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Determination of some trace elements in representatives of genus *Thymus* L. (*Lamiaceae*) by electrothermal atomic absorption spectrometry

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Determination results of trace elements: Cd, Co, Cr, Cu, Ni and Pb in some representatives of genus *Thymus* L. by electrothermal atomic absorption spectrometry are presented in this paper. Two procedures of mineralization (wet and dry) are suggested. Procedures were checked by a method of standard addition. Recovery values for examined elements ranged from 92.7% to 105.7% for wet and from 93.3% to 105.5% for dry procedure (except for Cd and Pb ranging from 67.4% to 87.9%). Representatives of genus *Thymus* in the flora of the Republic of Macedonia contain following trace elements: Cu > Cr > Ni > Pb > Co > Cd. Considerable variations in the amounts of some elements are probably dependent upon a particular season of the plant collection, site of its origin, as well as upon differences of the species included in the examination. Trace elements content probably depends upon climatic, ecological, pedological and other factors.

Keywords: *Thymus*, trace elements, Cd, Co, Cr, Cu, Ni, Pb, determination, atomic absorption spectrometry

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Recently, we have reported the data concerning the macroelements in the representatives of genus *Thymus* appearing in the flora of the Republic of Macedonia (1). The elements such as Ca, Mg, Na and K, which are commonly in higher quantities present in plants, were the most abundant elements in the samples of various taxa of genus *Thymus*. On the other hand, high amounts of Fe, Zn and Mn were also determined and a few taxa of genus *Thymus* from Macedonia showed a tendency to accumulate Fe (1). As it is well known, certain microelements are extremely important for some functions in human organism. Second part of our examination was devoted to the determination of microelements in Macedonian taxa of genus *Thymus*. The aim of present examination resulted from the fact that no data on microelements in Macedonian taxa of genus *Thymus* were found, and those concerning other taxa of genus *Thymus* are only few in number (2).

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For many years in Macedonian folk medicine some representatives of genus *Thymus* have been used against the abdominal throes or some pulmonary infections (3). Although the efficiency of these plants is probably due to some constituents of essential oil or some flavonoids, it is well known that the presence of some microelements could give a synergistic effect with those constituents (4).

The presence of trace elements in the plants was expected. As a result of the polluted environment, their concentration can be increased, and the elements, not present in the plant constitution, can be found. The most present contaminants are lead and cadmium. Their toxical reaction originates from their specific influence mechanism, and disturbance of which can prevent the hemoglobin synthesis and cause anemia (5).

Accordingly, the aim of this work is to determine some trace elements (Cd, Co, Cr, Cu, Ni and Pb) in the representatives of genus *Thymus* L. by use of electrothermal atomic absorption spectrometry (ETAAS).

EXPERIMENTAL

Apparatus

A Perkin-Elmer Model 303 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a deuterium background corrector and HGA-72 graphite furnace was used. Argon was used as purge gas. Optimal conditions for Cd, Co, Cr, Cu, Ni and Pb determinations by ETAAS are given in Table I.

Table I. Optimal conditions for the Cd, Co, Cr, Cu, Ni and Pb determination by ETAAS

Parameter	Cd	Co	Cr	Cu	Ni	Pb
Wavelength (nm)	228.8	240.7	357.9	324.8	232.0	283.3
Slit (nm)	0.7	0.2	0.7	0.7	0.7	0.7
Lamp current (mA)	4	30	25	15	25	10
Dry						
Temperature (°C)	100	100	100	100	100	100
Time (s)	20	20	20	20	20	20
Ramp time (s)	2	2	2	2	2	2
Char						
Temperature (°C)	300	900	1100	900	900	400
Time (s)	25	25	25	25	25	25
Ramp time (s)	1	1	1	1	1	1
Atomize						
Temperature (°C)	1800	2300	2400	2300	2500	2000
Time (s)	5	5	5	5	7	5
Ramp time (s)	0	0	0	0	0	0
Cleaning						
Temperature (°C)	2700	2700	2700	2700	2700	2700
Time (s)	3	3	3	3	3	3
Gas	argon	argon	argon	argon	argon	argon

Samples

Samples of different species of the genus *Thymus* were collected in different regions of Macedonia. Before examining, they were all milled.

Reagents

All reagents and standard substances were of analytical grade. Standard solutions were prepared with mass concentration of 1 g L^{-1} using commercial solutions of all investigated elements (Merck, Darmstadt, Germany). Standard solutions with lower concentrations were prepared by diluting with redistilled water.

