

*Original Article**Received: 14 November 2014**Revised: 8 December 2014**Accepted: 20 December 2014*

Accumulation of heavy metals in different parts of Russian thistle (*Salsola tragus*, *Chenopodiaceae*), a potential hyperaccumulator plant species

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Abstract:

Dragović, R., Zlatković, B., Dragović, S., Petrović, J., Janković Mandić, Lj.: Accumulation of heavy metals in different parts of Russian thistle (*Salsola tragus*, *Chenopodiaceae*), a potential hyperaccumulator plant species. *Biologica Nyssana*, 5 (2), December 2014: 83-90.

Distribution and accumulation of 10 heavy metals was observed in plant parts of species *S. tragus*, a native representative of Serbian flora. According to high bioaccumulation values (BAF), more than a half of the analyzed elements were bioaccumulated in roots, as well as in the aerial part of the plant. Translocation factor (TF) value was calculated, showing the ratio between the concentration of metals in roots and aerial parts of the plant. The results indicate that *S. tragus* may accumulate significant amounts of Cd, Co, Cr and Pb. Concentrations of some of the individual elements are shown to be statistically different depending on the sampled plant parts. Potential capacity of *S. tragus* for hyperaccumulation of heavy metals and possible utilization of this plant for phytoremediation is discussed.

Key words: *Salsola tragus*, heavy metals, plant parts, accumulation, Serbia

Apstrakt:

Dragović, R., Zlatković, B., Dragović, S., Petrović, J., Janković Mandić, Lj.: Akumulacija teških metala u različitim delovima sonjače (*Salsola tragus*, *Chenopodiaceae*), potencijalne hiperakumulatorske biljne vrste. *Biologica Nyssana*, 5 (2), Decembar 2014: 83-90.

Istraživana je distribucija i akumulacija 10 teških metala u delovima biljke *S. tragus*, autohtonog predstavnika flore Srbije. Prema visokim vrednostima faktora bioakumulacije (BAF), više od polovine analiziranih elemenata je bioakumulirano u korenu, kao i u nadzemnim delovima biljke. Izračunat je faktor translokacije (TF), koji predstavlja odnos koncentracije datog elementa u korenu i one u nadzemnim delovima biljke. Rezultati su pokazali da *S. tragus* može akumulirati značajne koncentracije Cd, Co, Cr i Pb. Pokazano je da se koncentracije elemenata statistički razlikuju u zavisnosti od analiziranih delova biljke. Diskutovan je potencijalni kapacitet hiperakumulacije teških metala ove biljke i mogućnost njene primene u fitoremedijaciji.

Ključne reči: *Salsola tragus*, teški metali, delovi biljke, akumulacija, Srbija

Introduction

Anthropogenic activities have resulted in increased deposition and migration of potentially toxic heavy metals, posing a major risk for both human health and the environment. Heavy metals may disrupt the physical, chemical and biological balance of the soil and therefore affect the growth of plants. However, many plant species have adapted to tolerate high concentrations of heavy metals without any detrimental effects on growth or survival (Brooks, 1998). A hyperaccumulator may be defined as a plant whose tissues and organs contain a metallic element at a concentration exceeding a specified threshold, when growing in nature (Van der Ent et al., 2013). The threshold concentration should be two or three orders of magnitude higher than in leaves of most species on normal soils, and at least one order of magnitude greater than the usual range found in plants from metalliferous soils. Based on these criteria, the proposed nominal threshold values (mg kg⁻¹ of dry leaf tissue) are: 100 for Cd, Se and Tl; 300 for Co, Cr and Cu; 1000 for As, Ni and Pb; 3000 for Zn and 10,000 for Mn (Van der Ent et al., 2013).

Salsola tragus L. (syn. *S. kali* subsp. *ruthenica*), commonly known as Russian thistle or tumbleweed, is one of the most common species of tumbleweed belonging to *S. kali* aggregate. It is an annual, weedy plant species, which at maturation forms spherical bushes approximately 1 m in diameter, detaches from the root system and tumbles in the wind, spreading its seed (Bargerón & Swearingen, 2010). Native distribution of the species overlaps large areas of Europe and Asia, but it is also naturalized in north, central and South America, Africa and Australia (Mosyakin, 2003). It has a comparatively wide distribution in Serbia, especially in the northern and northeastern parts of the country (Slavnić, 1972). It is generally distributed in sandy areas in coastal regions and semiarid and arid areas in lowlands, deserts, but it is also common at ruderal habitats and disturbed places in the settlements, across the roadsides, and cultivated fields. Due to its wide distribution, adequate life form and other biological traits, this taxon is a good candidate for a biomonitoring species. It has been shown that tumbleweed is able to capture elements from the ambient air and that has capabilities for being used as biomonitor of environmental pollution by heavy metals (Benitez, 2009). The tumbleweed is also able to grow in areas polluted by radionuclides (Warren, 2001). The aim this study was to assess the hyperaccumulating capacity of aerial as well as underground parts of tumbleweed (*S. tragus*)

collected in the urban and sub-urban area of the city of Niš, southeast Serbia. In this study we demonstrated distribution and accumulation of cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) and vanadium (V) in the tissues of *S. tragus*.

