Original research paper;
UDC: 582.929.4-119(497.11)
ID: 203787276

Acta Agriculturae Serbica, Vol. XVIII, 36(2013) 101-110

Acta Agriculturae Serbica FACULTY OF AGRONOMY ČAČAK

Content and allocation of nickel, chromium, cobalt, copper and zinc in *Teucrium montanum* L. from serpentine habitats in Serbia

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Received:9.10.2013. / Accepted:29.11.2013.

Abstract: *Teucrium montanum* L. is a commonly used plant in traditional medicine throughout Balkan Peninsula. Populations on various geological bedrocks, including serpentine, are included in its distribution. Due to antimicrobial, antioxidant, anticancer and other properties, it has been in focus of many pharmacological studies in past few decades. The economic potential of intensive production and usage of *T. montanum* draws attention to defining its phytochemical components and pharmacological activity. Serpentine soils are known for elevated heavy metal load; therefore they may pose a threat that can compromise mineral content of this healing plant's organs. To preview the state of two serpentine populations of this species we sampled and scanned their roots, stems and leaves for Ni, Cr, Co, Cu, and Zn. Our results indicate that the sampled populations did not show elevated levels of these metals, and can be considered safe for use. This is a good and supporting result for this medicinal plant which is already widely used as a popular healing herb. Because mineral composition and soil conditions that govern metal mobility vary greatly in soils, metal accumulation still has a potential of reaching potentially hazardous levels. Therefore precautionary measures of *in situ* checkups are strongly encouraged so that safe healing extracts of this plant can be produced.
Key words: *Teucrium montanum*, serpentine, heavy metals.

Introduction

Mountain germander – *Teucrium montanum* L. (Lamiaceae) – is a widely used popular healing and pharmaceutical plant, found to be beneficial primarily against digestive and respiratory disorders, but also against abscesses, gout and conjunctivitis. It is also shown to stimulate fat and cellulite decomposition, and exhibit anti-inflammatory, anti-oxidative, anti-microbial, anti-fungal, anti-diabetic and antihelminthic properties. Infusions of mountain germander are considered a good dietary source of some micronutrients (e.g. Zn, Fe, Mn), in addition to the known beneficial impact of its organic compounds (essential oils, flavonoids, saponins, etc). Cold tea of *T. montanum* is used against a wide range of internal illnesses, mostly liver disturbances (Pavlović 1975, Redžić 2007, Vuković *et al*. 2008, Stankovic *et al*. 2011a, Juranović Cindrić *et al*. 2013).

T. montanum is a semi-woody small herb with xeromorphic constitution and evergreen leaves. The main root is long, oriented downwards, while side roots are somewhat thinner and spreading sideways as well. Shoots are 10 to 30 cm long, with many side branches, either crawling or upright. Small linear leaves have a short petiole and straight edges curved towards abaxial side. Flowers that bloom from summer till autumn are whitish-yellow. *T. montanum* is distributed throughout South and Central Europe, till Netherlands to north, and till West Ukraine and Crimea in the east. It is also found in Algeria, and Asia Minor (Pavlović 1975, Lakušić 2000). In central Balkans it is mostly found between

500 and 1000 m altitude, but can reach up to 2100 m in the alpine region. It is very common in all parts of the Balkan Peninsula, and can be found on various geological bedrocks, serpentine included (Lakušić 2000).

Serpentine areas in Serbia are frequent and generally found in alpine regions (Stevanović *et al*. 2003). Since *T. montanum* is a soil indifferent plant – i.e. equally frequent on and off serpentine – its alpine serpentine populations (e.g. from Mt. Zlatibor & region) are also readily employed in popular medicine and pharmacy. Serpentine soils are loaded with certain heavy metals, and are known to host and give rise to metal hyperaccumulating plants (Reeves *et al*. 1999). It is fair to assume a risk that well adapted plant such as *T. montanum* could be capable of holding increased amounts of serpentine soil-defining metals (Ni, Cr, Co), or other potentially toxic metals (Cu, Zn). This is why considerations about mineral composition of *T. montanum* populations growing on metal-loaded serpentine soils are particularly interesting. *In situ* and industrial-input content control is supported by the study from serpentine soils in Bulgaria, where *T. montanum*, *T. chamaedrys* and *T. polium* were found unsuitable for pharmaceutical purposes due to metal concentrations that exceeded the permissible levels for such usage (Pavlova and Karadjova 2012). Simultaneously, ecotypes or species growing on serpentine could have altered (i.e. higher) antimicrobial activity than their relatives or populations growing in terrains underlain by other type of bedrock (Rajakaruna *et al*. 2002). Well known antimicrobial properties of *T. montanum* were also investigated intensively on other bedrocks (Vuković *et al*. 2008). The antioxidant activity of plant extracts is due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Data that promotes some use of *T. montanum* in traditional medicine, confirmed a free-radical scavenging activity of flavonoids and phenolic acids in extracts made by different solvents (Djilas *et al*. 2006). Recently, anticancer properties of plant extracts and compounds isolated from *T. montanum* were examined, which most probably lay in anti-proliferative effect that is exerted (Stankovic *et al*. 2011b). Despite the overall anti-mutagenic effect, the protective effect of *T. montanum* extract from serpentine decreased with the increase of extract concentration (Milošević-Djordjević *et al*. 2013). Extracting organic compounds with various solvents yields significant free radical scavenging, hydroxyl radical scavenging and antioxidant activity *in vitro* (Panovska *et al*. 2005). These natural features of *T. montanum*, can be hypothesized to be a part of stress tolerance that enables thriving of their population in serpentine soils.

