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Trace elements in aerial parts and rhizosphere of *Thymus pannonicus* All.

Research Article

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Abstract: This paper brings out the results of the study on the levels of selected trace elements (Cu, Fe, Mn, Zn and Cr) in aerial parts of *Thymus pannonicus* All. (Lamiaceae) and rhizosphere soil from twelve locations in Serbia. Prior to assays by flame and flameless atomic absorption spectrometry, samples were subjected to microwave-assisted acid digestion. Real and potential acidity of soil samples were also measured. Obtained results for soil samples, although slightly higher for some elements (Cu: 12.38–45.18 mg/kg; Fe: 22102–46193 mg/kg; Mn: 776.95–4901.27 mg/kg; Zn: 62.27–214.02 mg/kg; Cr: 48.86–69.13 mg/kg), were found to fit into biogeochemical background. Element contents in plant samples differed depending on collecting site (Cu: 5.26–14.07 mg/kg; Fe: 25.92–1454.07 mg/kg; Mn: 89.29–278.25 mg/kg; Zn: 1.81–10.64 mg/kg; Cr: 1.11–3.51 mg/kg), which can be partly explainable by different nutrient availability influenced by soil acidity. Zinc levels in *T. pannonicus* were below expected and seem to be strongly influenced by plant physiological properties.

Keywords: *Thymus pannonicus • Trace elements • Rhizosphere soil • Plant tissues • AAS*

1. Introduction

Soil is the main source for both organic and inorganic plant nutrients. Depending on plant species and soil type, there is a variety of different chemical environments influencing biological availability, absorption, distribution and accumulation of each element in many ways. Seventeen elements are considered to be essential for plants: C, H, O, N, K, Ca, Mg, P, S, B, Cl, Cu, Fe, Mn, Mo, Ni and Zn. These elements are involved in various processes of primary and secondary plant metabolism. With respect to concentration levels in plant tissues, they are classified as major, minor and trace elements. Beside a common knowledge, essentiality of certain elements is not proven yet, but some beneficial effects on plant growth have been noticed (e.g. the effects of Cr) [1].

Elemental pattern in medicinal herbs and soils could be discussed in a few different ways. Growing on a soil rich in potentially toxic heavy elements can lead to accumulation of higher amounts of these elements in plant tissues. Bearing in mind that plants are an essential part of regular diet, questions of their safety for human health have frequently arisen [2,3]. On the other hand, holding a focus on two environmental compartments (both soil and plant tissues), a close insight on the elemental composition could be reached, which can prove itself significant in highlighting its influence on biosynthetic pathways and production of secondary metabolites, e.g. essential oils [4]. Both approaches justify the objectives of this work.

The genus *Thymus* comprises more than 200 species native for Europe, Asia, North Africa and the Canary Islands. Apart from *T. vulgaris* L., *T. zygis* Loefl. ex L. and *T. serpyllum* L., which are recognized in a majority of the world's pharmacopoeias as biological sources of official herbal drugs, the other representatives of this genus are widely used in traditional medicine, or as culinary herbs and ornamentals [5]. To the best of our knowledge, several authors have reported values for a number of major, minor, and trace elements in

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aerial parts of *Thymus* species so far [6-10], but only a few have investigated levels and relationships between metal concentrations in soil and herb [11-13].

Hungarian or Eurasian thyme (*Thymus pannonicus* All., Lamiaceae) is an herbaceous perennial plant growing wild in the Pannonian plain and surrounding areas. The area of distribution of *T. pannonicus* in Serbia includes eastern and southeastern parts of the country, as well as the whole Vojvodina province [14]. In eastern Serbia, on Mts. Stol, Ozren and Rtanj, Hungarian thyme grows on the soils derived from underlying limestone [15]. Citral *T. pannonicus* chemotype from Mt. Vršačke planine in Vojvodina province, grows on acidic soils derived on albite-muscovite schist and granite gneiss. In this region, Hungarian thyme is traditionally used for treatment of respiratory and gastrointestinal complaints, as well as a flavoring agent because of its lemon-like scent [16]. Hungarian thymol/*p*-cymen *T. pannonicus* chemotype, as Pluhar *et al.* reported recently, can develop on variety of soil substrates and tolerate a wide soil pH range as well [17].

