

METALLOPHYTES IN THE LJUBIJA MINE AREA AND THEIR POTENTIAL IN PHYTOREMEDIATION

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Abstract: This study focused on a landfill flotation tailing pond of the “Ljubija” iron ore mine, which is habitat covered by sparse vegetation consisted by specific plant groups. The following plant species were selected: *Rumex hydrolapathum*, *Silene vulgaris*, *Tussilago farfara*, *Arabis hirsuta*, *Acer pseudoplatanus* and *Populus alba*. The plants were collected and processed for the determination of the content of toxic metals (Fe, Zn, Cu, Mn, Ni, and Cr) aiming to evaluate their phytoremediation potential. At the same time, the content of toxic metals in the soil was analyzed to determine the degree of loading and the possibility of cleaning the area with phytoremediation techniques. The sequence of bioaccumulation in the tissue of all examined species decreased in the order Fe > Mn > Zn > Cu > Ni > Cr. The values of phytoaccumulation factors BCF and BAC for all investigated elements were below 1, except for Fe and Mn, which indicates a medium to poor accumulation potential of the investigated species. An exception was recorded in the plant *A. hirsuta* where BCF > 1 for Cr. The translocation coefficient (TA > 1) for Cu and Mn was determined for *R. hydrolapathum*, for Cu and Zn in *A. pseudoplatanus*, for Cu, Zn, and Ni in *T. farfara*, and Zn in *A. hirsuta*, which classifies these species as effective in techniques of phytostabilization of the researched area. These data are the first research in the area of the Iron Ore Mine Ljubija, which is of great interest to determine the level of contamination of soil and plants with toxic metals, and to remediate the same with phytoremediation techniques.

Keywords: mine, metallophytes, toxic metals, phytoremediation

1. INTRODUCTION

In the last few decades, due to the increasing industrialization, urbanization and overpopulation, the degradation and pollution of the environment has occurred, which has encouraged many experts from the field of natural sciences to find the most efficient solution for cleaning the environment (Kabata-Pendias, 2001; Pilon-Smits, 2005; Singh et al., 2012; Galal & Shehata, 2015). High concentrations of toxic metals in the environment represent a serious threat as they are not biodegradable and easily travel through the food chain and can accumulate for many years in the highest links of this chain (Singh et al., 2012). A large number of metals in very low concentrations are essential micronutrients for plants such as Cu, Zn, Mn, Fe, Ni, Mo, Se, and Co (Milčić

et al., 2019; Yan et al., 2020), and probably Cr, Ni and V (Sekulić et al., 2003). On the other hand, at least 20 metals are classified as toxic, which can lead to serious health problems in many species, including humans (Akpor & Muchie, 2010). It has been observed that certain plant species growing on metal-laden soils have developed the ability to adapt and accumulate large amounts of metals in their tissues without showing toxicity to their metabolism. These plants are called hyperaccumulators because of their ability to tolerate, absorb and translocate high concentrations of some toxic metals, which are otherwise toxic to other organisms (Pilon-Smits, 2005; Yan et al., 2020). These plants can adopt 100 - 1000 times higher concentrations of toxic metals than those usual for plants, i.e. more than 10 mg·g⁻¹ (1 %) for Mn and Zn, more than 1 mg·g⁻¹ (0.1 %) for As,

Co, Cr, Cu, Ni, Pb, Sb, Se and Ti and more than 0.1 mg·g⁻¹ (0.01%) for Cd (Zavoda et al., 2001). According to literature data, approximately 400 plant species are characterized as metal hyperaccumulators (Prasad & Freitas, 2003; Pilon-Smits, 2005). Individual species of higher plants differ from each other in their ability to concentrate toxic metals under the same pollution conditions, and differences also exist between individual varieties of the same species (Pilon-Smits, 2005). In the last decade, there has been an intensive search for species and genotypes that are characterized by the ability to accumulate higher concentrations of toxic metals so that they can be used for cleaning and rehabilitation of land and that could be grown on contaminated land without ecological consequences. One of these solutions is phytoremediation technology (Milčić et al., 2019). Phytoremediation is a relatively new, economically viable, and environmentally friendly technological process using hyperaccumulator plant species to eliminate toxic metals from the environment (Pilon-Smits, 2005; Singh et al., 2012; Sharma et al., 2020; Yan et al., 2020). Phytoextraction is used to remediate land with toxic metals, in which fast-growing plants, which have increased tolerance to the intake of toxic metals, enable faster transport from the roots and accumulation in plant shoots are primarily used (Kanwal et al., 2021). For mining areas, phytostabilization is more commonly applied to prevent the further spread of pollution. Species chosen for this purpose typically have a high tolerance to metals, a widespread and dense root system, reduced metal transport and accumulation within the roots, or immobilization in the rhizosphere by precipitation, complexation, or reduction (Milčić et al., 2019).