Procedures

Dry procedure. – Milled drug (5 g) was transferred into a porcelain crucible and heated on a hot plate for three hours, then in muffle furnace at 150 °C for 30 minutes and finally, at 500 °C for 8 hours. After cooling at room temperature, the obtained mineral residue was dissolved in 100 mL of 4% (m/m) solution of HNO_3.

Wet procedure. – Milled drug (5 g) was transferred into a small Erlenmeyer flask, wetted with 30 mL of concentrated HNO_3 and heated in a boiling water-bath for 8 hours, covered with glass-watch. Then, the heating was continued on hot plate until it almost dried. The obtained mineral residue was dissolved in 100 mL 4% (m/m) solution of HNO_3.

RESULTS AND DISCUSSION

During the process of mineral determination, the important phase is the mineralization of organic substance. There are different approaches to the process of mineralization, but dry (2, 6, 7) and wet (8, 9) procedures are generally applied. Since one may obtain different results when applying one or the other procedure, the examinations were performed with both procedures. For a complete dry mineralization procedure about twelve hours were necessary, whereas for the wet procedure it took two days. Since a homogeneous state of the plant material was needed, an agate mill was used so as to avoid the contamination. The results defining the trace elements in one of the samples of genus *Thymus* (*Thymus moesiacus*, Popova Šapka, 1994), obtained by using both the proposed procedures are given in Table II.

For relative standard deviation (RSD, %) satisfactory values were obtained *i.e.* for Cu by dry and wet procedure 2.93% and 1.64%, respectively. For Ni, Pb and Co much better results were obtained by using dry procedure, whereas for Cr and Cd by using wet procedure (Table II).

The proposed procedures were checked by the standard addition method (see Table III).

The values of recovery ranged from 93.3 to 105.5% for dry procedure (excluding Pb and Cd for which it ranged from 67.4 to 87.9%), and from 92.7 to 105.7% for wet procedure. Low values of recovery for Pb and Cd determined by dry procedure were prob-

Table II. Content of Cd, Co, Cr, Cu, Ni and Pb in the representatives of Thymus *genus by ETAAS, including dry and wet procedures for mineralization of samples*

Element	Dry procedure		Wet procedure	
	Content ($\mu g\ g^{-1}$)	RSD (%)*	Content ($\mu g\ g^{-1}$)	RSD (%)*
Cd	0.082	2.68	0.210	5.89
Co	0.866	4.02	0.846	3.50
Cr	0.653	3.84	0.638	4.62
Cu	3.75	2.93	3.67	1.64
Ni	6.00	4.52	6.12	1.70
Pb	1.16	4.32	1.45	1.43

*RSD – relative standard deviation

ably due to the high burning temperature, needed for the isolation of other elements. As it can be seen, recovery values varied from one procedure to the other, the same as from one element to the other. This variation may be ascribed to the differences in interference of the matrix on the examined elements (10). From the results given in Table III, one may conclude that both described procedures can be used for determining microelements (except for Pb and Cd) in samples of genus *Thymus*. Due to the evaporating features of the elements Pb and Cd, all further examinations of the microelements were carried out by using the wet mineralization procedure.

The trace elements Cd, Co, Cr, Cu, Ni and Pb were determined in thirty different samples of various representatives of genus *Thymus* collected in different areas in the Republic of Macedonia. The results of this examination are given in Table IV.

From Table IV one may conclude that Cu prevailed over other elements (7.49–29.21 $\mu g\ g^{-1}$). The content of Cr was ranging from 2.06 to 22.92 $\mu g\ g^{-1}$. Ni was represented with the range of 2.19–16.59 $\mu g\ g^{-1}$, with one sample being the exception which contained 47.53 $\mu g\ g^{-1}$. The content of Pb was ranging from 0.03 to 4.35 $\mu g\ g^{-1}$, with the exception of one sample containing 13.83 $\mu g\ g^{-1}$. The two remaining elements (Co and Cd) were present in very small concentrations. Co was ranging from 0.15 to 1.31 $\mu g\ g^{-1}$, with the exception of one sample which contained 5.19 $\mu g\ g^{-1}$. Cd was present in the range from 0.08 to 0.39 $\mu g\ g^{-1}$, with the exception of one sample containing 1.20 $\mu g\ g^{-1}$.

