

Evaluation of heavy metals accumulation potential of hemp (*Cannabis sativa* L.)

Procjena potencijala konoplje (*Cannabis sativa* L.) za akumulaciju teških metala

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ABSTRACT

Heavy metals accumulation in crops and soils poses a significant threat to the human health. A study was carried out in 2016 in order to assess hemp (*Cannabis sativa* L.) ability to accumulate heavy metals and to reveal its possibility as a phytoaccumulator or phytostabilizer. Two soil types from Croatia were used in experimental pots: Gleysoils (alkaline soil) and Stagnic Luvisol (acid soil). Majority of the varieties accumulated more heavy metals in roots than in above-ground biomass. Removal of Cd, Ni, Pb, Hg, Co, Mo and As was higher in acid soil. Potential ability for phytostabilization was observed in alkaline soil in order Cu>Cr>Cd>Mo>Hg>Zn>Ni>Co>As>Pb, while for acid soil in order Zn>Cd>Cr>Ni>Hg>Cu>Mo>As>Co>Pb. Some varieties exhibited a translocation coefficient (TC) more than 1 and shown the ability of hyper-accumulation for Zn, Hg, Mo and Cd. Higher accumulation of heavy metals in some varieties could lead to their general application for phytoaccumulation of heavy metals from polluted soils.

Keywords: bioaccumulation coefficient, pH, phytoaccumulation, soil types, translocation coefficient, uptake

SAŽETAK

Akumulacija teških metala u usjevima i tlima predstavlja značajnu prijetnju ljudskom zdravlju. Istraživanje je provedeno 2016. godine kako bi se procijenila sposobnost konoplje (*Cannabis sativa* L.) za akumulaciju teških metala i njene mogućnosti kao fitokumulatora ili fitostabilizatora. U eksperimentalnim posudama upotrijebljene su dvije vrste tla iz Hrvatske: glejno (alkalno tlo) i luvisol (kiselom tlo). Većina vrsta nakupila je više teških metala u korijenu nego u biomasi iznad zemlje. Akumulacija Cd, Ni, Pb, Hg, Co, Mo i As bila je veća u kiselom tlu. Potencijalna sposobnost za fitostabilizaciju zabilježena je u alkalnom tlu kako slijedi: Cu>Cr>Cd>Mo>Hg>Zn>Ni>Co>As>Pb, dok je za kiselom tlo kako slijedi: Zn>Cd>Cr>Ni>Hg>Cu>Mo>As>Co>Pb. Neke su vrste pokazale koeficijent translokacije (TC) veći od 1 i sposobnost hiperakumulacije za Zn, Hg, Mo i Cd. Veća nakupljanja teških metala u nekim vrstama mogla bi dovesti do njihove opće primjene za fitoakumulaciju teških metala iz zagađenih tala.

Ključne riječi: fitoakumulacija, koeficijent bioakumulacije, koeficijent translokacije, pH, tipovi tla, unos

INTRODUCTION

Soil contamination has increased because of human activities such as release of industrial effluents, municipal wastes, and waste sludge enriched with heavy metals that contaminate environment (Arik and Yaldiz, 2010; Sehbardan et al., 2013; Zhang and Shao, 2013). Contaminated soil could be remediated by chemical, physical or biological techniques (Soleimani and Jaber, 2014). Biological decontamination methods are considered safe for removing metals, particularly from water and soil (Lone et al., 2008). Important components of ecosystems in remediation of metals in the environment are plants (Prasad and Freitas, 2003) but also other organisms like mushrooms which are known to accumulate high concentrations of toxic metallic elements and metalloids (Svoboda et al., 2006; Borovicka et al., 2010). Accordingly, Siric et al. (2016) and Siric et al. (2017) investigated bioaccumulation potential in mushrooms from Croatia and found bioaccumulation ability of Cd and Hg for all examined species. The ability to accumulate heavy metals varies between species and among cultivars within species, based on their genetic, morphological, physiological and anatomical characteristics (Farid et al., 2014). Therefore, many plants (e.g. *Cannabis sativa* L., *Polygonum aviculare* L., *Panicum virgatum* L., *Brassica juncea* L., *Thlaspi spp.* L.) have the potential to accumulate high concentrations of heavy metals in their tissues because of their ability to grow on contaminated sites (Bothe, 2011; Chen et al., 2011; Tangahu et al., 2011). *Cannabis sativa* L. is an annual herbaceous plant (Suurkuusk, 2010) which grows in the mild climate. It can grow from 2 to 5 meters depending on variety. Hemp species appear to be a good choice for metal accumulation, since they have large above-ground plant mass and a developed bush root. The main root can develop to a depth of 2 meters in the loose soils, while the secondary root forms the majority of the root system at a depth of 10-60 cm (Bouloc et al., 2013). Leaves are long and slender, often with pronounced serrations with different number of limbs. These tolerant species can grow in most harsh conditions and give a good amount of biomass as a secondary product (Sanghamitra et al., 2011). They possess a very high capability to absorb