It is known that species of the genus *Arabis* sp. grow successfully on highly contaminated soils and have the ability to hyperaccumulate toxic metals (Mou et al., 2021). *Silene vulgaris* is cited as a very good indicator of the presence of zinc and cadmium (Brown et al., 1995). Research (Zhao et al., 2001; Gawronski & Gawronska, 2007) on *Tussilago farfara* showed that this species, regardless of the fact that it does not produce a large biomass, can be successfully used in the phytoremediation technique for the removal of Cu, Zn, Pb, Cd, Cr and Ni. According to literature data (Muhammad et al., 2013; Yashim et al., 2014) species of the genus *Rumex* are used as biofilters for the extraction of toxic metals from different types of waste water and land purification. Unlike herbaceous species, woody species are more economical because they have the possibility of several cycles of decontamination without having to harvest the entire plant and re-sow every year, and

after planting, maintenance costs are relatively low (Kebert, 2014). It is known that poplars and willows are woody species that are most often used in the phytoremediation of water and land polluted by toxic metals due to their high growth rate, high biomass, and yield, and because they possess certain physiological adaptations and have excellent predispositions for use in many climatic zones and degraded environmental conditions (Kuzovkina & Quigley, 2005; Barbes et al., 2014; Suo et al., 2021; Štrbac et al., 2022). Also, species of the genus *Acer* show great potential in phytoremediation, primarily in phytostabilization and bioindication of toxic metals (Pulford & Watson, 2003; André et al., 2006).

In order to determine the degree of loading of soil and plants with toxic metals, research was carried out on the tailings pond of the „Ljubija“ iron ore mine, all with the aim of establishing the degree of bioaccumulation and translocation of toxic metals between the roots and leaves of herbaceous plants (*Rumex hydrolapathum*, *Silene vulgaris*, *Tussilago farfara* and *Arabis hirsuta*) and woody plants (*Acer pseudoplatanus*, *Populus alba*) in order to assess their phytoremediation potential and the choice of species in the rehabilitation of degraded areas.

2. MATERIALS AND METHODS

2.1. Plant material and soil

The plant material was sampled during the growing season in the area of the Ljubija iron ore mine (Figure 1), not far from the town of Prijedor (44°51'42.8"N 16°34'43.6"E). The Ljubija mine is considered the most important deposit of iron ore in the area of the former Yugoslavia, and the soil in this area is rich in toxic metals. During the past few decades, a landfill was formed for the disposal of mine waste, characterized by the sporadic presence of typical pioneer species of metallophytes and the absence of other plant communities. The following plant species were selected based on their density in the study area: *Rumex hydrolapathum*, *Silene vulgaris*, *Tussilago farfara*, *Arabis hirsuta*, *Acer pseudoplatanus*, and *Populus alba*. Fresh plant material was transferred to the laboratory where species determination was occurred. After sampling, the leaves and roots were separated, washed with tap and deionized water, and dried in a dryer at a temperature of 60 °C until a constant mass was achieved. After drying, the material was ground in a laboratory mill to a fine powder structure of 2 mm. Along with the plant material, soil samples were taken at several points from the study area at a depth of 10 - 15 cm. Approximately 1 kg of soil was

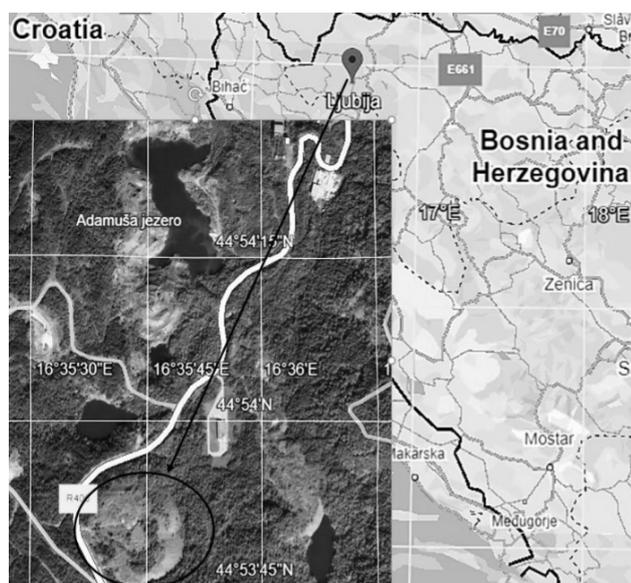


Figure 1. The sampling location at the study area.

collected and delivered to the laboratory. The sample was sieved to promote homogenization, then dried, sieved again, and prepared for further chemical analyses to determine the content of toxic metals (Cu, Zn, Fe, Mn, Ni, Cr), pH values, and organic matter.

2.2. Chemical analysis of soil and plant material

Chemical analysis of soil and plant material included acid digestion and measurement of their concentrations by atomic absorption spectrophotometry (AAS) using Perkin Elmer AAnalyst 400. Soil samples were digested according to a modified method ISO 11466 (1995), which included the method of sample destruction by acid digestion with nitric acid with the addition of 30 % hydrogen peroxide according to standard methods (US EPA, 1996). Destruction of plant material was done with concentrated HNO₃ with the addition of 30 % H₂O₂ and 70 % HClO₄ (Pequerul et al., 1993). The single element stock solutions (1000 mg·L⁻¹, Perkin Elmer, USA) was used as a standard. Extracts, obtained after acid digestion of the samples, were filtered through quantitative filter paper into measuring vessels, where they were stored at 4°C until analysis. The quantification of analyzed metals was performed using the atomic absorption method spectrophotometry (AAnalyst 400 Perkin Elmer®, USA).