Our goals were to survey selected metal content of two serpentine populations of *T. montanum* from Central and Western Serbia. We aimed to define if the metal levels determined within their tissues are close to or exceed the thresholds that would put their medicinal and nutritive properties in question. All five elements reported in this survey (Ni, Cr, Co, Cu, Zn) represent essential

nutrients, which can however turn out to be toxic, if their levels surpass the usual toxicity thresholds. Furthermore, serpentinophytes are known to employ several mechanisms of dealing with excess metal in soil: exclusion at root, toxicity tolerance, or sequestration through (hyper)accumulation to different organs (O'Dell and Rajakaruna 2011).

Materials and methods

Two serpentine populations of *T. montanum* were sampled from serpentine outcrops in Central and Western Serbia: from Brđani Gorge near the town of Gornji Milanovac, and from village Kremna in the region of Mt. Zlatibor. A thorough description of collection locations can be found in Vicić *et al*. (in press). Five root and stem samples, and 15 leaf samples were taken from both collection locations. Freshly-picked samples were washed with distilled water, air-dried, ground to fine powder, then re-dried at 105 °C for 3 h, and weighed to 0.5000 g (± 0.0001). Samples were then digested in 2:1 mixture of HNO_3 (65% p.a. Carlo Erba Reagents, Italy) and H_2O_2 (30% p.a. VWR International, USA), in three successive digestions. During the process glass beakers for digestions were heated on a hotplate. The clear extracts were brought to 50 ml volume with double-distilled water, and later on quantified for Cd, Pb, Zn, Cu, Ni, Cr, and Co using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectroflame, Germany).

Results and Discussion

No cadmium or lead was detected in any of the taken samples. Unlike Hg, As, Pb or Cd, other potentially-toxic metals in plants, such as the ones surveyed here (which are not in top-five metal contaminants and hazards), are surveyed more seldom, hence no such strict regulations exist (Juranović Cindrić *et al*. 2013). In both populations of *T. montanum*, the highest amounts of Ni and Cr were found in roots (27.7 and 3.9 mg kg^{-1}, respectively). There was no obvious preference for allocation to a certain organ for Co, Cu, and Zn (Table 1; Figures 1 and 2). Considering that bioavailable amounts of metals are abundant in surveyed soils – Ni (208–217 mg kg^{-1}), Cr (0.9–1.3 mg kg^{-1}), Co (38–112 mg kg^{-1}), Cu (3.7–3.9 mg kg^{-1}), Zn (4.6–10.6 mg kg^{-1}) (Vicić *et al*. – in press and unpublished data) – populations of *T. montanum* sampled in this study confirm that the mechanism employed is metal exclusion, even if the amounts of Cr and Ni were somewhat higher in the roots (Table 1). Harmful amounts of these metals were effectively prevented from being accumulated in the stem or the photosynthetic leaf tissue.

Thresholds set for Ni (60 mg kg^{-1}) or Cr (50 mg kg^{-1}), remained far from being reached (Kosalec *et al*. 2009, Pavlova and Karadjova 2012), suggesting a safe metal level in here surveyed populations of this natively picked herbs.

