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PHYTOREMEDIATION OF POTENTIAL TOXIC ELEMENTS BY NATIVE TREE SPECIES IN MINED- SPOILED SOILS IN MÁTRASZENTIMRE, HUNGARY

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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 (4) endemic tree species that are predominantly growing in an abandoned mining spoil sites in Mátra mountains in Hungary. Plant and soil samples were collected in the field and analyzed using ICP-OES. Results showed that the soil was highly contaminated with heavy metals, largely Pb, As, and Zn which were 10x to 60x more than the typical non-contaminated Hungarian soil. Among the trees evaluated, Carpinus betulus showed the highest potential for Pb dendroremediation, having a mean concentration value of 4071.67 mg kg^{-1}dry weight in roots, 439.06 mg kg^{-1}dry weight in stems and 92.53 mg kg^{-1} dry weight in leaves. Betula pendula and Salix caprea bioaccumulated 475.8 and 395. 97 mg kg^{-1} dry weight of Zn in their leaf biomass. Both trees had a Bioconcentration Factor (BCF) value of >1.0 but < 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)

INTRODUCTION

Ecological restoration after an enormous anthropogenic disturbance like mining operation is a difficult task. Aside from negative impacts it created in micro-climatic environment, the metal extraction activity laid waste 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 through food chain or pollution of ground and surface waters (Pulford et al., 2002). Physical and chemical methods of soil metal decontamination of polluted soil ecosystems destroy soil structure and render the soil biologically dead (Mc Grath, 2001). Engineering methods like excavation, transport and dumping of contaminated soils were too expensive (Salt et al., 1995). In US, the estimated cost was 1 million dollars per acre (Raskin et al., 1997) or a total cost of 400 B US dollars to 1.7 trillion US dollars (Stomp et al., 1994).

Phytoremediation an emerging phytotechnology which is the use of 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 (Stomp et al., 1994). Plant species that are commonly used in the bioremediation are called hyperaccumulator (Baker, 1981). 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 (Baker and Brooks, 1989).

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 and at the same time able to produce large amount of above ground biomass. Among the most cited research work on phytoremediation were on Thlaspi caerulescens, a known hyperaccumulator Cd and Zn (Baker and Brooks, 1989), Pteris vittata, a fern species found hyper accumulator of arsenic (Ma et al., 2001), Brassica juncea for Pb and Zn (Marchiol et al., 2004, Gisbert et al., 2006), and Pitygramma calomenalos for As phytoremediation (Visoottiviseth et al., 2002).

Most reported hyperaccumulators are slow growing and produce limited biomass. On a per hectare basis and per year growth rate, the total accumulated potential toxic elements is 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 cheaper and more environmentally acceptable technology (Evangelou et al., 2013). Although heavy metal uptake of trees is not as high as metallophytes but due to its greater biomass yield, the removal of metals from the soil could be more effective with respect to hyper accumulating plants (Greger and Lundberg, 2006). Moreover, trees have long term ecological values. Trees can live for many years even growing in highly contaminated soils (William, 2008).

The most commonly studied tree in Europe for phytoremediation were willows (Salix viminalis) and poplar trees (Populus alba), primarily because these trees are fast growing, produced 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 at apparently for bio-energy production (Greger and Lundberg, 2006). A tree species, Betula alnoides is highly recommended species for the reforestation of mining sites with high levels of Pb and Zn in several countries (Wang et al., 2015).

Trees vary in their ability to grow in highly contaminated soil. The most ideal tree species are those species naturally growing in mine tailing areas as they 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. Moreover, sources for mass propagation are accessible and readily available. The search for a nobel tree species for phytoremediation of potential toxic elements is the need of time. This field research activity was conducted to determine the phytoextracting ability of tree species growing in heavy metal contaminated soils in an abandoned mining site in Mátraszentimre, Hungary.

