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Phytoaccumulation of heavy metals in native plants growing on soils in the Spreča river valley, Bosnia and Herzegovina

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Abstract: This study evaluated the phytoremediation potential of eight native plant species on heavy metal polluted soils along the Spreča river valley (the northeast region of Bosnia and Herzegovina). Plants selected for screening were: ryegrass (*Lolium perenne* L.), common nettle (*Urtica dioica* L.), mugwort (*Artemisia vulgaris* L.), wild mint (*Mentha arvensis* L.), white clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), dwarf nettle (*Urtica urens* L.) and yarrow (*Achillea millefolium* L.). All aboveground parts of selected native plants and their associated soil samples were collected and analysed for total concentration of Ni, Cr, Cd, Pb, Zn and Cu. The bioaccumulation factor for each element was also calculated. The levels of Cr (90.9–171.1 mg/kg) and Ni (80.1–390.5 mg/kg) in the studied soil plots were generally higher than limits prescribed by European standards, indicating that the soils in the Spreča river valley are polluted by Cr and Ni. Among the eight screened plant species, no hyperaccumulators for toxic heavy metals Ni, Cr, Cd and Pb were identified. However, the concentrations of toxic heavy metals in the above-ground parts of *Artemisia vulgaris* L. and *Trifolium repens* L. were significantly higher than in the other studied plants, indicating that both plant species are useful for heavy metal removal.

Keywords: contamination; environment; floods; soil pollution; toxic elements

The exploitation of natural resources, as well as industries involving heavy metals, are the main causes of soil pollution in the Tuzla Canton located in the north-eastern part of Bosnia and Herzegovina. Soil pollution by heavy metals not only degrades the soil fertility but also negatively affects human health and well-being through the food chain (Břendová et al. 2015). This problem is particularly pronounced through the Spreča river valley in north-eastern Bosnia. During its course, the river Spreča, with its tributaries, is exposed to various heavy metals emission sources, but the facilities of the chemical industry such as the Global Ispat Coking Industry Lukavac (GIKIL) coke plant and Sisecam Soda are certainly one of the main sources of these pollutants. Overall, the Spreča river is the largest recipient of most untreated or inadequately treated wastewater from Tuzla Canton and supplies water to the largest artificial lake in Bosnia and Herzegovina, Modrac (Ahmetović et al. 2020). Due to poorly developed infrastructure for flood

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protection, flooding of soils in the Spreča river valley is frequent, especially in the coastal area downstream of the accumulation Modrac, where water after intensive release from the dam of the Modrac lake floods larger coastal areas, thus increasing the risk of soil pollution by heavy metals.

In order to protect the soils as well as other compartments of the environment in the above-mentioned area, special attention should be given to remediation techniques for heavy metal contaminated soils. The overall aim of any soil remediation technique is to reduce the total and/or bioavailable forms of heavy metals and their subsequent accumulation in the food crops. The key factors that may influence the selection of remediation technique are: (*i*) cost; (*ii*) long-term effectiveness; (*iii*) commercial availability, and (*iv*) acceptability to the public and regulators (Wuana and Okieimen 2011). However, various techniques exist for the remediation of heavy metal-contaminated soil: physical, chemical and biological. Physical remediation techniques include soil replacement, soil isolation, vitrification and electro-kinetic remediation, while immobilisation technique (i.e., remediation by adding immobilising agents to the contaminated soils), encapsulation (i.e., the mixing of the contaminated soils with some chemical products), and soil washing (i.e., the use of solvents to remove heavy metals from the soil) belong to basic chemical remediation techniques. Despite high efficiency, the majority of these remediation techniques are expensive, environmentally destructive, harmful to soil fertility and therefore not well accepted by the public. Hence, the use of nature-friendly and cost-effective biological remediation techniques is a more acceptable approach to remediate the contaminated soils (Petelka et al. 2019).

Among the biological remediation techniques, phytoextraction is one of the most widespread techniques for remediation of heavy metal-contaminated soils. Phytoextraction makes use of the capacity of some plants to take up and translocate heavy metals from soil to aboveground plant tissues. In addition to serving as remediation tools, these plants can provide an added economic value if used, for instance, in energy production and/or in high-value metal recovery. Furthermore, phytoextraction is a non-invasive remediation technique and provides a long-term solution (Ruley et al. 2019).

