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

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LIST OF ABBREVIATIONS

Potential Toxic Elements

As – Arsenic

Ca - Calcium

Cd - Cadmium

Cr - Chromium

Cu - Copper

Fe - Iron

Hg - Mercury

Mn - Manganese

Ni - Nickel

Pb – Lead

Se- Selenium

Zn - Zinc

Measurements

mg - milligram

g - gram

kg - kilogram

mm – millimetre

nm-nanometer

cm - centimeter

ha – hectare

wt - weight

bdl- below detection limits

Chemical Compounds

HNO_3 - Nitric Acid

H_2O_2 - Hydrogen peroxide

Other:

BCF- Bio-concentration Factor

Tf- Translocation Factor

1.0 INTRODUCTION

Ecological restoration after an enormous anthropogenic disturbance like mining operation is a difficult task. Mining creates negative impacts in micro-climatic environment and metal waste in the soil ecosystem laden with high amounts of potential toxic elements (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 (KRAEMER 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 tasks 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 LITERATURE OVERVIEW

2.1 Potential Toxic Elements As Environmental Pollution

The existence of potential toxic elements in soil and water environment above its normal level is generally a direct result of human activities. Anthropogenic activities like fossil fuel energy production, mining, utilization of municipal sewage sludge in agricultural farms, herbicides and pesticides application, the unlimited usage of inorganic fertilizers pollutes the soil (KABATA-PENDIAS and PENDIAS 2001, LASAT 1999, VASSILEV et al. 2004).

The most common types of potential toxic elements were As. Cd, Cu, Hg, Mn, Ni, Pb and Zn. Heavy metals refers to a series of metals with an atomic mass greater than 20 and specific gravity of more than 5.0 g cm^{-3}. (LIU et al. 2013, ACKOVA 2018). Potential toxic elements cannot be degraded organically by microbial organisms or biologically destroyed but can be transformed from one oxidation state to another (ZACCHINI et al. 2009). Some microorganism like bacteria and mycorrhiza living in the root zone could change metal speciation but not able to biologically degrade the metal (VASSILEV et al. 2004). Thus, it persists in soils for a long period of time but it is possible to alter or stabilize their chemical form and to change their solubility in water and hence, availability to plants (GARBISU et al. 2001, LIU et al. 2013).

Elevated concentrations of potential toxic elements in soils may result to phytotoxicity and consequently give direct hazards to human health (PULFORD et al. 2002). It could later result in exacerbated effects when it enters the food chain via ground and surface water contamination (EVANGELOU et al. 2013, PULFORD 2006).

Restoration of the damaged ecosystems as a direct result of potential toxic elements contamination is one of the most difficult or recalcitrant task (DOBSON and BAKER 1997). Conventional or traditional method of remediation of toxic element contaminated soils is uneconomical since it is labor and capital intensive (LASAT 1999, ALKORTA et al.2004). There are many efforts done by government agencies and private companies to reduce the concentrations of potential toxic elements in soil and water environment. The processes involved in the removal of potential toxic elements from polluted soils do not mean to eliminate all toxic elements but to decrease its concentration to an acceptable level based on future use (PULFORD and WATSON, 2003). Accepted technologies have been developed for the cleaning of potential toxic elements but often these are economically expensive or have some negative deleterious effects (RASKIN et al. 1997). Soil washing techniques to remove potential toxic elements from the soil have detrimental effects on the physical and biological properties (PULFORD and WATSON, 2003). Physical and chemical methods of soil toxic elements decontamination of polluted soil ecosystems damaged soil structure and could result the soil to be biologically dead (MC GRATH et al. 2001, DICKINSON and PULFORD 2005). The waste products of soil washing which contain high potential toxic elements content also requires another careful disposal (PULFORD and WATSON, 2003). Engineering based technologies are typical method to clean up polluted soils. The technology normally excavates the soil and disposed it to landfills. This method does not alleviate the environmental hazards of potential toxic elements. The pollution problem is simply relocated to different space and time (SALT et al. 1995). Technologies like soil excavation and transport and later dumping in designated areas will are also prohibitively expensive (SALT et al. 1998). In US, the estimated cost is 1M US 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).

This large economic cost associated with cleaning the area from potential toxic elements apparently resulted that most mining companies to just ignore the problem.

2.2 Phytoremediation

One of the emerging technologies that is environmentally acceptable in cleaning the soil and water pollutants is phytoremediation. Phytoremediation is the use of plants to extract or sequester potential toxic elements from contaminated soil or polluted water ecosystem (SALT et al. 1995, LASAT et al. 1995, SIMON et al. 2006). It is the use of photosynthetic plants to remove environmental contaminants from the environment to render it harmless (ZACCHINI et al. 2009). It is non-intrusive, less damaging, and relatively inexpensive way of remediating polluted soils (LASAT 1999, GONZÁLEZ-OREJA et al. 2008). Phytoremediation aside from its lower costs were able to generate recyclable materials, applicable to a wider range of toxic elements, has minimum ecological disturbance and high social acceptability (ENSLEY et al. 1997). Phytoremediation requires simple non labor intensive technology and does not require establishment of special techniques to implement. The process can be managed to a wide extent where other techniques prove to be very expensive and inefficient.

GLASS (1999) estimated that the total cost for phytoremediation technology is 50% to 80% lower than engineering method. SALT et al. (1995), estimated that using phytoextraction technologies, to clean contaminated soil at 50 cm soil depth will cost 60,000-100,000 US dollars per acre, much cheaper using excavation and storage technology that will cost at least 1 Million US dollars per acre (RASKIN et al. 1997, SALT et al. 1998). The phytoextraction of heavy metals represents one of the largest economic opportunities for phytoremediation because of the size and scope of environmental problems associated with metal contaminated soils and the competitive advantage offered by plant-based remediation technology (RASKIN et al. 1997).

Phytoremediation as a natural, aesthetically acceptable technology acknowledge by human entities and regulatory agencies, as potentially and highly promising green technology (PAJEVIC et al.

2016). Phytoremediation limits landscape alteration and preserves the ecosystem. Phytoremediation could improve ecological landscape and create a more healthful soil environment. WEIR and DOTY (2016), highlighted the interdisciplinary considerations in the utilization of phytoremediation as a technology. KOELMEL et al. (2014) conducted a bibliometric analysis that provided a global review of research and applications in phytoremediation, underscoring the increasing interest and investment in this field, particularly research studies concerning hyperaccumulator plants. FARRAJI et al. (2020), also emphasized the green technology of phytoremediation for improving both aquatic and terrestrial environments showing the broad scope of applications for hyperaccumulator plants. In addition, NAEEM et al. (2020), presented a comprehensive review of phytoremediation technologies, including phytoextraction and rhizofiltration, underscoring the diverse strategies involving hyperaccumulator plants. ASHRAFI et al. (2011), furthermore, investigated the molecular aspects of potential toxic elements transporters enabling organisms to cope with toxic elements pollution.

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.

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. Several research works showed that Cadmium and Zinc are more mobile in the plant root system as compared to Copper and Lead.

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. However, some plant species showed tolerance to high concentration of potential toxic elements in soil. Phytoremediation process utilizes complex collections of phyto technologies that employs both natural as well as genetically modified plant species for extraction of potential toxic elements in the environment (KUSHWAHA, et al. (2018).

There are different types of phytoremediation system in the rhizosphere. It could be phyto extraction, phyto stabilization, rhizofiltration and phyto volatilization.

2.2.1 Phytoextraction

Phytoextraction is the general technique of using plant tolerant species or plant hyper accumulators for the absorption of potential toxic elements from the soil and water environments (MARCHIO et al. 2004, DICKISON and PULFORD 2005). This technology generally used plants classified as hyper accumulators or metallophytes.

For the last two decades, research works have identified several plant species that could be explored for phytoextration of potential toxic elements. These plant species that generally grow in ultramafic soils that develop tolerance to potential toxic elements (BAKER and BROOKS, 1989). Hyper accumulators were defined as plants commonly grown on metalliferous soils and able to complete its life cycle without showing any toxicity symptoms to potential toxic elements. Majority of hyper accumulators are rare species and could be found growing in specific geographical locations (PULFORD 2006). Tolerance and avoidance is the process where plant utilizes root cells to restrict and limit the uptake and movement of potential toxic elements into the plant tissues (DALVI and BHALERAO, 2013).

Most of the plant accumulators of potential toxic elements were discovered in the tropical soils (ENSLEY et al. 1997).

Metallophytes or hyper accumulators are plants that have the capacity to survive on metal rich laden soils. Some known endemic plants were considered as hyper accumulators. BAKER et al (1989*)*, stated that the high biomass production and the accumulation of target metals in harvestable organs is critical in efficient phytoremediation.

A certain species plant was classified to be as hyper accumulators, if plant species should be able to accumulate above 100 mg/kg dry weight of Cd, more than 1000 mg/kg dry weight of Ni, Cu, Co, Pb and more than 10,000 mg/kg dry weight of Mn and Zn (BAKER and REEVES, 1988).

The most studied as hyperaccumulators were *Thlaspi caeruluscens* for for Cd and Zn, *Thlaspi rotundifolia* for Pb and Zn (BAKER and REEVES, 1988), *Pteris vittata* for As (MA et al. 2001), and *Brassica juncea*, the species which has the greatest potential for absorption of Pb and Zn (GISBERT et al. 2006). *Sebertia acuminata*, a tree that grow in New Caledonia is a phyto accumulator of Ni (BAKER and BROOKS, 1989). *Allysum murale* can accumulate 3% Ni in its leaf biomass (KRAEMER et al 1996). *Allysum montanum* is reported to have increase of free histidine in proportion to its capacity to absorb nickel. TORRE et al. (2018), elucidated the role of ferrofortins in nickel hyper accummulators. FERNANDO et al. (2014*)* found out that *Rinorea niccolifera* a hyperaccumulating shrub in Luzon Island, Philippines can accumulate more than 18,000 mg kg dry weight of Ni in its leaf tissues. *Pitygramma calomenalos* a fern species is a phytoremediator of As (VISOOTIVISETH et al. 2002) from a study conducted in Thailand.