MATERIAL AND METHODS

The Research Area. This research work was conducted in the abandoned mined spoil dumping site located in Mátraszentimre, Heves county, Hungary (Figure 1). The mean annual temperature is 5.9°C, mean annual rainfall is 670-750 mm per year, the highest rainfall is observed at the end of fall and at the beginning of winter (Odor et al, 1998). The natural vegetation surrounding the dump sites are mixed beech and evergreen forest with oak trees interspersed with bushy and grassy spaces. Pine plantations can also be normally observed in plantations (Odor et al, 1998).

The mining operation, a closed type of mining, ceased in decades ago due to falling prices of metals in the world market. The mining dump site serve 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.

Soil Sampling and Analysis

Soil samples were collected in eight (8) sampling points along the main dump sites (Figure 1). One sampling point (E), being the farthest from the main dump site served as control and considered to be not contaminated with heavy metals from mining operation.

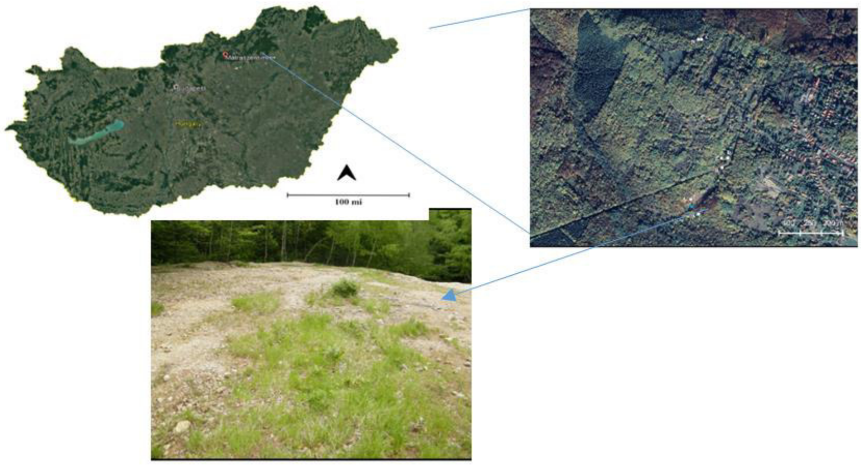

Figure 1. Sampling Area (Mátraszentimre, Mátra Mountains, Hungary), maps taken from Google Earth.

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 1kg composite sample from each sampling point was taken and brought for laboratory analysis in Hungarian University of Agriculture and Life Sciences, Institute of Environmental Sciences, Gödöllő, Hungary. Before analysis, samples were air dried, grounded and sieved in using 2mm 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 ICP-OES based on Hungarian standards (MSZ-21470-50-2006).

Plant Sampling and Analysis

Samples of tree species were collected around the 8 sampling points in the main dump site. Four (4) endemic tree species identified in the area were assessed in this study. The native tree species were Betula pendula (Birch), Carpinus betulus (Hornbean), Fagus sylvatica (Beech), and Salix caprea (Goat willow).

Whole plant samples of trees with height two feet and below were collected in this work. Plant samples were washed initially with tap water and later with distilled water to remove soil contaminants. The samples were air dried for 2 weeks and cut into 1cm pieces. Root, stem and leaf samples for each tree species were prepared separately. 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).

Bioconcentration Factor (BCF)

The BCF value of all 4 trees species studied in this research was calculated. Bioconcentration factor 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 / Concentration of heavy metals in soil

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. Plant with BCF greater than 10, as hyper accumulators, between 1 and 10 as accumulators, and with a value of less than 1 as excluders (Zayed et al., 1998). It will also measure the phytoremediating capacity of plant species even at lower concentrations of potential toxic elements in soil environment.

Translocation Factor (Tf)
Another important parameter, to determine the phytoextracting ability of trees is 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.

