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ENVIRONMENTAL SCIENCE

PhD PROGRAM

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

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

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1.0 BACKGROUND OF THE WORK AND ITS AIMS

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 (GONZÁLEZ-OREJA et al. 2008). Potentially toxic elements are generally non-biodegradable and difficult to manage due to their mobility in the soil and water environment. Increased concentrations of potentially toxic elements in the soils and water generally result in phytotoxicity in plant and animal, directly threatening human health and can have exacerbated effects when these elements enter the food chain as ground and water contaminants (EVANGELOU et al. 2013). Phytotoxicity problem as a consequence of elevated concentration of potential toxic elements in soils have also indirect hazards to human health (PULFORD et al. 2002). Several physiological and biochemical processes in plants can be severely affected by these potential toxic elements, however, their toxicity varies with plant species, and the chemical form and concentration of the element (ACKOVA 2018).

Restoration of the ecosystem through soil decontamination from potential toxic elements is the most recalcitrant problem (DOBSON and BAKER 1997). Traditional methods of removing potential toxic elements in water and soil are generally expensive and less successful (SALT et al. 1998, LASAT 1999, PAJEVIC et al. 2016). Excavation of mining wastes and disposing them to landfills do not alleviate its hazards. The pollution problem is simply relocated in space and time (RASKIN et al. 1997). Being prohibitively expensive, most of the mining companies ignore the problem and abandon the mining site after the operation ceases (SALT et al. 1998).

One of the methods in cleaning the abandoned mining sites is the use of phytoremediation. Phytoremediation is an emerging phytotechnology which uses plants in the removal, reduction, immobilization and degradation of potential toxic elements in the soil (LASAT, 1999). Some of the plants can accumulate the toxic elements in stem, shoot and leaves, other can produce enzymes, such as dehalogenase and oxygenase that can degrade the toxic elements in their tissues, while some can stabilize the metal through immobilization at the interface of roots and soil (KRAMER et al. 1996).

It is economically, ecologically, socially acceptable and has aesthetic value as phytotechnology (PULFORD and WATSON 2003, WILLIAM, 2008, STANKOVIC and DEVATAKOVIC, 2016).

Plant species that are commonly used in the bioremediation are called hyper accumulators. This group of plants are capable of accumulating and tolerating considerable level of potential toxic elements. The potential toxic elements are absorbed from the soil, then translocated and accumulated in plant biomass (BAKER, 1981). BABU et al. (2021), reviewed the mechanism and strategies to enhance phytoremediation rate, emphasizing the sustainable and ecofriendly nature of phytoremediation using hyper accumulator plants.

Phytoremediation is a process to purify and stabilize potential toxic elements from the toxic soil environment naturally executed by plants (PAZ-ALBERTO et al. 2013). 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). MAHARDIKA et al. (2018), emphasized the used of sunflower (*Helianthus annuus* L.) in phytoremediation of potential toxic elements specifically copper. This further strengthen the practical applications of hypera ccumulator plants in environmental remediation.

Most reported hyper accumulators 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.

Use of trees known as dendromediation is preferentially recommended in removing potential toxic elements not only in abandoned mining sites but also in industrial areas for conversion into residential communities. 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 LANDBERG 1999). 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 LANDBERG 1999). In Sweden, willows are largely cultivated for the phytoextraction of Cd and Zn and bio-energy production (GREGER and LANDBERG 1999). 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 plant 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 abandoned mining dump site in Mátraszentimre, Hungary serves as source of potential toxic elements and poses danger to the downstream ecosystems like rivers, farms and resident communities (ODOR et al. 1998). Phytoremediation activity is needed in the area to lessen the downward mobility of potential toxic elements and to protect the soil ecosystems and water sources. However, the specific plant species to be utilized to phytoremediate a specific potential toxic elements is needed to be identified. Hence, these research work.

The main objective of this study was to determine the phyto extracting ability of endemic tree and grass species naturally growing in toxic elements contaminated soils in an abandoned mining site in Mátraszentimre, Hungary.

The following research aims were done to accomplish the main objective.

The first (1) research task was to collect soil and plant samples naturally growing in the area. The second (2) task was to determine the concentration levels of As, Cd, Cu, Pb and Zn in soil samples. The third (3) task was to determine the concentration levels of As, Cd, Cu, Pb and Zn in roots, stem, and leaves of plant samples. The fourth (4) task is to determine the phytoextracting potential of each plant species by calculating the bio concentration factor of (BCF} of each potential toxic elements per plant species.

The fifth task (5) was to determine the translocation factor (Tf) of each potential toxic elements per plant species and determine which tree/grass species is a potential candidate for the phytostabilization of the abandoned mine site.

2.0 MATERIALS AND METHODS

2.1 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 several decades 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).

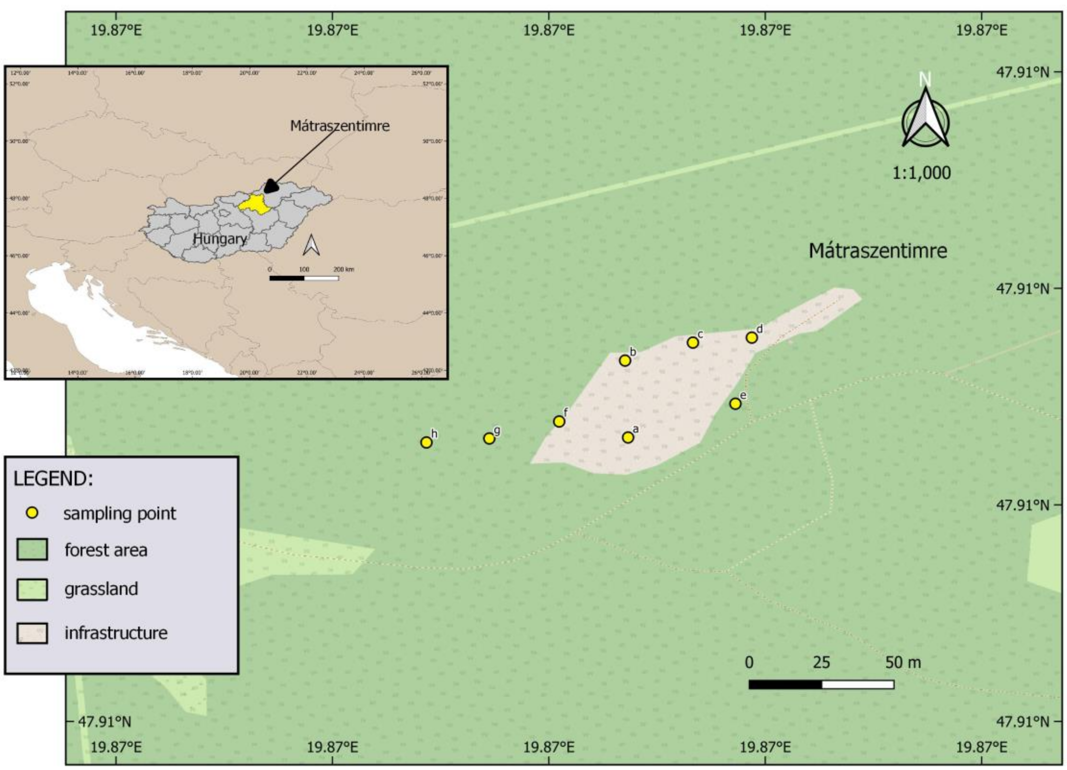


Figure 1. Sampling Area (Mátraszentimre, Mátra Mountains, Hungary), maps taken from Google Earth.