Lack of reliable data for elemental content and patterns in *T. pannonicus* tissues and soil where it was grown, adds to the overall importance of conducted research.

2. Experimental Procedures

All chemicals were of analytical grade. In preparation of all solutions, double distilled water was used. Stock solutions of Cu, Mn, Zn, Fe and Cr salts (1 g/l) were purchased from Merck (Darmstadt, Germany). Working solutions were produced by suitable dilution of corresponding stock solution with 2.5% nitric acid (HNO_3). Nitric acid (65%, v/v) was provided by Merck and solution of H_2O_2 (30%, v/v) and KCl from Zorka Pharma Šabac (Serbia).

Aerial parts of plants and rhizosphere soils were collected during the flowering period from Mts. Rtanj and Ozren, two locations on Mt. Stol (near the city of Bor) and eight locations on the Mt. Vršačke planine in 2008 (Table 1). For each location three representative

No.	Location	Description	Coordinates	Bedrock [15]
1	Mt. Vršačke planine	Mountain lodge	N 45° 07’ 37.7’’ E 21° 20’ 31.9’’ H 357 m	Amygdaloidal and Augen gneiss
2	Mt. Vršačke planine	Path	N 45° 07’ 11.7’’ E 21° 19’ 47.1’’ H 293 m	Amygdaloidal and Augen gneiss
3	Mt. Vršačke planine	Hill side road	N 45° 07’ 06.4’’ E 21° 19’ 47.9’’ H 271 m	Amygdaloidal and Augen gneiss
4	Mt. Vršačke planine – Kapela	Meadow	N 45° 06’ 59.1’’ E 21° 19’ 43.2’’ H 249 m	Amygdaloidal and Augen gneiss
5	Mt. Vršačke planine – Đakov vrh	Meadow	N 45° 07’ 18.5’’ E 21° 21’ 10.0’’ H 418 m	Amygdaloidal and Augen gneiss
6	Mt. Vršačke planine – Mesić village	Meadow	N 45° 06’ 34.6’’ E 21° 24’ 26.6’’ H 202 m	Claystone
7	Mt. Vršačke planine – Fizeš stream	Meadow	N 45° 05’ 36.3’’ E 21° 27’ 36.2’’ H 139 m	Albite-muscovite schist
8	Mt. Vršačke planine – Sočica village	Meadow (pasture)	N 45° 05’ 31.3’’ E 21° 27’ 23.5’’ H 191 m	Albite-muscovite schist
9	Mt. Stol	Meadow	N 44° 10’ 15,8’’ E 21° 07’ 25,2’’ H 878 m	Limestone
10	Mt. Stol	Hillside	N 44° 10’ 33,7’’ E 22° 07’ 50,0’’ H 1081 m	Limestone
11	Mt. Ozren	Hill side road	N 43° 35’ 28,8’’ E 21° 53’ 15,8’’ H 954 m	Limestone
12	Mt. Rtanj – Vrmdža village	Hill side road	N 43° 43’ 34,4’’ E 21° 50’ 45,6’’ H 651 m	Limestone

Table 1. Description of sampling locations.

samples of both plants and soils were collected and analyzed. The soil was sampled from the root zone, in a depth of approximately 5–15 cm. The identification of plant material was confirmed by Ivan Šoštarić from the Department of Botany, Faculty of Agriculture, Belgrade based on voucher specimens deposited there. Both plant and soil samples were air-dried and grounded before subsequent procedures.

Acidity was measured using a PHM240 pH meter (Radiometer). For microwave-assisted acid digestion of plants and soil samples, a close-vessel high-pressure microwave digesters CEM MDS-2000 model was used.

The determination of Cu, Zn, Mn, and Fe was performed with a Perkin–Elmer Model 5000 atomic absorption spectrophotometer under optimized measurement conditions using suitable hollow cathode lamps. Signals were measured with background correction (deuterium lamp) at the optimal flame (A–Ac) height [18].