The efficiency of phytoextraction relies on a few factors such as plant selection, heavy metal bioavailability, and soil chemical and physical properties. However, the selection of appropriate heavy metal-tolerant plant species is the most important criterion for successful phytoextraction (Suman et al. 2018). Yoon et al. (2006) noted that native plants should be selected as phytoextraction tools because these plants are evolutionary products of that environment. Over the long-term, native plants are adapted to their homeland environment and thus better able to survive, grow and reproduce under the environmental extremes of the local area compared to plants introduced from other environments.

Therefore, the main objectives of this study are (1) to identify native plant species thriving on heavy metal polluted soils in the Spreča river coastal area and (2) to evaluate their phytoextraction potential.

MATERIAL AND METHODS

Study area. A field study was conducted at three sites in the Spreča river valley downstream from Modrac lake (Figure 1). Each sampling site was approximately 50–100 m on both river banks and included three soil plots. The soil plots (an area of 200 m^2) at each sampling site were within close distance to each other (up to 300 m).

According to Köppen and Geiger classification (Kottek et al. 2006), the climate in the Spreča river valley is classified as Cfb (temperate oceanic climate). In general, the climate here is warm and temperate, with a lot of rain even in the driest month. At the time of heavy rainfall occurring almost every year in spring and autumn, the Spreča river floods soils in its coastal area, which has a major impact on contamination of soils. Therefore, these soils were selected for the research.

Soil sampling and analysis. A total of 9 average samples were collected from three research sites in March 2020, at the depths of 0–30 cm using stainless steel shovel. The average soil sample from each soil plot was made by physically mixing five individual soil cores (from north, south, east, west and centre of the plot) into one homogenous sample. All of the composite soil samples were placed in polythene bags and transported to the laboratory on the day of sampling. Each soil sample was cleared of impurities, air-dried at ambient temperature, ground with mortar and pestle, and finally sieved through a 1-mm sieve for organic matter, pH and heavy metals analysis.

Soil pH was measured in a 1:2.5 soil-solution ratio in 1 mol/L KCl (ISO 10390, 2005), and soil organic

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Figure 1. Map of the Spreča river basin downstream from Modrac lake and the sampling sites

carbon was determined by the H_2SO_4-$K_2Cr_2O_7$ oxidation method (ISO 14235, 1998).

Extraction of heavy metals from each soil sample was performed by mixing 3 g of the soil with 21 mL *aqua regia* solution for 16 h (overnight) in a fume hood. The mixture was then heated to its boiling point on a hotplate under reflux for 2 h. After cooling, the obtained solution was filtered through quantitative filter paper into a 100 mL flask and filled to the mark with deionised water (ISO 11466, 1995).

Heavy metals concentrations in the obtained extracts were analysed by atomic absorption spectrometry (ISO 11047, 1998) using a Shimadzu AA-7000 spectrophotometer (Tokyo, Japan). The standard stock reagents (Merck, Darmstadt, Germany) containing 1 000 mg/kg of tested heavy metals were used for the preparation of calibration standards. The correlation coefficients of the calibration curve for all tested heavy metals were higher than 0.99, which assured linearity of response over the range of concentrations used.

Vegetation survey. Vegetation surveys were conducted in July 2020. Quadrat sampling method was used to identify and record native plant species. Five quadrats (1 m × 1 m each) were established at each of the study sites. The following data were recorded for each quadrat: cover, species density and species frequency. The plant density was calculated by counting the number of individual plants of a species and dividing it by the quadrat's area, while the plant cover was calculated as the relation between the area covered by such species and the total area sampled. The cover classes and percentage cover ranges used in this study were: + = 0%; 1 = 1–10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100% coverage. Species frequency involves calculating the percentage of quadrats that contain each plant species (Braun-Blanquet 1964). A total of 71 plant species were identified in the Klokotnica village, but only two plant species, i.e. yarrow (*Achillea millefolium* L.) and dwarf nettle (*Urtica urens* L.), are characterised by high-density value (> 50 plants/m^2) and high plant cover characteristics (cover class 5). Accordingly, these two plants were selected for further investigation, that is, for evaluating their ability to remove heavy metals from studied soils.