and accumulate heavy metals like lead, nickel, cadmium, zinc, and chromium (Linger et al., 2002; Kos and Lestan, 2003; Citterio et al., 2005). Uptake and accumulation of heavy metals through plants are influenced by several soil factors, including pH, soil organic matter content, redox potential, clay content, cation exchange capacity, nutrient balance, concentrations of other trace elements in soil, soil moisture and soil temperature (Qishlaqi and Moore, 2007; Singh et al., 2011; Neilson and Rajakaruna, 2012; Tang et al., 2012; Gall and Rajakaruna, 2013). Soil pH is important because most heavy metals, including Cd, Cr, Cu, Ni, Pb, and Zn, become more bioavailable under acidic soil conditions (McLaughlin, 2002; Rajakaruna and Boyd, 2008). In some cases, however, a decrease in soil pH may not necessarily result in an increase in metal bioavailability (Volesky, 1995).

Different varieties of the same plant species can have contrasting abilities for metals uptake. The aim of this research was to determinate the possibility of using four varieties of hemp (*Cannabis sativa* L.) as phytoaccumulating or phytostabilizing plants and their potential to accumulate heavy metals in different soil types and reaction.

MATERIALS AND METHODS

Two soil types were used in experimental pots for cultivation of hemp. The first (alkaline) soil sample was taken in Rasa which is located in Istria peninsula (45°3' N; 14°2' E; average elevation – 2 m below sea level). Soil is classified as *silty loam colluvium soils* or Gleysoils (Bogunovic et al., 2017). The second (acid) soil sample was taken near Daruvar (N 45°33'54.66", E 17°01'43.89", at an altitude of 133 m). Based on IUSS (2014) classification, this type of soil is defined as *Stagnic Luvisols*. In this research, pristine soils without agrochemicals were used. An experiment was set up in 2016 in the open greenhouse of the General Agronomy Department, Faculty of Agriculture, University of Zagreb.

Soil samples were taken for analysis at the beginning of the experiment. Samples were air-dried, milled, sieved (<2 mm) and homogenized (HRN ISO 11464:2009). The

texture was determined according to ISO 11277:2009. Content of soil organic matter (OM) was determined using Tjurin titrimetric method (wet digestion). The soil pH was measured using the electrometric method with the Beckman pH-meter 72, in 1 M KCl in the ratio of 1:2.5, in compliance with the modified protocol HRN ISO 10390:2005 (Capka et al., 2009). Average values of pH and organic matter (g/kg) for alkaline and acid soil with coefficient of variation (%) are presented in Table 1.

Table 1. Average values of pH and organic matter (g/kg) for alkaline and acid soil with coefficient of variation (%)

	pH	CV (%)	OM (g/kg)	CV (%)	n
Alkaline soil	7.79	1.03	30.3	12.1	24
Acid soil	5.29	18.84	13.3	15.61	24

CV - coefficient of variation; OM - organic matter.

Heavy metals were extracted in aqua regia (HRN ISO 11466:2004) and measured with ICP-OES (inductively coupled plasma optical emission spectrometry) (HRN ISO 11885:2010). Table 2 represents average content of heavy metals (mg/kg) in alkaline and acid soil.