They are plants which naturally contain very high concentration of specific metals. They were also termed as green "liver" of the earth. (GONZÁLEZ-OREJA et al. 2008, EVANGELOU et al. 2013). *Larrea tridentata*, creosote bush species, growing around a heavy metal contaminated area was able to absorb and stored in its roots, stems, and leaves of 953 mg/kg, 493 mg/kg and 370 mg/kg of copper, respectively (GARDEA-TORRESDEY 1996).

In another study, CHANG and SHU (2015), reported that *Nicotiana tabacum* can absorb 277 mg kg^{-1} Pb in roots and 97 mg kg^{-1} Pb in shoots and leaves. *Carduus nutans* and *Phlomis* is reported to be good phytoremediator of mining soils contaminated by Cd (PALUTOGLU et al. 2018). *Nerium oleander* a tropical shrub is highly tolerant to Cu and Pb (LIU et al. 2013).

The capacity to remove potential toxic elements by plant species is generally measured using bioconcentration factor (BCF). Phytoextractor plants have genenarally high BCF index. The BCF values is highly dependent on the type of growth medium and selection of plant species. Hyperaccumulators usually grow roots in soil of high metal concentrations, having high concentration levels of uptake in root cell symplasm and reduced vacoular transport (MC NAIR, 2003). Hyperaccumulators have specific chracteristics such as BCF greater than 1, greater than 1 shoot root ratio of potential toxic elements and high tolerance to metals making them effective in detoxification of contaminated soils (PUSZ, et al 2021). Hyperaccumulators metallophytes can accumulate potential toxic elements in their upper biomass in concentrations between 100-500 times more other plants without negative effects on their development and physiological function (SHEORAM et al. 2016). It is further demonstrated that hyperaccumulators used in successful phytoextraction have high bioconcentration factor (BCF) and trandlocation factor (TF). These factors with increasing effect into epidermal or cortical cells, or export from pericycle or xylem parenchyma cells into stellar apoplast and converts the potential toxic elements into less harmful state (BAKER and BROOKS, 1989).

2.2.2 Phytostabilization

Phytostabilization is a technology which involves the utilization of plants to remediate polluted soils and water to reduce heavy metals mobility and toxicity within the root zone. It is the use of plants to reduce the bio- availability of pollutants in the environment according to ALKORTA et al. (2004). Phytostabilization involves the establishment of a plant cover on the surface of contaminated soils with potential toxic elements with the aim of reducing the mobility of the toxic elements within the vadose zone through accumulation of roots or immobilization within the rhizosphere (BOLAN et al. 2011).

Plants could also have associations with beneficial fungi or bacteria that reduce the bioavailability and neutralize the harmful effects of the potential toxic elements on the environment (LI et al. 2016). Phyto immobilization methods try to limit the solubility of metals in the soil and water environment. Addition of organic plant materials were also used for phytostabilization like rice hull which could reduce the bioavailability of Cu by 33% in soil (JEON, 2011). Rice straw biochar reduces the mobility of Zn by 39.33%, Pb by 27.64%, 23.54% of Cu, 23.25% of Cd (LU et al. 2017), and other organic ammendments. In Hungary, phytostabilization research works were also conducted. SIMON et al. (2012a), uses various soil ammendments that can lower the uptake of potential toxic elements. However, the addition of organic materials temporarily delayed the release of phytotoxic elements in soil but not totally removed it.

There were also scientific reports that root association with arbuscular mycorrhizal fungi decrease the mobility of heavy metals. GUO et al. (2013), reported that *Glomus visiforme* and *Glomus mosseae* decrease the uptake of potential toxic elements by trees in China. Arbuscular mycorrhizal fungi increase the rice tolerance to Cd (LI et al. 2016). It decreases Cd concentration in both shoots and roots of rice. *Glomus mosseae* have high affinity in the phytoextraction of Zn and stabilize in roots of trees (ALKORTA 2004). *Festuca rubra*, a grass species in association with *Glomus indrices* is recommended for phytostabilization (SIMON et al. 2006). Fungal sheath of mycorrhiza can function as barrier for toxic metals, (HAGEMEYER 1999).

EVANGELOU et al (2013), investigated that some tree species (poplar and willow) are more suitable for phytostabilization. Poplar and willow are better species for phytostabilization of Cu and Pb. Birch tree (*Betula pendula*) could be suitable for phytostabilization of Cd and Zn, but not for Cu and Pb (BOJARCZUK et al. 2002). Plants with low translocation factor (Tf) from root to shoot biomass are considered as good phytostabilizers of heavy metals. Phytostabilization is a way to avoid the transfer of potential toxic elements to other media specifically the food chain. Tree species for phytostabilization should not hyperaccumulate the concern potential toxic elements in harvestable tissues like stem and leaves (MENDEZ and MAIER 2008). In Australia, KING et al. (2008), observed that *Eucalyptus cladocalyx* is an ideal tree candidate for perennial stabilization of arsenic.

Elevated evapotranspiration using trees reduces the flow of water in the soil profile and therefore could reduce the amount of metal that leach from the soil to the ground and water surfaces (PULFORD and WATSON 2003).

2.2.3 Rhizofiltration

Rhizofiltration is another important technique in phytoremediation. It is the use of plants to absorb potential toxic elements in an aquaeous environment. The technology normally employed mature aquatic plants grown in a green house. After the root system is well developed, plants were placed in contaminated water environment. The plants in contaminated were made to absorb potential toxic elements until the roots is fully saturated at which point plant were harvested for disposal (ZHU et.al 1999). Rhizofiltration utilizes plant roots to acquire potential contaminants to prevent movement of potential toxic elements into another ecosystem (MIDHAT et al. 2019). *Arundo* *donax*, is reported to be very effective in rhizofiltration of a Bourdeux mix effluent (OUSTRIERE et al. 2017). The most common plant species employed for rhizofiltration of potential toxic elements were water hyacinth, *Azolla*, and duckweed, because of their bio accumulating capacity, high biomass production and high tolerance (HOODA, 2007). Duckweed (*Lemna minor*), an aquatic plant species is a phytoremediator of Cu and water velvet (*Azolla pinnata*) can uptake large amount of Fe and Cu (JAIN et al. 1989). Duckweed can bioaccumulate 15,000 mg kg^{-1} Cu in its plant tissues (ZAYED et al. 1998). PINTO et al. (1997), also demonstrated that water hyacinth (*Eichornia crassipes*) can uptake 8000 mg kg^{-1} of silver. *Eichornia crassipes, Salvinia molesta, and Pistia stratiotes* are promising aquatic plant species for rhizofiltration of industrial sewage in constructed wetlands (KODITUWAKKU, et al. 2020). The three aquatic species are good candidates in removing Cu, Cr, Cd, Ni, and Zn for industrial sewage sludge. The greatest challenge in rhizofiltration work is how to dispose the potential toxic elements in plant matter after it had bioaccumulated in plant tissues. 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). CUI et al. (2021) recommended heat treatment such as incineration, pyrolysis and gasification.

2.2.4 Phytovolatization

Phytovolatization is another type of phytoremediation technology applied to highly toxic elements like Hg. Plants take up contaminant toxic elements from the soil and eliminates it in the atmosphere through transpiration. Some plants have the capacity to convert metals into volatile chemical forms (BOLAN et al. 2011). In a study of FLECK et al. (1999), Hg in red pine (*Pinus resinosa*), these trees may used for the volatilization of Hg and Se. Hg is not derived from soil but from the atmosphere that enters the stomatal cells of pine trees. At present, there are less research work conducted with regards to this phytotechnology.

2.3 Limitations of Hyperaccumulors Used in Phytoextraction

Regrettably, most studied hyperaccumulators to be used for phytoextraction are of small size, have slow growth, and lack the necessary technology for their cultivation at large scale schemes. There are also limitations in understanding the biological and environmental factors involved in potential toxic elements accumulation.

Some plants can bioaccumulate higher concentations of potential toxic elements but they do not produce large enough biomass compared to trees. Phytoremediation capacities of *Salix viminalis* is five times higher than *Thlaspi caerulescens* or *Alyssum murale* due to high biomass production and transport of Cd to the shoot (GREGER and LANDBERG 1999). Metal uptake of trees per dry wt. basis is small, but on a per hectare basis is more effective in respect to hyperaccumulators due to high biomass production (GREGER and LANDBERG 1999). ENSLEY et al. (1997*)*, stated that to clean up one acre contaminated with Zn and Ni with sludge, it will take 13-14 years of continuous cultivation of *Thlaspi caerulescens.* The potential removal of 400 kg ha^{-1} of potential toxic elements require 100 tons per hectare of biomass (LASAT 1999).

The research emphasis has shifted on phytoextracting ability of high biomass plant species like trees that can be mass propagated, easily managed, with established silvicultural technologies.

The main criteria for the selection of plants with good phytoremediation potential are their potential to bioaccumulate pollutants and their ability to translocate them in their above ground harvestable biomass. Successful phytoextraction program can be achieved by the selection of the most suitable plant species for specific potential elements (GONZÁLEZ-OREJA et al. 2008).

2.4 Trees for Phytoremediation

Many researchers have suggested that using trees (compared to smaller plant species) as much suitable species. The use of trees to clean up polluted soil and water is termed as dendromediation. There are many advantages of using trees than some known plant species termed as hyperaccumulators. Some tree species are relatively tolerant to high concentration of the metals and can accumulate reasonably high quantities of metals. Trees have rapid growth rate and can produce notable biomass in the field with much important economic value (PULFORD and WATSON, 2003). Trees produce woody biomass for energy production. It is cleaner, cheaper, and more environmentally acceptable approach to energy production than coal and fossil fuel (DICKINSON 2000). Trees produced wood for paper production and charcoal.

Trees have deep and profuse root systems which are generally more suitable for phytostabilization than shallow rooted plant species (EVANGELOU et al. 2013) specifically in a hydrologically impaired soils. Shallow roots have less access to water and more likely suffer to drought that will impair its phytoremediation potential (VOGELEV et al. 2001). Basal tolerance of trees have higher coping ability and metal tolerance than agricultural crops (RIDDEL-BLACK 1993).

Among tree species mostly studied trees for phytoremediation were willows and poplar.

The use of trees as a vegetation cover for the phytoremediation of land contaminated by heavy metals does seem to have considerable potential. Trees can survive under these adverse conditions. The rate of growth may be less than on the uncontaminated sites.