The correlation between the obtained results was not as good as it was expected. The best correlation was found in the quantity of Co, the presence of which was within the range of 0.15–1.31 $\mu g\ g^{-1}$ (*Thymus albanus*, collected in Popova Šapka in 1995 contained 5.19 $\mu g\ g^{-1}$ of Co). The presence of other elements varied considerably depending on the species, origin and the season of the collection. The contents of Co, Cr, Cu and Ni, which are the components physiologically present in the plants, were expected. There was the accordance with the values obtained from the literature. According to the results of Šovljanski *et al.* (2) in the samples of some *Thymus* species collected around Bački Petrovac, Vojvodina, the quantity of Cu was found up to 14.5 $\mu g\ g^{-1}$. Simmons *et al.* (11) determined Cu in corn (2.18 $\mu g\ g^{-1}$), clover (5.26 $\mu g\ g^{-1}$), pea (6.46 $\mu g\ g^{-1}$) and some other plant material. The presence of Pb and Cd indicates a sort of certain contamination. The concentration of Cd in uncontaminated plants and food product ranges from 0.01 to 1.0 $\mu g\ g^{-1}$ (12). The content of Cd in all the samples we examined was

Table III. Results of Cd, Co, Cr, Cu, Ni and Pb determination in the representatives of Thymus *genus by standard addition method (results are given in* $\mu g\ g^{-1}$*)*

Element	Procedure	Added	Calculated	Determined	Recovery (%)
Cd	Dry	–	–	0.08	–
		0.1	0.18	0.16	87.9
		0.2	0.28	0.22	78.0
		0.3	0.38	0.28	73.3
	Wet	–	–	0.12	–
		0.1	0.22	0.21	95.5
		0.2	0.32	0.30	93.8
Co	Dry	–	–	1.20	–
		0.2	1.40	1.35	96.4
		0.4	1.60	1.74	105.4
		0.8	2.00	2.11	105.5
	Wet	–	–	1.15	–
		0.2	1.39	1.35	96.7
		0.4	1.56	1.55	99.3
		0.8	1.94	1.95	100.5
Cr	Dry	–	–	0.65	–
		0.2	0.85	0.82	97.0
		0.4	1.05	0.98	93.3
		0.8	1.45	1.47	101.3
	Wet	–	–	0.64	–
		0.2	0.84	0.79	94.1
		0.4	1.04	0.96	92.7
Cu	Dry	–	–	3.75	–
		1	4.75	4.44	93.5
		2	5.75	5.52	96.0
		3	6.75	6.72	99.6
	Wet	–	–	3.67	–
		1	4.67	4.43	94.9
		2	5.67	5.54	97.7
		3	6.67	6.70	100.4
Ni	Dry	–	–	6.00	–
		1	7.0	7.04	100.6
		2	8.0	7.82	97.8
		3	9.0	9.40	104.4
	Wet	–	–	6.12	–
		1	7.12	6.67	93.7
		2	8.12	7.82	96.3
Pb	Dry	–	–	1.15	–
		0.2	1.35	1.15	85.2
		0.4	1.55	1.20	77.4
		1.0	2.15	1.45	67.4
	Wet	–	–	1.52	–
		0.2	1.72	1.60	93.0
		0.4	1.92	2.03	105.7
		1.0	2.52	2.45	97.2

Table IV. The content of Cd, Co, Cr, Cu, Ni and Mn in representatives of Thymus *genus determined by electrothermal atomic absorption spectrometry in* $\mu g\ g^{-1}$