Due to the average content of total heavy metals (mg/kg), maximum allowable concentration (MAC) in soil for a certain soil texture and level of heavy metals contamination in soils (%) was determined according to the Croatian Legislative (Ministry of Agriculture, Forestry and Water Management, 1992, 2014). So (contamination level) was calculated as:

$$\text{So (\%)} = (\text{total heavy metals content in soil} / \text{MAC}) \times 100$$

Four different varieties of hemp were used: *Fedora 17* (variety I), *Fibrol* (variety II), *Futura 75* (variety III) and *Santhica 27* (variety IV). An experiment was carried out in three replications for each variety. The trial pot area was 0.03 m³ with 9 kg of soil. Ten seeds of hemp were sown into each pot on May 4, 2016 and harvested on September 12, 2016. The germinability ranged from 70-80% on neutral soils to 10-30% on acid soils. In accordance with weather conditions, water was added approximately four times a week based on the plant appearance and by checking the soil moisture. Standard agro-technical practices were applied to the pots-fertilization and chemical protection against. For plant material analysis, composite samples of roots, leaves and stems were taken separately at the

Table 2. Average content of heavy metals in alkaline and acid soil (mg/kg), MAC of heavy metals in soil for a certain soil texture and level of heavy metals contamination in soils (%)

	pH, So (%) and MAC									
	pH>7									
Alkaline soil	Cd	Cu	Ni	Pb	Zn	Cr	Hg	Co	Mo	As
		0.3	19.02	77.74	13.65	78.21	113.5	0.04	10.24	0.2
Acid soil	pH<7									
	Cd	Cu	Ni	Pb	Zn	Cr	Hg	Co	Mo	As
	0.18	17.5	27	18.25	46.25	18.5	0.04	12.25	0.2	9.25
Alkaline soil	So (%)									
	30	21.1	155.5	13.7	52.1	141.9	4	20.5	2	42.7
Acid soil	18	19.4	54	18.3	30.8	23.1	4	24.5	2	46.3
MAC (NN 09/14; NN 15/92)	1	90	50	100	150	80	1	50	10	20

So - contamination level; MAC - maximum allowable concentration.

end of the experiment. Plants samples were oven-dried at 70 °C to constant weight and milled, after which heavy metals were extracted by microwave digestion and determined by atomic absorption spectrometry (HRN EN ISO 12846:2012; HRN EN ISO 14084:2005). In this research, hemp varieties that are in the Common Catalogue of the European Union were used. In order to establish the relationship between heavy metal concentrations in plants and soil (Kabata-Pendias and Pendias, 2001), Biological Adsorption Coefficient (BAC) was calculated according to the formula:

$$\text{BAC} = c(\text{plant part}) / c(\text{soil})$$

In this research, BAC was calculated for the root, stem and leaf considering the content of heavy metals in the soil.

Translocation coefficient (TC) is calculated according to Mattina et al. (2003) as the ratio between the metal concentrations in the above - ground biomass (stem and leaves) and the metal concentration in the root. TC was calculated according to the formula:

$$\text{TC} = c(\text{above - ground biomass}) / c(\text{root})$$

Based on the determined concentrations of heavy metals in plant parts, removal of individual elements in plants for all varieties and both soils type were calculated.

RESULTS AND DISCUSSION

Metal content in plants

Metal content in plants cultivated on alkaline and acid soil are shown in Table 3. All the results are in mg/kg dry weight.

According to Table 3, higher metal concentration in roots prevails in all varieties cultivated on acid soil in comparison with alkaline soil. Roots of variety I in acid soil contains 2.4 times more metals than roots of variety I in alkaline soil, as well as roots of variety II (1.1), variety III (3.1) and variety IV (1.2) times more metals in acid soil than in alkaline soil. The highest metal content was recorded in the plant roots: in acid soil, the highest metal content was accumulated by variety III (Zn=688.6 mg/kg) and clearly shows that major determining factor affecting

Zn distribution is soil pH, which affects Zn solubility and mobility in soil solution. In alkaline soil, the highest metal content was accumulated by variety II (Cr=272.1 mg*kg⁻¹) where Cr concentration in plant mainly depends on Cr concentration in soil and variety of hemp, while its bioavailability in soil is not inversely related to the soil pH. This research is in agreement with the result of Citterio et al. (2003) about heavy metal tolerance and metals accumulation by *Cannabis sativa* L., which concluded that metals were preferentially accumulated in the roots and only partially translocated to the above-ground tissues. In Irshad et al. (2014) research most of the 13 investigated species including *Cannabis sativa* L. exhibited higher heavy metals composition in the root as compared to shoot.