The determination of Cr was performed by using a Perkin–Elmer Model 5000 atomic absorption spectrophotometer with a graphite furnace HGA 400 Automatic Burner Control, with pyrolytic graphite tubes according to the temeperature program, with optimal temperatures for drying, pyrolysis, atomisation and cleaning: 110, 1650, 2500 and 2650°C, respectively [19]. Wavelengths and method detection limits used for measuring both in FAAS and GF-AAS are given in Table 2.

Acidity of soil samples was determined in both KCl solution (potential acidity) and aqueous suspensions (real acidity). To 10.00 g of soil samples, 25.00 ml of 1 M KCl or 25.00 ml of redistilled water were added. Suspensions were stirred occasionally, and acidity measured after 30 min.

Prior to elemental analysis, plant and soil samples were subjected to microwave-assisted acid digestion, according to the procedure performed by Ražić *et al.* [19]. Each sample (about 0.5 g dry weight) was digested with 2 ml H_2O_2 (30%, v/v) and 7 ml HNO_3 in a microwave oven. After filtration, the obtained solutions were transferred into 25 ml volumetric flasks and diluted to the volume with redistilled water. One blank sample, containing the same amounts of acid and oxidant, was processed along with each set of analyzed samples.

Accuracy of the methods applied for determination of Cu, Zn, Cr, Mn and Fe, after microwave-assisted acid digestion of plants and soil samples, was checked by analysis of SRMs: NIST SRM 1547 - Peach Leaves and NIST SRM 2711 - Montana II Soil (Table 3). Quantification of Cu, Zn, Mn, Fe and Cr was performed by external calibration, after adequate regression and

Element	λ[a] [nm]	λ[b] [nm]	MDL [mg/kg]	Range[c] [mg/kg]
Cu	324.7	/	0.05	4
Fe	248.3	/	0.05	10
Mn	279.5	/	0.05	2
Zn	213.9	/	0.05	1
Cr	/	357.6	0.005	0.2

Table 2. Operating conditions.

[a]FAAS, [b]GF-AAS, [c]Working range
MDL – method detection limit

Element	NIST SRM 1547 - Peach Leaves			NIST SRM 2711 - Montana II Soil		
	Found [µg/g]	Ceritified value [µg/g]	Recovery [%]	Found	Ceritified value	Recovery [%]
Cu	4.2 ± 0.5	3.7 ± 0.4	113.5	120 ± 3 µg/g	114 ± 2 µg/g	95.0
Fe	199 ± 12	218 ± 14	91.2	2.67 ± 0.08%	2.89 ± 0.06%	92.4
Mn	113 ± 8	98 ± 3	115.3	685 ± 30 µg/g	638 ± 28 µg/g	107.4
Zn	19.6 ± 0.5	17.9 ± 0.4	109.5	325.1 ± 3.2 µg/g	350.4 ± 4.8 µg/g	92.8
Cr	0.89	1	89	51 µg/g	47 µg/g	108.5

Table 3. Analysis of standard reference materials.

correlation analyses (Table 4). The precision for all the results was on average less than 2% (RSD).

3. Results and Discussion

Rhizosphere soil and aerial parts of *T. pannonicus* were collected from twelve locations in Serbia. The results of preliminary investigations in soils such as innorganic composition, total organic carbon (TOC), soil type, as well as real and potential acidity of rhizosphere soil samples are presented in Table 5. The obtained results on element content of rhizosphere soil and aerial parts of *T. pannonicus* are given in the Table 6. Additionally, Pearson correlation coefficients for element concentrations in soil and plant samples were calculated and data are shown in Table 7.

All samples from mountains in eastern Serbia (Stol, Ozren and Rtanj), with the exception of a sample from location 9 (pH 5.91), have acidity in pH range between 7.13 and 7.62. For samples from Mt. Vršačke planine, the acidity was below 7 and ranged from 5.24 to 6.71. According to pH values, two groups of samples could be recognized: the first one, including samples No. 1–4 with real acidity 5.24–6.09 and the second one, with samples 5–8 and pH 6.14–6.71. Content of total organic carbon (TOC), P_2O_5 and K_2O, was within the expected ranges for all samples [20], with the exception of Mt. Vršačke planine, which was a bit higher and could be explained by increased usage of fertilizers to supply plant nutritients or amend soil fertility in that region.