2.2 Soil Sampling and Analysis

Soil samples were collected in eight sampling points along the main dump sites (**Figure 1**) 17th June, 2019. Soil samples were collected between 19.86935 to 19.86994 longitude and 47.90729 to 47.90777 latitude.

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, Gödöllő, 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).

2.3 Plant Sampling and Analysis

Samples of tree species were collected around the eight 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 two sampling points. Seven to ten plant samples for each species were taken from each sampling point. Whole plant samples of trees with height 0.60 m and below growing within one meter diameter distance around the soil sampling point were collected.

Grass samples were also collected in the same sampling site. There were limited grass samples collected, since they are located only in one or two sampling sites. Ten grass samples were collected in each sampling points. Grass species that were collected are *Holcus lanatus*, *Poa augustifolia*, *Dactylis glomerata*, *Arrhenatherum elatius* and *Poa nemoralis*. One rush species were also observed which was *Luzula albida*. Tree species and grasses were identified by Dr. János György Nagy, Institute of Botany and Ecophysiology, MATE.

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).

2.4 Bio-concentration Factor (BCF)

The capacity to remove potential toxic elements by plant species is generally measured using bioconcentration 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 plant species was calculated using the following formula *(*ZAYED et al. 1998*):*

$$\text{BCF}=\frac{\text{Concentration of potential toxic elements in plant tissues}}{\text{Concentration of potential toxic elements in soil}}$$

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. 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.

2.5 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.

$$\text{TF} = \frac{\text{Concentration of potential toxic elements in stem + leaves}}{\text{Concentration of potential toxic elements in roots}}$$

2.6 Statistical Analysis

All statistical analysis were carried out by means using JASP version 16.2.1

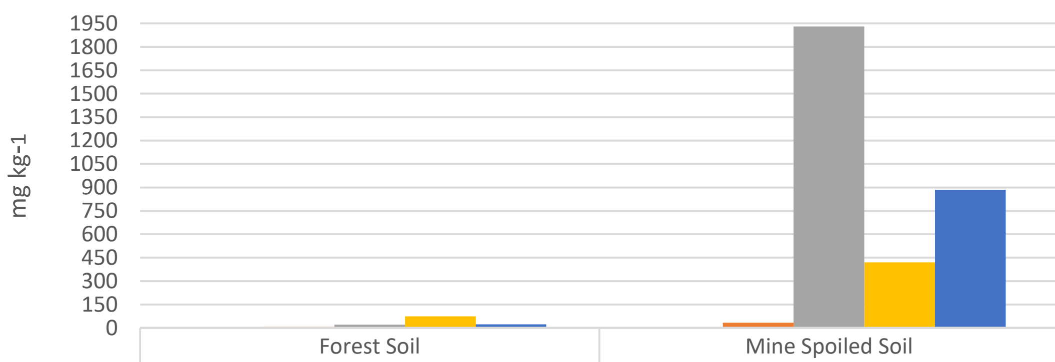
Analysis of Variance (ANOVA) was done to determine if there are significant differences in the absorption capacity of potential toxic elements among tree species. Analysis of Variance were not implemented for grasses due to limited plant samples. Differences were considered significant if the value of $p < 0.05$.

3.0 RESULTS

3.1 Concentrations of Heavy Metals in Soil Samples from the Mining Site

The mined spoil soil located in Mátraszentimre, Northern Hungary had elevated concentrations of As, Cd, Cu, Pb and Zn (**Figure 2**). The contamination of the mine spoiled soils can be attributed to mining activities conducted in the area several decades ago. Concentration of Cd is 4.77±0.28 times higher than the normal soil observed in the forest and above the acceptable limit when compared to a normal Hungarian soil (Hungarian Joint Decree No 10/2000 *Gazdag and Sipter 2008*)

. 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.



	Forest Soil	Mine Spoiled Soil
Cadmium	0.01	4.77
Copper	7.71	32.81
Lead	20.9	1929.71
Zink	74.6	421.26
Arsenic	22.48	885.57

Figure 2. Concentrations of potential toxic (mg kg^{-1}) elements in mined –spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

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.

3.2 Concentrations of Potential Toxic Elements in Tree Samples in the Mining Site

3.2.1 Arsenic.

Total As in different tree species were not significantly different (**Figure 3, Table 1**) 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 phytoaccumulator of As.

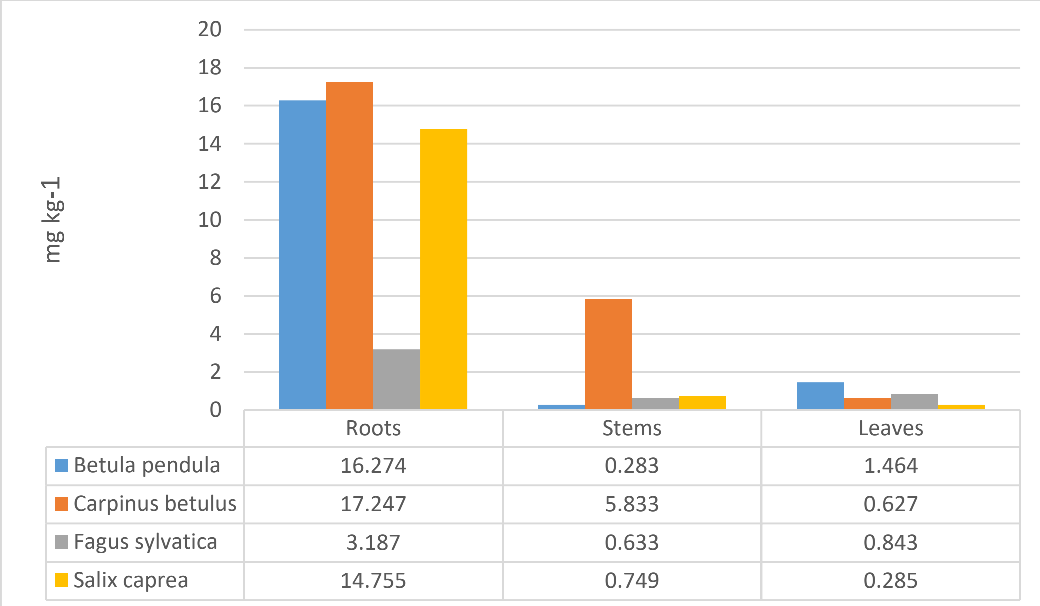


Figure 3. Concentration levels (mg kg^{-1}) of As in roots, stem and leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Table 1. 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**
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***	0.760ns

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

3.2.2 Cadmium

Significant levels of Cd were observed in stems and leaves 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 (**Figure 4, Table2**). 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 with a concentration value of 0.158±0.011 mg kg^{-1} .