In stem, higher metal concentration prevails in all varieties cultivated on acid soil in comparison with alkaline soil, as well as in roots. Stem of variety I in acid soil contains 5.8 times more metals than stem of variety I in alkaline soil, as well as stem of variety II (17.9), variety III (8.7) and variety IV (2.4) times more metals in acid soil than in alkaline soil. Content of heavy metals through relationship between soil type and varieties is clearly expressed in stem, where in acid soil higher metal content prevails in variety II and lower metal content prevails in variety IV what is inversely in alkaline soil.

Higher metal concentration in leaves also prevails in acid soil for all varieties of hemp. Leaves of variety I in acid soil contains 3.1 times more metals than leaves of variety I in alkaline soil, as well as leaves of variety II (6.6), variety III (6.5) and variety IV (1.2) times more metals in acid soil than in alkaline soil. The highest metal content was accumulated by variety II (Zn=440.7 mg/kg) in acid soil which is similar to Zerihun et al. (2015) who investigated about metal levels in *Cannabis sativa* L. leaves and found concentration of Zn in rates from 315–380 mg/kg. In alkaline soil, the highest metal content was accumulated by variety IV (Zn=69.2 mg/kg). Two to four times higher Mo content in leaves prevails in alkaline soil. Content of Ni and Cd in alkaline soil is in consistence with leaves of cannabis samples from Zehra et al. (2009) research.

Removal of individual elements through the plant

Although plants require certain heavy metals for their growth, excessive amounts of these metals can become toxic to them (Chibuike and Obiora, 2014). Soil acidity is one of the major growth-limiting factors for plants (Jayasundara et al., 1998). Total uptake of heavy metals from the investigated soils and their removal through harvested biomass of the tested plants are the most important factors for the final calculation (Hamid, 2011). Total removal by plant biomass was calculated as average from concentrations of elements in above - ground biomass with respect to the ratio of individual plant parts and their yield. In this research, above - ground biomass included stems and leaves. Figure 1a-d present the removal of Zn, Cr, Ni and Cu (kg/ha) through hemp varieties from alkaline and acid soil.

Removal of most elements was higher in acid soil where a lower above - ground plant biomass was recorded, with an exception of Zn (var IV), Cr (var III and IV) and Cu (var I). In this research, hemp had ability to remove the largest quantities of Zn (up to 27.1 kg/ha) while for others elements lower removal was recorded: Cr (0.15–0.35 kg/ha), Ni (0.08–0.73 kg/ha) and Cu (0.14–0.52 kg/ha). *Nicotiana tabacum* L. which predominantly accumulates Cd and Cu, and *Zea mays* L. are discussed as effective plants because of high production of above - ground biomass with a relatively high uptake of elements. Comparing these species, *Zea mays* L. is able to remove 18 kg/ha more Zn than *Nicotiana tabacum* L. (Wenger et al., 2002). Management of biomass containing large amounts of toxic metals also poses a problem because after their removal, heavy metals can partially remain in