A total of 67 plant species were recorded in the Donja Lohinja village, but only 6 native plant species had high-density value and high plant cover characteristics. These native plant species were as follows: ryegrass (*Lolium perenne* L.), common nettle (*Urtica dioica* L.), mugwort (*Artemisia vulgaris* L.), wild mint (*Mentha arvensis* L.), white clover (*Trifolium repens* L.) and alfalfa (*Medicago sativa* L.). *Lolium perenne* L. had the highest density (187 plants/m^2), followed by *Trifolium repens* L. (131 plants/m^2), *Urtica dioica* L. (69 plants/m^2), *Medicago sativa* L. (41 plants/m^2), *Mentha arvensis* L. (33 plants/m^2) and *Artemisia vulgaris* L. (27 plants/m^2).

Vegetation survey also revealed that plant habitats along the Spreča river margins and banks in the Lukavac town are greatly affected by riverine and coastal floods. A total of 34 different plant species were recorded at this site; however, none of them

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with high or medium density and cover value. Due to the reasons mentioned above, the assessment of the phytoremediation potential of native plants at this site has not been performed.

Plant sampling and analysis. Native herbaceous plants from examined soil plots were collected during the summer of 2020 at the stage when plants have reached their maximum growth in height. For each of the dominant native herbaceous plants, three samples were randomly chosen within the study area (1 × 1 m quadrat). Each plant sample consisted of five healthy individuals without obvious symptoms of pathology or herbivore attack. Above-ground parts of selected plants were collected very carefully, then placed in sealed plastic bags and transported to the laboratory.

The washed plant samples were separately dried in an oven at 60 °C for 48 h, ground into powder using a dry leaf grinder machine, then transferred into small paper bags and stored at room temperature until analysis.

Heavy metals in the plant samples were extracted with a mixture of HNO_3 and H_2SO_4 in the ratio of 2.5 : 1 *v*/*v*. Briefly, 1 g of dried and grinded plant material was placed into 100 mL flat bottom flask, then 10 mL HNO_3 and 4 mL H_2SO_4 were added. The flasks were left for few hours at room temperature in a fume hood and then heated to its boiling point on a hotplate under reflux for 30 min. After cooling, the mixture was filtered through quantitative filter paper into a 50 mL flask and filled to the mark with deionised water (Lisjak et al. 2009). The heavy metal concentrations in the obtained extract were also determined by atomic absorption spectrophotometry.

Bioaccumulation factor. Bioaccumulation factor (BAF) refers to the efficiency of a plant species to accumulate heavy metals into its tissue from the surrounding environment (Ladislas et al. 2012). As indicated in Dessalew et al. (2018), BAF was calculated using the following equation:

$$\text{BAF} = \frac{C_{\text{plant}}}{C_{\text{soil}}} \quad (1)$$

where: C plant and C soil – heavy metal concentration in the harvestable aboveground plant material and in the soils, respectively.

Plants with BAF values higher than 1 demonstrate the potential success of a plant species for phytoremediation purposes (Ramírez et al. 2021).

Statistical analysis. All experimental measurements were performed at least in triplicate, and the results were presented as mean ± standard deviation. The one-way analysis of variance (ANOVA) and least significance difference test (*LSD*) with a 95% confidence interval ($P < 0.05$) were applied to identify significant differences between the means using Microsoft Excel 2010 (Office 2010, Redmond, WA, USA)

RESULTS

Total heavy metal concentrations and selected chemical properties of studied soils. pH value, soil organic carbon (SOC) and levels of heavy metals in soil samples collected from the Spreča river valley are presented in Table 1.

