elements observed, Cu and Zn were considered as essential elements. Cd and Pb are highly reactive elements. The level of potential toxic elements in roots, leaves, and stems observed in this research work is crucial in the choice of tree species for phytostabilization establishment (*King et al. 2008*).

Arsenic (As). All of the trees evaluated in this study bioaccumulated low levels of As. The levels of As was higher in roots compared to stems and leaves. Levels of As ranged from 3.187±0.560 mg kg^{-1} dry weight in the roots of *Fagus sylvatica* to 17.247±2.294 mg kg^{-1} dry wt. in the roots of *Carpinus betulus*. The BCF ratio for all trees was <1.0 indicating that these trees are heavy metal excluders. Trees are not known as hyperaccumulator of As compared to ferns, grasses and sunflower. Trees tend to absorb little amount of As compared to its biomass. In a research report, willow can absorb only around 1.92 to 2.11 mg kg^{-1} As (*Simon et al. 2012*). The result of the study is considered below compared to other plant species which absorb more than 1000 mg kg^{-1} dry weight of As. The reported most popular hyperaccumulator of As is *Pteris vittata*, a fern species which can bioaccumulate above 10,000 mg kg^{-1} dry wt (*Ma et al. 2001*).

Cadmium (Cd). Cd is a non-essential element and considered to be one of the most hazardous elements or heavy metals (*William 2008*). Cd is highly mobile in the biological systems and present potential risks to human health (*Adriano 2001*). Cd generally inhibit tree growth resulting in low biomass production (*Robinson et al. 2000*). Among the tree species evaluated, *Salix caprea* showed the highest potential for phytoremediation of Cd. *Salix caprea* had very high concentration levels of 4.702±1.579 mg kg^{-1} dry wt. in leaves and 4.302±0.017 mg kg^{-1} dry wt. in stems, resulting to significant TF and BCF.

The TF indicated that the Cd in harvestable parts of *Salix caprea* is more than three times higher than concentration value of Cd in roots. The bioconcentration factor for Cd were also highest in *Salix caprea* with a mean concentration value of 1.577±0.346 mg kg^{-1} in roots, 4.299±2.516 mg kg^{-1} in stems and 4.387±1.134 mg kg^{-1} in leaves. This showed that the phytoextraction of Cd using *Salix caprea* could restore soils in Cd contaminated areas in Hungary.

The other tree species that absorbed high levels of Cd was *Betula pendula* with a concentration value 1.39 mg kg^{-1} dry wt. of Cd in their leaf biomass but it had low BCF and Tf values. This level of Cd which is above 1.0 mg kg^{-1} Cd is considered toxic (*Kabata-Pendias and Pendias 2001*) and above the acceptable Hungarian standard of 1.0 mg kg^{-1} dry wt. Other tree species evaluated showed <1.0 mg kg^{-1} dry wt. of Cd in their biomass.

In a similar research work conducted in Sudety, Mountains in Poland, higher results were observed also in *Salix caprea* which accumulated 54.1 mg kg^{-1} Cd, while *Betula pendula* accumulated 11.8 mg kg^{-1} Cd in leaves (*Wislocka et al. 2006*). *Wieshammer* (*2007*) showed that *Salix caprea* can absorb 116 mg kg^{-1} dry wt. of Cd in a pot experiment. Other research work showed that *Salix* contain more Cd leaves than in stems (*Hammer* *et al. 2003*). *Fagus sylvatica* was reported to have high amount of Cd (*Zupunski et al. 2015*). However, it gave a lower result value for Cd uptake in this study. This could be attributed to a lower Cd concentration value in soil in the study area relative to their study area.

Some other trees were reported good in absorbing Cd. A study in France reported that poplar can bioaccumulate up to 200 mg kg^{-1} Cd while in New Zealand, willows showed phytoextracting potential in cleaning Cd contaminated soils (*Robinson 2000*). *Salix viminalis* (Willow) can uptake 5.7 mg kg^{-1} dry wt. of Cd in leaves (*Greger and Lundberg 2006*). Two studies, (*Pietrini et al. 2015 and Zupunski et al. 2015*), however, indicated that willows were more tolerant to Cd than poplar.

Copper (Cu). All tree species bioaccumulated Cu within range of normal requirements in plants. Levels of Cu ranges from 2.578±0.018 mg kg^{-1} dry weight in the roots of *Fagus sylvatica* to 11.490±0.240 mg kg^{-1} dry wt. observed in roots of *Carpinus betulus* These levels of Cu are considered non-toxic. The normal concentration levels of Cu in plants ranges from 10-30 mg kg^{-1} (*Marschner 1995*). The BCF and TF values for Cu were also low. BCF values for Cu for all leaves of trees were less than 1.0. These tree species, therefore, have low potential to be candidate for phytoextraction of Cu. The concentration levels of Cu in soil is low, which influence the low absorption of Cu among trees. The translocation factor among trees is above 1.0 and the highest was observed in *Betula pendula* with a value of 2.430±3.00. This showed low mobility of Cu from roots to shoots of birch tree. The low mobility of Cu in plants is due to xylem binding of Cu (*Nissen and Lepp 1997*). Cu is preferentially bounded in xylem with less mobility compared to Zn (*Nissen and Lepp 1997*). Cu mobility is restricted and large proportion of absorbed Cu is retained in roots (*Kopponen et al. 2001*).

On the contrary, some trees were reported good in absorbing Cu. *Eucalyptus camaldulensis* can uptake 297.8 mg kg^{-1} of Cu (*Assareh et al. 2008*) while *Phragmites australis* known as aquatic reed can uptake 849-1,154 mg kg^{-1} dry weight of Cu (*Ait-Ali et al. 2004*).