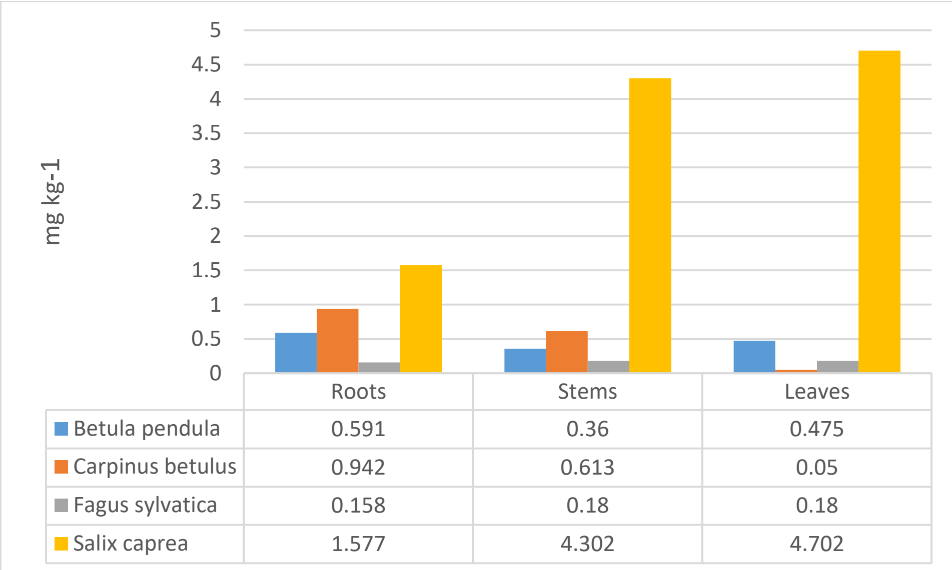


Figure 4. Concentration levels (mg kg^{-1}) of Cd in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Table 2. 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**
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	0.120ns	<0.010**	<0.001***

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

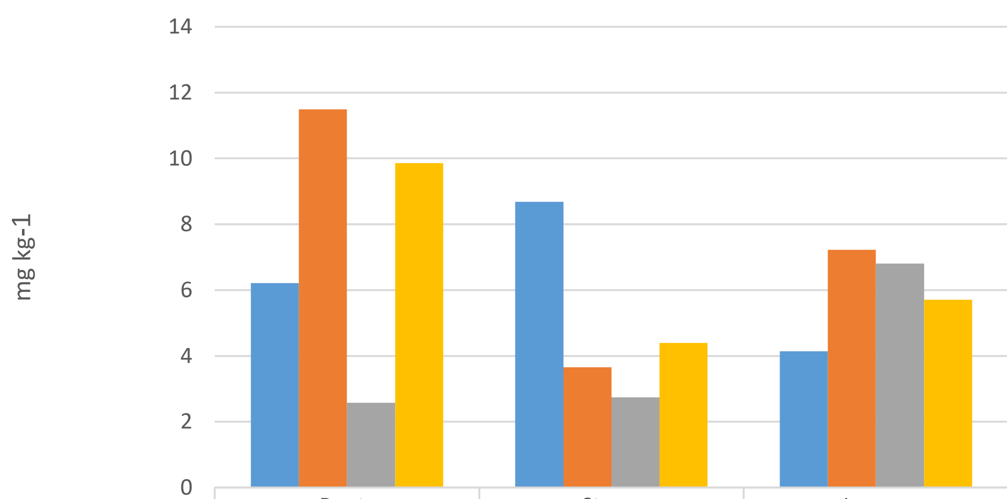
ns-not significant

bdl-below detection limits

*significant at 0.05
**significant at 0.01
***highly significant at 0.01

3.2.3 Copper

No significant differences were observed on the concentration levels of Cu in roots, stem and leaves of the four tree species studied (**Figure 5, Table 3**). *Carpinus betulus* contained highest level of Cu in the roots and leaf 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 *Betula 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.



	Roots	Stems	Leaves
Betula pendula	6.209	8.684	4.145
Carpinus betulus	11.49	3.658	7.226
Fagus sylvatica	2.578	2.737	6.801
Salix caprea	9.861	4.398	5.708

Figure 5. 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.

Table 3. 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**
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 Salix	2.578±0.018.a	2.737±0.098 a	6.801±0.057 a
caprea	9.861±0.070 a	4.398±0.018 a	5.708±0.229 a
p-value	0.272ns	0.310ns	0.718ns

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

4.2.4 Lead

The concentration levels of Pb on trees were significantly different among tree species (**Figure 6, Table 4**). It was also significantly different in roots, stems and leaves. The tree species that contains highest level of Pb in roots, stem and leaves 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 2.500±0.133 mg kg^{-1} dry wt. in leaves.

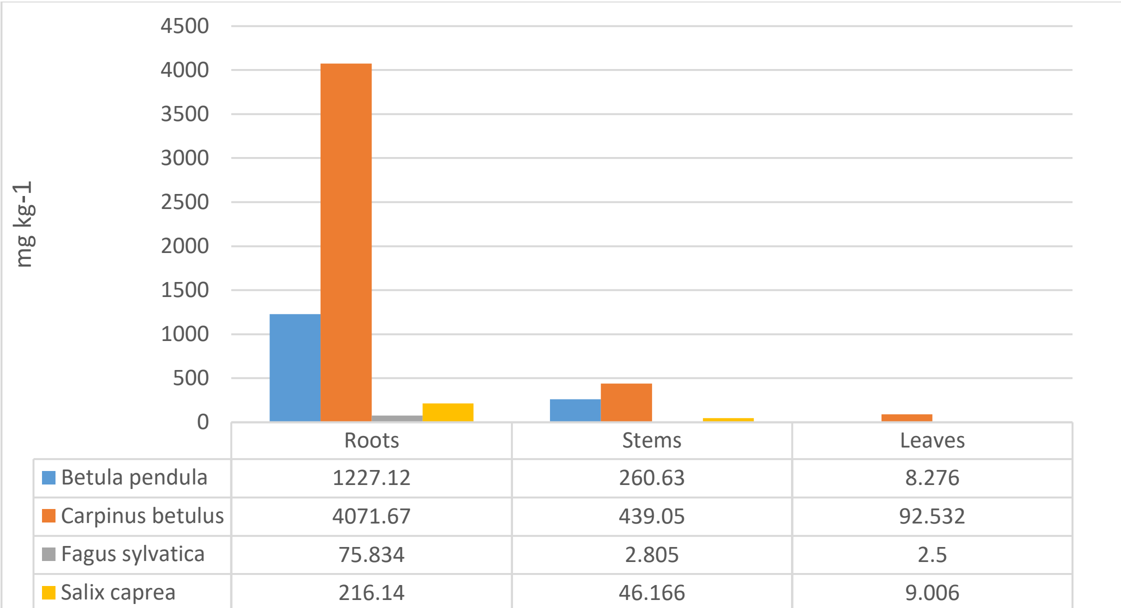


Figure 6. Concentration levels (mg kg^{-1}) of Pb in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Table 4. 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**
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*

Mean ± Standard Deviation D). 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

4.2.5 Zinc

The tree species that contained the highest level of Zn in roots was found in *Carpinus betulus* with a concentration value of 335.320±4.439 mg kg^{-1} of Zn. It was followed by Zn concentration levels in roots of *Betula pendula* (Birch tree) (**Figure 7, Table 5**) with a value of 243.975±1.504 mg kg^{-1} of Zn. It was followed by concentration levels of Zn in *Salix caprea* with a value 216.055±0.292

mg kg^{-1} dry wt. in roots. The lowest value of Zn were observed in roots of *Fagus sylvatica* with mean levels of 133.947±1.362 mg kg^{-1} dry wt. in roots. The concentration levels of Zn in roots of trees were statistically not significant.