RESULTS

Concentrations of potential toxic elements of mined spoil soils located in Matraszentimre, Northern Hungary were summarized in Table 1. The soil had elevated concentrations of As, Cd, Cu, Pb and Zn. The contamination of the mine spoiled soils can be attributed to mining activities conducted in the area decades of years ago. Concentration of Cd is 4.77 times higher than the normal soil in the forest. The mean concentration level of Cu in mined spoil soils is 32.81 mg kg^{-1} dry weight of soil which is 4.25 times higher than normal soil. The concentration levels of Pb like Cd were also above the normal soils. The concentration levels of Pb had a mean value of 1929.71 mg kg^{-1} dry weight of soil, more than 92x of Pb in the normal soil. The concentration levels of Zn at the site were nearly 6x of Zn in a typical Hungarian soil. It had a mean value of 421.26 mg kg^{-1} dry weight of soil. Likewise, As concentration levels at the site were very high. The As mean concentration value of 885.57 mg kg^{-1} dry weight of soil was 39x of As in normal forest soil in the research area.

Table 1. Concentrations of potential toxic elements in mined –spoil soils in Mátraszentimre, Mátra Mountains, Hungary

Potential Toxic Elements	Forest Soil (A) Mean (mg kg-1)	Mine Spoiled Soil (B) Mean (mg kg-1)	Concentration Levels Ratio (B/A)
Cadmium (Cd)	Bdl*	4.77 ±0.28	4.77
Copper (Cu)	7.71±0.04	32.81 ±0.07	4.25
Lead (Pb)	20.9±1.5	1929.71±6.4	92.33
Zinc (Zn)	74.6±0.5	421.26	5.65
Arsenic (As)	22.48±0.88	885.57±9.56	39.53

*bdl-below detection limits (< 0.001 mg kg^{-1})

CONCENTRATIONS OF HEAVY METALS IN TREE SPECIES IN THE MINING SITE

Arsenic (As)
The amount of As in tree biomass was summarized in 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 17.25 mg kg^{-1}, 16.27 mg kg^{-1}, and 14.75 mg kg^{-1}, respectively were significantly higher than As in roots of Fagus sylvatica, having the lowest results with a value of 3.187 mg kg^{-1} in their roots. The same significant differences were noted in the stem

but all values in the stem were much lower than those in 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 values was obtained in *Betula pedula* (1.46 mg kg^{-1} As in leaves). The levels of As were below the detected value compared to other plant species considered phyto accumulator of As.

Table 2. Mean 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	As in Roots	As in Stems	As in Leaves
Betula pendula	16.274±0.831 A	0.283±0.141 B	1.464±1.67 A
Carpinus betulus	17.247±2.294 A	5.833±2.025 A	0.627±0.24 A
Fagus sylvatica	3.187±0.560 B	0.633±0.612 B	0.843±0.018 A
Salix caprea	14.755±0.953 A	0.749±0.109 B	0.285±0.070 A
p-value	0.044	<0.001	0.760
Remarks	Significant	Significant	Not Significant

* Mean ± Standard Division (SD).
Mean values in the same row with different letters as statistically different (P<0.05) u
Tukey's test

Cadmium (Cd)

Table 3. Mean 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	Cd in Roots	Cd in Stems	Cd in Leaves
Betula pendula	0.591±0.06 A	0.360±0.09 B	0.475±0.76 B
Carpinus betulus	0.942±0.06 A	0.613±0.01 B	0.050±0.015 B
Fagus sylvatica	0.158± 0.011 A	Bdl	Bdl
Salix caprea	1.577±0.024 A	4.302±0.017 A	4.702 ±1.579 A
p-value	0.120	<0.010	<0.001
Remarks	Not Significant	Significant	Significant

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

Table 4. Mean 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	Cu in Roots	Cu in Stems	Cu in Leaves
Betula pendula	6.209 ±0.176 A	8.684 .±0.061 A	4.145 ±0.040 A
Carpinus betulus	11.490 ±0.240 A	3.658 ±0.143 A	7.226 ±0.24 A
Fagus sylvatica	2.578 ± 0.018.A	2.737 ±0.098 A	6.801 ±0.057 A
Salix caprea	9.861 ±0.070 A	4.398 ± 0.018 A	5.708±0.229 A
p-value	0.272	0.310	0.718
Remarks	Not Significant	Not Significant	Not Significant

* Mean ± Standard Division (SD).
Mean values in the same row with different letters as statistically different (P<0.05) using Tul
test

Lead (Pb)

The levels of Pb present in tree biomass were presented in Table 5. The concentration levels of Pb on trees were significantly different on 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 mg kg^{-1}dry weight in roots, 439.057 mg kg^{-1}dry weight in stems and 92.532 mg kg^{-1}dry wt. in leaves.