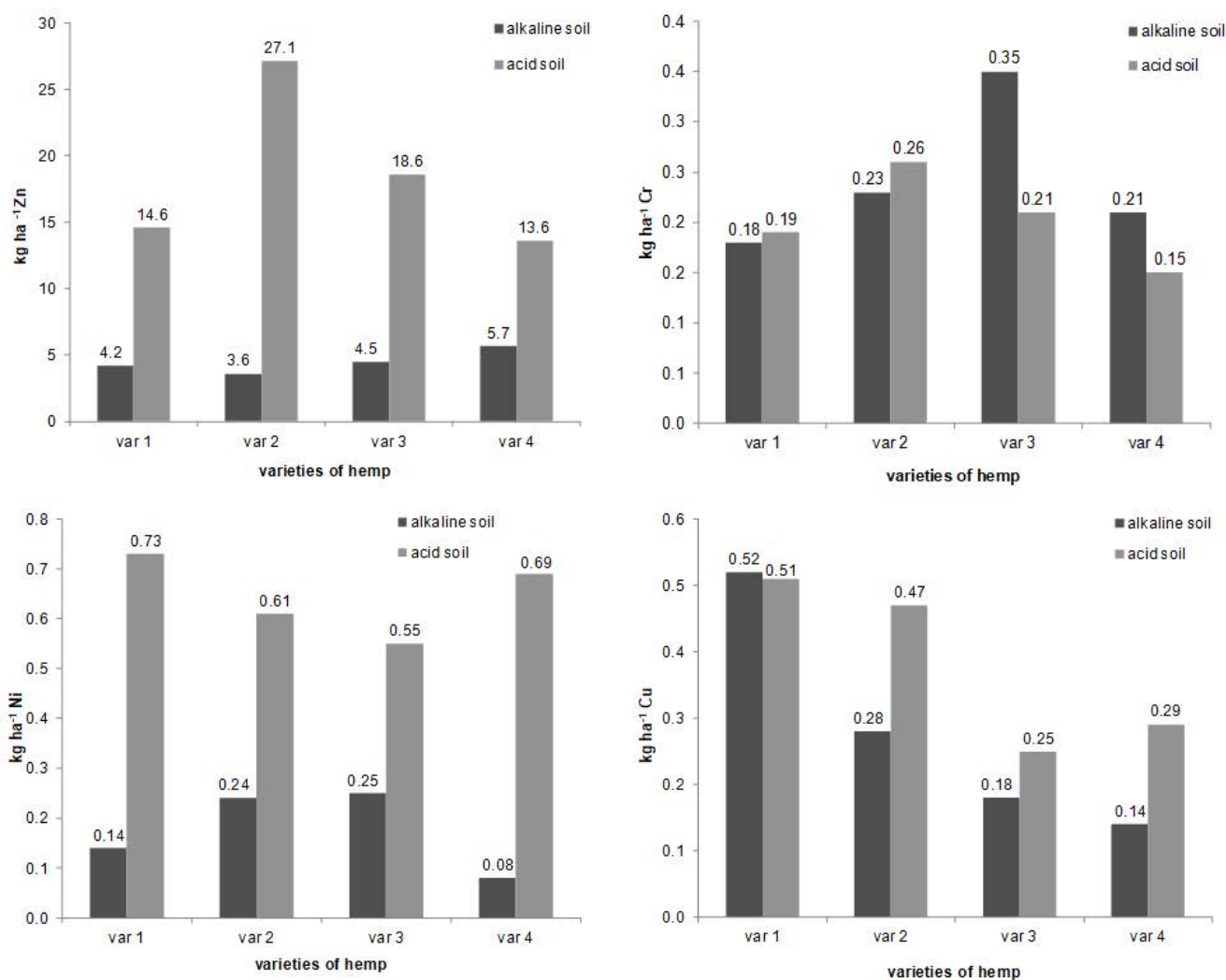


Figure 1a-d. Removal of Zn, Cr, Ni and Cu (kg/ha) by varieties of hemp

the soil in plant roots that contain higher levels of heavy metals than harvested above - ground plant parts (Ernst, 2000; Sas-Nowosielska et al., 2004).

Biological Adsorption Coefficient (BAC)

The BAC values for each variety of hemp in alkaline and acid soil are listed in Table 4. Coefficients are calculated for different plant parts (root, stem and leaves) regarding to the concentration of heavy metals in soil. Cd (root and stem), Zn (root, stem and leaf) and Cr (root) BAC reached extreme values in acid soil, while in alkaline soil, their BAC values were low, medium or very high (only in the roots). It can be seen that bioavailability of most metals in soils rapidly reduces when soil pH value is above 7. For almost all varieties of plants, the highest BAC values were recorded in the roots, with exception of Mo in alkaline soil where the highest BAC was revealed in leaves.