The phytoremediation potential of plants was obtained taking into account the bioconcentration factor (BCF), bioaccumulation factor (BAC) and translocation factor (TA) (Yoon et al., 2006). BCF is the ratio between the toxic metal content in the roots and in the soil, while BAC represents the ratio

between toxic metals in the leaves and in the soil. Depending on the BCF value, the accumulation efficiency was evaluated using the scale: BCF >1 (intense), 1 to 0.1 (moderate) 0.1 to 0.01 (no accumulation) (Kabata-Pendias & Pendias, 1999). TA is considered as the plant's ability to translocate metals from the roots to the leaves and is evaluated according to the scale: TA > 1 - successful transfer, TA < 1 - not efficient transfer (Baker & Brooks, 1989).

$$BCF = CM_r/CM_s \quad (1)$$

$$BAC = CM_l/CM_s \quad (2)$$

$$TA = CM_l/CM_r \quad (3)$$

where: CM_r - root metal concentration; CM_s - soil metal concentration; CM_l - leaves metal concentration.

2.3. Statistical processing

The obtained results were statistically processed in the statistical software program SPSS 22 (IBM 2013) and Origin 7.5. All results are presented as average values of three replicates ± standard deviation (SD). Different lowercase letters (*a, b, c, d, e, f*) indicate a statistically significant difference between the toxic metal contents in roots and leaves, according to the LSD test ($p > 0.05$). Principal component analysis (PCA) was conducted taking into account all analyzed metal contents to determine their correlations and grouping patterns.

3. RESULTS AND DISCUSSION

3.1. Toxic metal content in the soil

Mining is a key activity for the development of countries where it has a strong direct and indirect impact on exploited areas and the wider environment. Many mining methods involve moving large amounts of land, which creates changes in the environment and a large number of tailings pond and waste (da Silva et al., 2022). Tailings ponds from iron mines are characterized by high pH values due to excessive concentrations of iron and manganese oxides, causing a certain level of their unavailability for plant uptake (da Silva et al., 2022). The uptake of toxic metals depends on plant species and their metabolic strategy for metal transport from roots to leaves (Zhao et al., 2019; Mou et al., 2021). Many authors believe that plants performing this function extract various elements and accumulate them in their organs and tissues, thereby preventing the spread of contamination in the environment (Yan et al., 2020).

The concentration of metals in the soil and plants of the investigated mining area was significantly different from the concentrations of the same elements in control soils and plants (Uroic et al., 2011). In the tailings pond samples, the highest content of Fe (283958.0 mg·kg⁻¹) and Mn (21388.5 mg·kg⁻¹) was determined, significantly lower concentrations were recorded for Zn (98.21 mg·kg⁻¹), while the content of Cu, Ni and Cr was uniform without significant statistical differences (Figure 2). The studied site in the area of the Ljubija mine was not burdened with toxic metal content (according to RS Official Gazette, 64/2019). However, according to Kabata-Pendias (2001), the total and available amounts of Fe and Mn were extremely high, while the Zn content indicated moderate pollution. The content of toxic metals in the tailings pond decreased in the following sequence: Fe > Mn > Zn > Cu > Cr > Ni. This result could be attributed to mining activities in the researched area, the influence of the pH of the soil solution, the content of organic matter, microbiological activity (Ke et al., 2007) as well as the presence of metallophytes, which are known for their accumulator properties (Yan et al., 2020). Similar results were published by Abbaslou et al. (2018) in the area of iron ore mines in Iran, and research by da Silva et al. (2022) in mine tailings pond from Brazil.

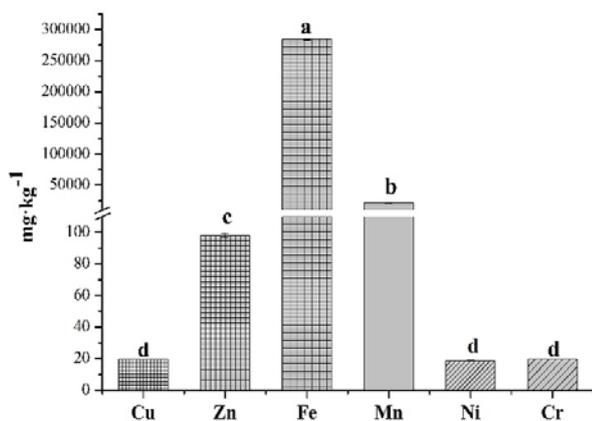


Figure 2. Content of toxic metals in tailings pond (mg·kg⁻¹) in the researched area. Different lowercase letters (a, b, c, d, e, f) indicate a statistically significant difference in toxic metal concentrations according to the LSD test ($p > 0.05$).

The pH value of the tailings pond was slightly alkaline (7.58), which is one of the important parameters that affects the solubility of salts, increasing or decreasing the availability of metals (Uroic et al., 2011), as well as for greater retention of some toxic metals in the soil (Fe and Mn), while more significant translocation of certain elements

intissue of the researched species (Cr) can be attributed to antagonism between ions and different strategies and defense mechanisms.