2.4.1 Phytoremediation potential of Willows (*Salix spp*.)

One of the highly studied trees for phytoremediation are willows specifically *Salix viminalis*. It is most suited in soils high in Cd, Cu, Pb and Zn (HERMLE et al. 2006, BEDELL et al. 2009, MLECZEK et al., 2010). Willows produced high biomass, easy to propagate, fast growing, has deep root systems to reach the deeper soil horizons, and has the ability to uptake large amount of heavy metals.

Willows are not hyperaccumulators (DICKINSON and PULFORD 2005, ELTROP et al. 1991). Willows have massive growth potential and it can uptake large amount of potentially toxic elements. It is one of the desirable tree species if soil remediation is likely to be achieved. Willows can produce 10 to 15 tons of biomass per hectare in Sweden (GREGER and LANDBERG 1999). Thus, it is accepted is more efficient as phytoextractor compared to *Thlaspi caerulescens* and *Assylum murale,* two known plant species of hyperaccumulator (BAKER and BROOKS 1989). FISCHEROVA et al. (2006), have shown that *Salix dasyclados*, another willow species, can uptake Cd and Zn, similar to *Arabidopsis halleri* and *T. caerulescens.*

Willows are not proven to be suitable for rehabilitation of heavily contaminated soils but they could be effective in remediation of moderately contaminated soils. Increased uptake of Cd and deposited in lower stems and roots of *Salix viminalis* under clone condition results in Mg and Fe deficiency (BORISEV et al. 2016). This could be attributed to competition in the binding sites of metals between Cd, Mg and Fe (COSIO et al. 2006). Phytoextracting ability of willow for Cd in France could reach 200 mg kg^{-1} (ROBINSON et al. 2000). He further reported that 20 tons of biomass of willow could remove 1.06 kg per ha of Cd in New Zealand. It can also remove 5-27 kg per hectare of Zn (MEERS et al. 2007). Cadmium in ash of willow in a contaminated sites contain 10 times more Cd from ash of trees from normal forest (GREGER and LANDBERG 1999).

Variable results had been reported in the phytoextracting ability of Willows for Pb.

Lead is mostly retained in the root systems (HERMLE et al. 2006). GREGER and LANDBERG (1999) reported that willow can absorb 5.7 mg kg^{-1} of Cd and 22 mg kg^{-1} of Zn per dry wt. basis. KOPPONEN et al. (2001) also showed that willows can absorbed 20-60 mg kg $^{-1}$ of Cu. UNTERBRUNNER et al. (2007), reported that *Salix caprea* (Goat willow) can absorb 116 mg kg^{-1} Cd and 4680 mg kg^{-1} Zn. In another study, HAMMER et al. (2003), Salix can uptake 70 g of Cd per ha and 134 g of Zn per ha. Salix performed better in acidic soils than in calcareous soil.

Willows can be frequently harvested by coppicing. It has erect stems, rapid growth and good rooting ability (DICKINSON and PULFORD, 2005).

2.4.2 Poplar (*Populus alba*)

Poplar is another tree species popularly studied for phytoremediation. However, most of the research work done to determine the phyto extracting ability of poplar were limited to pot experiment, hydroponics, or under laboratory condition.

In a research work done by DI LONARDO et al. (2011) which investigated the phytoextraction ability of *Populus alba* for As, Cd, Cu, and Zn. He found out that the metal content was generally higher in roots than in shoots of poplar. Accumulation rates is around 125% to 200%.

MERTENS et al. (2007), illustrated that poplar have highest concentration of Cd and Zn in leaves compared to the stems. Poplar were able to uptake 1306.5 mg kg^{-1} Zn and 4 g mg kg^{-1} Cd. Other species like *Quercus robus* (Oak), *Fraxinus excelsior* (Ash), *Acer pseudoplanatus* (Maple) contain normal concentartions of Cd and Zn.

RAFATI et al. (2011) have also shown that *Populus alba* and *Morus alba* can be suitable for phytoextraction of Cd and Ni in a pot experiment conducted in Iran. *Populus deltoides* is a poplar species extensively used as reforestation species have shown high absorption of Cu (WANG et al 2015). *Populus alba* is also used for phytoremediation of riparian forests (MADEJON et al. 2004).

2.4.3 Birch (*Betula pendula*)

Betula pendula (birch tree) is good bio-indicator of heavy metals in contaminated environment of natural or of human origins (GJENGEDAL et STEINNER 1994, KOZLOV et al. 2000). Birch tree can uptake 11.8 mg/kg Cd in leaves (WISLOCKA et al. 2006). RIDDEL-BLACK (1993) demonstrated that *B. pendula* can bioaccumulate 20-60 mg kg of Cu in leaves. Cd concentration in leaves is 3-5 times higher in leaves as compared to stems. Birch grown in calcareous soils has the highest uptake of Zn and Cu (HERMLE et al. 2006). A tree species, *Betula alnoides* is highly suggested species for the reforestation of mining sites with high levels of Pb and Zn (WANG et al. 2015). It can uptake 1.8 mg kg^{-1} Pb and 3.7 mg kg^{-1} Zn.

In an experimental study of EVANGELOU et al. (2013) the highest Pb accumulation occurred in in birch with up to 135 mg kg^{-1} dry weight in wood and 75 mg kg^{-1} in foliage. ROSSELLI et al. 2003, in a pot experiment reported that *Betula* transferred higher concentrations of zinc and cadmium in their leaves and twigs compared to *Alnus*, *Fraxinus* and *Sorbus* which excluded them in their above ground biomass. *Betula* is also known to highly bioaccumulate high concentration of Zn, Cd, and Pb in their leaves (BORGEGARD and RYDIN 1989).

2.4.4 Other Tree Species for Phytoremediation

Eucalyptus camaldulensis, a known reforestation species is also a potential tree species for phytoremediation (PIETRINI et al. 2015). *Eucalyptus camaldulensis* is a tree suitable for phytoextraction of Pb and Zn. ASSAREH et al. (2008), observed that *Eucalyptus camaldulensis* can uptake 297. 8 mg kg^{-1} Cu and 253.0 mg kg^{-1} Zn. It exhibited high tolerance to Al (SILVA et al. 2004). They also reported that *Acacia mangium* showed greater tolerance to Al. *Pinus sylvestris* is a temperate tree for phytoremediation of Zn and Cu (UCUN et al. 2009). They reported that this tree can absorb 67% of Cu in soil. *Alnus acuminata* is used for phytoremediation of Cr and Pb in South America (ESCOBAR and DUSCAN 2016). Similarly, Scots pine is reported to be good in phytoextraction of Cu in Southwestern Finland (HELMISAARI et al. 1995). *Eucalyptus globus* is used for the phytoremediation of Pb according to GERARDO and KIKUCHI (2009).

Picea abies is highly suited for reclamation of calcareous soil contaminated with Zn accompanied by Cd, Cu, and Pb (HERMLE et al. 2006).

Alnus nepalensis can uptake 4.7 mg kg^{-1} Pb and 6.72 mg kg^{-1} Zn (WANG et al. 2015). In Peru, COATES (2005), observed that *Tamarix asphylla* and *Acacia saligna* have high tolerance to Cu.

Vitex parviflora, *Samanea saman*, and *Pongamia pinnata* , three native trees in Marinduque Island Philippines, was used in pot experiment, can uptake 1776, 953, 1219 mg kg^{-1} of Cu (TULOD et al. 2012). However, the results should be further verified in field experiments. ZUPUNSKI et al. (2015), showed that black locust is potential tree candidate for phytoremediation of Cd and Ni. It can absorb 5.73 mg kg^{-1} Cd and 1442.93 mg kg^{-1} Ni. Black locust had shown high tolerance index to toxic elements. Similarly, *Paulownia fortunni* accumulated 1179 mg kg^{-1} Pb (AZARELLO et al. 2012), *Broussonetia papyrifera* accumulated 973.3 mg kg^{-1} Pb, and *Zenia insigni* can uptake 1968 mg kg^{-1} of Zn (ZHAO et al. 2013). A mangrove tree species (*Rhizopora mucronata*) can absorb high levels of Mn. KAEWTUBTIM et al. (2016), reported that this tree species can absorb 257.8 mg kg^{-1}, 255.4 mg kg^{-1}, 230 mg kg^{-1} of Mn in shoots, leaf and roots, respectively. HOFFMAN et al. (2003) reported that *Phyllanthus balgooyi*, growing in ultramatic sites in Palawan Island, Philippines and Sabah, Malaysia contains 16,000 mg kg^{-1} or 1.6 % of Ni. It exudes jade-green sap and contain 90,000 mg kg^{-1} of Ni in phloem tissues. *Phyllanthus securinegoides* can uptake 34750 mg kg^{-1} Ni, according to REEVES and BAKER (1995). This tree can be found in Mindanao Island Philippines. NKRUMAH et al. (2018), highlighted the contrasting hyperaccumulation nickel and zinc in subspecies of *Dichapetalum gelanoides,* shedding light on the specific mechanisms of hyperaccumulation in different plant species. *Dichapetalum gelanoidesb* from Palawan Island, Philippines could accumulate 10732 mg kg^{-1} of Ni inside their plant tissues. *Dichapetalum gelanoides sub species tuberculatum* could hyperaccumulate 30260 mg kg^{-1} of Ni, a potential plant species for phytomining.

Mahogany (*Swietenia macrophylla*), a timber producing tree, can uptake 154 mg kg^{-1} Cd as demonstrated by FAN et al. (2011), in a pot experiment in China. *Quercus robus* (Oak tree) is used for phytoremediation of Pb and Zn (WISNIEWSKI et DICKINSON 2003, SHI et al. 2016).

2.5 Grasses for Phytoremediation

Some grass species were also identified as hyper accumulators. In a similar research work conducted in Australia, three grass species were identified as potential use species for phytostabilization. ARCHER et CALDWELL (2004), selected *Cynodon dactylon* (couch), *Juncus usitatus* (Common rush), *Lomandra longifolia* (spiny-headed mat rush), for these three plant species can bioaccumulate high levels of lead (Pb) in their harvestable plant tissues. *Cynodon* *dactylon* (couch), *Juncus usitatus* (Common rush), *Lomandra longifolia* (spiny-headed mat rush), can uptake 723 mg kg^{-1}Pb, 864 mg kg^{-1} Pb, and 302 mg kg^{-1} Pb, respectively.