In general, the content of measured elements in investigated soil samples was obtained in the ranges of expected biogeochemical background [20]. However, the obtained results for certain elements varied within slightly broader limits. Iron levels were relatively consistent, ranging between 22102 and 46193 mg/kg. Manganese was found to be increased in all soil samples (above 776.95 mg/kg), with the highest values measured in soils from mountains in eastern Serbia, particularly Ozren (location 11) and Stol (location 10) – 4901.27 and 4209.98 mg/kg, respectively. Levels of zinc were also higher in the majority of samples (62.27–214.02 mg/kg), while the content of Cu was determined in the range 12.38–45.18 mg/kg. The highest concentrations of Zn and Cu were measured in samples from mountains in eastern Serbia; similar observations were valid for manganese, too. Chromium levels in rhizosphere soil samples have not varied significantly, and ranged between 48.86–69.13 mg/kg.

In contrast to relatively uniform levels of Fe in soil samples, determined concentrations in aerial parts of plants varied significantly. The highest concentrations were recorded in samples from locations 6, 7 and 8 on Mt.

	Calibration curve	R^2	S_a	S_b
Cu	y = 0.1282x – 0.0260	0.9931	0.0075	0.0174
Fe	y = 0.0301x + 0.0089	0.9972	0.0009	0.0047
Mn	y = 0.1325x – 0.0088	0.9991	0.0023	0.0025
Zn	y = 0.5227x + 0.0132	0.9958	0.0340	0.0215
Cr	y = 0.0034x + 0.0305	0.9969	0.0001	0.0155

Table 4. The results of regression analysis.

S_a – standard error of slope, S_b – standard error of interrcept, R^2 – regression coefficient

Location		TOC %	P_2O_5 mg/100 g	K_2O mg/100 g	Total nitrogen %	Soil type	pH (H_2O)	pH (KCl)
1 – 8	Mt. Vršačke planine*	3.41	12.39	29.46	0.31	n.a.	5.24-6.71	4.09-6.00
9	Mt. Stol	4.53	2.86	55.00	n.a.	Vertisol	5.91	4.93
10	Mt. Stol	7.72	2.96	30.00	n.a.	Calcimelanosol	7.13	6.47
11	Mt. Ozren	2.70	6.10	20.30	n.a.	n.a.	7.37	6.80
12	Mt. Rtanj	4.15	2.19	24.06	n.a.	Fluvisol	7.62	7.12

Table 5. Preliminary analysis of investigated soils - inorganic composition, total organic carbon (TOC), pH and soil type according to particular site.

** - average values for several locations; n.a. – not analyzed*

	Elements (mg/kg)									
	Cu		Fe		Mn		Zn		Cr	
Location	plant	soil	plant	soil	plant	soil	plant	soil	plant	soil
1	8.79	22.97	262.97	35917	250.78	980.20	5.72	125.87	1.83	65.86
2	10.46	21.74	509.22	35226	198.14	832.87	4.25	120.92	2.58	56.99
3	8.48	29.53	646.71	33580	209.86	803.53	2.17	112.43	2.55	54.19
4	6.87	18.44	506.30	36318	209.66	776.95	6.08	129.99	2.25	54.53
5	10.94	13.67	796.60	29313	238.33	924.78	10.64	93.38	2.20	55.71
6	11.61	14.63	1454.07	22102	278.25	1675.78	4.78	62.78	3.51	48.93
7	7.75	12.38	1375.14	26560	164.87	927.37	4.00	63.29	3.23	48.86
8	8.43	14.58	960.45	32977	201.19	1401.88	3.15	62.27	3.05	63.94
9	13.77	32.42	25.97	30899	148.09	2238.66	4.37	100.86	1.32	58.31
10	14.07	45.18	254.68	46193	95.92	4209.98	2.27	160.57	1.78	54.22
11	5.26	34.17	25.92	38073	124.33	4901.27	3.67	134.06	1.11	65.55
12	6.21	28.62	267.00	27106	89.29	1754.74	1.81	214.02	1.42	69.13
Mean	9.39	24.03	590.42	32855	184.06	1785.67	4.41	115.04	2.23	58.02
SD	2.83	10.14	478.61	6305	60.35	1380.59	2.38	44.19	0.78	6.68
Max	14.07	45.18	1454.07	46193	278.25	4901.27	10.64	214.02	3.51	69.13
Min	5.26	12.38	25.92	22102	89.29	776.95	1.81	62.27	1.11	48.86
Max/Min	2.68	3.65	56.10	2.09	3.12	6.31	5.88	3.44	3.17	1.41

Table 6. Avarage content of the elements in aerial plant parts of *T. pannonicus* and rhizosphere soil samples (n=3).