No.	Sample	Element											
		Cd	RSD (%)*	Co	RSD (%)*	Cr	RSD (%)*	Cu	RSD (%)*	Ni	RSD (%)*	Pb	RSD (%)*
	Thymus tosevii Velen. ssp *tosevii* var. *tosevii*												
1	– v. Nikolič, 1994	0.20	0.06	0.29	4.17	3.97	3.18	14.84	1.31	2.19	1.06	4.35	4.33
2	– v. Laki, 1994	0.10	8.68	0.29	4.17	2.21	3.82	13.91	5.28	4.85	0.08	1.28	7.63
3	– v. Vitačevo, 1995	0.30	5.99	0.29	4.17	7.93	1.21	11.79	2.88	8.55	0.09	0.83	4.38
4	– v. Tajmište, 1995	0.15	0.19	0.57	0.92	16.08	0.81	11.79	4.33	10.04	0.37	2.68	2.84
5	– v. Majdan, 1995	0.15	3.46	0.29	4.17	2.06	4.10	9.83	2.82	5.40	0.22	0.22	2.50
6	– v. Rajko Žinzifov, 1995	1.20	0.48	0.46	0.99	8.01	0.91	11.45	1.49	10.37	0.22	13.83	3.94
	Thymus tosevii Velen. ssp. *tosevii* var. *longifrons* Ronn.												
7	– Karadžica, 1994	0.32	8.08	0.46	0.99	8.60	0.61	17.29	3.12	8.02	0.75	0.03	5.25
	Thymus tosevii Velen. ssp. *substriatus* (Borb) Matevski												
8	– v. Nikolič, 1994	0.08	8.11	0.46	1.77	5.89	0.39	10.99	4.48	3.09	5.91	2.99	5.65
9	– v. Vitačevo, 1994	0.19	2.26	0.17	3.64	2.77	0.63	11.04	3.71	3.50	0.13	0.03	6.93
10	– v. Vitačevo, 1995	0.17	0.92	0.29	4.17	7.93	1.23	17.89	1.03	6.84	0.16	1.05	0.22
	Thymus tosevii Velen. ssp *tosevii* var. *degenii* (H. Br.) Ronn.												
11	– Preslap, 1994	0.39	1.24	0.29	4.17	5.51	0.39	29.21	8.28	5.63	0.99	0.37	6.30
12	– Lazaropole, 1995	0.21	1.94	0.57	0.92	14.05	0.52	11.22	8.75	10.0	0.53	0.03	6.93
13	– Vrben, 1995	0.23	1.80	0.54	2.57	7.49	0.68	16.15	1.43	6.0	1.52	0.16	3.39
	Thymus alsharensis Ronn.												
14	– v. Majdan, 1994	0.22	3.93	0.40	3.56	4.04	1.87	13.56	9.23	5.33	0.13	2.33	3.75
15	– v. Majdan, 1995	0.13	6.19	0.20	5.67	4.99	1.27	10.98	3.68	5.12	0.18	0.91	2.73
16	– v. Majdan, 1995	0.15	8.60	0.15	1.54	4.13	4.49	13.69	3.4	6.52	1.67	0.29	3.80
	Thymus longidens Velen. var. *lanicaulis* Ronn.												
17	– v. Sonje, 1994	0.33	1.52	0.46	1.77	6.02	4.01	13.47	2.02	5.28	1.00	3.51	2.30
18	– v. Banjani, 1994	0.21	2.34	0.69	1.83	17.34	0.67	14.09	2.63	10.70	5.39	0.79	5.86
19	– v. Banjani, 1995	0.26	6.82	0.69	1.83	15.59	1.00	12.14	5.69	10.93	0.58	1.93	2.39
	Thymus longidens Velen. var. *dessareticus* Ronn.												
20	– Karadžica, 1994	0.32	1.54	0.90	0.64	16.52	0.58	10.69	5.31	10.02	1.12	0.93	0.69
21	– Karadžica, 1995	0.18	8.24	0.31	3.52	18.89	0.44	7.49	5.76	11.72	0.47	0.91	2.73
	Thymus ciliatopubescens												
22	– v. Sonje, 1994	0.42	2.11	0.46	0.99	4.48	0.90	10.63	4.56	4.12	3.24	1.07	3.17
	Thymus macedonicus (Degen et Urumov) Ronn.												
23	– v. Laki (in bloom), 1994	0.10	7.20	0.29	4.17	3.94	5.48	9.44	4.08	3.33	0.12	1.52	5.27
24	– v. Laki (before bloom), 1994	0.16	2.64	0.46	1.77	4.16	2.38	14.10	6.59	4.01	1.09	0.56	5.18
25	– v. Laki (in bloom), 1995	0.09	6.50	0.29	4.17	5.56	0.67	13.61	4.99	3.60	0.17	0.41	0.43
	Thymus moesiacus Velen.												
26	– Popova Šapka, 1994	0.32	7.24	1.31	0.53	14.44	0.64	13.19	6.79	12.03	0.51	1.05	0.77
27	– Bistra, 1994	0.34	1.48	0.46	0.99	4.20	3.20	11.28	1.46	7.66	0.53	0.41	0.17
28	– Popova Šapka, 1995	0.10	5.71	0.60	1.06	12.93	2.71	10.52	0.88	10.03	1.08	0.64	6.39
29	– Bistra, 1995	0.28	4.56	1.26	0.84	19.02	0.34	15.69	1.84	16.59	0.63	0.68	3.82
	Thymus albanus H. Braun												
30	– Popova Šapka, 1995	0.33	7.85	5.19	0.71	22.92	0.31	21.4	5.94	47.53	0.14	2.82	1.43

v. – village; * $n = 5$

within these limits. The exception makes *Thymus tosevii* ssp. *tosevii* collected in the area Rajko Žinzifov near Veles, 1995, with the Cd concentration of 1.20 μg g^{-1}. This increase of concentration might originate not only from the air and earth contamination (13), but as well from the possibility of the plant to accumulate this element (14, 15). At the same time, the increase of Cd in the plant tissue can be used as a kind of toxicological monitoring (16).