Table 1. Heavy metal concentrations (mg/kg), pH and soil organic carbon content (SOC, %) of studied soils

Research site		pH_{KCl}	SOC	Cr	Cd	Pb	Zn	Cu	Ni
S1 Lukavac	soil 1	7.2	2.4	169.9	0.0	8.5	41.9	13.3	84.3
	soil 2	7.0	2.1	151.2	0.0	8.1	33.1	16.1	80.1
	soil 3	7.4	2.5	144.1	0.0	9.0	37.2	15.9	103.3
S2 Donja Lohinja	soil 1	7.2	2.8	169.6	0.2	16.0	42.7	27.6	380.1
	soil 2	7.1	2.7	171.1	0.1	14.5	48.4	28.2	390.5
	soil 3	7.2	2.9	181.1	0.1	15.1	45.1	27.9	386.4
S3 Klokotnica	soil 1	7.3	3.2	104.1	0.0	10.1	58.1	14.1	201.1
	soil 2	7.1	3.3	90.9	0.0	10.6	65.3	16.5	206.3
	soil 3	7.0	3.3	106.2	0.0	10.8	70.1	17.1	194.2
World mean*				50	0.4	15	40	12	25
Limit value**				100	1.5	100	200	100	70

*World average concentrations reported by Berrow and Reaves (1984); **limit value proposed by European Commission (Gawlik and Bidoglio 2006)

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The pH of the soil samples analysed ranged from 7.0 to 7.4, showing that the soils in the study area are neutral to slightly alkaline in nature. All studied soils have a medium organic carbon content.

The highest total concentration was recorded for Ni, followed by Cr, Zn, Cu, Pb and Cd concentration being the lowest one. The results also showed that the concentrations of Cr and Ni in the soil plots located in Donja Lohinja and Klokotnica were much higher than those in Lukavac town. According to World Reference Base for Soil Resources (IUSS 2015), the studied soils in Donja Lohinja and Klokotnica were classified as Fluvisol, humic (humofluvisol). The pedogenetic processes in humofluvisol are affected by the humus accumulation underneath the meadow vegetation as well as wetting the surface soil horizons through the atmospheric, ground and occasionally floodwaters. Generally, humofluvisols have a mainly fine-loamy granulometric composition with a good balance between air-porosity and water-holding capacity. Considering these parameters, humofluvisols have good characteristics for plant growth and development, thus contributing remediation efficiencies.

Heavy metal concentrations in plants growing on soils in the Spreča River valley. Heavy metal concentrations (Cr, Cd, Pb, Zn, Cu and Ni) in the above-ground parts (stem and leaves) of selected eight plants from the studied soil plots are presented in Table 2.

Heavy metals' levels revealed significant variations in the eight studied native plants. Cr and Ni levels were found to be highest in white clover (*Trifolium repens* L.) and mugwort (*Artemisia vulgaris* L.), while the lowest quantity of those was found in common nettle (*Urtica dioica* L.). Pb and Cd levels were found to be highest in the above-ground parts of *Artemisia vulgaris* L., whereas the lowest concentration was recorded in *Lolium perenne* L. The results showed no significant differences in Cu and Zn concentrations within the above-ground parts of studied native plants.

Bioaccumulation factor. Bioaccumulation factor values of Cr, Cd, Pb, Zn, Cu and Ni in selected native plants from the study area are shown in Table 3.

According to BAF data, plant species differ greatly in their ability to accumulate heavy metals from studied soils. The highest BAF value for Cr and Ni were found in *Trifolium repens* L. and *Artemisia vulgaris* L., while the lowest BAF of most of the elements was observed in *Urtica dioica* L. and *Lolium perenne* L. In this study, however, none of the studied plant species reached acceptable BAF values for hyperaccumulation.

DISCUSSION

The levels of Ni and Cr in soils collected from the sampling sites were much higher than the threshold values proposed by European Commission, 70 and 100 mg/kg, respectively. Considering the Cr and Ni are present in high concentrations in soils developed on ultramafic rocks (Quantin et al. 2008), these findings lead to the hypothesis that the parent material of the studied soils is characterised by ultramafic bedrocks and their minerals. This hypothesis has, in fact, been confirmed by many scientists (Cipurković et al. 2011, Babajić et al. 2017).

