Agronomy Research **17**(1), 234–244, 2019
https://doi.org/10.15159/AR.19.005

Assessment of wild plants for phytoremediation of heavy metals in soils surrounding the thermal power station

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Abstract. The present investigation was carried out to evaluate the phytoextraction potential of three main wild plant species: annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) that grow spontaneously in heavy metal contaminated areas near the thermal power station in Kakanj, Bosnia and Herzegovina. Analyses of the heavy metal content (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in soil and plant samples taken from the examined area were performed using atomic absorption spectrophotometry. The results obtained revealed that the examined soils are polluted by Ni and Pb and contain relatively high value of Cr and Fe. Annual nettle, daisy fleabane and yarrow have not shown high efficiency in the absorption and accumulation of heavy metals from polluted soils, and therefore these plants are not be considered as potential phytoremediators of soils on the examined area. Furthermore, the results of the study undoubtedly confirm the fact that the total content of heavy metals in soils is not a sufficient parameter for estimating the toxicity of heavy metals in soils and consequently for their transfer and accumulation in plants.

Key words: environment, heavy metals, pollution.

INTRODUCTION

Thermal power station in Kakanj (TPS) uses coal for electricity generation and is one of the largest environmental polluters in Bosnia and Herzegovina. Environmental deterioration is attributed to emission of large amount of carbon dioxide (CO_2), sulphur dioxide (SO_2), nitric oxide (NO), particulate matter (PM) and heavy metals as result of coal burning (Guttikunda & Jawahar, 2014). These hazardous materials are accumulated in environment and lead to severe environmental and health impacts, causing respiratory and related ailments to humans and animals and leading to disorder in physiological processes in the plant (Nagajyoti et al., 2010). Soil and water contaminated with heavy metals may pose risks and hazards to human health through: consumption of fruits and vegetables grown on these soils, drinking of contaminated ground water, reduction in land usability for agricultural production, etc. (Morais et al., 2012).

The release of heavy metals from TPS and their subsequent deposition in soil is a growing global concern that requires much greater attention of society. The necessity for harmonization of thermal engineering with environment for meeting the needs of the living and future generations has become obvious. One of the ways in which above mentioned harmonization can be achieved is restoration of heavy metal contaminated soils and investment in modern and environmentally-friendly technology, thus reducing environmental pollution due to TPS activity. Different approaches are used for the restoration of heavy metal contaminated soils: physical, biological and chemical (Sharma et al., 2018). Most physical and chemical approach are expensive and do not make soil acceptable for plant growth. In contrast, biological approach (phytoremediation) offers green technology solution for the heavy metal contamination problem without any negative impact on the environment (Marques et al., 2009).

Phytoremediation represents a group of innovative technologies that use plants to remove, detoxify, or stabilize persistent heavy metals and other contaminants in soils by inactivating contaminants in the rhizosphere or translocating them in the above-ground parts (Lone et al., 2008).

However, the ability to remediate heavy metals in soils varies significantly between plants, as different mechanisms of heavy metal absorption are operative in each plant species, based on genetic background of plants, and their morphological, anatomical, and physiological characteristics (Liu et al., 2013).

According to Yoon et al. (2006), wild plants should be preferred for phytoremediation since these plants are often better in terms of growth, development, reproduction and generally survival under stress conditions compared to plants introduced from other environments.

Some wild plants such as annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) spontaneously growing on soils around TPS in high density and therefore these plants could be very interesting candidates as potential phytoaccumulator or phytostabilizer of heavy metals from contaminated soils on the examined area. These plants, as annual plants, could be used to remove heavy metals from contaminated soils by harvesting/coppicing (Jacobs et al., 2017). However, up to now, there is no systematic investigation in Bosnia and Herzegovina related to the possibility of using these plant species for that purpose, especially not in soils surrounding the thermal power stations.

The main objectives of this study were: 1) to identify the wild plants that grow in high density in soils around TPS; 2) to determine the content of heavy metals (Cu, Zn, Pb, Co, Cr, Fe, Ni and Mn) in examined soil and plant samples 3) to evaluate ability of selected wild plants to remove or stabilize heavy metals based on the determined soil-plant transfer factor (TF).