3.2. Content of toxic metals in tissues of *Rumex hydrolapathum*

Numerous studies have shown that plants adapted to growing in mining areas have a high level of tolerance to toxic metals, even though their adaptability and resistance strategies are species-specific (Zhao et al., 2019; Jakovljević et al., 2019; Brković et al., 2021; Mou et al., 2021), which we also stated in this paper. The high content of toxic metals in certain organs (root and shoot) is considered one of the indicators of soil quality and pollution (Ciftci et al., 2021). The risk assessment of metal toxicity should consider not only their soil content but also their mobility (Remon et al., 2005).

In the tissue of the *Rumex* sp. all examined metals showed significant variations both in the leaf and in the root. The content of Fe (908.41 mg·kg⁻¹) was the highest in the roots, followed by Mn (150.06 mg·kg⁻¹), while the lowest concentrations were determined for Ni and Cr in both examined tissues without clear statistical differences (Figure 3A). The content of Zn in both tissues was uniform (22.50 mg·kg⁻¹). When it comes to the mobility of toxic metals in the soil-plant relationship, it was determined that their concentrations in the tissues of the examined species were significantly lower compared to their content in the soil, which was also documented in the paper by Lovynska et al. (2023).

It is important to emphasize that this species adopted and transported less of all the analyzed elements compared to the other analyzed species, which can be explained by its good avoidance strategy (e.g. sequestration in vacuoles) (Millaleo et al., 2010). Antagonism with Cd, retention in the root zone by metal complexes, and interactions with nutrient elements (e.g., K and Mg) are cited as the interpretation of low uptake in previous works (Alam et al., 2005). Content of all tested toxic metals in *Rumex* sp. tissue was higher in the root, except for Cu and Zn, whose content was higher in the leaf, which was contrary to the results of Zehra et al. (2009) and Yashim et al. (2014) who recorded a higher content in the roots. In general, manganese tends to accumulate more in plant shoots than in roots (Millaleo et al., 2010), which was contrary to our results for this species. Better translocation of Cu and Zn in the leaves is probably the result of specific plant interactions with the presence of other elements. However, the influence of pH and plant nutrients significantly regulate Zn and Cu intake and

antagonism with other ions (Kabata-Pendias & Pendias, 1999; Zahirnejad et al., 2017). The sequence of bioaccumulation in both tissues of *R. hydrolapathum* decreased in the order Fe > Mn > Zn > Cu > Ni > Cr.

The values of BAC and BCF (above 0.1) indicate that this plant shows a medium accumulation potential, where the root of this species has a good ability to mobilize and assimilate metals from the soil, which was also noted by other authors (Zehra et al., 2009; Yashim et al., 2014). BAC and BCF for Fe and Mn was less than 0.01, which indicates poor accumulation potential. TA is an important indicator that enables the assessment of the mobility of toxic metals in plants (Yashim et al., 2014). High TA (above 1) was determined for Cu, Zn, Mn, which implies that *R. hydrolapathum* can be a hyperaccumulator of these metals, while for Fe, Ni and Cr it was less than 1, which indicates a good accumulation potential. The obtained results agree with the study conducted by Yashim et al. (2014). It is interesting to note that the seeds of *Rumex japonicus* species taken from the Cu mine area on Tonglushan Hill in Hubei Province, China, grown hydroponically, shows a high tolerance to Cu intake compared to seeds of the same species taken from an uncontaminated area (Ke et al., 2007), and effectively excludes it from the roots. Knowing the strategy of copper intake, accumulation, and tolerance (Dahmani-Muller et al., 2000), it can be determined that this species can be used as a metal exclusion agent in the phytostabilization of areas with high Cu content.

3.3. Toxic metal content in tissues of *Acer pseudoplatanus*

It is known that woody plants can be more efficient at toxic metal uptake compared to herbaceous plants due to their higher biomass (Mleczek et al., 2017). This is explained by the deep root system and higher biomass yield (Capuana, 2011). However, the results obtained in this paper show the opposite. Accumulation of toxic metals in leaves and roots of *A. pseudoplatanus* decreased in the following order: Fe > Mn > Zn > Cu > Ni > Cr (Figure 3B). The obtained results of the concentration of toxic metals showed a higher content in the roots compared to the leaves, which indicates a weak translocation to the shoot, which was stated by Mleczek et al. (2017) in six tree species grown on mining sludge. Regarding the migration of toxic metals in the soil-plant system, it was determined that the concentrations of toxic metals in the tissue of the examined species were significantly lower compared

to their content in the soil, which was also documented in the paper of Lovynska et al. (2023). The calculated bioaccumulation coefficients for Cu and Zn were less than 1, for Ni and Cr less than 0.1, and for Mn and Fe less than 0.01, which indicates a medium to weak accumulation potential for this species. For this species, TA > 1 was determined for Zn (1,964) and Cu (1,126), while for Fe, Mn, Ni and Cr, TA was below 1. High TA for Zn and lower BCF was documented in the research of Mleczek et al. (2017), which indicates the possibility of tolerance, adoption, and transmission Zn in the shoot which classifies this species as the most suitable in the phytostabilization technique. Of all the metals analyzed, Ni and Cr were the elements that showed the weakest translocation and ability to move from root to shoot.