Table 2. Heavy metal concentrations (mg/kg) in the above-ground biomass of plants

Selected native plants	Research site	Cr	Cd	Pb	Zn	Cu	Ni
Lolium perenne L.	S2 Donja Lohinja	4.2 ± 1.3^{d}	0.012 ± 0.008^{c}	0.03 ± 0.02^{d}	35.9 ± 7.1	12.32 ± 6.1	6.0 ± 2.1^{d}
Urtica dioica L.		0.9 ± 0.8^{d}	0.022 ± 0.012^{b}	0.04 ± 0.03^{d}	31.8 ± 6.5	13.81 ± 5.3	1.7 ± 1.9^{d}
Artemisia vulgaris L.		28.1 ± 7.1^{b}	0.034 ± 0.011^{a}	3.36 ± 0.58^{a}	34.5 ± 6.2	16.92 ± 6.8	42.9 ± 9.1^{b}
Mentha arvensis L		2.1 ± 0.9^{d}	0.019 ± 0.004^{b}	1.31 ± 0.50^{c}	32.2 ± 5.9	13.87 ± 6.3	7.6 ± 4.8^{d}
Trifolium repens L.		47.8 ± 11.6^{a}	0.033 ± 0.006^{a}	2.43 ± 0.69^{b}	31.3 ± 7.2	16.46 ± 7.1	88.2 ± 14.5^{a}
Medicago sativa L.		14.5 ± 2.4^{c}	0.024 ± 0.008^{b}	1.12 ± 0.84^{c}	30.08 ± 8.1	15.28 ± 6.6	28.8 ± 11.5^{c}
Urtica urens L.	S3 Klokotnica	1.6 ± 1.6^{d}	nd	nd	38.9 ± 8.3	12.9 ± 5.1	5.2 ± 3.5^{d}
Achillea millefolium L.		2.1 ± 1.4^{d}	nd	nd	36.2 ± 7.6	12.1 ± 7.3	6.1 ± 4.1^{d}
$LSD_{0.05}$*		4.41	0.007	0.42	–	–	7.25

nd – below the detection limit. *Averages denoted by the same letter indicate no significant difference ($P \leq 0.05$); *LSD* – least significant difference

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Table 3. Bioaccumulation factor values (BAF) for the heavy metal transfer from soils to plants

Selected native plants	Research site	BAF					
		Cr	Cd	Pb	Zn	Cu	Ni
Lolium perenne L.	S2 Donja Lohinja	0.025	0.080	0.002	0.843	0.442	0.016
Urtica dioica L.		0.005	0.147	0.002	0.747	0.489	0.004
Artemisia vulgaris L.		0.164	0.243	0.232	0.764	0.606	0.110
Mentha arvensis L		0.011	0.136	0.087	0.754	0.492	0.020
Trifolium repens L.		0.264	0.220	0.161	0.733	0.584	0.228
Medicago sativa L.		0.080	0.160	0.074	0.704	0.548	0.075
Urtica urens L.	S3 Klokotnica	0.015	–	–	0.670	0.754	0.025
Achillea millefolium L.		0.020	–	–	0.623	0.707	0.031

Pb, Zn, Cd and Cu concentrations in the studied soils were much lower than the threshold values proposed by European Commission, indicating that there is no pollution by Pb, Zn, Cd and Cu in the research sites.

Problems with heavy metals pollution of soil include phytotoxic effects of heavy metals as well as their transfer to the food chain, causing a potential health risk to humans and animals (Puschenreiter et al. 2005). Among heavy metals, Cr and Ni are highly toxic metals to living organisms, and therefore contamination of the food chain with these elements deserves special attention. However, there are no simple solutions for efficient and quick remediation of Cr and Ni polluted soils or for the control of its negative influence on living organisms. Many scientists agree that phytoextraction is the less destructive and drastic process among remediation techniques (Wyszkowska et al. 2019, Nedjimi 2021); therefore, the present study focuses on remediation of Cr and Ni polluted soils in the Spreča river valley using phytoextraction as a remediation technique. In this study, eight native plant species, i.e., ryegrass (*Lolium perenne* L.), common nettle (*Urtica dioica* L.), mugwort (*Artemisia vulgaris* L.), wild mint (*Mentha arvensis* L.), white clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), dwarf nettle (*Urtica urens* L.) and yarrow (*Achillea millefolium* L.), were chosen in terms of evaluating their practical phytoextraction potentials. Although numerous studies have confirmed that the above-mentioned native plants have the ability to accumulate heavy metals in very high concentrations (Gajić et al. 2018, Antoniadis et al. 2021), their potential for removal heavy metals from soils in the studied area has not been sufficiently investigated.