		Plant					Soil			
		Cu	Fe	Mn	Zn	Cr	Cu	Fe	Mn	Zn
Plant	Fe	0,01	/	/	/	/	/	/	/	/
	Mn	0,09	0,55	/	/	/	/	/	/	/
	Zn	0,14	0,17	**0,58**	/	/	/	/	/	/
	Cr	0,07	**0,95**	**0,61**	0,08	/	/	/	/	/
Soil	Cu	0,25	**-0,76**	**-0,70**	-0,53	**-0,72**	/	/	/	/
	Fe	0,12	**-0,63**	-0,38	-0,20	-0,47	**0,68**	/	/	/
	Mn	0,09	-0,49	**-0,63**	-0,34	-0,56	**0,74**	0,51	/	/
	Zn	-0,21	**-0,71**	**-0,65**	-0,34	**-0,72**	**0,65**	0,40	0,33	/
	Cr	-0,44	**-0,64**	-0,35	-0,20	**-0,64**	0,27	0,21	0,24	0,55

Table 7. Correlation matrix (Pearson correlation) for the element concentrations in plants and soil.

Bolded figures indicate significant correlation at the 0.05 level for n=12

Vršačke planine, 1454.07, 1375.14 and 960.45 mg/kg, respectively. Manganese concentration was slightly elevated in several samples, but never exceeded physiological ranges for upper plant parts [20]. Plants growing wild on mountains in eastern Serbia had significantly lower concentrations of Mn (89.29–148.09 mg/kg) in comparison to samples from Mt. Vršačke planine (164.87–278.25 mg/kg). All plant samples contained Zn in amounts lower than expected, ranging from 1.81 to 10.64 mg/kg. The highest Cu concentrations were determined in sampling sites from Mt. Stol (13.77 and 14.07 mg/kg), but the lowest in plants from Mts. Ozren and Rtanj (5.26 and 6.21 mg/kg, respectively). On the other hand, the samples from Mt. Vršačke planine contained 6.87–11.61 mg/kg of Cu. Determined Cr levels were found to be within expected range (1.11–3.51 mg/kg). Samples from Mt. Vršačke planine contained higher amounts of this element than plants from mountains in eastern Serbia. The highest values were measured in samples from sampling sites 6, 7 and 8 (3.51, 3.23 and 3.05 mg/kg, respectively).

In general, soil composition is the result of complex processes influenced by several factors, such as nature and composition of parent material, climate, topography, type of vegetation and presence of other living organisms and depends on all these natural influences. However, in the last decades anthropological factors appeared to be a hazard and shape our environment in different ways. So, in industrial and urban areas, as well as in agricultural land, there is an impact of human activities with direct and/or indirect influence on inorganic composition of the soil. The availability of minerals to the plant is governed by soil and plant geochemical and biological properties. Diversity, often induced by environmental conditions directly govern nutrient absorption and translocation of specific elements in plants.

Among different influences that create a chemical environment in which the plants can grow, rhizosphere soil acidity is one of the most important. Nutrient uptake by plants is usually influenced by a combination of numerous chemical processes and equlibria such as precipitation/dissolution, adsorption/desorption, complexation/dissociation and oxidation/reduction [21]. Soil samples from Mt. Vršačke planine, derived on gneiss, schist and claystone, are slightly acidic resulting in good metal availability. Soils with underlying limestone on Mts. Stol, Ozren and Rtanj showed alkaline reaction and buffering capacity lower than soils from Mt. Vršačke planine, with the exception of sample collected on location 9 on Mt. Stol. The latter is situated in northeastern Serbia, in the vicinity of the town of Bor. This district is a part of Carpatho-Balkan arc, a metallogenic province rich in Cu and Au-Ag, Pb-Zn, and Mo deposits [22], which are mostly of high sulphidation type. Therefore, the processing of ore in Copper Mining and Smelting Complex in Bor causes massive air contamination by sulphur dioxide. Although sulphuric acid factories utilize much of released SO_2, significant amounts still escape into the atmosphere. Slightly acidic reaction of soil collected from sampling site 9 (pH 5.91) could be explained by retention of acid rainfall and also with increased concentration of SO_2 in the atmosphere.