Lead (Pb). Another potential toxic element observed at high level of concentrations among trees in the research work was Pb. High levels of Pb were observed in roots of *Carpinus betulus* and *Betula pendula* with a mean value of 4071.67±45.713 mg kg^{-1} and 1227.120±4.917 mg kg^{-1} dry wt Pb, respectively. These values of concentration levels were both above 1000 mg kg^{-1} dry wt. Pb which could classify these tree species as hyperaccumulator of Pb (*Baker and Brooks 1989*).

The levels of Pb in stems and leaves were also high in *Carpinus betulus* with a mean concentration value of 439.05±1.061 mg kg^{-1} dry wt Pb in stems and 92.532±0.730 mg kg^{-1} dry wt Pb in leaves but much lower than in roots. Both *Carpinus betulus* and *Betula pendula* showed that Pb can be easily accumulated in roots compared to stem and leaves. Other tree species have been reported with similar response and were considered as good Pb accumulators. The Pb levels of *Betula pendula* were 135 mg kg^{-1} dry wt Pb in stems and 78 mg kg^{-1} dry wt Pb in leaves (*Evangelou et al. 2013*). *Paulownia fortunni* accumulated Pb up to 1179 mg kg-1 while *Bronssonetia papyrifera* (L.) accumulated 973.3 mg kg^{-1} Pb in the leaves (*Zhao et al. 2013*). E*ucalyptus camaldulensis* was suitable for phytoextraction of Pb (*Coupe et al. 2013*). Poplar is also reported to have high tolerance to Pb (*Evangelou et al. 2013*).

Although high Pb values were obtained in the plant analyses, BCF value for all trees for Pb were also below 1.0. This means that the high concentration value of Pb in trees is due to the concentration value of Pb in the soil samples. Translocation factor for Pb for all trees were also below 1.0. This showed that Pb has low mobility from roots to shoots. Most of the Pb absorbed were retained in the roots of *Carpinus betulus* and *Betula pendula*, which are advantageous in using these trees for phytostabilization of Pb contaminated soils. Low BCF value could not categorize these trees as phytoextractor of Pb but essential for phytostabilization purposes.

Zinc (Zn). The normal range for Zn requirement in trees is around 15-20 mg kg^{-1} dry wt. (*Broadley et al. 2006*). *Betula pendula* and *Salix caprea* can bioaccumulate high levels of Zn in their biomass. Betula pendula absorb 583.180±1.504 mg kg-1 Zn in stems and 475.575±2.219 Zn mg kg^{-1} in leaves while Salix caprea had 285.825±1.217 mg kg^{-1} Zn in stems and 395. 970±1.43 mg kg-1 Zn in leaves.

Highest BCF value were also observed in *Betula pendula* with a mean value of 4.396±5.459 in stems and 3.030±1.284 in leaves followed by leaves of Salix caprea at 2.071±0.205. Translocation factor for Zn were also high in *Betula pendula* and *Salix caprea*. These values indicate that the two tree species could be phytoextractor of Zn. High Tf value is desirable. However, if the plantsare food for animals, high translocation factor of metals can pose risks. It could lead to higher transfer of Zn to other animals in the ecosystem at toxic levels (*Pulford et al. 2002*).

Betula pendula was reported to contain higher Cd and Zn concentrations in leaves or stems (*Zupunski et al. 2015*). It is not known as hyperaccumulator of Zn since it absorbed less than 10,000 mg kg^{-1} dry wt. of Zn (*Baker and Brooks 1989*), but it is a Zn metal tolerant tree (*Kopponen et al. 2001*).

The association of birch with mycorrhiza provided larger surface area that promotes greater absorption of Zn in *Betula pendula* (*Roselli et al. 2003*). Inoculation with arbuscular mycorrhizal fungi in trees increased the concentration of heavy metals in roots but decreased in the shoot (*Guo et al. 2013*).

Salix caprea has been reported to absorb high amount of Zn. A study showed that *Salix caprea* can absorb 4,680 mg kg^{-1} dry wt. of Zn in a pot experiment (*Weishammer et al. 2007*) while another study indicated 2020 mg kg^{-1} dry wt. of Zn in leaves (*Fernandez et al. 2017*).

Some trees were reported good for Zn removal. Willows can remove 5-27 kg ha^{-1} yr^{-1} of Zn (*Meers et al. 2005*) while *Eucalyptus camaldulensis* can uptake 253 mg kg-1 dry wt. of Zn (*Assareh et al. 2008*) and *Salix viminalis* can bioaccumulate 200 mg kg^{-1} of Zn in their leaves (*Hermle et al. 2006*).

CONCLUSION AND RECOMMENDATIONS

The tree species that demonstrated highest potential for hyperaccumulation of Pb is *Carpinus betulus* since the absorbed value was more than 1,000 mg kg^{-1} dry wt. of Pb in roots. It is therefore, a good candidate for the phytostabilization of Pb contaminated sites. It gave a TF value less than 1.0. showing less mobility of Pb in the harvestable parts. *Salix caprea* is a potential tree for phytoextraction of Cd and Zn while *Betula pendula* has shown potential as phytoextractor of Zn. Further research work is still needed in the physiological and chemical mechanism of uptake of these potential toxic elements. Pot experiments are needed to determine the growth rates of each tree species and their capacity to absorb other toxic elements per unit time for the future restoration of heavy metal contaminated soils.

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