In the present study, the highest bioaccumulation of Cr and Ni was noticed in *Trifolium repens* L. and *Artemisia vulgaris* L. Earlier studies also revealed that *Trifolium repens* L. and *Artemisia vulgaris* L. could accumulate Ni and Cr effectively (Mleczek et al. 2018, Lin et al. 2021). Furthermore, both plant species have the ability to adapt to different environments, produce high biomass and offer multiple harvests in a single growth period. Taken together, these data suggest that *Trifolium repens* L. and *Artemisia vulgaris* L. have a great potential for retrieving Cr and Ni from studied soils.

However, the results obtained by BAF analyses have not fully confirmed these findings. BAF value of Cr and Ni for both plant species were less than 1, indicating that *Trifolium repens* L. and *Artemisia vulgaris* L. could not be considered as suitable plants for Cr and Ni phytoextraction from studied soils. However, a number of scientists argued that the criterion of BAF > 1 does not necessarily have to be achieved by plants to qualify as heavy metal hyperaccumulating plants (Proc et al. 2021).

Sheoran et al. (2016) reported that the primary requirement for retrieving heavy metals from soils is the solubility of heavy metal in the soil solution. Namely, it is very difficult to remove heavy metals from soils using plants if the heavy metals are not at least slightly soluble, regardless of plant genotype. Therefore, BAF value is commonly used to evaluate heavy metal mobility in soils.

There are, in fact, many factors that are important in the evaluation of plants for phytoextraction purposes, such as plant genetic background, soil chemical and physical properties and heavy metal levels in the soil. Among soil properties, soil pH is considered as one of the most important factors influencing the bioavailability of metals in the soil for plant uptake. Usually, the bioavailability of heavy metals increases as pH decreases because H^+ ions contributes to displacing the cations from the adsorption soil complex, reducing the cation exchange capacity, and thus increasing the mobility of heavy metals in the soil solution (Kupka et al. 2021). Our assumption is that specific chemical properties of the studied soils, i.e. high pH value caused a decrease in heavy metals mobility in the soil solution, resulting in lower metal-accumulating capacities of plants. Regardless, this study showed that BAF value for hazardous heavy metals Ni, Cr, Cd and Pb in *Trifolium repens* L. and *Artemisia vulgaris* L. was much higher than those in the other studied plants. These findings are consistent with the hypothesis that the efficiency of heavy metal removal by plants depends strongly on the plants' genetic background (Koźmińska et al. 2018).

An interesting finding of this study was that all studied plants most efficiently took up Zn and Cu from soils. The BAF ranges obtained for Zn and Cu were 0.623–0.843 and 0.442–0.754, respectively. This pattern of translocation of Zn and Cu from soil to above-ground parts of plants was not surprising because Cu and Zn are essential nutrients for many physiological processes, including photosynthesis and growth hormone production; therefore, plants tend to translocate them to leaves (Roschzttardtz et al. 2019).

Among the heavy metals, BAF value was found to be the lowest for Pb, regardless of plant species. The lowest BAF value for Pb was also not surprising because Pb is known to be the least bioavailable heavy metal (Agnieszka et al. 2014). Furthermore, Pb is a toxic element for plants even at low concentrations, and therefore plants activate different mechanisms to prevent or reduce Pb uptake and its translocation from roots to the above-ground part of plants. Some of these mechanisms are: limiting uptake of Pb and other toxic heavy metals through immobilisation of metals by mycorrhizal association or complexation by exuding organic compounds from roots, metal sequestration and compartmentalisation in vacuoles, metal binding to the cell wall, etc. (Usman et al. 2020).

Overall, the results of this study revealed that the studied soils in the Spreča river valley are polluted by Cr and Ni. Among the 8 screened plant species, no hyperaccumulators for Cr, Cd, Ni and Pb were identified. Low BAF value for all tested toxic heavy metals was probably associated with the chemical properties of studied soils that were unsuitable for heavy metals mobility, and thus their availability to plants. However, the concentrations of toxic heavy metals, i.e., Cr, Ni, Cd and Pb in the above-ground parts of *Trifolium repens* L. and *Artemisia vulgaris* L. were significantly higher than those in other studied plants, indicating that both plant species represent a good tool for a phytoremediation approach on polluted soils.

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