In China, *Vetiver,* a grass a highly utilized grass species for erosion control could remediate Cd polluted soils (CHEN et al., 2004). In Japan, *Crotolaria juncea* – a tropical weed species was discovered to be a phytoextractor of Cu (URUGACHI et al. 2006). Plants that have high capacity to absorb Cu has also high content of metallothioneine in their plant tissues (ROBINSON et al. 1993).

Another research work in Hungary, reed (*Phragmatis australis*) are potential phytoextractor of Cr (KERESZTURI et al. 2001). Reed as reported by AIT ALI et al. (2004), can absorb 287.5-1193 mg kg^{-1} Cd, 849-1554 mg kg^{-1} Cu and 166-915 mg kg^{-1} Zn. *Holcus lanatus* is a grass species that is highly tolerant to As (MEHARG et al. 1993). *Commelina diffusa* is a good phytoextractor of Cu (WANG et al. 2004). In another research work, *Pennisetum purpureum* (Napier) has showed to be a good grass species for phyto accumulation of Cd (YANG et al. (2020). In another related research work in China, YIN et al. (2016), in a field experiment had reported that *Solanum nigrum* is a tolerant plant species in cadmium contaminated soils.

2.6 Tree Species Examined

2.6.1 *Betula pendula* (Birch tree)

Betula pendula or Birch tree is a pioneer tree species and native to Europe. It is fast growing tree, medium sized, deciduous tree growing up to 20 meters.

Figure 1. *Betula pendula*

2.6.2 *Caprinus betulus*

Carpinus betulus is the European or common hornbeam is species of tree in the birch family Betulaceae. It is native to Southern Europe and Western Asia. This grows up to 60 ft. It grows slowly, shallow rooted, deciduous, and commonly used as hedgerows.

https://www.boethingtreeland.com/plants/carpinus-betulus-fastigiata/

Figure 2. *Carpinus betulus*

2.6.3 *Fagus sylvatica*

Fagus sylvatica (European beech) is a common beech belongs to Family Fagaceae. It is a drought tolerant tree. It has simple leaves, alternately arranged with oval shaped crowns.

https://www.boethingtreeland.com/plants/fagus)

Figure 3. *Fagus sylvatica*

2.6.4 *Salix caprea*

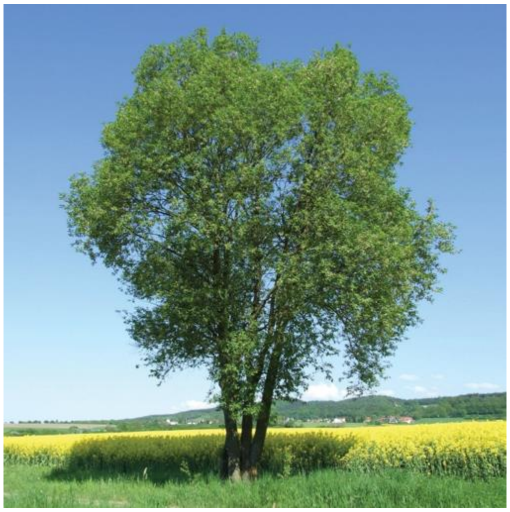


Figure 4. *Salix caprea*

https://www.boethingtreeland.com/plants/Salix caprea/

2.7 Grasses Species Examined

2.7.1 *Holcus lanatus*

Holcus lanatus (Velvet grass or meadow soft grass) is a perennial plant belonging to Family Poaceae. It is a wooly plant with hairy plant's texture.

Figure 5. *Holcus lanatus*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Holcus_lanatus

2.7.2 *Poa augustifolia*

Poa augustifolia (blue grass) is a narrow leaved meadow grass. It is native to Europe and grows 50-70 cm in height. Its basal leaf blades has wing bristle structure and flowers from April to August.

Figure 6. *Poa augusttifolia*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Poa augustifolia/

2.7.3 *Dactylis glomerata*

Dactylis glomerata (cock's foot grass or cat grass) is a species of flowering plant under family Poaceae. It is a cool season season perennial grass tufted grass native to Europe and tremperate Asia. It grows from 20 -160 cm and it has flattened stem base which distinguishes it from other grasses.

Figure 7. *Dactylis glomerata*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Dactylis glomerata/

2.7.4 *Arrhenatherum elatius*

Arrhenatherum elatius (false oat grass or tuber oat grass) is a perennial grass species under family Poaceae. It is native to Europe and Western Asia. It is a tall erect tussock forming perennial grass.

Figure 8. *Arrhenatherum elatius*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Arrhenatherum elatius

2.7.5 *Poa nemoralis*

Poa nemoralis (the wood blue grass) is a perennial plant under family Poaceae. It grows from 30-60 cm ad grenerally flowers from June to August. It is native to Europe.

Figure 9. *Poa nemoralis*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Poa nemoralis

2.7.6 *Luzula albida*

Luzula albida (Wood rush) is a perennial plant species belonging to family Juncaceae. It is a perennial and grows primarily in temperate biomes. It grows from 40-70 cm tall, grows in clumps with cylindrical stems and narrow leaves.

Figure 10. *Luzula albida*

https://upload.wikimedia.org/wikipedia/commons/4/4c/Luzula albida

2.8 Limitations of Trees As Phytoextractor

Although trees have high potential as phytoextractors there were also some limitations. The major limitation of using trees as phytoremediator is that majority of trees are not classified as hyperaccumulator (GREGER and LANDBERG 1999). One hectare planted with willow that produce 20 tons of biomass per year could only remove 1.06 kg of Cd per ha. (GREGER and LANDBERG 1999). They also reported that decontamination of Cd polluted soils will take 12 years to remove the Cd accumulated in Sweden soils. Trees absorb relatively small amount of toxic elements in its biomass. Some trees are selective in the uptake of heavy metals. FELIX (1997), calculated that it will take 77 years for a hybrid willow to reduce Cd to an acceptable level of 0.8 mg kg^{-1} Cd. This species of willow could remove 0.22 mg kg^{-1} Cd per hectare per year. Aside from these, there are also some minor limitations of trees as phytoremediator.

Another limitation of trees is some species have low translocation factor (Tf). Metals can be complex and sequestered in vacuoles that inhibits the transfer to upper harvestable parts (LASAT, 1999). Translocation factor is the ratio between concentration of heavy metals between the shoot (leaves + stems) and the root (ZAYED et al. 1998). STANKOVIC and DEVETAKOVIC (2016), reported that Pb, Cr, and Cu are usually immobilized in roots while Cd, Ni and Zn can move easily to harvestable parts.

Tree species have different ability to translocate heavy metals from root to shoot. Willows are good phytoextractor of Cd and Zn but not for As contaminated sites. It can translocate large amount of Cd and Zn from roots to above ground biomass while Cu and Pb are predominantly stored in roots (HERMLE et al. 2006, EVANGELOU et al. 2013). In an experiment conducted by

ROSSELLI et al. (2003), results showed that *Salix* and *Betula spp* transferred Cd and Zn to harvestable parts (leaves and twigs) while *Alnus*, *Fraxinus* and *Sorbus* excluded these metals in above biomass. These showed that trees have some degree of specificity in the translocation of heavy metals. Poplar has shown poor translocation factor of Cd to its harvestable parts. Phytochelations and metallothionines formation in plants also helps in detoxifying heavily contaminated soils (COBBETT and GOLDSBROUGH 2002).

High level of toxicity of heavy metals limits tree growth. High concentration of heavy metals has shown to inhibit growth and establishment of trees in highly contaminated soils specifically in mining spoil wastes. Several physiological and biochemical processes in plants can be severely affected by these metals, however, their toxicity varies with plant species, and the chemical form and concentration of the element (ACKOVA, 2018). Absorbed metal can be loaded to xylem vessels and binded by amino acids to be transported to shoots (ZACCHINI et al. 2009). PIETRENI et al. (2015), reported that too much Cd destroyed the thylakoids limiting the photosynthetic potential of trees. Cadmium toxicity results in chlorosis, growth inhibition and reduction in photosynthesis in willows and Poplar (ZACCHINI et al. 2009). Sites with high metal contamination can cause toxicity symptoms that significantly reduced biomass production (CHANEY et al. 1997). Metal toxicity displaced absorption of essential nutrients. Ni can displace Mg and Ca is displaced by Cd (ZUPUNSKI et al. 2015).

Willows are reported to be more tolerant to Cd when compared to poplar (ZACCHINI et al. 2009). Cadmium toxicity inhibits root length compared to other metals as treatment to *Salix viminalis* (PRASAD 1995). There is 10% growth reduction in growth of Salix if Cd concentration was increased into 10-20 mg kg^{-1} in soil. Excessive amount of Cd limits the growth of *Eucalyptus* *camaldulensis* and results to chlorosis (PIETRINI et al. 2015). Excess Cd and Cu causes chlorosis while excess in Zn, results in loss of turgor pressure in plants (HAGEMEYER 1999).

TRIPATHI et al. (1999), found out that leaf area and biomass production of *Albizzia lebbek* seedlings were negatively affected by Cr (VI). It also resulted to necrosis in leaves. Similarly, leaf area and dry weight biomass of *Leucaena leucocephala* treated with tannery effluent were significantly reduced (KARUNYA et al. 1994). High levels of Cr in soils reduced N levels in plant tissues of oats (ZAYED et al.1998). Zinc toxicity in most trees is 141 mg kg^{-1} Zn that significantly reduced leaf and root dry weight (LIU et al. 2013).

Low mobility of heavy metals or bioavailability is other limitation of phytoextraction using trees.

Other threats of using trees as phytoextractor were poor soil physical and chemical properties in the mining sites, long duration process of phytoextraction compared to excavation and dumping, and danger of transfer of contaminants to other members of the food chain like herbivores. The other limitations that inhibit trees to exhibit its full potential as phytoextractor were the poor soil physical and chemical conditions in the soil contaminated sites. MOFATT et al. (2001), identified these limitations as macronutrient deficiencies, poor water holding capacity, N deficiency, low organic matter, high soil acidity and salinity.