Material and methods

Study area

The study area covers the urban and suburban area of the city of Niš, i.e. the peripheral parts of Niš valley, a small part of the Sićevo Gorge and alluvial terraces of Nišava River and the South Morava River at their confluence. Niš is a city in southern Serbia, situated in the composite valley of the river Nišava near its confluence with the South Morava. The city of Niš has an area of 597 km² including Niška Banja and 68 suburban and rural settlements. With 393,357 inhabitants (census of 2011) it is the second largest city in Serbia and one of the most important industrial centers in Serbia (electronics, mechanical engineering, textile and tobacco industry). The Nišava valley is located at the contact of young and accumulated layers of broken rocks belonging to the Carpatho-Balkanides and Paleozoic formations belonging to Rhodopian crystalline mass. In Niš basin the following rock types are distributed: magmatites, metamorphites, carbonate rocks, clastites and non-consolidated or partly consolidated Neogene sediments (Krstić et al., 1980; Rakić et al., 1973). At the rims of Niš basin, above these rocks the alluvial sediments are deposited. The alluvial soils dominated in the area. Sićevo Gorge is formed by vertical fluvial erosion in the Pliocene limestones between the southern slopes of the Svrljig Mountains and the mountain Suva Planina. Sampling locations in Sićevo Gorge are located next to the main road junction with high traffic frequency (Vujsić et al., 1980). At the study sites, there are no significant deposits of heavy metals.

Sampling and sample preparation

The samples of tumbleweed plants and underlying soils were collected in 2013 from 15 locations with different intensity and types of anthropogenic influence. The selection of sampling sites was influenced by spatial distribution of tumbleweed populations in the investigated area. Underground (roots) and aerial parts (stems, leaves and inflorescences) of plants from the same location were separated and washed in a series of fresh and de-ionized water to remove any particles of soil. The

material was chopped, dried at room temperature for two weeks and finally stored in paper bags, prior to chemical analysis. Voucher specimens are deposited at the Department of Biology and Ecology (HMN), Faculty of Science and Mathematics, University of Niš, under the acquisition numbers 7315-7324.

The mineralization of soil samples was performed by slightly modified methods described by Edge11 (1988). Approximately 1-3 g (depending of expected total metal concentrations in soil) of air-dried, ground and sieved (< 0.2 mm) soil was weighed and transferred into cuvettes. Each sample was weighed in two replications. The 10 ml of HNO₃ was added and the samples were heated on the heating plate gradually, until no brown fumes were emitted. The solution was allowed to evaporate approximately to 5 mL. After cooling, 2 mL of water and 3 mL of 30% H₂O₂ were added and heated until effervescence subsided and the solution cooled. Additional H₂O₂ was added until effervescence ceased (but no more than 10 ml H₂O₂ was added). The heating was continuing, and the solution was allowed to evaporate to approximately 5 mL.

Dried and ground plant materials (root and aerial parts, separately) were weighed (approximately 0.5 g) and transferred into cuvettes for mineralization. Each sample was weighed in two replications. The mineralization was performed by wet procedure, i.e. with the mixture of HNO₃ (65%) and H₂SO₄ (min. 95%) (ISO 6636/2: 1981).



Fig. 1. *S. tragus* at the collection site in suburban area of the city of Niš

Both soil and plant extract were cooled, filtered through Satorius Stedim Biotech filter paper (quantitative grade 391) and adjusted to the appropriate volume with HNO₃/H₂O (3:1) solution.

Analytical methods

Soil and plant samples were analyzed by using an atomic absorption spectrometer Shimadzu AA-7000. Measurements was performed by flame technique with the detection limits (3σ) being approximately 0.1 ppm for V, 0.6 ppm for Cr, 0.01 ppm for Cu, Zn and Mn, 0.2 ppm for Ni, 0.07 ppm for Pb, 0.7 ppm for Fe, 0.3 ppm for Co and 0.03 for Cd. The Deuterium Lamp (D2) method was used as background correction function. Control software WizAard was used for Shimadzu atomic absorption spectrophotometer. Reference standard materials NIST 1515 (apple leaves) and BIPEA quality control sample (Soil 90-0115-0106) were used for the verification of the measurement.