MATERIALS AND METHODS

Study Area

TPS (44°5′26″ N, 18°6′51″ E) is located on the left bank of the river Bosnia, five kilometers upstream of Kakanj town, in Central Bosnia. The TPS includes three units with a total installed capacity of 450 MW. The climate in this area is classified as Cfb by Köppen and Geiger. The average annual temperature in Kakanj is 10.7 °C, and precipitation here averages 960 mm.

Species richness estimation

Species richness (i.e., the number of species) is the simplest concept for evaluating vegetation community in some area.

Since the plants were not equally distributed around the TPS, only the areas where the individual plants grew in high density were the subject of research. Three study plots (each 100 m^2) with these characteristics were established in the immediate vicinity of TPS. A 1 m x 1 m quadrat was used to study plant population and each experimental plot had three quadrats. High density of dominant plant species and cover characteristics was criteria for quadrat selection.

Plant density was determined by counting the number of individuals of plant species in uniformly sized sample plots within a site. The dominant plant species on the experimental plot 1, 2 and 3 was annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.), respectively (Table 1).

Table 1. Density of dominant plant species at the experimental soil plots

Plant species	Number of individuals in each quadrat			Total number of individuals (S)	Total number of quadrat studies (Q)	Density S/Q
	I	II	III			
Urtica urens L. (soil plot 1)	62	56	59	177	3	59
Stenactis annua (L.) Ness. (soil plot 2)	112	96	108	316	3	105.33
Achillea millefolium L. (soil plot 3)	123	100	101	324	3	108

Since these plants have the capacity to successfully growth on the soils surrounding the TPS, they were studied as potential plant species for soil remediation.

Soil sampling and preparation

Soil sampling was carried out in March 2018 from each of the three examined soil plots in the immediate vicinity of a thermal power station. At each plot (area of 100 m^2) soil samples were collected from five spots (north, south, east, west and centre of the plot) that were thoroughly mixed to make the average sample. Samples were taken at a depth of 0–30 cm using a soil sampler probe.

Estimation of basic chemical properties of soil

The following basic chemical properties of soil were subject of analysis: soil acidity (pH), organic matter (OM), content of available forms of phosphorus (P_2O_5) and potassium (K_2O) and $CaCO_3$ content. Soil pH was determined by the potentiometric method according to ISO 10390 method (2005), OM by oxidation with potassium dichromate in the presence of sulphuric acid according to ISO 14235 method (1998), the content of available phosphorus and potassium by AL – method (Egnér et al., 1960), and $CaCO_3$ content by volumetric method (Allison & Moodie, 1965).

Extraction of heavy metals from soil samples

Extraction of heavy metals from the soil was carried out with aqua regia solution (a mixture of HCl and HNO_3 in a ratio of 3:1) as follows: 3 g of the air-dried soil was placed in 250 mL round bottom flask, and 28 mL of aqua regia (21 mL HCl and 7 mL HNO_3) was added. The flask was covered with a watch glass and allowed to stand 16 h (overnight) at room temperature. After, the mixture was heated on hotplate under reflux for two hours and cooled to room temperature. At the end, the mixture was filtered through quantitative filter paper into 100 mL Erlenmeyer flasks and diluted to the mark with deionized water according to ISO 11466 method (1995).

Plant sampling and preparation

A 1 m x 1 m quadrat was used to study vegetation cover in the examined area. Each examined soil plot had three quadrats. Quadrats were established subjectively within a study site (high density of dominant plant species and cover characteristics were criteria for quadrat selection). Plants were collected in July 2018 at the flowering stage when plants have reached their maximum height. Whole plants (with root) were sampled very carefully, then placed in clean plastic bags and transported to the laboratory. Five individual plants of dominant plant species from each quadrant were taken for analysis. The plants were carefully washed with distilled water, thereafter each plant was separately dried and grinded, and then stored in bags until extraction.

Extraction of heavy metals from plant samples

Extraction of heavy metals from the plant material was performed as follows: 1 g of air-dried and grinded plant material was placed into 100 mL round bottom flask, then 10 mL HNO_3 and 4 mL H_2SO_4 was added. The flasks were left for few hours at a room temperature and then heated gently on a hot plate for thirty minutes. After cooling to room temperature, the solution in flask was filtered through quantitative filter paper in 50 mL flask and diluted to the mark with deionized water (Lisjak et al., 2009).