Phytoextraction using trees involves long time period. RIDDLE-BLACK (1993), calculated that willows can clean Cd in Swedish contaminated soils will take 20 to 25 years before reducing the Cd below the natural levels. A soil containing 5 mg kg^{-1} Cd in 100 mm depth contains 6 kg of Cd per ha, a four cropping of willow is required that will reduce Cd burden to 2.35 mg kg^{-1} below the threshold level of 3.0 mg kg^{-1}. Biomass production of 20 tons per ha could only remove 1.06 kg of Cd per year (ROBINSON et al. 2000).

Aside from its phytoextracting ability, willows are generally tree species utilized to clean municipal waste water and sludge, hence the risk of transfer of contaminants could possibly increase. Consumption of plant parts such as fruits and leaves by animals and humans could result in some risks (EVANGELOU et al. 2013). TURNER and DICKINSON (1993), reported that sycamore trees grown in contaminated soil, most Pb are not retain in roots and was translocated to stem. Most Pb are stored in leaves. This phenomenon poses risk for the transfer of heavy metals to food chain. Phytostabilizing tree species is suggested to be planted in metal rich soils. Tree species with fruits and leaves not palatable to wildlife is considered.

Aside from its beneficial uses as phytoremediator, there are other benefits that can be derived in using trees. Trees have long term values because trees can remain healthy for many years even growing in highly contaminated soils. Trees can easily ameliorate damaged land ecosystems. Trees can be mixed with high other absorbing plant species (WIESHAMMER et al. 2007). Phytoextraction of Cd and Zn from agricultural soils were implemented by intercropping willow (*Salix caprea*) and phytoextractor *Arabidopsis halleri*. Willows were found to absorbed 2020 mg kg^{-1} of Zn (WIESHAMMER et al. 2007).

Thus, association with other hyperaccumulators will hasten the removal of toxic elements. Trees improve soil environment by addition of organic matter. Increased soil water retention and nutrient availability (WANG et al. 2015).

3.0 MATERIALS AND METHODS

3.1 Study Area

This research work was conducted in the abandoned mined spoil dumping site located in Mátraszentimre, Heves county, Hungary (**Figure 11**). 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).

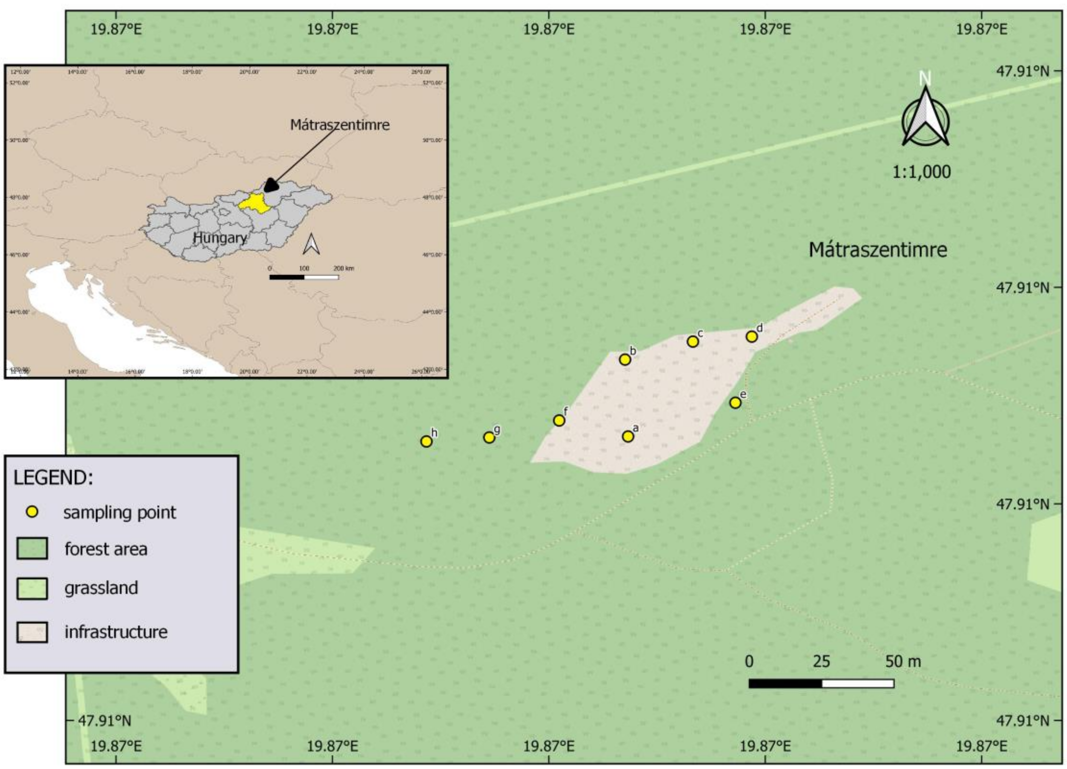


Figure 11. Sampling Area (Mátraszentimre, Mátra Mountains, Hungary), maps taken from Google Earth.

3.2 Soil Sampling and Analysis

Soil samples were collected in eight sampling points along the main dump sites (**Figure 11**) 17th June, 2019. The coordinates of sampling points were summarized in **Table 1**. 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.

Table 1. Coordinates of points where soil and plant samples were collected in Mátraszentimre, Mátra Mountains, Hungary

Sampling Points	Longitude	Latitude
A	19.86937	47.90731
B	19.86935	47.90767
C	19.86967	47.90775
D	19.86994	47.90777
E	19.86986	47.90747
F	19.86905	47.90738
G	19.86873	47.90731
H	19.86843	47.90729

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). The wave length used for Cd was 228.802 nm, 324. 754 nm for Cu, 220.353 nm for Pb, and 213.857 for Zn, respectively. This is the standard protocol for environmental soil test for determination of total and soluble toxic elements, heavy metals, and chromium (VI).

3.3 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).

3.4 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 written in Equation 1 (ZAYED et al. 1998)

$$[1]\ \text{BCF}=\frac{\text{Concentration of potential toxic elements in plant tissues}}{\text{Concentration of potential toxic elements in soil}}$$

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.

3.5 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 written in Equation 2 (ZAYED et al. 1998). It measures the mobility of potential toxic elements from roots to harvestable organs.

$$[2]\ \text{TF}=\frac{\text{Concentration of potential toxic elements in stem} + \text{leaves}}{\text{Concentration of potential toxic elements in roots}}$$

3.6 Statistical Analysis

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

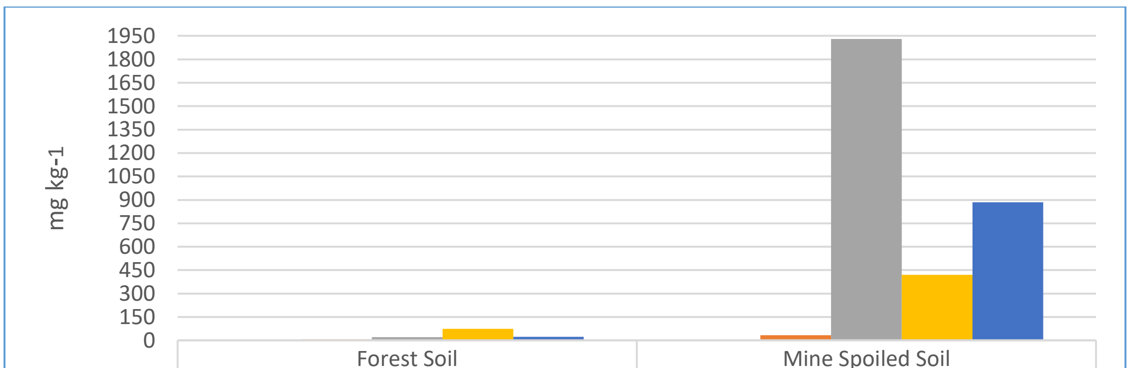
Analysis of Variance (ANOVA) was done to determine if there are significant differences in the absorption capacity of potential toxic elements among tree species. Analysis of Variance were not implemented for grasses due to limited plant samples. Differences were considered significant if the value of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

4.0 RESULTS AND THEIR DISCUSSION

4.1 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 12**, **Appendix Table 1 and Appendix Table 2, resp.**). 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

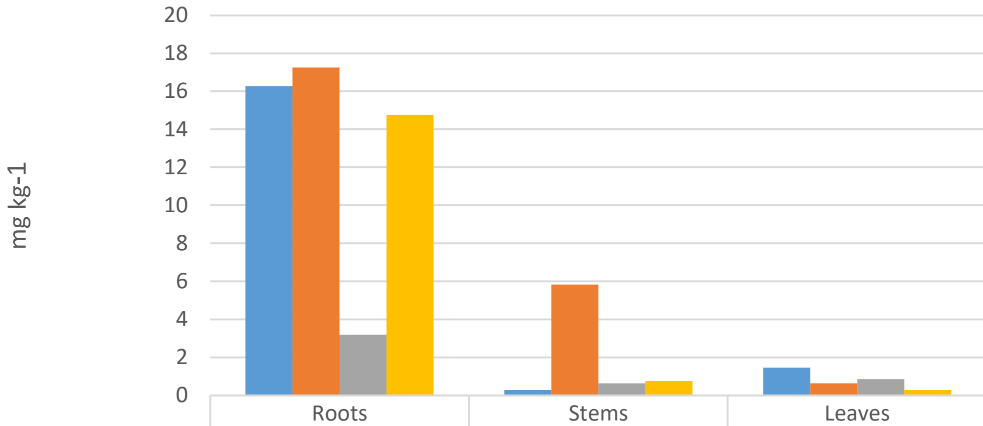
Figure 12. Concentrations of potential toxic (mg kg^{-1}) elements in mined –spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

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.

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

4.2.1 Arsenic.

Total As in different tree species were not significantly different (**Figure 13 and Appendix Tables 3-6, resp.**) 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.