Betula pendula has the highest concentration levels of Zn in stems with a mean observed value of 583.180±1.504 mg kg^{-1} dry wt. of Zn in stems. It was followed by Zn concentration levels found in *Carpinus betulus* with an observed mean value of 351.660±2.216 mg kg^{-1} dry wt. in stems, respectively. *Salix caprea* had an observed value of 285. 825±1.217 mg kg^{-1} dry wt. in stems while lowest was observed in *Fagus sylvatica* with mean level of 171. 427±1.332 mg kg^{-1} dry wt. in stems. Similarly, the observed value of concentration levels of Zn in stems of trees were found to be statistically not significant.

Concentration levels of Zn in leaves was found significant. Highest concentration levels of Zn in leaves were demonstrated in *Betula pendula* with a value of 475.575±2.219 mg kg^{-1} dry wt. in leaves.

Salix caprea had a concentration levels of 395. 970±1.43 mg kg^{-1} Zn in leaves while *Carpinus betulus* had an observed mean value of 111.200 ±o.561 mg kg^{-1} dry wt. in stems. Lowest value was observed in *Fagus sylvatica* with mean levels 71.285±0.709 mg kg^{-1} Zn dry wt. in leaves.

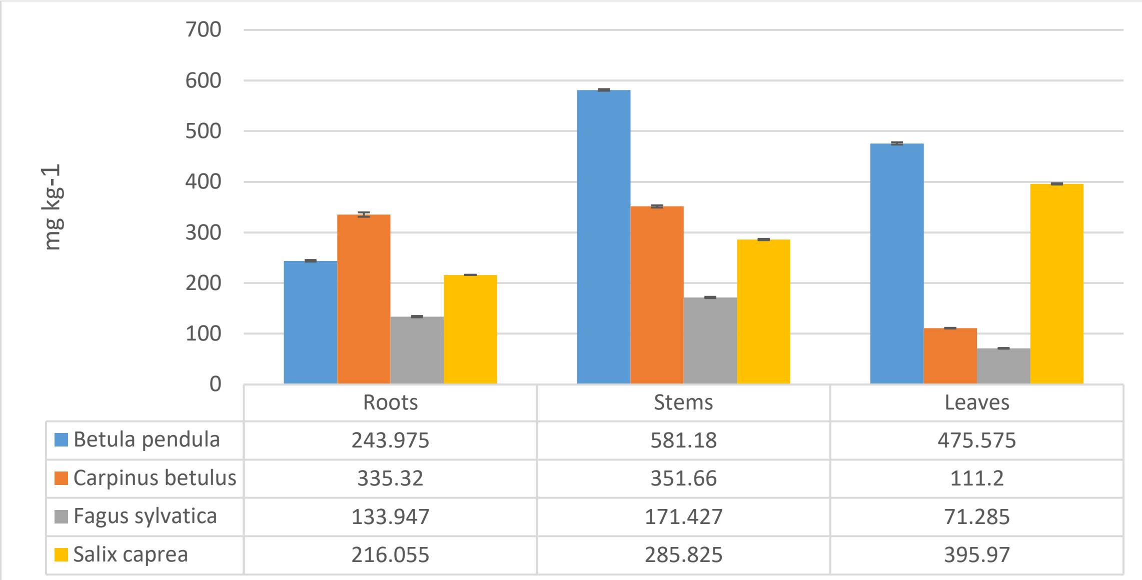


Figure 7. Concentration levels (mg kg^{-1}) of Zn in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Table 5. 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
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±1.43 a
p-value	0.634ns	0.128ns	<.001***

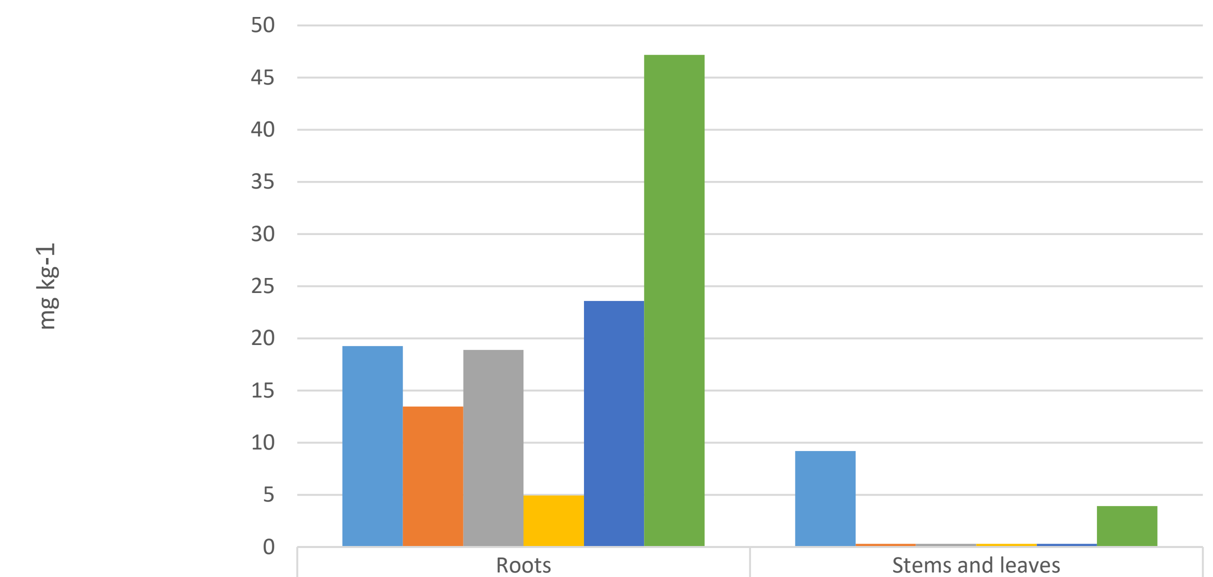
Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test ns-not significant

*significant at 0.05
**significant at 0.01
***highly significant at 0.01

4.3 Concentrations of Potential Toxic Elements in Grass Species in the Mining Site

4.3.1 Arsenic

Concentration levels of As in different grass species were summarized in **Figure 8.**