The bioaccumulation factor is calculated as follows (Feijtel et al., 1997; Gobas & Morrison, 2000; Mackay & Fraser, 2000):

$$BF_i = C_i/C_s \quad (1)$$

where C_i is the mean concentration of metal in roots ($i=a$) or aerial ($i=b$) parts of tumbleweed (mg kg^{-1}) and C_s is the mean metal concentration in underlying soil (mg kg^{-1}). The plant could be considered as hyperaccumulator if the BAF is higher than 1.

Translocation factor (TF) is calculated as ratio of the metal concentration in aerial parts of the plants to those in the roots (Cui et al., 2007; Li et al., 2007; Sousa et al., 2008; Malik et al., 2010):

$$TF = C_b/C_a \quad (2)$$

where C_b is the mean metal concentration in aerial parts (mg kg^{-1}) and C_a is the mean metal concentration in roots of plant (mg kg^{-1}). The $TF > 1$ indicates that the plant translocates metals effectively from the roots to its aerial parts (Baker & Brooks, 1989).

Statistical methods

Analysis of variance (ANOVA): one-way ANOVA was used in order to determine whether there are any differences in concentrations of heavy metals among different examined parts of the plant, and among the localities. The results of this analysis should also pinpoint the most important variables regarding concentrations of metal in tissues of two plant parts. Correlation analysis: In order to affirm relationships between heavy metal concentrations in roots and aerial parts of tumbleweed, Pearson's correlation analysis was applied. Statistical data processing was carried out by STATISTICA 8 software.

Table 1. Mean concentrations of heavy metals in underground (C_a) and aerial (C_b) parts of tumbleweed and underlying soil C_s (mg kg^{-1} d.w.) and mean values of bioaccumulation (BAF) and transfer (TF) factors

Parameter	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	V
C_a	5.56	79.7	86.4	8.12	6120	19.9	75.2	185	14.9	140
C_b	5.92	85.9	102	10.4	6470	43.5	72.2	188	28.7	87.7
C_s	0.88	14.7	41.6	54.7	27310	320	41.7	101	63.0	43.4
BAF _a	6.32	5.42	2.08	0.15	0.22	0.06	1.80	1.82	0.24	3.18
BAF _b	6.73	5.85	2.46	0.19	0.24	0.14	1.73	1.85	0.46	2.02
TF	1.06	1.08	1.18	1.27	1.06	2.18	0.96	1.02	1.92	0.63

Results and discussion

The mean concentrations of heavy metals in different parts of tumbleweed and in underlying soils and values of bioaccumulation and transfer factors are presented in **Tab. 1**.

Different plant species and genera grown on the same soil may display different metal uptake and movement in plant (Miles & Parker, 1979). In addition to availability of metals in soil to plants, there are several factors that cause metal uptake and transport through the plant. The biological factors, including the capability to transport particular metals through transport systems, strongly affect the accumulation behavior of the plant. Heavy metal absorption and accumulation varies according to plant taxa and presence of suitable metal forms for the plant uptake in the soil. The studies of De la Rosa et al. (2004) and Gardea-Torresday et al. (2005) suggested that tumbleweeds could be considered as potential hyperaccumulating species for cadmium and chromium, respectively. One of the species, *S. soda* has also found to be hyperaccumulator for lead (Lorestani et al., 2011; 2013) and zinc (Lorestani et al., 2013) and therefore suitable candidate for phytoremediation of contaminated soils.

The obtained BAF values pointed out the tendency of *S. tragus* to accumulate Cd, Co, Cr, Ni, Pb and V in both underground and aerial parts of the plant (**Tab. 1**). The highest bioaccumulation was observed for Cd, with BAF values of 6.32 and 6.73 in underground and aerial parts of the plant, respectively. For most studied elements, the plants have shown higher bioaccumulation capacity in their upper parts, except for V, which was better represented in roots than in aerial parts. However, the number of analyzed cases with favorable transport of absorbed metals from root zone into the aerial parts of plant, shown as translocation factor (TF), was much lower. In this study, high values of translocation factor were recorded only for Cd, Co, Cr and Pb, in cases of realistic possibility of uniform

accumulation of these elements in aerial parts of plant species *S. tragus*.