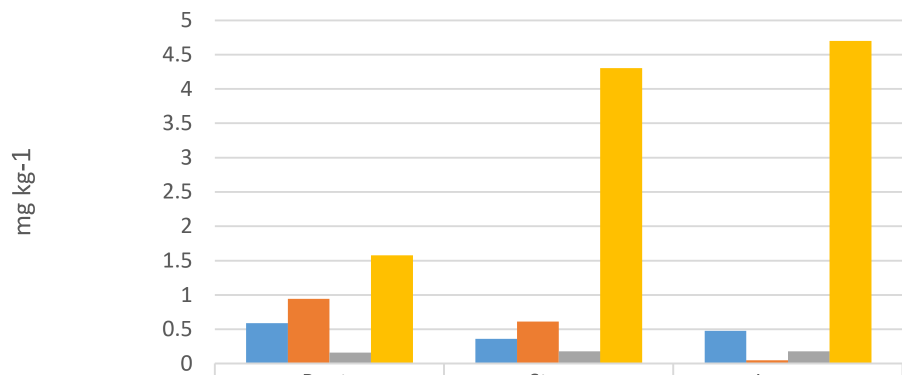


	Roots	Stems	Leaves
Betula pendula	16.274	0.283	1.464
Carpinus betulus	17.247	5.833	0.627
Fagus sylvatica	3.187	0.633	0.843
Salix caprea	14.755	0.749	0.285

Figure 13. 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.

4.2.2 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 14 and Appendix Tables 7-10, resp.**). 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} .



	Roots	Stems	Leaves
Betula pendula	0.591	0.36	0.475
Carpinus betulus	0.942	0.613	0.05
Fagus sylvatica	0.158	0.18	0.18
Salix caprea	1.577	4.302	4.702

Figure 14. 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.

4.2.3 Copper

No significant differences were observed on the concentration levels of Cu in roots, stem and leaves of the four tree species studied (**Figure 15 and Appendix Tables 11-14, resp.**). *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.

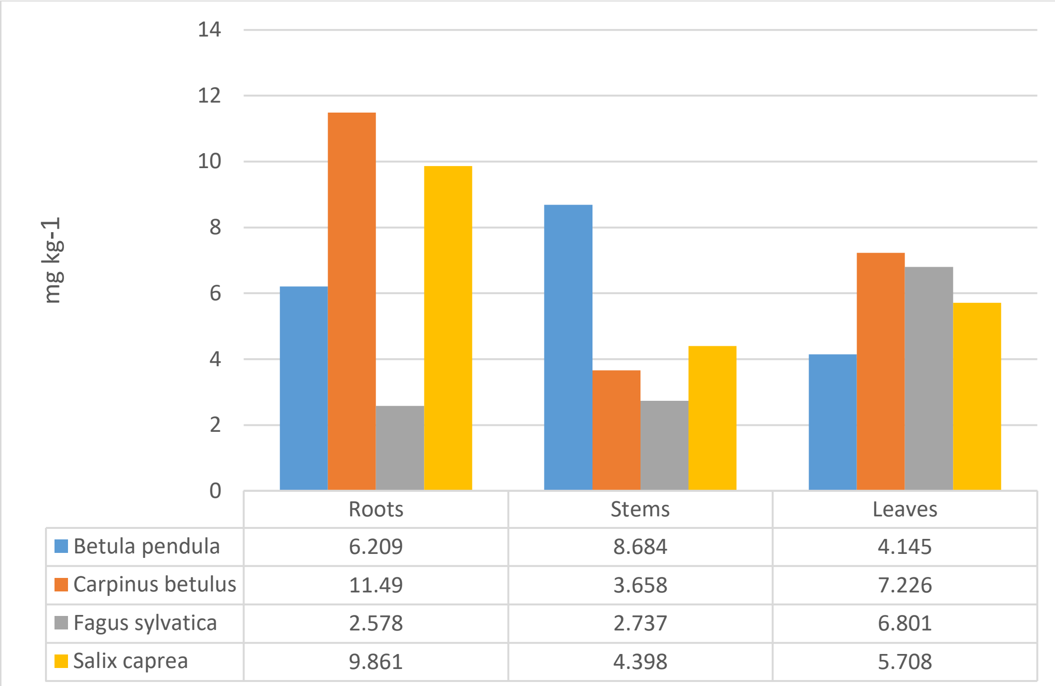
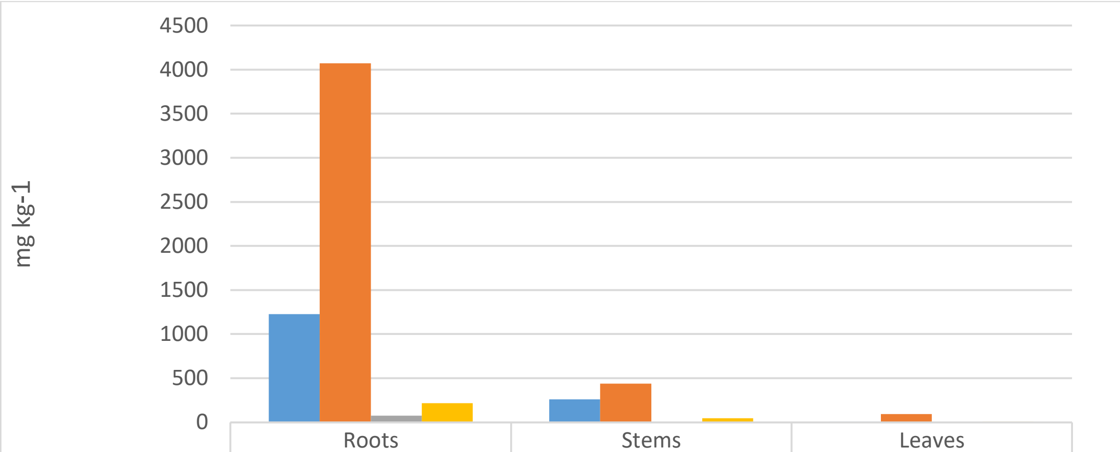


Figure 15. Concentration levels (mg kg^{-1}) of Cu in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

4.2.4 Lead

The concentration levels of Pb on trees were significantly different among tree species (**Figure 16 and Appendix Tables 15-18, resp.**). 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.



	Roots	Stems	Leaves
Betula pendula	1227.12	260.63	8.276
Carpinus betulus	4071.67	439.05	92.532
Fagus sylvatica	75.834	2.805	2.5
Salix caprea	216.14	46.166	9.006

Figure 16. 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.

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 17 and Appendix Tables 19-22, resp.**) 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.

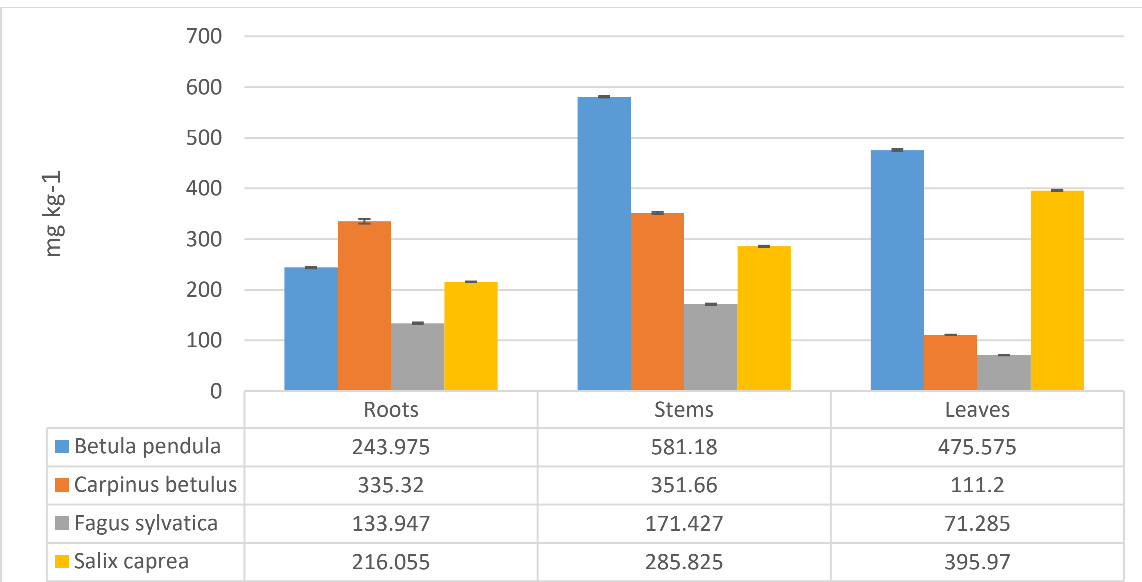
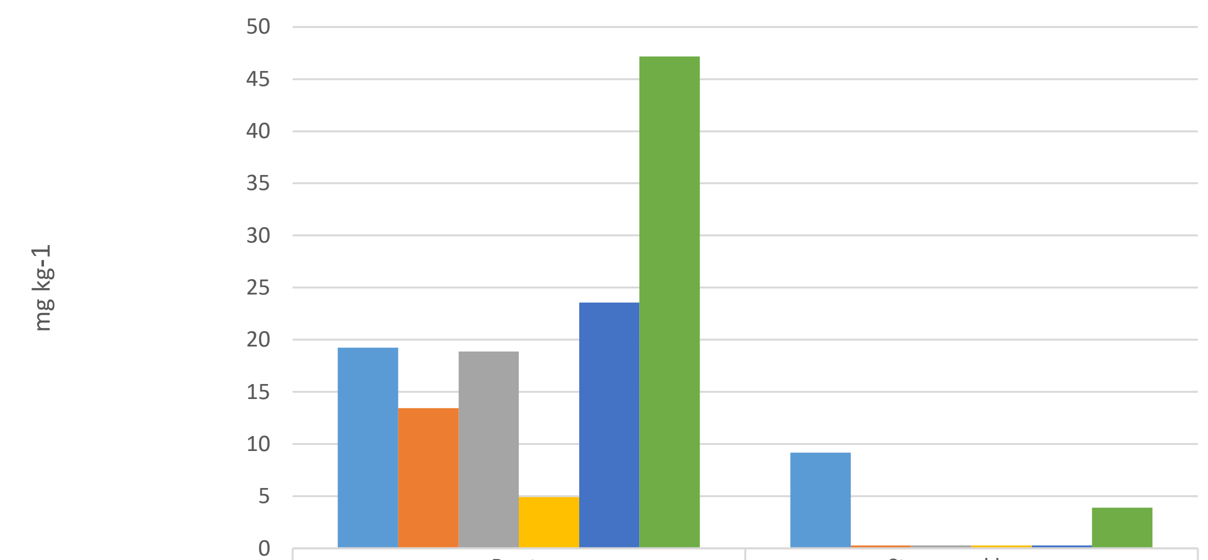