Table 5. Mean 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	Pb in Roots	Pb in Stems	Pb in Leaves
Betula pendula	1227.120 ± 4.917 B	260. 630±1.857 B	8.276 ±0.267 B
Carpinus betulus	4071.67± 45.713 A	439.05 ± 1.061 A	92.532 ± 0.730 A
Fagus sylvatica	75.834 ± 1.101 D	2.805 ± 0.024 D	2.5000± 0.133 D
Salix caprea	216.14 ±0.966 C	46.166 ±0.720 C	9.006 ±0.054 C
p-value	0.004	0.014	0.014
Remarks	Significant	Significant	Significant

* Mean ± Standard Division (SD).
Mean values in the same row with different letters as statistically different (P<0.05) using Tul test

It was followed by Pb concentrations observed in Betula pendula with a mean value of 1227.12 mg kg^{-1} dry wt in roots, 260.63 mg kg^{-1} dry wt in stems and 8.28 mg kg^{-1} dry wt in leaves of Pb. The mean concentration levels of Pb in *Salix caprea* were 216.14 mg kg^{-1} dry wt in roots, 46.166 mg kg^{-1} dry wt in stems, and 9.006 mg kg^{-1} dry wt in leaves. Lowest concentration levels of Pb were observed in *Fagus sylvatica* with a mean value of 75.834 mg kg^{-1} dry wt. in roots, 6.801 mg kg^{-1} dry wt. in stems, and 2.805 mg kg^{-1} dry wt. in leaves.

Zinc (Zn)

The levels of Zn present in tree biomass were presented in Table 6. The tree species that contains highest level of Zn in the biomass were in the stems of *Betula pendula* (Birch tree). The mean observed value of Zn were 243.975 mg kg^{-1} dry wt. in roots, 583.180 mg kg^{-1} dry wt. in stems, and 475.575 mg kg^{-1} dry wt. in leaves. It was followed Zn concentration levels found in *Carpinus betulus* with an observed mean value of 335.320 mg kg^{-1} dry wt. in roots, 351.660 mg kg^{-1} dry wt. in stems, and 111.200 mg kg^{-1} dry wt. in leaves of Zn.

Table 6. Mean 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	Zn in Roots	Zn in Stems	Zn in Leaves
Betula pendula	243..975±1.504 A	583.180 ±1.504 A	475.575 ±2.219 A
Carpinus betulus	335.320 ± 4.439 A	351.660±2.216 A	111.200 ±0.561 B
Fagus sylvatica	133.947 ±1.362 A	171. 427 ±1.332 A	71.285 ±0.709 B
Salix caprea	216.055 ±0.292 A	285. 825 ±1.217 A	395. 970 ± 1.436 A
p-value	0.634	0.128	<.001
Remarks	Not Significant	Not Significant	Significant

* Mean ± Standard Division (SD).
Mean values in the same row with different letters as statistically different (P<0.05) using Tul test

High Zn value were also observed on the leaves of *Salix caprea* with concentration levels of 395.970 mg kg^{-1} Zn in leaves, 285.825 mg kg^{-1} dry wt. in stems, and 216.055 mg kg^{-1} dry wt. in roots. Lowest observed value was observed in *Fagus sylvatica* with an observed mean levels of 133.947 mg kg^{-1} dry wt. in roots, 171.427 mg kg^{-1} dry wt. in stems, and 71.285 mg kg^{-1} dry wt. in leaves.