Determination of heavy metals

Content of heavy metals in soil and plant samples were determined by atomic absorption spectrophotometer (AA 7000, Shimadzu, Japan), according to the instructions specified in the ISO 11047 method (1998). The standard solutions of examined heavy metals were prepared by dilution of standard stock solutions (Merck AAS solutions) with deionized water.

Determination of transfer factor soil-plant

Transfer Factor (TF) is an index used to assess the mobility of heavy metal from soil to plant. TF was calculated by dividing the content of heavy metals in the plant by the total heavy metals content in the soil (Cui et al., 2004; Osu & Ogoko, 2014).

$$TF = C\ plant / C\ Soil$$

where C plant and C soil represents the toxic metal content in the soil and plant samples on dry weight basis, respectively. If the plants have TF values higher than one, they can be potentially used for phytoextraction.

Statistical analysis

All experimental measurements with plant material were carried out in triplicate and the results were presented as mean ± standard deviation. The results were processed statistically using one-way ANOVA and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$. Means that differed at $P \leq 0.05$ were considered as significantly different.

Statistical analysis was performed using Microsoft Excel software and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Heavy metal contents (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in examined soil plots are given in Table 2.

The content of toxic elements Cd, Cr, Cu, and Zn in examined soil samples did not exceed the maximum permissible value (Table 2), indicating that examined soils is not polluted by these elements. Permissible value of Fe and Mn in soils is not established by legislative, because they are not directly contaminant elements. However, the content of Mn in examined soils was lower than toxic level of Mn in soils (850 mg kg^{-1}) reported by Pais & Jones (1997), while the Fe content in analysed soils was higher than the average value of these elements in soil (0.6–1.2%) reported by Kabata-Pendias & Pendias (2001).

Table 2. Content of heavy metals in soil (mg kg^{-1} dry mass)

Soil plot	Heavy metal							
	Ni	Fe	Cr	Cu	Zn	Cd	Pb	Mn
1	106.6	19,110.6	71.9	37.1	58.8	0.3	141.3	257.9
2	111.1	1,9012.5	59.1	44.1	55.1	0.1	138.4	303.1
3	102.1	20,001.1	65.1	45.2	51.1	0.2	156.1	255.2
Permissible value[1]	50	-	100	80	200	1.5	100	-

[1]Permissible value prescribed by legislation in BIH; *Abbreviations: - = not listed in legislation.

In the present study, it was found that the content of toxic heavy metals Ni and Pb in all examined soil samples exceeded the maximum permissible value for agricultural soil prescribed by legislation in Bosnia and Herzegovina (Official Gazette of FBIH, 2009), and by legislation in some European countries (Pérez et al., 2002).

The content of heavy metals (Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn) in dominant plant species is presented in Table 3.

Table 3. Content of heavy metals in plant samples (mg kg^{-1} dry mass)

Heavy metal	Plant species			F test	$LSD_{0.05}$
	Urtica urens L.	*Stenactis annua* (L.) Ness.	*Achillea millefolium* L.		
Ni	0.71 ± 0.33	0.9 ± 0.15	0.89 ± 0.16	n. s.	-
Fe	172.55 ± 10.22^{c}	275.03 ± 16.54^{b}	295.7 ± 14.94^{a}	s.	14.33
Cr	0.87 ± 0.32	1.17 ± 0.41	1.33 ± 0.32	n. s.	-
Cu	8.53 ± 0.92^{a}	5.7 ± 1.51^{b}	7.04 ± 1.35^{a}	s.	2.02
Zn	24.65 ± 2.92^{a}	17.24 ± 5.31^{b}	19.45 ± 3.91^{b}	s.	4.19
Cd	n. d.	n. d.	n. d.	-	-
Pb	0.21 ± 0.11	0.24 ± 0.09	0.31 ± 0.14	n. s.	
Mn	18.97 ± 3.11	15.65 ± 4.14	13.34 ± 3.22	n. s.	

Means in rows followed by the same letter are not significantly different at $P = 0.05$; *Abbreviations: n. d. = not determined; n. s. = no significant; s. = significant; - = not analysed.

Annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) are the spontaneous plants growing in high density on the examined area, and therefore the purpose of this study was to examine their ability to accumulate heavy metals from soils, especially Ni, Pb and Cr. If these plants have ability to absorb these elements in high amounts, then there is an objective possibility of their use to remove heavy metals from soils around TPS.

Verbruggen et al. (2009) reported that plants can be considered as a plant for soil remediation, if the content of heavy metals in their harvestable parts is approximately 1% or more of Zn, 0.1% or more of Co, Cr, Cu, Ni, Pb, Sb and 0.01% or more of Cd.

Except of ability to take up large amounts of heavy metals from soil, each plant for soil remediation must be adaptive to soil and climate characteristics on the examined area. Also, the roots of plants must fit the spatial distribution of heavy metals in soil (Keller et al., 2003). de Kroon & Hutchings (1995) noted that the root system possesses a certain level of plasticity that allows plants to cope with a wide range of soil factors, thus enhancing their ability to accumulate heavy metals from soils. The ability of plant to absorb heavy metals from soil is also dependent on a number of plant factors. Plant seasonality, the characteristics and behaviour of an individual plant species or variety are also very important in evaluating the possiblity of plants to absorb large amounts of heavy metals from soils (Mondol et al., 2011).

Unfortunately, in this study the amounts of heavy metals absorbed by above mentioned plants were significantly less than the criteria required for classifying these plants as potential hyper-accumulators. Moreover, the content of Ni, Pb, Cr and other heavy metals in tested plants; annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) was lower or in the range with normal value for these elements in plants. The normal range of Ni, Fe, Cr, Cu, Zn, Cd, Pb, Mn in plants are 0.02–50 mg kg^{-1}, 30–300 mg kg^{-1}, 0.1–1 mg kg^{-1}, 5–20 mg kg^{-1}, 20–100 mg kg^{-1}, 0.1–2.4 mg kg^{-1}, 0.5–30 mg kg^{-1}, 15–150 mg kg^{-1} respectively (Chaney, 1989; Kastori et al., 1997).

The main reasons for the low level of heavy metals in plants are closely related to the chemical properties of soils, primarily to the soil reaction (Chibuike & Obiora, 2014). Wang et al. (2006) reported that the availability of most heavy metals, especially Cd and Zn, decreased with increases in soil pH. Namely, in alkaline soils, heavy metal ions bind with hydroxyl ions and carbonates to forms that have extremely low solubilities, thus becoming unavailable or low available to plants (Tack, 2010). This conclusion is consistent with some other studies that also reported the low availability of heavy metals in neutral and alkaline soils (Alkorta et al., 2004; Lenart & Wolny-Koładka, 2013).

As shown in Table 4, the all examined soil plots had an alkaline reaction, and relatively high content of $CaCO_3$ that negatively affected on the uptake of heavy metals by root of plants, so these results confirmed above mentioned hypothesis.

Table 4. Basic chemical properties of soils

Soil plot	pH (H_2O)	pH (KCl)	OM (%)	P_2O_5 (mg $100g^{-1}$)	K_2O (mg $100g^{-1}$)	$CaCO_3$ (%)
1	7.7	7.1	3.54	6.1	22.1	9.62
2	7.5	7.2	3.59	5.5	18.4	9.11
3	7.4	7.0	3.81	5.9	21.8	8.96

Except soil reaction, several other soil properties have a high influence on the availability of heavy metal in soils such as texture, content of organic matter, soil oxidation-reduction potential, activity of microorganisms, antagonistic and synergistic behaviours among heavy metals (Salgare & Acharekar, 1992; Gadd, 2004).

Also, seasonal variation of heavy metal content in soils significantly affect the ability of the plant to absorb heavy metals from the soil. Rainfall may facilitate the leaching of heavy metals from the surface layer of contaminated soils so it can be assumed that the average content of heavy metals during the dry season in the root zone is higher and this hypothesis has, in fact, been confirmed by many scientists (Rahman et al., 2012; Rouane-Hacene et al., 2018).

Generally, plants may react differently to the highly presence of heavy metals in soils, and the same plant under different conditions may exhibit a completely different efficiency in absorption of heavy metals from polluted soil. Available literature data on the ability of annual nettle (*Urtica urens* L.), daisy fleabane (*Stenactis annua* (L.) Ness.) and yarrow (*Achillea millefolium* L.) to absorb heavy metals from soils are quite limited. That was an additional reason why these plants were the subject of this research.