Figure 17. Concentration levels (mg kg^{-1}) of Zn in roots, stem and leaves of tree species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

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 18 and Appendix Tables 23, respectively.**



	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 18. 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 19 and Appendix Table 24, respectively.**

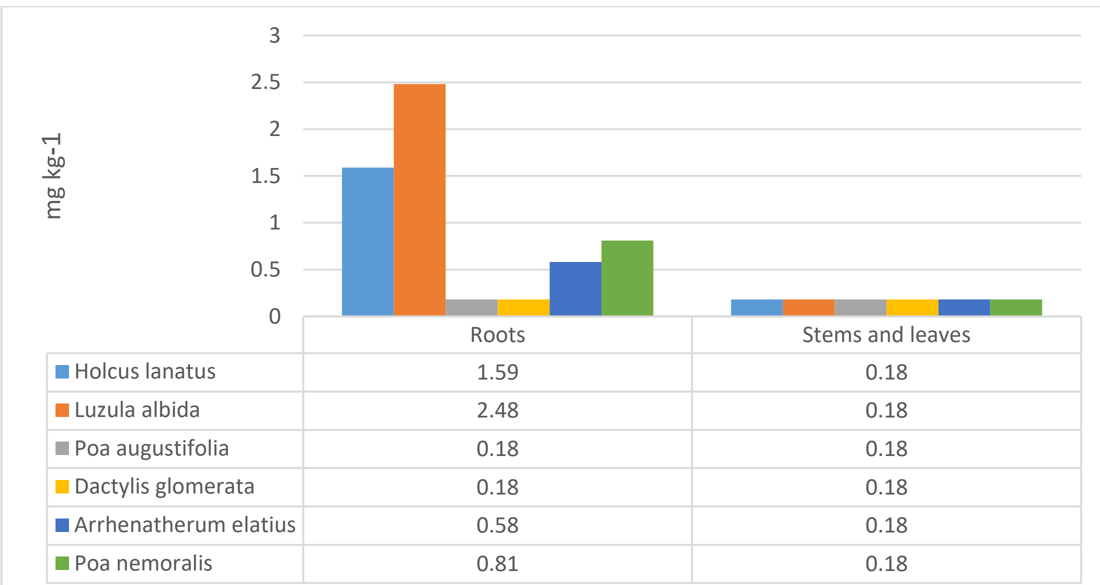
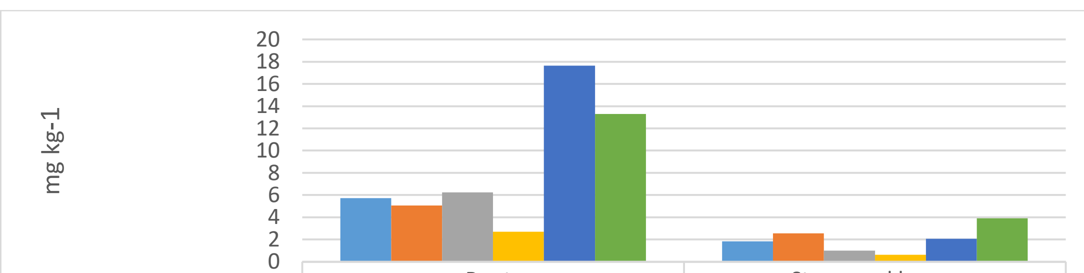


Figure 19. Concentration levels (mg kg^{-1}) of Cd in roots, stem and leaves of grass species growing in mined spoil soils in Mátraszentimre, Mátra Mountains, Hungary.

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 20 and Appendix Table 25, resp.**) . *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.



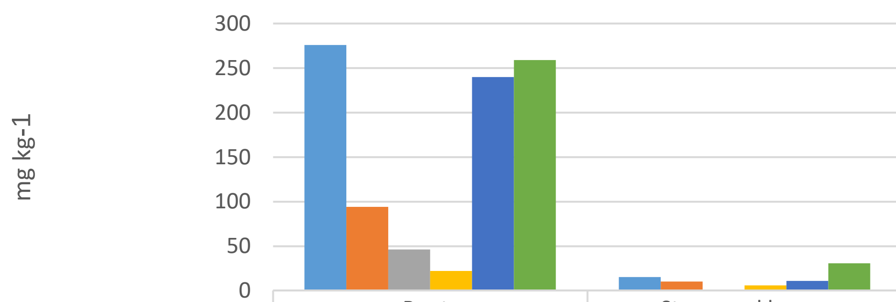
	Roots	Stems and leaves
Holcus lanatus	5.725	1.825
Luzula albida	5.07	2.55
Poa augustifolia	6.25	0.99
Dactylis glomerata	2.7	0.61
Arrhenatherum elatius	17.65	2.06
Poa nemoralis	13.31	3.89

Figure 20. 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 21 and Appendix Table 26, respectively).**

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.



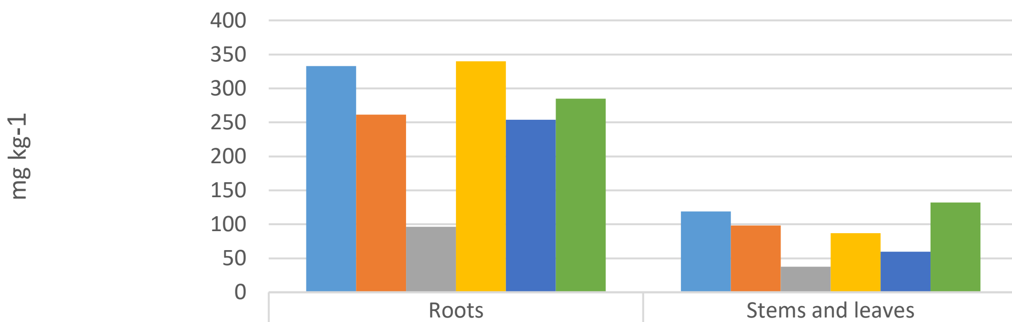
	Roots	Stems and leaves
Holcus lanatus	275.9	15.23
Luzula albida	94.05	10.285
Poa augustifolia	46.1	0.15
Dactylis glomerata	22.1	6
Arrhenatherum elatius	240	11.07
Poa nemoralis	259.04	30.9

Figure 21. 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 22, Appendix Table 27 resp.**).



	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 22. 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 2**. 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 2. 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 3**.

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 3. 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

4.5 Translocation Factor (TF)

4.5.1 Translocation Factor (TF) in Trees

Table 4. 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 (**Table 4**). 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.

4.5.2 Translocation Factor (TF) in Grasses

Table 5. 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 (**Table 5**). 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.

5.0 DISCUSSION

5.1 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.

5.2 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).

5.2.1 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).

5.2.2 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} Cd in leaves (WISLOCKA et al. 2006). WIESHAMMER, (2007) showed that *Salix caprea* can absorb 116 mg kg^{-1} dry wt. of Cd in a pot experiment. Other research work showed that *Salix* contain more Cd leaves than in stems (HAMMER et al. 2003). *Fagus sylvatica* was reported to have high amount of Cd (ZUPUNSKI et al. 2015). However, it gave a lower result value for Cd uptake in this study. This could be attributed to a lower Cd concentration value in soil in the study area relative to their study area.

Some other trees were reported good in absorbing Cd. A study in France reported that poplar can bioaccumulate up to 200 mg kg^{-1} Cd while in New Zealand, willows showed phytoextracting potential in cleaning Cd contaminated soils (ROBINSON 2000). *Salix viminalis* (Willow) can uptake 5.7 mg kg^{-1} dry wt. of Cd in leaves (GREGER and LUNDBERG 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.

5.2.3 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).

5.2.4 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).

5.2.5 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* (ROSSELLI 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).

6.0 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.

7.0 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.

New Results of Soil Analysis:

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.

New 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.

8.0 SUMMARY

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. There are various methods in the restoration of soil ecosystems laden with potential toxic elements. However, physical and chemical methods of cleaning this environment are very expensive and prohibitibly uneconomial.

Phytoremediation is the most practical method of restring this damage ecosystem. The process is tedious and requires long period of time. However, this technology is environmentally, economically and the most socially acceptable. This tehnology does not only restore the soil environment but also restore the damage landscapes, improve the macro climate, and produce economically important biomass. The search for the most appropriate native plant species is the need of time.

This research work evaluated the phyto extracting ability for potential toxic elements of endemic tree species and grass species that are 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 are *Holcus lanatus*, *Poa augustifolia*, *Dactylis glomerata*, *Arrhenatherum elatius* and *Poa nemoralis*. One rush species were also observed which was *Luzula albida*. These plant species are naturally growing in the area.

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

Results showed that the soil was highly contaminated with potential toxic elements, such as Pb, As, and Zn which were 10 to 60 times more than the typical non-contaminated Hungarian soil. Cd is 4 to six times higher than the acceptable limit. Cu is within the acceptable when compared with Hungarian standards.

Among the trees evaluated, *Carpinus betulus* showed the highest potential for Pb dendroremediation, with a mean concentration value of 4071.67±45.71 mg kg^{-1} dry weight in roots, 439.05±1.06 mg kg^{-1} dry weight in stems and 92.53±0.73 mg kg^{-1} dry weight in leaves. *Betula pendula* and *Salix caprea* bioaccumulated 475.575±2.219 and 395.97±1.43 mg kg^{-1} dry weight of Zn in their leaf biomass. Both trees had a Bio-concentration Factor (BCF) value of greater than 1.0 but less than 10 which classified them as potential phytoextractors of Zn. Salix caprea gave the highest Translocation Factor (Tf) for Cd while Betula pendula gave the highest Tf for Zn.

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.