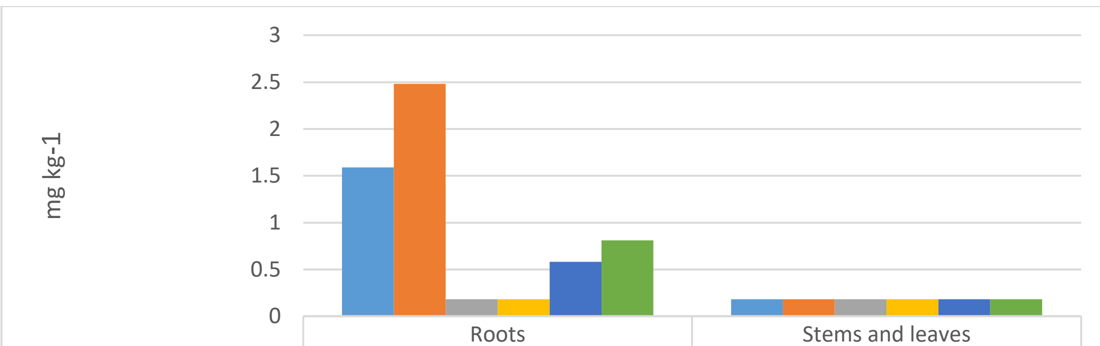
	Roots	Stems and leaves
Holcus lanatus	19.26	9.2
Luzula albida	13.44	0.28
Poa augustifolia	18.89	0.28
Dactylis glomerata	4.91	0.28
Arrhenatherum elatius	23.58	0.28
Poa nemoralis	47.16	3.91

Figure 8. Concentration levels (mg kg^{-1}) of As in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

The levels of As in roots varied among grass species. Highest As in roots of *Poa nemoralis* with mean values of 47.16±1.84 mg kg^{-1} in roots. It was followed by levels of As in roots of *Holcus lanatus* and *Arrhenatherum elatus* with a total concentration value of 28.46 mg kg^{-1} and 23.58 mg kg^{-1}, respectively. Lowest As value was observed in *Dactylis glomerata* with a mean value of 4.91±0.73 mg kg^{-1} in roots. Concentration of As were only detected in stems and leaves of *Holcus lanatus* and *Poa nemoralis* with a value of 9.2±1.0 mg kg^{-1} and 3.91±0.34 mg kg^{-1}. Most of the levels of As in stems and leaves in other grass species were below the detection limits.

4.3.2 Cadmium

Concentration levels of Cd in grasses were shown in **Figure 9, respectively.**



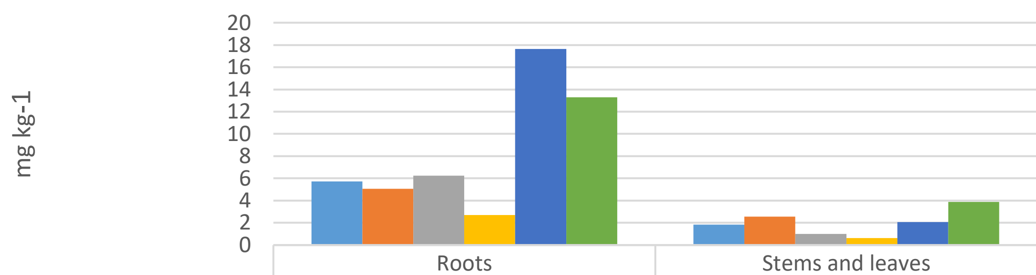
	Roots	Stems and leaves
Holcus lanatus	1.59	0.18
Luzula albida	2.48	0.18
Poa augustifolia	0.18	0.18
Dactylis glomerata	0.18	0.18
Arrhenatherum elatius	0.58	0.18
Poa nemoralis	0.81	0.18

Figure 9. Concentration levels (mg kg^{-1}) of Cd in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

Highest level of Cd were observed in roots of *Luzula albida* with value of 2.48±0.84 mg kg^{-1} Cd. It was followed by *Holcus lanatus*, *Poa nemoralis*, and *Arrhenatum elatius* with a mean value of 1.59±0.04 mg kg^{-1} Cd, 0.81±0.02 mg kg^{-1} Cd and 0.58±0.02 mg kg^{-1} Cd, respectively. The concentration levels of Cd in roots of Poa augustifolia and Dactylis glomerata were below the detection limits. Concentration levels of Cd in stem and leaves of all grasses sampled in the area were below the detection limits (bdl).

4.3.3 Copper

Highest value for Cu uptake were observed in roots of *Arrhenatherum elatius* with concentration levels of 17.65±0.23 mg kg^{-1} in roots (**Figure 10**) . *Poa nemoralis* had a concentration levels of 13.31±0.18 mg kg^{-1} in roots. Lowest concentration of Cu value were observed from the roots of *Dactylis glomerata* with a mean value of 2.7±0.08 mg kg^{-1} in roots. For the total copper uptake, highest concentration were also observed in *Arrhenatherum elatius* with concentration levels of 19.11 mg kg^{-1} in roots. Lowest total concentration levels of Cu value were observed from *Dactylis glomerata* with a mean value of 2.7 mg kg^{-1} in roots.



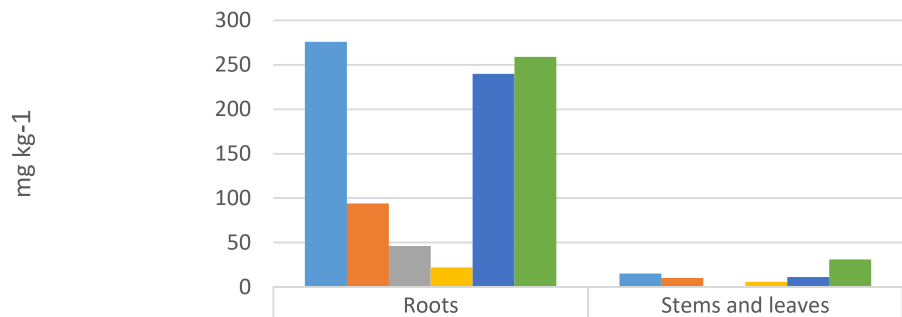
	Roots	Stems and leaves
Holcus lanatus	5.725	1.825
Luzula albida	5.07	2.55
Poa augustifolia	6.25	0.99
Dactylis glomerata	2.7	0.61
Arrhenatherum elatius	17.65	2.06
Poa nemoralis	13.31	3.89

Figure 10. Concentration levels (mg kg^{-1}) of Cu in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

4.3.4 Lead

Holcus lanatus gave the highest total concentration value of Pb in plant tissue followed by *Poa nemoralis* and *Arrhehatherum elatius* (**Figure 11).**

The observed total concentration value of Pb were 291.13 mg kg^{-1} in *Holcus lanatus*, 289.94 mg kg^{-1} in *Poa nemoralis* and 251.07 mg kg^{-1} of Pb in *Arrhehatherum elatius*, respectively. The same grass species gave the highest concentration values of Pb in roots. High concentration levels of Pb were found in roots of *Holcus lanatus* and *Poa nemoralis* with a value of 275.9±1.6 mg kg^{-1} and 259.04±1.51 mg kg^{-1}, respectively.