Table 1. Allocation and accumulation of Ni, Cr, Co, Cu, and Zn in organs of *T. montanum*; mg kg^{-1} dry weight; mean ± SE; nd – not detected.

location	**organ**	**Ni**	**Cr**	**Co**	**Cu**	**Zn**
Brđani Gorge	root	27.7±7.4	3.9±1.2	nd	10.0±2.6	41.0±6.5
	stem	4.2±1.6	1.6±0.5	0.5±0.5	4.9±1.1	32.6±2.1
	leaf	12.1±1.3	1.5±0.5	0.8±0.4	3.4±0.9	44.3±2.2
Kremna	root	33.0±2.5	5.9±1.4	1.6±1.1	6.9±0.8	58.7±5.2
	stem	7.0±3.9	1.5±0.6	nd	10.7±2.0	37.5±2.0
	leaf	13.2±1.7	3.1±0.7	1.6±0.8	9.8±1.2	49.0±2.2

Compared to values determined in aerial parts of *T. montanum* from serpentine areas in Bulgaria (Ni: 19.8–63 mg kg^{-1}, Cr: 2.3–28 mg kg^{-1}, Co: 0.4–4.1 mg kg^{-1}), our results show significantly lower levels of serpentine typical Ni, Cr, and Co within the tissues – see Table 1. Similar amounts were determined for Zn (29–70 mg kg^{-1}), and only slightly lower for Cu (5.7–15.4 mg kg^{-1}). Among two other *Teucrium* species (*T. chamaedrys, T. polium*) from Bulgarian serpentine soils, *T. montanum* was shown to accumulate the least Fe, Pb, Ni, and Cr of three congeners (Pavlova and Karadjova 2012). Conversely, Ni levels in Croatian population sampled from calcareous bedrock were significantly higher: 72–125 mg kg^{-1} (Juranović Cindrić *et al*. 2013). This finding is hard to explain when metal content in soil is unknown, but typically calcareous soils are not particularly rich in Ni. Yet, the final water infusion analyzed in that study showed significantly lower amounts of Ni, therefore not questioning the beneficial health effect of the infusion. The amounts of metals determined in our study suggest that *T. montanum* from the named collection locations is probably safe for use (in terms of the amounts of accumulated Ni, Cr, Co, Cu, and Zn).

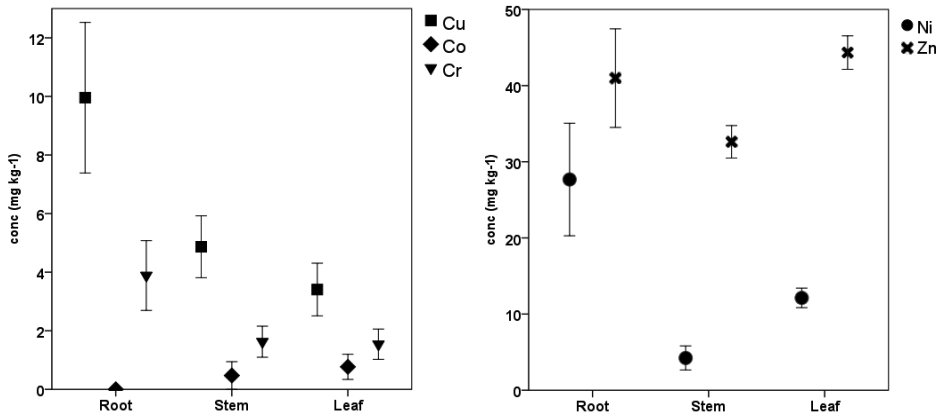

Figure 1. Root, stem and leaf concentration of Cu, Co, Cr (left), Ni, and Zn (right) in *T. montanum* from Brđani Gorge locality; mg kg^{-1}; mean ± SE.

In a study of both native and cultivated individuals of *T. montanum* from Croatia, it was determined that more nutrients such as Ca, K or Mn were accumulated in cultivated samples, while more Ni, Co, Cu Fe, Mg, and Zn were found in native-growing individuals (Jurišić *et al*. 2001a,b). This indicates that abilities of handling metal cations are not specific for serpentine populations, but can also be found in non-serpentine populations.