One of the samples, *Thymus albanus*, collected from Popova Šapka, 1995, could be recognized by many interesting microelements it contains. Actually, the presence of nearly all the elements is greatly increased if compared to the same ones in other samples. A considerable increase in concentration was noticed for the elements Cr (22.92 μg g^{-1}) and Ni (47.53 μg g^{-1}). This data could indicate that the concentration of these elements is most probably under the influence of geochemical factors (17) or it depended upon the plant capability to accumulate them (18).

CONCLUSION

Electrothermal atomic absorption spectrometry is a conventional analytical method for the determination of trace elements in the representatives of genus *Thymus* L. Mineralization of plant materials could be provided by dry or wet procedure. According to the values obtained for recovery, it can be concluded that both the procedures can be useful for the determination of Co, Cr, Cu and Ni in the representatives of genus *Thymus*.

Since the elements Pb and Cd are easily evaporated, for further examinations of microelements the wet procedure of mineralization is highly recommended.

The content of trace elements Cd, Co, Cr, Cu, Ni and Pb in thirty different samples of various representatives of genus *Thymus* collected all over the Republic of Macedonia, probably depends upon the area they are collected from, then geological, climate and other factors, as well as the botanical *Thymus* species they belong to.

REFERENCES

1. T. Kadifkova Panovska, T. Stafilov, S. Bauer, S. Kulevanova, and K. Dorevski, *Pharmacia (Sofia)*, in press.
2. R. Šovljanski, S. Lazić, V. Maćko, and S. Obradović, *Herba Hung.* 29 (1990) 59.
3. V. Dervendži, *Sovremeno lekuvanje so lekoviti bilki*, Tabernakul, Skopje 1992.
4. B. Okhotski, in *Propolis*, Apimondia, Bucharest 1978, 39.
5. *Casarett and Doull's Toxicology, The Basic Science of Poisons*, 3rd ed., Macmillan Publishing Company, New York 1986, pp. 609–619.
6. R. J. Haynes, *Commun. Soil Sci. Plant Anal.* 11 (1980) 459.
7. R. A. Isaac and W. C. Johnson, *J. Assoc. Off. Anal. Chem.* 58 (1975) 436.
8. S. S. Chao and E. E. Pickett, *Anal. Chem.* 52 (1980) 335.
9. G. Ogner, *Commun. Soil Sci. Plant Anal.* 14 (1983) 937.
10. V. Rizova, T. Stafilov, and L. Sivakov, *God. Zb. Zemjod. Fak. Skopje* 38 (1993) 59.
11. W. J. Simmons and J. F. Loneragan, *Anal. Chem.* 47 (1975) 566.
12. T. J. Ganje and A. L. Page, *At. Absorpt. Newslett.* 13 (1974) 131.

13. M. K. Wong, P. Tan, and Y. C. Wee, *Biol. Trace Elem.* **36** (1993) 135.
14. B. H. Olson, D. C. Hill, and M. G. Rigby, *Trace Subst. Environ. Health* **13** (1979) 124.
15. L. Wajda and P. Malec, *Acta Physiol. Plant.* **14** (1992) 93.
16. K. Robards and P. Worsfold, *Analyst* **116** (1991) 549.
17. R. M. Welch and E. E. Cary, *J. Agric. Food Chem.* **23** (1975) 479.
18. O. V. Gambi, R. Gabbrielli, and L. Pancaro, *Acta Oecol.* [*Ser.*]; *Oecol. Plant.* **3** (1982) 291.

SAŽETAK

Određivanje nekih elemenata u tragovima kod predstavnika roda *Thymus* L. (*Lamiaceae*) elektrotermičkom atomsko-apsorpcijskom spektrometrijom

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U ovom su radu prikazani rezultati određivanja nekolicine elemenata u tragovima (Cd, Co, Cr, Cu, Ni i Pb) u nekim predstavnicima roda *Thymus* L. elektrotermičkom atomsko-apsorpcijskom spektrometrijom. Predložena su dva postupka mineralizacije (mokra i suha). Ovi su postupci provjereni metodom standardne adicije. Vrijednosti povrata za ispitivane elemente kretale su se između 92,7 i 105,7% za mokru digestiju i između 93,3 i 105,5% za suhu digestiju osim za Cd i Pb za koje su se vrijednosti kretale između 67,4 i 87,9%. Predstavnici roda *Thymus* prisutni u flori Republike Makedonije sadrže slijedeće elemente u tragovima: Cu > Cr > Ni > Pb > Co > Cd. Značajne varijacije u sadržaju nekih elemenata vjerojatno su posljedica perioda sakupljanja biljnog materijala, njegovog porijekla kao i razlika između specija uključenih u ispitivanje. Sadržaj elemenata u tragovima ovisi o klimatskim, ekološkim, pedološkim i drugim faktorima.

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