9.0 APPENDICES

9.1 Bibliography

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9.2. Appendix Tables

Appendix Table 1. Concentration levels (mg kg^{-1}) of potential toxic elements in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Potential Toxic Elements				
	Arsenic (As)	**Cadmium (Cd)**	**Copper (Cu)**	**Lead (Pb)**	**Zinc (Zn)**
A	556	bdl	21.7	755	82
B	308	bdl	40.1	797	162.6
C	258	bdl	19.24	606	224
D	270	7.89	62.23	**4349**	**1239**
E (Uncontaminated)	22.48	bdl	7.71	20.9	74.6
F	381.7	5.91	36.99	**6043**	**1045**
G	**1844**	1.13	29.93	66.7	119.9
H	**2580**	4.17	19.47	86.3	86.3
Mean (Contaminated Soil)	**885.57**	**4.77**	**32.81**	**1929.71**	**421.6**

bdl: below the detection limit

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

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

Mann-Whitney U test was used due to non-homogeneity of variance

*significant

** Hungarian Joint Decree No 10/2000 (*Gazdag and Sipter 2008*)

Appendix Table 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.

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

Appendix Table 4. Concentration levels (mg kg^{-1}) of As in roots of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	14.170±1.490	12.64±3.38	0.980±0.130	n.d.
B	15.941±0.384	n.d.	2.656±0.612	n.d.
C	33.400±1.080	12.114±0.67	n.d.	5.644±0.179
D	n.d.	21.550±2.830	n.d.	23.847±1.726
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	50.560±1.820	n.d.	n.d.
G	16.370±0.370	12.640±1.310	8.980±0.990	n.d.
H	n.d.	7.895±0.008	n.d.	n.d.

n.d.: no data

Appendix Table 5. Concentration levels (mg kg^{-1}) of As stem of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	bdl	bdl	1.34±1.37	n.d.
B	bdl	n.d.	bdl	n.d.
C	bdl	bdl	n.d.	0.13±0.10
D	n.d.	bdl	n.d.	1.37±0.12
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	6.38±2.5	n.d.	n.d.
G	bdl	5.28±1.51	bdl	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 6. Concentration levels (mg kg^{-1}) of As in leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	bdl	bdl	bdl	n.d.
B	0.243±0.04	n.d.	bdl	n.d.
C	bdl	kha	n.d.	bdl
D	n.d.	1.32±0.24	n.d.	bdl
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	bdl	n.d.	n.d.
G	3.870±0.329	bdl	1.97±0.42	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 7. 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

Appendix Table 8. Concentration levels (mg kg^{-1}) of Cd in roots of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	bdl	1.062±0.027	bdl	n.d.
B	0.363±0.11	n.d.	bdl	n.d.
C	1.310±0.01	1.25±0.02	n.d.	1.332±0.016
D	n.d.	12.539±0.132	n.d.	1.822±0.032
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	bdl	0.513±0.003	0.437±0.111	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 9. Concentration levels (mg kg^{-1}) of Cd in stems of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	bdl	bdl	Bdl	n.d.
B	1.042±0.009	n.d.	bdl	n.d.
C	bdl	0.401±0.006	n.d.	2.526±0.412
D	n.d.	0.425±0.006	n.d.	6.078±0.067
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	bdl	1.013±0.017	bdl	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 10. Concentration levels (mg kg^{-1}) of Cd in leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	bdl	0.114±0.015	bdl	n.d.
B	1.389±0.076	n.d.	bdl	n.d.
C	bdl	bdl	n.d.	3.586±0.027
D	n.d.	bdl	n.d.	5.819±0.006
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	bdl	bdl	bdl	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 11. 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

Appendix Table 12. Concentration levels (mg kg^{-1}) of Cu in roots of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	2.88±0.09	3.9±0.03	0.85±0.03	n.d.
B	6.955±0.032	n.d.	4.355±0.034	n.d.
C	12.48±0.19	8.895±0.057	n.d.	6.858±0.004
D	n.d.	19.67±0.31	n.d.	12.864±0.129
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	2.52±0.10	24.271±0.210	2.55±0.01	n.d.
H	n.d.	7.551±0.057	n.d.	n.d.

n.d.: no data

Appendix Table 13. Concentration levels (mg kg^{-1}) of Cu in stems of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary. *bdl: below the detection limit*

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	14.77±0.11	0.900±0.05	1.70±0.11	n.d.
B	2.995±0.010	n.d.	42.995±0.010	n.d.
C	kha	4.968±0.601	n.d.	3.305±0.015
D	n.d.	3.379±0.001	n.d.	5.762±0.023
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	kha	4.568±0.040	kha	n.d.
H	n.d.	4.476±0.023	n.d.	n.d.

n.d.: no data

Appendix Table 14. Concentration levels (mg kg^{-1}) of Cu in leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	2.16±0.01	1.15±0.05	1.94±0.02	n.d.
B	4.296±0.050	n.d.	4.817±0.027	n.d.
C	2.23±0.01	5.227±0.021	n.d.	5.508±0.044
D	n.d.	0.41±0.04	n.d.	5.907±0.018
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	6.8±0.10	15.3±0.620	4.400±0.13	n.d.
H	n.d.	n.d.	n.d.	n.d.

n.d.: no data

Appendix Table 15. 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

Appendix Table 16. Concentration levels (mg kg^{-1}) of Pb in roots of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	201±1	327±2	128±2	n.d.
B	203.36±0.075	n.d.	18.502±0.304	n.d.
C	3277±13	170.47±1.098	n.d.	79.25±0.410
D	n.d.	5998±17	n.d.	77.176±1.148
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	5890±118.14	n.d.	n.d.
G	2.21±0.06	327±1	81±1	n.d.
H	n.d.	194.77±3.138	n.d.	n.d.

n.d.: no data

Appendix Table 17. Concentration levels (mg kg^{-1}) of Pb in stems of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary. *bdl: below the detection limit*

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	66.6±0.500	97±1	98±2	n.d.
B	380.29±4.073	n.d.	97.843±1.805	n.d.
C	335±1	77.069±0.827	n.d.	174.170±0.410
D	n.d.	n.d.	n.d.	258.11±0.182
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	1143.1±0.1.354	n.d.	n.d.
G	kha	n.d.	206±1	n.d.
H	n.d.	57.213±0.326	n.d.	n.d.

n.d.: no data

Appendix Table 18. Concentration levels (mg kg^{-1}) of Pb in leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	3.8±0.300	97±1	bdl	bdl
B	8.729±0.477	n.d.	bdl	bdl
C	12.3±0.02	40.956±0.189	n.d.	7.534±0.08
D	bdl	34.9±0.04	n.d.	10.478±0.025
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	bdl	n.d.	7.2±0.10	n.d.
H	n.d.	bdl	n.d.	n.d.

bdl: below the detection limit
n.d.: no data

Appendix Table 19. 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

Appendix Table 20. Concentration levels (mg kg^{-1}) of Zn in roots of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	Tree Species			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	227±3	142±1	98±2	n.d.
B	163.9±1.017	n.d.	97.843±1.805	n.d.
C	412±1	124.63±0.552	n.d.	174.170±0.410
D	n.d.	329.1±0.40	n.d.	258.11±0.182
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	173±1	745.55±15.805	206±1	n.d.
H	n.d.	175.24±0.254	n.d.	n.d.

n.d.: no data

Appendix Table 21. Concentration levels (mg kg^{-1}) of Zn in stems of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	871±10	336±1	62.77±0.27	n.d.
B	347.54±1.918	n.d.	158.44±1.014	n.d.
C	412±1xx	197.22±1.465	n.d.	207.290±2.254
D	n.d.	24.9±0.245	n.d.	364.26±0.178
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	531±1	521.67±4.183	92.1±1	n.d.
H	n.d.	293.07±2.645xx	n.d.	n.d.

n.d.: no data

Appendix Table 22. Concentration levels (mg kg^{-1}) of Zn in leaves of tree species growing in mined out soils in Mátraszentimre, Mátra Mountains, Hungary.

Sampling Points	**Tree Species**			
	Betula pendula	*Carpinus betulus*	*Fagus sylvatica*	*Salix caprea*
A	227±3	142±1	98±2	n.d.
B	163.9±1.017	n.d.	97.843±1.805	n.d.
C	412±1	124.63±0.552	n.d.	174.170±0.410
D	n.d.	329.1±0.40	n.d.	258.11±0.182
E	n.d.	n.d.	n.d.	n.d.
F	n.d.	n.d.	n.d.	n.d.
G	173±1	745.55±15.805	206±1	n.d.
H	n.d.	175.24±0.254	n.d.	n.d.

n.d.: no data

Appendix Table 23. 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.

Grass Species	Roots	Stems and Leaves
Holcus lanatus	19.26±2.405	9.2±1
Luzula albida	13.44±0.67	bdl
Poa augustifolia	18.89±1.4	bdl
Dactylis glomerata	4.91±0.73	bdl
Arrhenatherum elatius	23.58±3.35	bdl
Poa nemoralis	47.16±1.84	3.91±0.34

*bdl-below detection limits

Appendix Table 24. 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.

Grass Species	Roots	Stem and Leaves
Holcus lanatus	1.59±0.02	Bdl
Luzula albida	2.48±0.04	bdl
Poa augustifolia	bdl	bdl
Dactylis glomerata	bdl	bdl
Arrhenatherum elatius	0.58±0.02	bdl
Poa nemoralis	0.81±0.02	bdl

*bdl- below detection limits

Appendix Table 25. 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.

Grass Species	Roots	Stem and Leaves
Holcus lanatus	5.725±0.11	1.825±0.02
Luzula albida	5.07±0.11	2.55±0.08
Poa augustifolia	6.25±0.12	0.99±0.04
Dactylis glomerata	2.7±0.08	0. 61±0.01
Arrhenatherum elatius	17.65±0.23	2.06±0.05
Poa nemoralis	13.31±0.18	3.89±0.15

Appendix Table 26. . 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.

Grass Species	Roots	Stems and Leaves
Holcus lanatus	275.9±1.6	15.23±0.03
Luzula albida	94.05±0.65	10.285±0.01
Poa augustifolia	46.1±0.10	bdl
Dactylis glomerata	22.1±0.30	6±0.3
Arrhenatherum elatius	240±1	11.07±0.3
Poa nemoralis	259.04±1.51	30.9±0.4

*bdl- below detection limits

Appendix Table 27 . 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.

Grass Species	Roots	Stems and Leaves
Holcus lanatus	333±1.65	118.9±0.41
Luzula albida	261.65±1.0	98.3±0.11
Poa augustifolia	96.6±0.02	37.6±0.10
Dactylis glomerata	340±1.0	87±1.0
Arrhenatherum elatius	254±1.0	60±1
Poa nemoralis	284.75±0.55	132±2