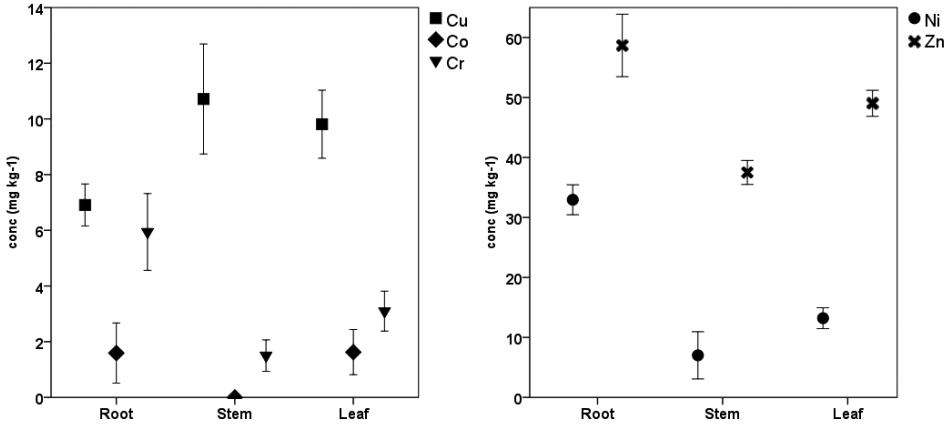

Figure 2. Root, stem and leaf concentration of Cu, Co, Cr (left), Ni, and Zn (right) in *T. montanum* from Kremna locality; mg kg^{-1}; mean ± SE.

For mountain germander picked from serpentine soils in Serbia it was also found that water is an efficient polar solvent of its phenolic contents. Leaves were found to hold most of the water-extracted phenolic compounds (Stankovic

et al. 2011a). Compared to the leaves of cultivated individuals, leaves of native populations were also higher in polyphenolic substances. Among other congeners, *T. montanum* from calcareous bedrock was also shown to hold higher concentrations of bioactive compounds (Grubešić *et al*. 2012). Combining data on organic compounds and activity of *T. montanum* with mineral composition and stress-handling in serpentine soil, could point to the further research on specific role of its anti-oxidative system in serpentine.

Conclusion

As a widely used herb in popular medicine and a source of pharmaceutical substances, *T. montanum* is a valuable part of the flora in Balkan Peninsula. Wide application and bioactive potential of its extracts insist on safety precautions. Therefore, it is necessary to evaluate the actual heavy metal content, especially so in populations growing on metal-loaded serpentine soil.

Serpentine populations of *T. montanum* encompassed by this study can be labeled *excluders*. This is confirmed by low levels of surveyed micronutrients in roots primarily, and in other organs as well. Our results suggest that Ni, Cr, Co, Cu, and Zn content of two surveyed populations of *T. montanum* growing in serpentine can be considered safe for usage in afore-mentioned ways. However, mineral composition and soil conditions that govern metal mobility vary greatly in serpentine soils, therefore their accumulation still has a potential of reaching potentially hazardous levels. Precautionary measures of metal content scanning are suggested for beneficial properties of this well-known and widely-used plant species to remain safe and uncompromised.

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Sadržaj i distribucija nikla, hroma, kobalta, bakra i cinka u organima *Teucrium montanum* L. sa serpentinitskih staništa u Srbiji

- originalni naučni rad -

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Rezime

Teucrium montanum L. – iva trava – je lekovita biljka sa dugom istorijom primene u tradicionalnoj medicini širom Balkanskog poluostrva. Raste na raznovrsnoj geološkoj podlozi, uključujući i serpentinit. Zbog svojih anti-mikrobnih, anti-oksidantnih, anti-kancerogenih i drugih lekovitih svojstava, proteklih decenija postala je predmet istraživanja brojnih farmakoloških studija. Ekonomski potencijal koji intenzivna proizvodnja i upotreba *T. montanum* nosi sa sobom, povlači i interesovanje za bližim određivanjem njenog fitohemijskog sastava i farmakološke aktivnosti. Serpentinitska zemljišta su poznata po povišenim nivoima metala koje sadrže, stoga mogu predstavljati faktor ugrožavanja kvaliteta mineralnog sadržaja u organima ove vrste. Kao osvrt na stanje kod dve serpentinitske populacije ove vrste, uzorkovali smo i kvantifikovali sadržaj nikla, hroma, kobalta, bakra i cinka u korenu, stablu i listu. Dobijeni rezultati ukazuju da nivo navedenih metala nije povišen u ovim populacijama, koje se stoga mogu smatrati bezbednim za upotrebu. Ovo je dobar i ohrabrujući rezultat za upotrebu ove lekovite biljke, koja se i sada široko koristi kao narodni lek. Zbog mineralnog sadržaja zemljišta, i varijabilnih edafskih uslova koji mogu u velikoj meri uticati na mobilnost prisutnih metala, akumulacija

metala u pre svega nadzemne organe ove biljke i dalje predstavlja potencijalnu pretnju. Stoga preventivni *in situ* pregledi mogu biti preporučeni kao mera u cilju održavanja bezbednosti lekovitih proizvoda na bazi ove biljke.

Ključne reči: *Teucrium montanum* L., serpentine, sadržaj teških metala.