3.4. Toxic metal content in tissue of *Populus alba*

Populus species are potentially suitable for phytoremediation purposes, given their known ability to accumulate toxic metals, which is documented in a large number of works (Guerra et al., 2011; El-Mahrouk et al., 2020; Brković et al., 2021; Tózsér et al., 2023). Our study showed that the main place of metal accumulation is in the root: Cu (12.83 mg·kg⁻¹), Zn (53.39 mg·kg⁻¹), Fe (13154.65 mg·kg⁻¹), Mn (1270.9 mg·kg⁻¹), Ni (6.84 mg·kg⁻¹) and Cr (10.48 mg·kg⁻¹). The leaves accumulated significantly lower metal contents: Cu (4.12 mg·kg⁻¹), Zn (48.39 mg·kg⁻¹), Fe (292.04 mg·kg⁻¹), Mn (274.17 mg·kg⁻¹), Ni (2.22 mg·kg⁻¹) and Cr (0.04 mg·kg⁻¹) (Figure 3C). This can be explained by the limited metal transport between the roots, stem and leaf, which is crucial for their exclusion in the metabolism process (Brković et al., 2021; Borišev et al., 2009). The results of our research (Figure 3C) also showed that the total mean concentrations of the tested metals were much higher than in the plant samples, which was in agreement with the results of Brković et al. (2021) of toxic metals in the tissue of the species decreased in this order: Fe > Mn > Zn > Cu > Ni > Cr (Figure 3C). Poplars are known for their significant potential to accumulate Zn, especially in the leaves, where the highest concentrations of this metal are found compared to other organ (Brković et al., 2021; Tózsér et al., 2023), which was contrary to our results. On the other side, Benyó et al. (2016) explain lower Zn translocation rate by the fact that the roots of *P. deltoides* and *P. canadensis* accumulate and prevent the metal transport in a medium contaminated with multiple Cu/Zn. Our study indicates that successful accumulation of Zn in *Populus alba* can be used in phytoextraction technology, which is in line with the