The hypothesis that these plants could not be considered as hyperaccumulators was also confirmed by the analysis of the results that refer to the transfer soil–plant factor (Table 5).

Table 5. Transfer soil–plant factor values of heavy metals from soil to plants

Heavy metal	Plant species		
	Urtica urens L.	*Stenactis annua* (L.) Ness.	*Achillea millefolium* L.
Ni	0.007	0.008	0.009
Fe	0.009	0.014	0.015
Cr	0.012	0.02	0.02
Cu	0.23	0.129	0.156
Zn	0.419	0.313	0.380
Pb	0.001	0.002	0.002
Mn	0.074	0.052	0.052

Transfer soil–plant factor (TF) of Zn was the highest (0.419), followed by Cu, Mn, Cr, Fe, Ni, and Pb, but TF value for all examined heavy metals were below one, regardless of which plant species were subjected to study. Moreover, the TF values of hazardous heavy metals Cd and Pb for all studied plants were below 0.01. This data indicates that tested plants: annual nettle, daisy fleabane and yarrow have a very poor ability to absorb heavy metals, especially Cd and Pb from the examined soils, so it is obvious that these plants could not be considered as hyper-accumulators of heavy metals under conditions of this study.

Although tested plants do not have a large ability to absorb heavy metals from examined soils, the results of this study have shown that there was a significantly difference in ability of plants to uptake heavy metals from examined soils. From Table 4 it is observed that among studied plants, annual nettle had a highest capability of absorbing and accumulating Zn, Cu and Mn. According to Viktorova et al. (2016) nettles belong in the group of zinc hyperaccumulating plant species, and the results of this study partially support this hypothesis.

Gounden et al. (2016) noted that Zn had an antagonistic effect on the Pb and Cd uptake by plant roots, which could be one of the reasons for the extremely low level of Pb and Cd in tested plants in our experiment. Similar results were obtained by other scientists (Pachura et al., 2016; Murtaza et al., 2017).

Interesting finding of this study was that the daisy fleabane and yarrow had a higher capability to absorb and accumulate Cr, Ni and Fe in comparison with annual nettle.

Irrespective of the fact that the examined plants have not shown high efficiency in relation to the absorption of heavy metals from polluted soils in examined area, their presence on these soils, as potentially stabilizers, is certainly desirable. Cui et al. (2007) reported that each plant with TF value lower than one can potentially be used as the stabilizer of polluted soils by heavy metals, of course, assuming that the plant can successfully develop on these soils. Furthermore, plants for successful use in soil stabilization should be able to prevent or reduce soil erosion and the distribution of the toxic heavy metals to other areas, and decrease water percolating through the soil, which may result in the formation of a hazardous leachate (Azubuike et al., 2016).

Considering that the growth and develop of annual nettle, daisy fleabane and yarrow are very successful on the soil around the thermoelectric power plant in Kakanj, and that the coverage of these soils with the examined plants is extremely large, it is assumed that these plants could have a positive effect in remediation of contaminated soils as stabilizers, but it is also obvious that their impact on the absorbing of heavy metals from examined soils is negligible.

The results of the present study also undoubtedly confirm the fact that the total content of heavy metals in soils is not suitable for estimate the solubility and mobility and consequently the availability of heavy metals in soils to plants (Pueyo et al., 2004).

CONCLUSIONS

The examined soils near the thermal power station in Kakanj are polluted with Ni and Pb and contain relatively high value of Cr and Fe. Annual nettle, daisy fleabane and yarrow have shown a very low capability to absorb and to accumulate heavy metals from examined soils, and hence phytoextraction by using these plants is not suitable for removing heavy metals from soils on the examined area. The present study also undoubtedly confirms the fact that the total content of heavy metals in soils is not a satisfactory parameter for estimating the toxicity of heavy metals in soil and consequently for their transfer and accumulation in plants. In the future, studies should focus on developing strategies to enhance phytoextraction efficiency, taking into consideration soils properties, heavy metal levels and characteristics, spatial distributions of heavy metals in soils, vegetation coverage and climatic conditions of the examined area.

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