BIOCONCENTRATION FACTOR (BCF)

Bioconcentration factor is a measure of how much potential toxic elements accumulated by each tree species based on the concentration of potential toxic elements present in plant tissues over the concentration values potential toxic elements in the contaminated soil were it is growing. A BCF value of >1.0 means, the plant can bio-accumulate potential toxic elements higher than the concentration levels of heavy metals present in the soil. A BCF value of <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 Salix caprea had BCF values of 1.58, 4.30 and 4.39, respectively while the roots of Carpinus betulus had BCF value of 1.13. For Zn, the root BCF of all tree species ranged from.1.11 to 1.37 while the stem of all except Fagus sylvatica were also above 1.0, ranging from 1.45 to 4.40. The Zn levels in the leaves of two tree species (Betula pendula and Salix caprea) were likewise above 1.0, at 3.03 and 2.07, 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 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 of Carpinus betulus and Fagus sylvanica.

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

Potential Toxic Elements	Tree Species					
	Betula pendula	Carpinus betulus	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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
Cd in Stem	0.359±0.590 B	0.332±0.140 B	Bdl	4.299±2.516 A	0.008	Significant
Cd in Leaves	0.475±0.792 B	0.050±0.055 B	Bdl	4.387±1.134 A	< 0.001	Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Not Significant
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	Significant

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

TRANSLOCATION FACTOR (TF)

Translocation Factor (Tf) is a measure of ratio between the concentrations levels of potential toxic elements present in stems and leaves to the concentration levels of potential toxic elements present in the roots. A value of Tf <1.0 means that the potential toxic elements has high mobility to the upper harvestable plant tissues. 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 1.60 to 4.68. 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 3.17. Significant Tf value for Zn was also observed on the harvestable tissues of Betula pendula and Salix caprea with a value of 4.682 and 3.171, respectively. Translocation factor for As, Cu, and Pb was found to be not significant (Table 8).

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

Potential Toxic Elements	Tree Species					
	Betula pendula	Carpinus betulus	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	Not Significant
Cd	0.838±0.932 B	0.563±0.145 B	Bdl	3.174 ±0.229 A	0.002	Significant
Cu	2.430±3.00 A	1.593±0.910 A	2.856±1.282 A	1.865±0.555 A	0.839	Not Significant
Pb	0.839±0.932 A	0.576±0.124 A	0.210±0.098 A	0.273±0.1267A	0. 476	Not Significant
Zn	4.682 ±1.402 A	2.091±1.019 B	1.5690±0.968 B	3.171 ±0.005A	0.037	Significant

* Mean ± Standard Division (SD). Mean values in the same row with different letters as statistically different ($P<0.05$) using Tukey's test

DISCUSSION

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 levels of 885.57 mg kg^{-1} dry weight were way above the 15 mg kg^{-1} As per dry weight of soil is 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. Similarly, 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 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 levels of Zn had value of 421.89 mg kg^{-1} dry weight of soil were also above the 200 mg kg^{-1} dry weight of soil which is acceptable level in soils in Hungary (Gazdag and Sipter, 2008). The concentration levels of Cu had a mean value of 32.28 mg kg^{-1} dry weight of soil were little higher the normal plant requirement ts 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} (Gazdag and Sipter, 2008).