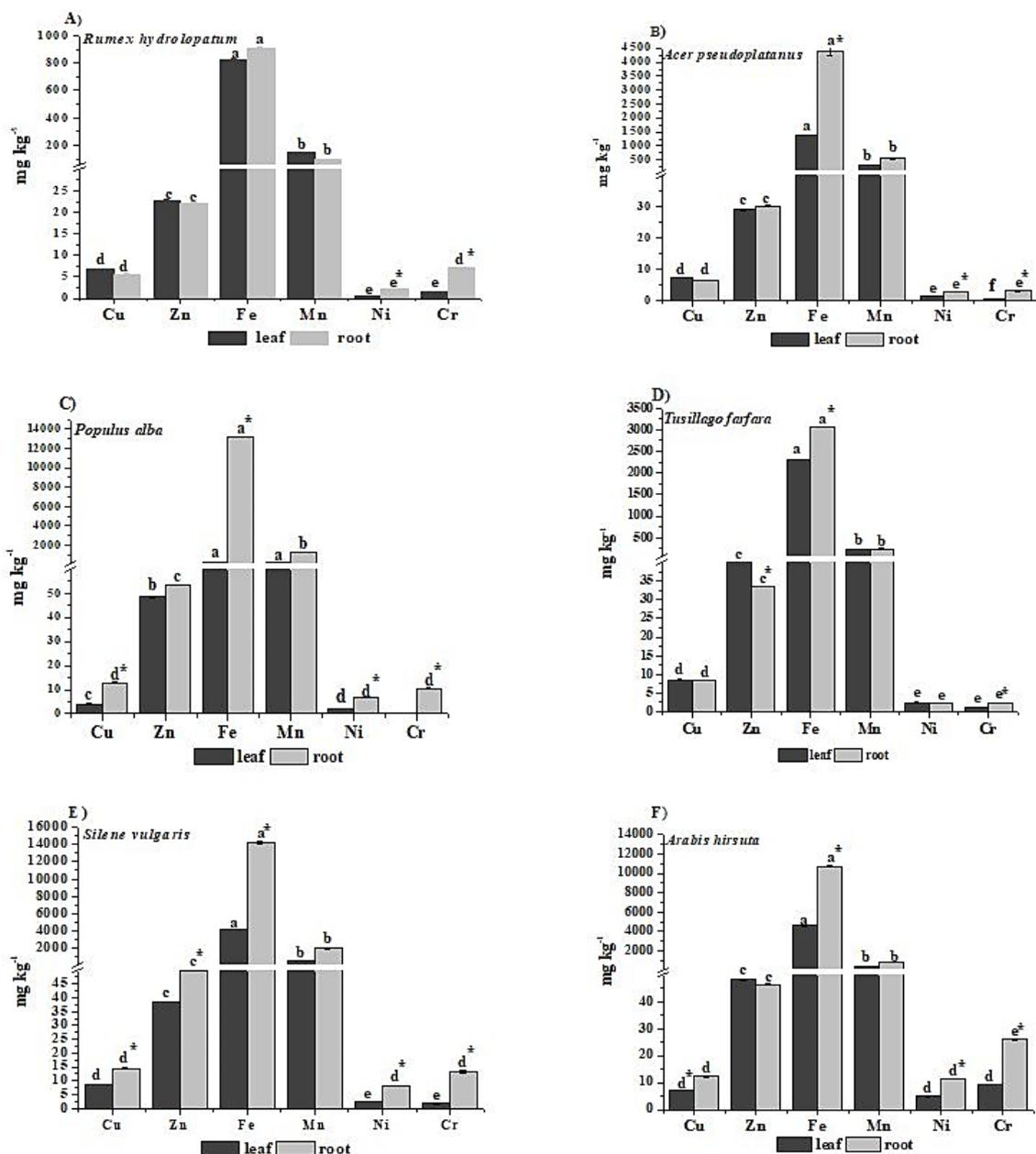


Figure 3. Toxic metal content (Cu, Zn, Fe, Mn, Ni and Cr) in the shoots and roots of herbaceous plants (A-*Rumex hydrolopatum*, E- *Silene vulgaris*, D- *Tussilago farfara* and F- *Arabis hirsuta*) and woody plants (B- *Acer pseudoplatanus* and C- *Populus alba*). Different lowercase letters (a, b, c, d, e, f) indicate a statistically significant difference between the concentrations of different toxic metals in the roots and leaves of plants, while * indicates a statistically significant difference in the concentration of one metal between the roots and leaves according to the LSD test ($p > 0.05$).

results of other authors (Guerra et al., 2011; Brković et al. 2021; Tózsér et al., 2023). Values of BAC and BCF > 1 for Cu, Zn and Ni, while for Fe, Mn and Cr were less than 0.1, which indicates medium to weak accumulation potential (Table 1). Contrary to ours, El-Mahrouk et al., (2020) noted in their research that the species *P. nigra* had BCF > 1 and TA > 1, and that it has a high phytoextraction capacity. A high TA was determined for Zn (0.905), followed by Ni (0.323) and Cu (0.320), Mn (0.210), Fe (0.002) and

then Cr (0.003), which was significantly lower compared to the research by Suo et al. (2021) on *P. deltooides*. The results of bioconcentration factors for this species indicate that it does not possess phytoextraction ability in metal accumulation, which was contrary to the results recorded by Brković et al. (2021). Phytotoxic concentrations of Fe were also determined in this species in both examined organs.

3.5. Toxic metal content in *Tussilago farfara*

This species is characterized by a selective distribution of metals; Fe, Mn and Cr were retained in the roots, while Cu, Zn and Ni accumulated more in the leaves (Figure 3D). This tendency to accumulate toxic metals was also noted by other researchers for this species (Jakovljević et al., 2019). The mean values of the metal concentrations of leaves and roots decreased in the following order: Fe > Mn > Zn > Cu > Ni > Cr, where significant statistical differences were recorded for all analyzed elements except for Ni and Cr (Figure 3D). Jakovljević et al. (2019) in their study on mine tailings pond found that *T. farfara* retains some toxic metals more in the leaves and some in the roots. The reason for this can be explained by the fact that *T. farfara* is a perennial plant, which can also be part of the detoxification strategy through the loss of leaves. On the other hand, a higher concentration of toxic metals in the roots also triggers different detoxification strategies, such as detoxifying the roots to prevent their mobility to the photosynthetically active tissue.

The values of all toxic metals were within the allowed concentrations except for Fe and Mn whose values were within the toxic limits according to Kabata-Pendias (2010). Successful phytoextraction primarily depends on the plant's ability to transfer metals from the soil (Li et al. 2007). BAC and BCF values of *Tussilago farfara* for Cu, Zn, Ni and Cr were less than 1, while for Mn and Fe they were below 0.01, which indicates a weak accumulation potential. Although *Tussilago farfara* is not suitable for phytoextraction due to toxic metal contents below hyperaccumulation thresholds, it plays an important role in soil revitalization and preventing the spread of trace elements to neighboring habitats. Contrary to ours, on the other hand, several studies have proven the good phytoextraction potential of *T. farfara* in places polluted by metals (Liu et al., 2014; Wechtler et al., 2019). TF is an important indicator that allows the assessment of the mobility of toxic metals from the roots to the aerial part (Zhao et al., 2001). Low values of BCF and TA greater than 1 were determined for Cu, Zn and Ni (Table 1), which implies that this plant can play a significant role in the phytostabilization technique of this area.

3.6. Toxic metal content in the tissue of *Silene vulgaris*

It is known that *Silene vulgaris* is a species resistant to toxic metals and successfully grows on the polluted soil of a copper mine dump (Bringezu et al., 1999), and efficiently accumulates significant

amounts of toxic metals in its roots and shoots (Ernst, 2003), which we also stated in this paper. For the *Silene vulgaris* species among the studied metals, the maximum content was determined for Fe and Mn, followed by Zn, a much lower content was found for Cu, while for Ni and Cr the lowest concentrations were determined without clear statistical differences (Figure 3E). It is important to point out that the root of this species accumulated more Fe, Mn and Cr compared to other researched species, and the values obtained were within the limits of phytotoxicity according to Kabata-Pendias (2010). The concentration of Mn in the roots, Fe in both organs exceeded the values considered toxic for plants (400 - 1000 mg kg⁻¹ for Mn and for Fe). For this species, a higher metal contents were determined in the root in comparison to the leaf, so it can be assumed that this is a type of strategy of this species that limits the transport of metal ions through endodermal Casparian strip (Yan et al., 2020), which was also noted by other researchers for this species (Brej & Fabiszewski, 2006; Đurović et al., 2016; Ozturk et al., 2019).