Data from Table 4 indicate very low to medium (1.25) Cd mobility from alkaline soil for all varieties and plant parts. In acid soil, Cd mobility reaches extreme values, especially for variety I (16.1) in the root where BAC was 17 times higher than BAC for variety in alkaline soil. For Cd, BAC in all varieties was in order root>stem>leaf on both types of soil. Investigation of Cd tolerance and accumulation in eight potential energy crops revealed that hemp is the best Cd accumulator and excellent candidate for phytoremediation or phytoaccumulation (Shi and Cai, 2009).

Bioaccumulation coefficient for Zn in acid soil was classified from low to extreme (14.9), while in alkaline soil, it was classified from very low (<1) to medium (1.01) depending on variety and plant part. The highest Zn mobility was recorded in acid soil at variety III in the root. The overall lowest values were recorded in the stem grown on alkaline soil. For Zn, bioaccumulation coefficient in all varieties was in order root>leaf>stem on both types of soil. Kos et al. (2003) investigated the phytoextraction potential for Pb, Zn and Cd on 14 different plants and discovered high phytoextraction of Zn from the control soil in all plants, which is not surprising since many plants are known to hyper-accumulate Zn.

Values for Cr in acid soil ranged from very high (2.46) to extreme (3.76) in the roots, while in the stem and leaves values were very low (<1). The stem and leaves from alkaline soil also recorded very low values of BAC for Cr. BAC value in the root of variety II in alkaline soil was very high (2.4), while in variety I and IV it was low, and for variety III medium (1.15). Cr bioaccumulation coefficient in all varieties was in order root>leaf>stem on both soil types.

Molybdenum in soil is less soluble under low pH levels (Kabata-Pendias and Pendias, 2001). Similarly, anionic forms of some heavy metals may become more bioavailable under increased pH levels. In accordance with this data, the highest BAC values in this research were recorded in plants grown on alkaline soil and ranged from low (1) to extreme (4), while in acid soil, values were low in all parts of the plant (1). In this research it was observed that Mo had the highest BAC values in the leaves of plants grown in alkaline soil, while all other metals had higher values in the roots. Mo bioaccumulation coefficient in all varieties was in order leaf>stem>root for alkaline soil, and root=stem=leaf for acid soil.

Bioaccumulation coefficients for Hg (2.22 - variety I) and Ni (2.06 - variety IV) indicated very high values calculated for the root in acid soil.

Translocation coefficient (TC)

Translocation coefficient (TC) is defined as the ratio of metal concentration towards above - ground plant biomass in consideration with metal concentration in the root. Its purpose is to determine the efficiency of the plant in translocation of heavy metals through the roots to other plant parts (Marchiol et al., 2004). Its values can indicate the movement and distribution of heavy metals in the plant. Hyper-accumulating plants are characterized by TC>1 (Cluis, 2004). Translocation coefficient for all metals and varieties in alkaline and acid soil is shown in Table 5.

Molybdenum had the highest TC of all elements in both soils (≥ 2), although higher values prevailed in alkaline soil (from 3.15 to 5). In comparison to all other

Table 4. BAC values for each variety of *Cannabis sativa* L. with different soil pH and its classification according to Kisic (2012)*

Plant part	Alkaline soil												Acid soil											
	Root				Stem				Leaf				Root				Stem				Leaf			
Varieties of hemp	var I	var II	var III	var IV	var I	var II	var III	var IV	var I	var II	var III	var IV	var I	var II	var III	var IV	var I	var II	var III	var IV	var I	var II	var III	var IV
Element																								
Cd		1.25	1.22										16.1	3.43	5.03	1.37	2.11	2.91	2.86	2.06	1.31	1.2	1.77	1.49
Cu		2.53	1.18										2.24		1.97	1.03								
Ni		1.28											1.61	1.35	1.99	2.06								
Pb																								
Zn		1.01											7.55	9.25	14.9	1.5	2.28	6.06	3.37		4.13	9.53	6.99	1.51
Cr		2.4	1.15										2.46	3.65	3.04	3.76								
Hg			1.25	1									2.22	1.11	1.67	1.39								
Co																								
Mo	1	1	1	1	1.15	1	1	1	2.65	2.65	4	2.15	1	1	1	1	1	1	1	1	1	1	1	1
As																								1.47

*Empty fields represent BAC values <1; =1 low; 1.01-1.5 medium; 1.51-2 high; 2.01-2.5 very high; ≥2.51 extreme.