The result 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 sampled in Shrewsbury, contains higher Pb, while contaminated soils located in Maaltheide, Belgium, contained higher Zn (Vangrosveld et al., 1995). Soils in smelter plant in Belgium contains very high Zn (Kopponen et al., 2001) and soil samples in a contaminated area in El Paso, Texas, USA have higher results on concentration levels of Pb and Cu (Gardea-Torresday, 1996). Kabata-Pendias and Pendias (2001), 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^{-} dry wt for Zn. Concentration of Zn between 100-900 mg could have phytotoxic effects on plants. Based on these criteria, the concentration levels of Cd, Cu and Zn under study is 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 more of tolerance rather than avoidance of metal toxicity (Dobson et al., 2007). 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, foliage, and stem were observed in this research work crucial in the choice of tree species for phytostabilization establishment (Mendez and Maeir, 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 ranges from 3.187 mg kg^{-1} dry weight in the roots of Fagus sylvatica to 17.247 mg kg^{-1} dry wt. observed in roots of Carpinus betulus. The BCF ratio for all trees was <1.0 indicating that these trees are heavy metal excluders (Baker, 1981). 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 most popular hyperaccumulator of As reported 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 element or heavy metal (William, 2008). Cd is highly mobile in the biological systems and present potential risks to human health (Adriano, 1986). Cd generally inhibits 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 4.70 mg kg^{-1} dry wt. in leaves and 4.302 mg kg^{-1} dry wt. in stems, resulting to significant Tf and BCF. The Tf indicated that the Cd in harvestable parts of S. caprea is more than 3 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 1.58 mg kg^{-1} in roots, 4.30 mg kg^{-1} in stems and 4.39 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. (Gazdag and Sipter, 2008). 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 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 well in absorbing Cd. A study in France, reported that poplar can bioaccumulate up to 200 mg kg^{-1} Cd (Robinson, 2000), 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 2.59 mg kg^{-1} dry weight in the roots of Fagus sylvatica to 11.49 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 kg1 (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 level 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. 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 849-1154 mg kg^{-1} dry weight of Cu (Ait-Ali et al., 2004). The creosote bush species, Larrea tridentata, creosote bush species, in a heavy metal contaminated area in Texas, USA absorb 953 mg kg^{-1} and 370 mg $kg^{-1}$of Cu in its roots, stem and leaves, respectively (Gardea-Torresday, 1996). The soil in this area also had elevated levels of Cu at around 4.993 mg kg^{-1} dry wt. Cu (Gardea –Torresday, 1996).

Birch leaves in North-western Russia, concentration of Cu in Betula pubescens trees near the Cu smelter plant was 6-12 times higher in than trees growing in normal soils (Koslov 1995). These results showed that the concentration levels of Cu in soils influences the absorption capacity of trees for Cu.

Lead (Pb)

Another potential toxic elements are 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 mg kg^{-1} and 1227.12 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 mg kg^{-1} dry wt Pb in stems and 92.532 mg kg^{-1} dry wt Pb in leaves but much lower than roots. Both *Carpinus betulus* and *Betula pendula* showed that Pb can be easily accumulated in roots compared to stem and leaves. Other trees species have been reported with similar response ad 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).

Very high levels of Pb can be tolerated by some tree species. Salix caprea can tolerate and grew in soils up to 17,000 mg kg^{-1} of Pb while *Betula pendula*, a pioneer tree species that can tolerate soils that contains as much as 29,000 mg kg^{-1} of Pb but the soil contains high levels of Ca which enhances the tree tolerance to Pb (Eltrop et al., 1991). 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 values 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 was retain 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 (Mendez et al., 2008).

Zinc (Zn)

The normal range for Zn requirement in trees is around 15-20 mg kg^{-1} dry wt. (Marschner, 1995). Results of this research work showed that *Betula pendula* and *Salix caprea* can bioaccumulate high levels of Zn in their biomass. *Betula pendula* absorb 583.18 mg kg^{-1} Zn in stems and 475.57 Zn mg kg^{-1} in leaves while *Salix caprea* had 285.83 mg kg^{-1} Zn in stems and 395. 97 mg kg^{-1} Zn in leaves. Highest BCF value were also observed in *Betula pendula* with a mean value of 4.40 in stems and 3.03 in leaves followed by leaves of Salix caprea at 2.07. 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 plants are 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, Assareh et al., 2008). *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 presence of mycorrhiza in Birch tree was found important factor that lead to high uptake and translocation of Zn (Brown and Wilkins, 1985).

The association with mycorrhiza provided larger surface area that that promotes greater absorption of Zn in *Betula pendula* (Brown and Wilkins, 1985). *Salix caprea* has been reported to absorb high amount of Zn. A study showed that *Salix caprea* can absorb 4680 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 well for Zn removal. Willows can remove 5-27 kg/ha/yr 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

The tree species that demonstrated highest potential for hyperaccumulation of Pb is *Carpinus betulus* since the absorbed value were more than 1000 mg kg^{-1} dry wt. of Pb in roots. It is therefore, a good candidate for the phytostabilization of Pb contaminated sites. It gave Tf value of less than 1.0 showing less mobility of Pb in the harvestable parts. *Salix caprea* is potential tree for phytoextraction of Cd and Zn while *Betula pendula* has shown as potential 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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