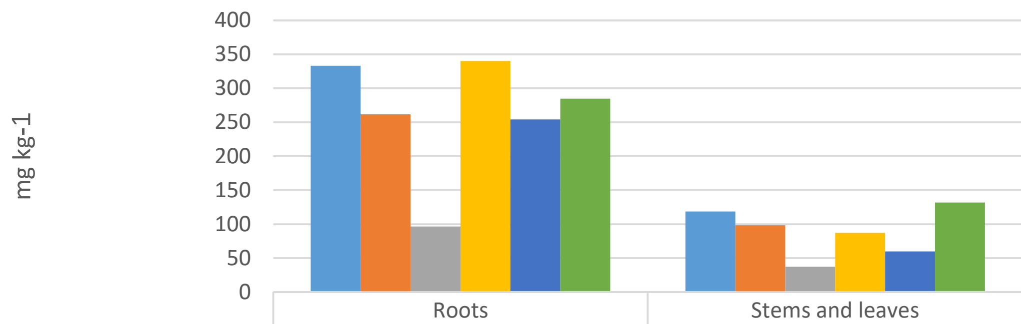


	Roots	Stems and leaves
Holcus lanatus	275.9	15.23
Luzula albida	94.05	10.285
Poa augustifolia	46.1	0.15
Dactylis glomerata	22.1	6
Arrhenatherum elatius	240	11.07
Poa nemoralis	259.04	30.9

Figure 11. Concentration levels (mg kg^{-1}) of Pb in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

4.3.5 Zinc

The grass species that contained the highest level of Zn in the total biomass was *Holcus lanatus*. (**Figure 12**).



	Roots	Stems and leaves
Holcus lanatus	333	118.9
Luzula albida	261.65	98.3
Poa augustifolia	96.6	37.6
Dactylis glomerata	340	87
Arrhenatherum elatius	254	60
Poa nemoralis	284.75	132

Figure 12. Concentration levels (mg kg^{-1}) of Zn in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

The mean observed values were 333±1.65 mg kg^{-1} of Zn in dry wt. of roots, 118.9±0.41 mg kg^{-1} dry wt. instems and leaves. It was followed by Zn concentration levels found in *Dactylis glomerata* with an observed mean value of 340±1.0 mg kg^{-1} dry wt. in roots, and 87±1.0 mg kg^{-1} dry wt. in stems and leaves of Zn. High Zn values were also observed on the leaves of *Luzula albida* with concentration levels of 98.3±0.11 mg kg^{-1} Zn in stem and leaves and 261.65 ±1.0 mg kg^{-1} dry weight.

4.4 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 potential toxic elements 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.

4.4.1 Bioconcentration Factor in Trees

The bioconcentration factor (BCF) of tree species were summarized in Table 6. 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 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*.

Table 6. Bioconcentration Factor of different tree species for the uptake of As, Cd, Cu. Pb, and Zn.

Potential Toxic Elements	Tree Species				p-value	Remarks
	Betula pendula	***Carpinus betulus***	***Fagus sylvatica***	***Salix caprea***		
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 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

4.4.2 Bioconcentration Factor (BCF) in Grasses

Bioconcentration factor of potential toxic elements (As, Cd, Cu, Pb and Zn) for roots and stem and leaves of grasses were summarized in Table 7.

Highest BCF value for Zn were shown by the roots of *Holcus lanatus* with an observed value of 4.06. It means that it absorbs 4.06 times more Zn inside its root biomass than in soil ecosystem.

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

Potential Toxic Elements	Grass Species					
	Holcus lanatus	*Luzula albida*	*Poa augustifolia*	*Dactylis glomerata*	*Arrhenatherum elatius*	*Poa nemoralis*
As in Roots	0.05	0.04	0.07	0.02	0.09	0.02
As in Stem and Leaves	0.02	bdl	bdl	bdl	bdl	0.01
Cd in Roots	bdl	bdl	bdl	bdl	0.07	0.19
Cd in Stem and Leaves	bdl	bdl	bdl	bdl	bdl	bdl
Cu in Roots	0.35	0.19	0.38	0.17	0.31	0.88
Cu in Stem and Leaves	0.08	0.06	0.05	0.03	0.03	0.20
Pb in Roots	0.38	0.13	0.08	0.04	0.06	**3.35**
Pb in Stem and Leaves	0.02	0.01	bdl	0.01	0.01	0.35
Zn in Roots	**4.06**	**1.61**	0.43	1.52	0.25	**3.30**
Zn in Stem and Leaves	1.45	0.61	0.17	0.39	0.05	**1.10**

bdl- below detection limits

3.5 Translocation Factor (TF)

3.5.1 Translocation Factor (TF) in Trees

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

Potential Toxic Elements	Tree Species				p-value	Remarks
	Betula pendula	***Carpinus betulus***	***Fagus sylvatica***	***Salix caprea***		
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 Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test
bdl-below detection limits
ns-not significant
*significant at 0.05
**significant at 0.01
***highly significant at 0.01

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.

3.5.2 Translocation Factor (TF) in Grasses

Table 9. Translocation Factor of different potential toxic elements in shoots and leaves of different grass species.

Potential Toxic Elements	**Grass Species**					
	Holcus lanatus	*Luzula albida*	*Poa augustifolia*	*Dactylis glomerata*	*Arrhenatherum elatius*	*Poa Nemoralis*
As	0.48	bdl	bdl	bdl	Bdl	0.08
Cd	bdl	bdl	bdl	bdl	bdl	bdl
Cu	0.32	0.50	0.16	0.23	0.12	0.29
Pb	0.06	0.11	bdl	0.27	0.05	0.12
Zn	0.35	0.38	0.39	0.26	0.24	0.46

bdl- below detection limits

Translocation factor (TF) values of less than 1.0 for As, Cd, Cu, Pb and Zn were observed in shoots and leaves for all six grass species. Highest TF values were shown by *Lucula albida* for the translocation of Cu from roots to stem and leaves with a value of 0.50. TF values for Zn were high for all grass species. *Poa nemoralis* had TF value of 0.46 for Zn. For Cd, all observed TF values were all below the detection limits.

DISCUSSION

4.1 Concentration of Potential Toxic Elements 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 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 of Zn 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 Gyöngyösoroszi (SIMON 2006) and the sediments in Toka Creek (ODOR et al. 1998) suggested their high potential toxicity in plants. Soils sampled in Gyöngyösoroszi contained 7.1 mg kg^{-1} Cd, 120 mg kg^{-1} Cu, 2154 mg kg^{-1} Pb and 605 mg kg^{-1} of Zn (SIMON 2006).

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.

4.2 Concentration of Potential Toxic Elements in Trees and Grasses

Screening and evaluation of plant species growing in soils polluted with potential toxic elements is very important in selecting the most suitable species for any successful revegetation program (GONZÁLEZ-OREJA et al. 2008). Plants growing in metalliferous soils cannot prevent metal uptake (BENDER et al, 1989). The strategy of survival is based 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).

4.2.1 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 potential toxic elements 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. 2012b). 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).