Table 5. Translocation coefficient for hemp in alkaline and acid soil

	Translocation coefficient									
	Alkaline soil									
	Cd	Cu	Ni	Pb	Zn	Cr	Hg	Co	Mo	As
Var I	0.29	0.52	0.05	0.8	1.14	0.04	0.67	0.02	3.8	0.57
Var II	0.22	0.11	0.05	0.24	0.88	0.02	1.33	0.01	3.65	0
Var III	0.28	0.11	0.05	0.33	1.13	0.03	0.6	0.02	5	0.6
Var IV	0.27	0.18	0.03	0.19	1.47	0.04	1	0.02	3.15	0.14
					0.18					
	Acid soil									
	Cd	Cu	Ni	Pb	Zn	Cr	Hg	Co	Mo	As
Var I	0.21	0.24	0.38	0.17	0.85	0.08	0.38	0.06	2	0.09
Var II	1.2	0.67	0.47	0.11	1.69	0.1	1	0.08	2	0.06
Var III	0.92	0.18	0.27	0.58	0.7	0.1	0.33	0.08	2	0.06
Var IV	2.58	0.45	0.35	0.29	1.47	0.06	0.06	0.17	2	0.04

metals, cobalt had the lowest TC recorded on alkaline soil (≥ 0.02). On acid soil, the lowest TC was recorded for As (from 0.04–0.09) and Cr (from 0.06–0.1). In general, TC was less than 1, except for Zn, Hg and Mo in some varieties in alkaline soil and Cd, Zn, Hg and Mo in some varieties in acid soil. This research is in accordance with a previous study (Al-Farraj et al., 2009) where $TC < 1$ for Cu and Pb in *Ochradenus baccatus* was observed and with a study where TC of hemp was > 1 for Zn (Malik et al., 2010). In a study from 2011 TC for cadmium and lead in *Aeluropus littoralis* showed a $TC > 1$, suggesting that Pb could be effectively translocated from the roots to the above - ground biomass (Rezvani and Zaefarian, 2011).

CONCLUSIONS

Uptake of studied metals (Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As) measured in the hemp root, stem and leaf were influenced by the soil pH. The current study concludes that hemp variety *Fibrol* can be used for the removal of heavy metals. Furthermore, hemp showed ability to remove the largest quantities of Zn (up to 27.1 kg/ha) while other elements recorded lower removal (< 0.8

kg/ha). Moreover, *Fibrol* had the highest accumulation ability in alkaline soil (2.65 - Mo), while in acid soil it was *Fedora 17* (16.1 - Cd). Based on BAC values, the potential ability for phytostabilization was observed in alkaline soil in order: $Cu > Cr > Cd > Mo > Hg > Zn > Ni > Co > As > Pb$, while in acid soil in order: $Zn > Cd > Cr > Ni > Hg > Cu > Mo > As > Co > Pb$. Some varieties of hemp exhibited a translocation coefficient (TC) more than 1 and shown the ability of hyper-accumulation of certain metals. On alkaline soil, *Fedora 17*, *Futura 75*, and *Santhica 27* proved to be hyper-accumulators of Zn; *Fibrol* and *Santhica 27* of Hg and all varieties hyper-accumulators of Mo. On acid soil, *Fibrol* and *Santhica 27* are hyper-accumulators of Cd; *Fibrol* and *Santhica 27* of Zn; *Fibrol* of Hg and all varieties hyper-accumulated Mo. Therefore, these results endorse hemp as a suitable candidate for phytostabilization or phytoaccumulation approaches.

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