While the translocation factor indicates the relative ratio of concentrations of a certain metal in root and aerial parts, the transport of metals through the plant is directly influenced by various ecological characteristics of the plant, primarily the type and level of development of transport tissues, xylem and phloem, or existence of special physiological mechanisms. The data collected on ten elements in both plant parts in all localities were used to determine the interaction among metal concentrations in different plant parts. In combination with the TF values this could provide additional information on possibility of their transport through plant tissues. The derived correlation coefficients, as a measure of linear strength of connection between a pair of observed factors, indicate strong connection between concentrations of Pb, Fe and Ni in roots and in aerial parts of plant (Table 2). There was also a strong and statistically highly significant ($p < 0.001$) positive correlation of concentrations of Fe and Pb in aerial and underground part of plant. At the same time other types of correlation between concentrations of other variables were also recorded. However, the values of the correlation coefficients were weak or medium-strength, indicating that in the species *S. tragus* there is a clear interaction between concentrations of adopted elements in aerial and underground parts of plant. Existence of favorable transport through the tissues and successful overcoming of the barriers that might potentially disrupt transport of heavy metals through plants, speaks in favor of the possibility that *S. tragus* performs hyperaccumulation of Pb, Fe and Ni in cases when these metals are present in the environment in greater concentrations.

Samples of roots and aerial parts of plant species *S. tragus* collected in the study area show variability in concentrations for all 10 analyzed elements. The original hypothesis assumed that, as

Table 2. Correlations between concentrations of heavy metals in roots and aerial parts of tumbleweed (high *r* values in bold)

	Cd_b	Co_b	Cr_b	Cu_b	Fe_b	Mn_b	Ni_b	Pb_b	Zn_b	V_b
Cd _a	0.72	0.17	0.21	-0.08	-0.32	-0.09	0.05	-0.25	0.19	-0.11
Co _a	0.29	0.42	0.10	-0.32	-0.41	-0.15	-0.26	-0.48	0.25	0.03
Cr _a	-0.16	-0.10	0.49	0.52	0.17	0.20	0.21	0.11	-0.21	-0.37
Cu _a	-0.48	0.02	-0.06	0.30	0.40	0.32	0.01	0.43	-0.11	-0.47
Fe _a	-0.35	-0.15	-0.14	0.29	0.94	0.71	0.18	0.96	-0.03	-0.49
Mn _a	-0.43	0.05	0.03	0.33	0.67	0.65	0.24	0.70	0.03	-0.54
Ni _a	-0.05	-0.23	0.23	0.27	0.35	0.41	0.84	0.38	0.05	-0.06
Pb _a	-0.42	-0.25	-0.13	0.32	0.94	0.62	0.24	0.98	-0.02	-0.48
Zn _a	-0.15	-0.04	-0.00	0.10	-0.03	0.10	0.07	-0.04	0.38	-0.08
V _a	-0.24	0.04	0.12	0.11	0.13	-0.21	-0.16	0.17	-0.56	0.10

a – roots, b-aerial parts

different parts of plant differ in anatomically-morphological and functional sense, the movement and potential accumulation of metals in tissues would also be different. Therefore the differences in mean values of measured concentrations of metals in various parts of plants were tested, and characters with greatest impact in differentiation of samples from aerial and underground plant parts were determined. The results of unifactorial analysis have shown that there are statistically significant differences in concentrations of heavy metals depending on plant organ, but only for some metals (**Tab. 3**). Comparison of aerial and underground parts of plant *S. tragus*, at level of 15 collected samples, shows statistically highly significant concentrations of Mn, Zn and V. The possibility of differentiation is most influenced by Mn, which is characterized by the highest F value among all statistically significant variables. The measured mean values of Mn were greater (more than double) in tissues of aerial part than in the root of the analyzed plant species. The manganese is one of the most important elements, influencing the process of photosynthesis and some other metabolic processes, but it is believed to be easy to transport through transport tissues and accumulated primarily in green parts of plants (Marschner, 1995). The manganese shows a general tendency of greater accumulation in shoots than in root of the same plant, as demonstrated in samples of a number of different plant species (Millaleo et al., 2010). It was proven that Mn is transported through xylem relatively easily, in contrast to transport in the opposite direction, through the phloem part of transport system toward the underground plant organs (Page & Feller, 2005). The aerial parts of *S. tragus* have included concentration of Zn almost double to that in the underground part. The zinc is similar to Mn in being an important nutrient

for plant organisms, playing a significant role in process of photosynthesis and shows tendency to accumulate in the aerial parts of plants. In experimental conditions it was proven that this metal accumulates in leaves, and in case of increased concentration in substrate there may be hyperaccumulation in leaves of a number of cultivated plant species (Sekara et al., 2005).

