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

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Abstract: *Teucrium montanum* L. is a commonly used plant in traditional medicine throughout the 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 the focus of many pharmacological studies in the 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 the mineral content in various organs of this healing plant. 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 can still reach 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 popular widely used 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, to the Netherlands in the north, and to West Ukraine and Crimea in the east. It is also found in Algeria, and Asia Minor (Pavlović 1975, Lakušić 2000). In the 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 the 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 the anti-proliferative effect 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 the 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) are 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 the Brđani Gorge near the town of Gornji Milanovac, and from the village of Kremna in the Mt. Zlatibor region. A thorough description of the sampling locations is provided by Vicić *et al.* (in press). Five root and stem samples, and 15 leaf samples were taken from both sampling 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 ($\pm$ 0.0001). Samples were then digested in 2:1 mixture of HNO₃ (65% p.a. Carlo Erba Reagents, Italy) and H₂O₂ (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 samples taken. Unlike Hg, As, Pb or Cd, other potentially toxic metals in plants, such as the ones surveyed here (which are not among the 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 the populations surveyed in this study.

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
Brdani 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 the values determined in the 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 the three congeners (Pavlova and Karadjova 2012). Conversely, Ni levels in the 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 sampling locations is probably safe for use (in terms of the amounts of accumulated Ni, Cr, Co, Cu, and Zn).

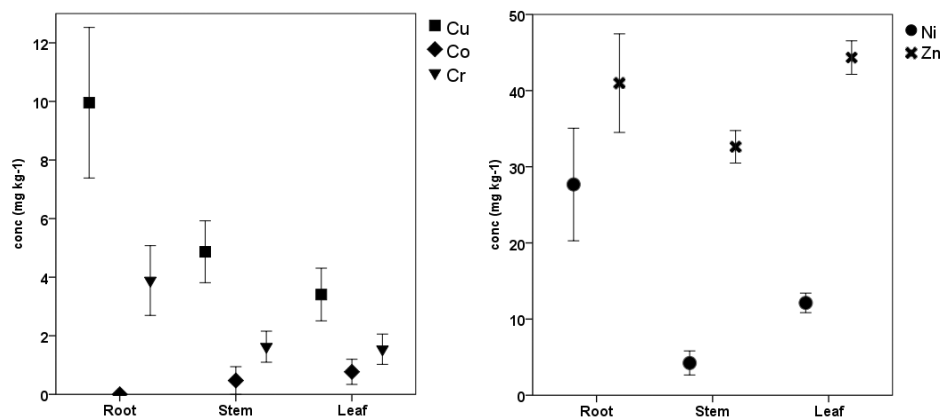


Figure 1. Root, stem and leaf concentration of Cu, Co, Cr (left), Ni, and Zn (right) in *T. montanum* from Brđani Gorge locality; mg kg⁻¹; mean $\pm$ SE.

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 the abilities of handling metal cations are not specific for serpentine populations, but can also be found in non-serpentine populations.

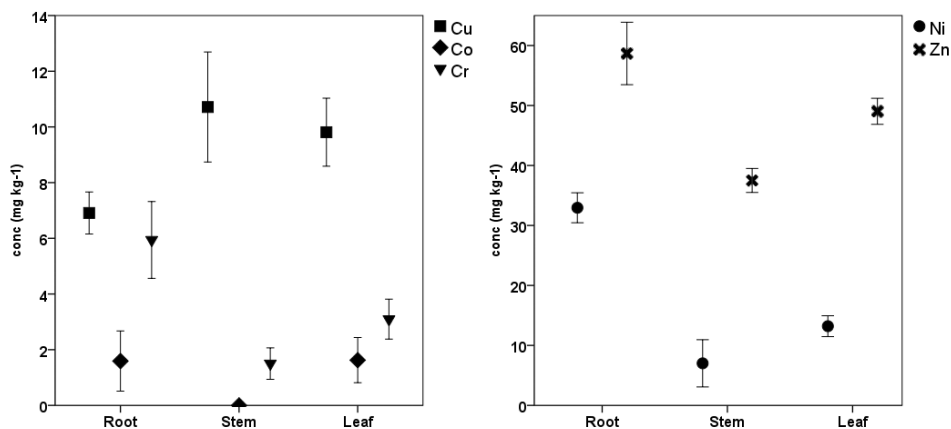


Figure 2. Root, stem and leaf concentrations of Cu, Co, Cr (left), Ni, and Zn (right) in *T. montanum* from Kremna locality; mg kg⁻¹; mean $\pm$ SE.

Another finding regarding the mountain germander picked from serpentine soils in Serbia is that water is an efficient polar solvent of its phenolic contents. Leaves were found to hold most of the water-extracted phenolic compounds (Stanković *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 can point to a further research on the 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 the Balkan Peninsula. The wide application and bioactive potential of its extracts insist on safety precautions. Therefore, it is necessary to evaluate the actual heavy metal content, especially 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 the low levels of the surveyed micronutrients in roots primarily, and in other organs as well. Our results suggest that the Ni, Cr, Co, Cu, and Zn content of the two populations of *T. montanum* growing in serpentine can be considered safe for use in the afore-mentioned ways. However, mineral composition and soil conditions that govern metal mobility vary greatly in serpentine soils, therefore their accumulation can still reach potentially hazardous levels. Precautionary measures such as scanning for metal content 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 – lekovita je 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. Serpentinitiska 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 serpentinitiske 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.

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