Results on concentration levels of As among grasses evaluated in this study showed that grasses bioaccumulated higher level of As compared to trees. Similar to trees, levels of As was higher in roots compared to stems and leaves. Levels of As ranged from 4.91 ±0.73 mg kg^{-1} dry weight in the roots of *Dactylis glomerata* to 33. 21 ±1.81 mg kg^{-1} dry wt. in the roots of *Poa nemoralis* The BCF ratio for all grasses was <1.0 indicating that these grasses are potential toxic elements excluders for As. Another fern species that is hyper accumulator of As is *Pitygramma calomenalos* on a research work conducted in Thailand (VISOOTTIVISETH et al. 2002). TF values for both grasses and trees were at low levels. Similarly in research work of TIWARI et al. (2015) found that TF values for As in *Chrysopogon zizanoides* in shoot were also at low level. This could be attributed possibly due to sequestration of root vacuole which inhibits the upward transport of potential toxic elements (ZHAO et al. 2013) . *Holcus lanatus* were considered highly tolerant to high concentration levels of As (DRADACH et al. 2020).

Festuca rubra is another grass species reported to have high tolerance to As (DRADACH et al. 2020, SIMON 2006). *Panicum maximum* were reported to have been negatively affected by high As with a decrease in nutrient use efficiency (RABELO et al. 2021). Grasses are more suited for phytostabilization of As in polluted soil ecosystem. In another research work, *Pteris vittata* is reported to be very efficient in the phytoextraction of Arsenic through inoculation of As resistant bacteria (LAMPI et al. 2015).

4.2.2 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} Cdin 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 1999). Two studies, (PIETRINI et al. 2015 and ZUPUNSKI et al. 2015), however, indicated that willows were more tolerant to Cd than poplar. Similarly, *Paulownia spp.* is a tree species that demonstrated as a good phytoaccumulation of Cd (TZVETKOVA et al. 2015).

The concentration levels of Cadmium in grasses were considered at low level concentration. It ranges from below detection limits to 2.48 mg kg^{-1} Cd in *Luzula albida*. The levels of Cd in stem and leaves were not detected. Likewise, all observed TF values were all below the detection limits. Low Cd concentration in soils influence the low Cd concentration of Cd in roots, stem and leaves of grasses. However, the concentration levels of Cd above 1.0 mg kg^{-1} is considered toxic to some plant species.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. Similarly, *Pennisetum purpureum* (Napier) is reported to be a good grass species for phyto accumulation of Cd (YANG et al. (2020). YIN et al. (2016), in a field experiment had shown that *Solanum nigrum* is a tolerant plant species in cadmium contaminated soils.

4.2.3 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 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±3.00. This showed low mobility of Cu from roots to shoots of birch tree.

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).

Cu in roots, stem and leaves of grasses were observed to be at lower concentration values as compared to trees. Highest value for Cu uptake were observed in roots of *Arrhenatherum elatius* showed highest concentration levels of Cu in roots at 19.11 mg kg^{-1}, folllowed by *Poa nemoralis* with concentration levels of 13.31±0.18 mg kg^{-1} in roots. The concentration levels is within the normal Cu requirements of plants and not classified at toxic level (MARSCHNER 1995). Lowest concentration of Cu value were observed from the roots of *Dactylis glomerata* with a mean value of 2.7 ±0.08 mg kg^{-1} in roots.

Highest TF values were shown by *Luzula albida* for the translocation of Cu from roots to stem and leaves with a value of 0.50. Most of the grasses has low BCF values for Cu. *Paspalum plicatum* is one grass species native in South American grasslands that was found to have greatest potential in phytostabilization of Cu contaminated sites (DE CONTI et al. 2020). The limited level of accumulation of Cu in plants is due their ability and efficiency to exclude Cu in their uptake (KUMAR et al. 2021).

In another study, GILABEL et al (2014) showed that *Panicum maximum* have high Cu concentrations in roots than in shoots. It shows low TF values of Cu in grasses. Similarly, sunflower demonstrated as hyperaccumulator of Cu as explored in a research work done by MAHARDIKA et al. (2018). *Pennisetum giganteum*, another grass species could absorb 453.3 g of Cu per year grown in Cu contaminated soil (ZHOU, 2016). POLECHONSKA and KLINK (2014) showed that *Phalaris arundinaceae* is another grass species that shown good potential for the phytoextraction of Cu and Zn.

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).

4.2.4 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). High levels of Pb in these trees could be attributed to high level of Pb in soils in the study area. These trees tend to tolerate the high level of potential toxic elements specifically Pb.

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.

Phytoremediation potential of grasses to Pb were also observed in this research work.

Holcus lanatus gave the highest total concentration value of Pb in plant tissue with an observed total concentration value of Pb were 291.13 mg kg^{-1}. The same grass species gave the highest concentration values of Pb in roots. BEGUM and MELAIRAJAN (2019) conducted an experiment showing *Holcus lanatus* and *Cynodon dactylon* is a good phytoextractor of Pb.

High concentration levels of Pb were also found in roots of *Poa nemoralis* and *Arrhehatherum elatius*. Results showed that the concentration levels of Pb was 289.94 mg kg^{-1} in *Poa nemoralis* and 251.07 mg kg^{-1} of Pb in *Arrhehatherum elatius*, respectively. *Dactylis glomerata* is tolerant to Pb (VISCONTI et al. 2020). Results of these study is higher than same experiment conducted in Matra Mountains. MURANYI and KODOBOCZ (2008) in an experiment reported that Sudan grass absorbed 106 mg kg^{-1} of Pb while *Sorghum* absorbed 132 mg kg^{-1} of Pb in their biomass.

Poa nemoralis had also the highest BCF in roots with an obtained value of 3.35. This showed that this grass are more efficient than other grass species in absorbing and storing Pb in their root system. TF values for all grasses were <1.0 for all grasses. Mobility of Pb from roots to shoots is at low level. Another tropical grass species that is highly tolerant to Pb was *Imperata cylindrica* (PENG et. al. 2006).

4.2.5 Zinc (Zn)

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. However, the obtained value of concentration of Zn in these trees cannot be classified them as hyperaccumulator of Zn.

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 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).

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 4680 mg kg^{-1} dry wt. of Zn in a pot experiment (WEISHAMMER et al. 2007) while another study in Spain indicated a concentration value of 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. 2007) 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).

The concentration levels of Zn on grasses were above the normal range for Zn requirement in plants which is around 15-20 mg kg^{-1} dry wt. (BROADLEY et al. 2007). Among grass species studied under this research work, *Holcus lanatus* absorbed 333±1.65 mg kg^{-1} of Zn in dry wt. of roots, 118.9±0.41 mg kg^{-1} dry wt. instems and leaves. *Dactylis glomerata* had an observed mean value of 340±1.0 mg kg^{-1} dry wt. Zn in roots, and 87±1.0 mg kg^{-1} dry wt. in stems and leaves of Zn.

High Zn values were also observed on the leaves of *Luzula albida* with concentration levels of 98.3±0.11 mg kg^{-1} Zn in stem and leaves and 261.65±1.0 mg kg^{-1} dry wt. in roots. Lowest value was observed in *Poa augustifolia* with mean levels of 96.6±0.02 mg kg^{-1} dry wt. in roots, and 37.6±0.10 mg kg^{-1} dry wt. in stems leaves. *Pennisetum purpureum* (Napier) has demonstrated to be a good grass species for phyto accumulation of Zn (YANG et al. (2020). High level of Zn could depress the growth of grasses. *Chloris barbata* was negatively affected by high levels of Zn in an experiment conducted (PATRA et al. 1994). Zn decrease root elongation of these grass species.