Like Mt. Stol, Mts. Ozren and Rtanj are also parts of the Carpatho-Balkan arc. This fact, along with higher acidity of soil samples from Mt. Vršačke planine, could explain higher concentrations of Cu, Zn and Mn in rhizosphere soil samples on mountains in eastern Serbia than on Mt. Vršačke planine. Among rhizosphere soil samples from Mt. Vršačke planine slightly lower concentrations of zinc and copper are measured in samples collected on locations 5–8 than on locations 1–4. Increased content of Zn and Cu on these sites which are located closer to the hill road, might be related to traffic flow (*i.e.* brake linings, tire tread rubber) [23].

For the majority of measured elements, higher contents were detected in plant samples from Mt. Vršačke planine than on Mts. Stol, Ozren and Rtanj. Although rhizosphere soil specimens, collected from the latter three mountains, were abounded with Fe and especially Mn, corresponding plant samples contained substantially lower amounts of these elements. Furthermore, their negative correlation was observed. In plants growing on well-aerated calcareous or alkaline soils, such as locations on mountains in eastern Serbia, low concentrations of Fe or even deficiency is not rare. Dicotiledonous and nongraminaceous monocotiledonous plants try to overcome this problem with so-called strategy I activities, mainly by enhancing enzymatic reduction of Fe^{3+} to Fe^{2+} and increasing release of hydrogen ions into the rhizosphere. Under such conditions the reduction of MnO_2 to Mn^{2+} is favored, increasing its availability in that way [24]. Mężyk and Więckowski (1999) also found that manganese contents in *T. serpyllum* from three collecting sites were very different. On the other hand, the same authors reported quite consistent zinc concentrations (mean value 24.1 mg/kg) [9], which is similar to findings of Remon *et al.* (2005) who found that leaves of *T. pulegioides* grown on two distant locations with different Zn content in the soil (545 and 288 mg/kg), contained 31.5 and 38.6 mg/kg of this element, respectively [11]. In contrast, upper plant parts of *T. praecox* from Mt. Uludağ, Turkey, collected from different directions and distances around the wolfram mine, contained Zn in range from 50±20 mg/kg to 6780±790 mg/kg [12]. Additionally, relatively high amounts of Zn (173–186 mg/kg), higher than in corresponding soils, were found in aerial parts of *T. zygis* [13].

According to the literature [20] Zn levels in soils should not be higher than 200–300 mg/kg. Symptoms of deficiency for a majority of plant species appear with concentrations of zinc in leaves in the range 20-30 mg/kg [20]. However, in our findings even though Zn levels in the rhizosphere were slightly higher, concentrations in all plant samples were below expected, indicating that uptake and translocation of this element in *T. pannonicus* are strongly controlled by inner plant mechanisms.

Unlike relatively uniform results for Cu content in aerial parts of *T. pannonicus* from Mt. Vršačke planine, its concentrations vary significantly in plants collected on other four locations. Alkaline reaction of soils from Ozren and Rtanj, by decreasing its availability, resulted in the lowest measured contents of Cu in plants sampled on those locations. On the other hand, both plant samples from Mt. Stol contained the highest Cu levels (14.07 and 13.77 mg/kg) without obvious influence of the soil acidity.

Significant correlation (Table 7) was observed between Fe and Cr contents in aerial plant parts of *T. pannonicus* (correlation coefficient 0.95). The highest measured chromium levels were found in samples which also contained the highest amounts of Fe. This is in accordance with reported findings that chromium translocation from lower to upper plant parts is related with plants ability to accumulate Fe [1].

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