Bioaccumulation factors (BAC) for Cu, Zn, Ni and Cr were less than 1, while the values for Fe and Mn were in the range of 0.1 - 0.01, which implies that the *Silene vulgaris* has a weak accumulation potential and that it retains metals more in the roots, which was also stated by other researchers (Kobierski et al., 2017). The mean value of BCF for Cu and Cr was close to 1, which indicates a strong uptake of Cu and Cr by plant roots, while for Zn and Ni it was below 1. Plants with higher BCF values and lower TA have a greater potential in phytostabilization (Yoon et al., 2006). BCF for Fe and Mn was below 0.1, suggesting that these elements have low level of availability, which was similar to results obtained by Nworie et al. (2019). The values of the TA factor were less than 1, and they varied in the order; Cu (0.59) > Fe (0.429) > Mn (0.519) > Ni (0.424) > Cr (0.351) (Table 1) which indicates a weak translocation and limited transport between the roots and the leaves, and that the metal accumulates in the roots.

3.7. Toxic metal content in the tissue of *Arabis hirsuta*

Species of *Brassicaceae* family belong to one of the best accumulators of toxic metals in nature (Knight et al., 1997; Gawronski & Gawronska, 2007), which we also noted in this paper. Species of the genus *Arabis* have long been recognized as the best accumulators of Zn (Ernst, 1974) but also hyperaccumulators of other toxic metals, so it is used as a model in numerous laboratory and field studies (Nanda & Abraham, 2013; Mou et al., 2021). They

are also known for their distinct ability to hyperaccumulate toxic metals (Ni, Cd, Cu, P and Zn) and grow successfully on highly contaminated soils without harmful effects on metabolism (Prasad & Freitas, 2003; Gawronski & Gawronska, 2007; Banášová et al., 2008). The obtained results showed that, on average, this species had the highest Fe content (15.2712 mg kg⁻¹) together with *Silene vulgaris*, and therefore it can be considered a hyperaccumulator for this metal.

Zn and Cr concentrations were higher in the leaves, while the content of Cu, Fe, Mn, and Ni was higher in the roots (Figure 3F). Species from Brassicaceae family are known to be a well-known hyperaccumulator Zn, which successfully accumulates Zn from metal and non-metal habitats to a level above 1% in leaves (Brooks & Robinson, 1998). This process can be explained by overexpression of Zn transmembrane transport regulating genes which can be connected with the results of our research (Assunção et al., 2001). Better translocation Zn in the shoot was also recorded by Fuksová et al. (2009) and Banášová et al. (2008) in *Thlaspi caerulescens* in contaminated soil. Mou et al. (2021) confirmed that several species of the genus *Arabis* hyperaccumulate Zn and Cd from the Pb - Zn mine area Huize.

Absorption, distribution and capacities of *A. hirsuta* are expressed by BCF (leaf/soil ratio) and leaf/root ratio (Table 1). BAC and BCF varied in the range of 1 - 0.1 indicating a medium accumulation potential for Cu, Zn, Ni and Cr. An exception is the BCF for Cr, whose value was 1.316, which indicates the ability of hyperaccumulation for this metal, as well as a good ability to move from the soil to the roots and a significant role in phytostabilization. TA greater than 1 (TF > 1) was found for Zn, indicating that this species remediates Zn by phytoextraction, accumulating it in the shoot. For all other metals, TA is less than 1, suggesting that this species remediates Cu, Zn, Ni, and Cr by phytostabilization, concentrating these metals in the roots. Research by Mou et al. (2021) showed that species of the genus *Arabis* have good transport capacities for Pb, Cu and Zn and that they naturally grow in mining areas as tolerant, which is of great importance for soil restoration and ecological restoration on such land.

3.8. The correlation between metal contents in different tissues of analyzed plant species

Principal components analysis indicated clear grouping and dispersion of studied species as well as analyzed elements per studied tissues (Figure 4). Principal components analysis explains 94.58 % of

variation in the data. Component 1 is 82.24 % and Component 2 is 12.34 % of variation. It is an indicative grouping of measured concentrations of analyzed toxic metals along predominantly first component in the positive direction. Along Component 2 the most indicative is the influence of Cr in the positive direction. The studied species and tissues are separated mostly along the second component i.e. root in the upper part of the graph and shoots in the lower part of the graph. The exception is the *Arabis* shoot which is grouped together with the shoots of the other plants. The extreme position of the *Arabis* root is also indicative. It is predominantly defined with high concentrations of Cr in the shoots and roots of the *Arabis*. Lower positions of *Populus* are predominantly defined with extremely low concentrations of Cr in shoots.