Table 3. Analysis of variance of heavy metal concentrations between the root and the aerial parts of tumbleweed (significant values in bold)

Effect	Root/Aerial part	Root/ Locality	Aerial part/ locality
Variable	F (df 1)	F (df 10)	F (df 10)
Cd	0.51	0.71	0.78
Co	1.01	1.16	1.22
Cr	0.30	2.14	1.04
Cu	1.84	1.40	0.62
Fe	0.11	3.22*	3.20*
Mn	44.92***	1.82	0.77
Ni	0.09	3.22*	6.95**
Pb	0.02	4.39**	4.18**
Zn	22.13***	0.69	1.32
V	12.68***	0.49	1.14

*The significance levels: *p<0.05; ** p<0.01; ***p<0.001

Distribution of V in parts of plant body is different than distribution of other elements. Depending on sampling locality, values of this element in roots of *S. tragus* were greater on average than the values for aerial parts (**Tab. 1**). The mean values of all analyzed samples show concentrations of V in roots to be more than 1.5 times greater than in rest of the plant. In contrast to Mn and Zn which play important roles in various physiological processes in plants, the phenomenon of accumulation and transport of V in plants is still

largely understudied. Most plants absorb V in very small amounts in comparison to its availability in soil. Results of several studies, including our research, indicate that this element is accumulated in the root and poorly transported toward the aerial organs of the plant (Sziklai et al., 1987; Nowakowsky, 1993). Possibility of stronger accumulation of V in the root than in stems, fruits and green leaves, was also proven experimentally (Gil et al., 1995; Vachirapatama et al., 2001). Increased concentration of this element in environment and in plant and animal organisms may have a toxic effect on consumers. Increase of level of V in water and soil is attributed to burning of coal and oil derivatives, as well as to use of phosphate fertilizers (Ringelband & Hehl, 2000). According to literature data, increased level of V was primarily recorded in plants growing on alluvium soil (Gil et al., 1995). It must be noted that almost all samples of species *S. tragus* analyzed in this study were collected at agricultural land or recently abandoned cultivated land, formed on alluvial deposits of river valleys. Although *S. tragus* is able to accumulate V, according to TF values for this element (0.63) it is similar to most other plants in not being able to hyperaccumulate it and transport it to aerial parts in favorable amounts.

Results of analyzing the concentrations of heavy metals in a single part of plant among 11 groups of localities defined according to the distance from the potential source of pollution (highway) paint a slightly different picture (Tab. 3). It is assumed that determination of important variables is crucially affected by contamination of soil with heavy metals, unlike the previous case where the important factor was affinity for accumulation in plant organs. If roots and aerial parts of plants are compared according to spatial distribution of sampling sites, concentrations of Fe, Ni and Pb become more important in differentiation of sample groups. Comparison of mean values shows that approximately identical concentrations of Ni and Pb were recorded in roots and aerial parts of plants, while Fe is characterized by a significantly higher concentration in the aerial part. The F value was most prominent for concentration of Pb, which was shown to be more variable and statistically more significant than concentration of other metals regarding the concentration of metals in the root. The lead belongs to metals most commonly mentioned as primary contaminants in the environment. In this case, the role of this metal in differentiation of samples probably accentuates the level of contamination in sampling sites, which were situated in vicinity of traffic routes as contaminant sources. The significance of species *S. tragus* as an

indicator organism for lead contamination of environment, as concentration of lead in plants is dependent on distance from traffic routes where lead gasses are emitted, was stressed by Sinegani (2007). Considering the aerial parts of plant, Ni is more significant and represents a more significant variable than either Pb or Fe. The nickel does not have any great role as a plant nutrient, and increase of its concentration in plants is mostly caused by emission from industrial sources or by prolonged use of mineral fertilizers and pesticides containing that element (Seregin & Kozhevnikova, 2006).

Conclusion

According to its ability to accumulate several heavy metals, *S. tragus* may represent an interesting model of phytoremediation or biomonitoring. This plant can uptake Cd, Co, Cr and Pb from the soil and make significant deposits in its aerial parts. Distribution and accumulation of heavy metals is not equal in all parts of a plant. Differences in concentrations of heavy metals between the underground and the aerial parts of a plant are mostly determined by concentrations of Mn, Zn and V. The levels of Mn and Zn were higher in the green parts of the plant, while concentration of V was higher in the roots. Possibility of differentiation in statistical sense was particularly high for concentration of Mn. The positive correlation was determined between the concentrations of Pb, Fe and Ni in the roots and their concentrations in upper parts of the plant.

Acknowledgements. This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (projects III43009 and 173030).

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