Highest BCF value for Zn were shown by the roots of *Holcus lanatus* with an observed value of 4.06 It means that it absorbs 4.06 times more Zn inside its root biomass than in soil ecosystem. Other grass species with high values of BCF for Zn were *Poa nemoralis* with a value of 3.30 in roots and 1.10 value in stem and leaves. *Luzula albida* had 1.61 BCF value for Zn.

These means that these three grass species has high tolerance to Zn and good candidate species for Zn contaminated soils.

TF values for Zn were high for all grass species. *Poa nemoralis* had TF value of 0.46 for Zn.

Poa nemoralis although not good phytoextractor with a TF value >1.0, can be used in cleaning Zn contaminated soils. Grasses are fast growing and could produce high biomass and easy to harvest.

Phytoremediation of potential toxic elements is generally affected by plant factors specifically plant species. There are plant species that can tolerate high concentration levels of potential toxic elements. Based on the results of these research work the type plant species influence the amount of potential toxic elements it can accumulate in its body. *Carpinus betulus* can tolerate high concentration levels of Pb in soils, *Salix caprea* can tolerate high concentration levels of Cd and Zn, while *Betula pendula* is tolerant to high levels of Zn. Plant species preferentially absorbed some potential toxic elements but discriminately reject other potential toxic elements.

The second factor that affect the extent on the amount of potential toxic elements that accumulated inside the plant body is the mobility of potential toxic elements. Although there is high levels of Pb and As in soils under this study, the mobility of these potential toxic elements is lower compared to Cd and Zn as shown in Bio-concentration factor (BCF) of each element.

The third factor that greatly influence the extent to which plant species can accumulate potential toxic is the concentration of potential toxic elements present in the soil. The higher the concentration level of toxic elements in soil increases the amount of potential toxic elements inside the plant body. This is evident in some plant samples collected in uncontaminated forest soil as compared to plants collected in contaminated soil. However, some plant species showed tolerance to high concentration of potential toxic elements in soil.

Among the potential toxic elements Pb is most abundant in roots of *Carpinus betulus*, Zn and Cd inside the stem and leaves of *Salix caprea* and Zn inside the stem and leaves of *Betula pendula*. Similarly, high concentration of Zn were observed in the plant tissues of *Holcus lanatus*, *Poa nemoralis* and *Luzula albida.*

The other most important concern of this research work is how to handle the potential toxic elements in plant matter. The most economical way is not to harvest the plant and trees in the area. Let the trees and grasses stabilized the potential toxic elements particularly Pb in their root zones. The other suggested treatments based on literature were heat treatment, extraction treatment, microbial treatment, the used of compressed landfill and synthesis of nano-materials (LIU and TRAN, 2021). Heat treatment includes incineration, pyrolysis and gasification (CUI, et al. 2021).

5.0 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.

Holcus lanatus, *Poa nemoralis* and *Luzula albida* had BCF value of more than 1.0 for Zn in their roots. These species are good for phytostabilization of Zn. Poa nemoralis with TF value of >1.0 is good candidate for phytoextraction of Zn. Among grass species *Poa nemoralis* is a potential phytoextractor of Pb. However, all grass species cannot be considered as hyperaccumulator, since their uptake is less 1,000 mg kg^{-1} dry wt. of Pb and less than 10,000 mg kg^{-1} dry wt. 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 contaminated soil ecosystem contaminated with potential toxic elements.

6.0 NEW SCIENTIFIC RESULTS

This research work evaluated the phyto extracting ability for potential toxic elements endemic tree and grass species predominantly growing in an abandoned mining spoil sites in Mátra Mountains, Hungary. The tree species studied were *Betula pendula* (Birch), *Carpinus betulus* (Hornbean), *Fagus sylvatica* (Beech), and *Salix caprea* (Goat willow). Grass species that were collected were *Holcus lanatus, Poa augustifolia, Dactylis glomerata, Arrhenatherum elatius* and *Poa nemoralis*. One rush species were also observed which was *Luzula albida*.

Plant soil samples collected in the field and analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-OES) method.

This research work results showed that 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. The concentration 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.

Based on the results of plant tissue analysis, 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. This showed that *Carpinus betulus* is a good candidate for phytostabilization of Pb for contaminated soils.

***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.**

Among the grasses evaluated, *Holcus lanatus*, *Poa nemoralis* and *Luzula albida* had high BCF value (>1) and are potential grass species for phytostabilization of Zn while *Poa nemoralis* is good candidate for phytoextraction of Pb and Zn with Tf value greater than 1.0. However, all grass species cannot be considered as hyperaccumulator for As, Cd, Cu, Pb and Zn since their Tf value is less than 10.

7.0 LIST OF PUBLICATIONS

1.0 Poster Presentation

“Philippine Water Resources: Issues and Challenges” presented in International Water Day Conference, March 22,2018; Szent Istvan University, Szarvas, Hungary

2.0 Paper Presented:

Opena JL., GE Halasz (2018). Assessment of plant species on copper contaminated soils as potential phytoremediators. 5th VUA Youth Scientific Session, 20 November,2018. SZIE, Godollo, Hungary.

Opena JL, (2019***).*** Growth and drought resistance of *Swietenia macrophylla* (King) as affected by arbuscular mycorrhizal fungi presented in International Water Day Conference, March 22,2019; Szent Istvan University, Szarvas, Hungary

Opeńa, JL**.** Halász Gábor Endre, Horváth Márk, Árgyelán József Tibor. Phytoremediation of Potential Toxic Elements by Native Tree Species in Mined- Spoiled Soils in Mátraszentimre, Hungary International Symposium on Biosphere & Environmental Safety: online conference on the 5-6t h of May, 2022: Budapest' Hungary

3.0 Publications

- Opena JL., GE Halasz (2018). Assessment of plant species on copper contaminated soils as potential phytoremediators. 5th VUA Youth Scientific Session, Conference Proceedings, (ISBN: 978-963-269-788-8) pp.130-135
- Opena JL, (2019***).*** Growth and drought resistance of *Swietenia macrophylla* (King) as affected by arbuscular mycorrhizal fungi presented in International Water Day Conference, March 22,2020; Szent Istvan University, Szarvas, Hungary (ISBN-978-963-269-892-2) pp 96-102
- Jovito L. OPENA, Gábor Endre HALÁSZ Phytoremediation of Potential Toxic Elements by Tree Species in Abandoned Mining Sites. Abstract Book -19th Alps Adria Scientific Workshop Wisła, Poland, 29.04

- Opena JL, (2019***).*** Growth and drought resistance of *Swietenia macrophylla* (King) as affected by arbuscular mycorrhizal fungi presented in International Water Day Conference, March 22,2019; Szent Istvan University, Szarvas, Hungary ISBN 978-963-269-892-2 Szarvas, 2020, pp 93-104

Peer Reviewed

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4.0 Citations:

The above Scopus Index paper was cited by:

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