4. CONCLUSIONS

This study represents the result of research into the possibility of using selected herbaceous and woody plants in biomonitoring of pollution and phytoremediation of contaminated land. The obtained results showed an uneven distribution of the content of toxic metals in the tailings and investigated species in the area of the Ljubija iron ore mine. The study area was contaminated with toxic metals in the following decreasing order: Fe > Mn > Zn > Cu ≈ Cr > Ni. The average concentration of trace elements in the tissue of the examined plant species was as follows in decreasing order: Fe > Mn > Zn > Cu > Ni > Cr. Comparing metal content (Cu, Zn, Fe, Mn, Ni and Cr) in most plants, metal accumulation was higher in the

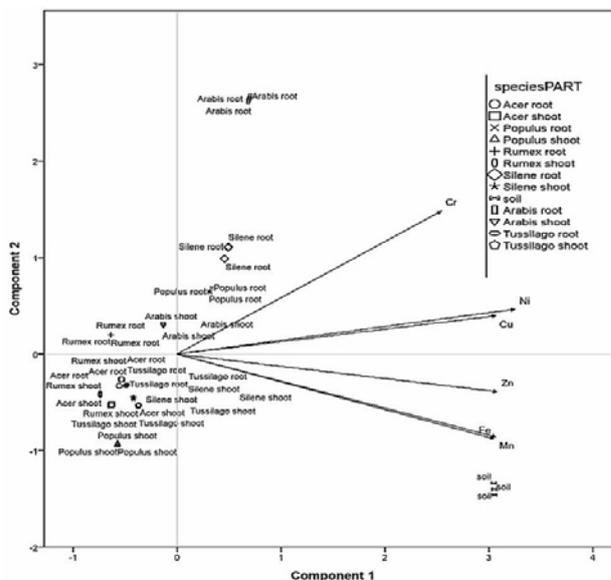


Figure 4. PCA analysis performed by comparing the obtained values of all analyzed metals in soil and in different organs of selected plant species.

Table 1. Determined bioaccumulation, bioconcentration and translocation factors of ragweed at the study area (BAC-bioaccumulation factor; BCF-bioconcentration factor; TA-translocation factor). Values >1 are marked in bold.

Species	Cu			Zn			Fe		
	BAC	BCF	TA	BAC	BCF	TA	BAC	BCF	TA
<i>R. hydrolopatum</i>	0.348	0.286	1.215	0.230	0.225	1.024	0.002	0.003	0.910
<i>A.pseudoplatanus</i>	0.367	0.325	1.126	0.295	0.306	1.964	0.004	0.015	0.311
<i>P. alba</i>	0.208	0.651	0.320	0.492	0.543	0.905	0.001	0.046	0.022
<i>T. farfara</i>	0.433	0.420	1.030	0.461	0.339	1.358	0.008	0.01	0.748
<i>S. vulgare</i>	0.436	0.728	0.598	0.393	0.505	0.778	0.014	0.049	0.290
<i>A. hirsuta</i>	0.372	0.626	0.594	0.488	0.470	1.038	0.016	0.037	0.429
Species	Mn			Ni			Cr		
	BAC	BCF	TA	BAC	BCF	TA	BAC	BCF	TA
<i>R. hydrolopatum</i>	0.007	0.004	1.579	0.035	0.111	0.324	0.072	0.357	0.203
<i>A.pseudoplatanus</i>	0.013	0.025	0.555	0.087	0.158	0.558	0.029	0.157	0.191
<i>P. alba</i>	0.012	0.059	0.215	0.118	0.365	0.323	0.002	0.532	0.003
<i>T. farfara</i>	0.010	0.011	0.932	0.130	0.115	1.125	0.062	0.112	0.593
<i>S. vulgare</i>	0.024	0.091	0.267	0.119	0.438	0.271	0.084	0.666	0.126
<i>A. hirsuta</i>	0.020	0.039	0.519	0.261	0.614	0.424	0.463	1.316	0.351

roots, while certain species had a higher accumulation in the leaves. In that sense, particularly Cu, Cr, and Zn accumulation was pronounced in *Rumex hydrolapathum*, Cu and Ni accumulation in *Tussilago farfara*, while *Arabis hirsuta* showed a high tendency for Zn uptake. Bioaccumulation factors were highly variable among the analyzed plant species. The results related to the BAC values showed that the *T. farfara*, *S. vulgaris* and *A. hirsuta* have the ability of Cu phytoextraction, while *P. alba*, *T. farfara* and *A. hirsuta* showed the ability of Zn phytoextraction. In addition, according to the BCF values *P. alba*, *S. vulgaris* and *A. hirsuta*, have the ability of Cu and Zn phytoextraction. Moreover, *A. hirsuta* with high BFC for phytoextraction of Cr and Ni especially stands out. According to the results of this study, priority should be given to planting herbaceous species for the revitalization of this area due to the lower ratio of BAC and BCF to TA, which makes these species suitable for phytostabilization and phytosanitization of the study area. Among the plants we examined, there is no typical hyperaccumulator, but certain levels of hyperaccumulation of iron and manganese, which are the most abundant elements in the soil of the research area, are especially pronounced in the leaves of *S. vulgaris* and *A. pseudoplatanus*. In this sense, these two species can be primarily proposed for the removal of Fe and Mn by the phytostabilization technique. Therefore, the study of plants that grow naturally on tailings pond with increased accumulation and tolerance to toxic metals is of great importance for land revitalization and ecological restoration of the mining area.

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