APPLICATION NOTES

DETERMINATION OF CADMIUM IN SPECIES OF GENUS *THYMUS (LAMIACEAE)* BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Abstract

A new method for determination of cadmium in plants, particularly in species of genus *Thymus (Lamiaceae)* using electrothermal atomic absorption spectrometry, has been established. The influence of the matrix present in the samples on the absorbance of cadmium was examined. In the samples with higher potassium concentration correction for cadmium was applied. In the analysed samples of genus *Thymus* cadmium ranges from 0.07 to 0.42 μg/g with some exceptions. High values of cadmium in some samples indicate air pollution originating from the existing industrial activity in this area.

Keywords: *Trace elements, Cadmium determination, Atomic absorption spectrometry, Thymus*

Introduction

Small quantities of cadmium occur in air, water, soil, and food. For most people, food is the primary source of cadmium exposure, since edible plants and animals tend to take up and retain cadmium. The largest source of cadmium is the burning of fossil fuels (such as coal or oil) or the incineration of refuse materials. Cadmium may also be emitted into the air from zinc, lead, or copper smelters. Smoking is another important source of cadmium [1]. Most people who smoke have significantly higher cadmium content than nonsmokers [2-8].

High values of cadmium in some samples indicate air pollution originating from the existing industrial activity in this area [9] and the analysis of cadmium concentration in plants presents useful toxicological monitoring [10].

Some species of genus *Thymus* have been used for many years in Macedonian folk medicine in the form of a tea for therapeutical purposes for both children and adults. Therefore, it is of great importance to determine the mineral content of thyme, especially the heavy metal concentrations, considering their harmful effect on human health. Continuing our investigations on the determination of some macroelements [11], trace elements [12] in species of genus *Thymus*, and their water extracts [13] by atomic absorption spectrometry, the aim of the present work was to determine cadmium in genus *Thymus* species by electrothermal atomic absorption spectrometry (ETAAS).

Today there are a number of techniques available for determination of trace metals in plant materials. The most frequently used techniques are based on ETAAS [14-21]. In this work special attention is paid to the establishing of a new method for determination of cadmium in this plant material.

Table 1. Optimal conditions for Cd determination by ETAAS (deuterium background correction)

Wavelength	228.8 nm
Spectral width slit	0.7 nm
Lamp current	4 mA
Calibration mode	Peak area
Gas	Argon

Parameter	Drying	Charring	Atomization	Cleaning
Temperature (°C)	100	300	1800	2700
Hold time (s)	20	25	5	3
Ramp time (s)	2	1	0	0

Experimental

Samples

During the summer of 1994 and of 1995, 30 samples of species of genus *Thymus* were collected, from different areas of Macedonia. The samples were air-dried, and then milled on agate mill.

Apparatus

A Perkin Elmer Model 703 atomic absorption spectrometer equipped with a deuterium background corrector and HGA-400 graphite furnace was used. Argon was used as purge gas. A pyrolitically graphite tube with L'vov platform was used without using modifiers. Optimal conditions for Cd determinations by ETAAS (Table 1) were established by extensive testing. Inductively coupled plasma–atomic emission spectrometric (ICP–AES) measurements were performed by Varian spectrometer Model Liberty 110.

Reagents

All reagents were of analytical grade. A stock standard solution of Cd with concentration of 1 g/dm^3 (Merck, Darmstadt, Germany) was used. Standard solutions with lower concentrations were prepared by diluting with redistilled water.

Procedures

Dry ashing

Milled drug (5 g) was transferred into a porcelain crucible and heated on a hot plate for 3 h, then in a muffle furnace at 150°C for 30 min and finally, at 500°C for 8 h. After cooling at room temperature, the obtained mineral residue was dissolved in 100 cm^3 of 4% (w/w) solution of HNO_3.

Wet ashing

Milled drug (5 g) was transferred into a small Erlenmeyer flask, then 30 cm^3 of concentrated HNO_3 was poured. The sample was covered with watch glass and heated in a boiling water bath for 8 h. Then, the heating was continued on a hot plate until almost dry. The obtained mineral residue was dissolved in 100 cm^3 4% (w/w) solution of HNO_3.

Results and Discussion

During the process of Cd determination, the most important phase is the mineralization of the organic substances. Both dry and wet ashing were applied, since there is a possibility of obtaining different results. For a complete dry mineralization procedure about twelve hours were necessary, whereas for the wet ashing it took two days. Since a homogeneous state of the plant material was needed, an agate mill was used as to avoid contamination. The results for the sample of *Thymus moesiacus* (Popova Sapka, 1994), obtained according to the proposed procedures, are given in Table 2.

Since satisfactory values for relative standard deviations (RSD) were obtained by applying either procedure, the next step in our examination was to check the proposed procedures by the method of standard additions for one of the samples of genus *Thymus* (*Thymus moesiacus*, Popova Sapka, 1994) (Table 3).

Values of recovery for cadmium ranged from 93.8 to 95.5% for wet ashing and from 73.3 to 87.9% for dry ashing. Low values of recovery for Cd determined by dry ashing were due to the high

Table 2. Content of Cd in species of *Thymus* genus (*Thymus moesiacus*, Popova Sapka, 1994) by ETAAS, including dry and wet ashing for mineralization of samples (n = 5)

Element	Dry ashing		Wet ashing	
	Content (μg/g)	RSD (%)	Content (μg/g)	RSD (%)
Cd	0.082	2.68	0.120	5.89

Table 3. Results of Cd determination in the species of *Thymus* genus (*Thymus moesiacus*, Popova Sapka, 1994) by the standard additions method (n = 5)

№	Added (μg/g)	Calculated (μg/g)	Determined (μg/g)	R (%)
1	–	–	0.08	–
2	0.1	0.18	0.16	87.9
3	0.2	0.28	0.22	78.0
4	0.3	0.38	0.28	73.3
1'	–	–	0.12	–
2'	0.1	0.22	0.21	95.5
3'	0.2	0.32	0.30	93.8

1, 2, 3 and 4 – dry ashing
1', 2' and 3' – wet ashing

temperature (Cd is volatile at temperature greater than 450°C), needed for the mineralization of the matrix. This shows that dry ashing is inadequate for Cd determination. Consequently, all further determination of cadmium in the examined samples has been performed by wet ashing.

From our previously reported data, higher concentrations of K, Ca and Mg were determined [11]. Therefore, the interferences of these elements in cadmium determination were investigated. Series of solutions with the same concentration of Cd and different concentrations of interfering elements were prepared so that the concentration of these elements was similar to the concentrations in the sample solutions. It was found that in case of higher concentration of potassium the value of cadmium absorbance decreased. To determine the influence of potassium on cadmium absorbance, several solutions with a constant mass concentration of cadmium (0.02 μg/cm^3) and mass concentration of potassium ranging from 100 to 750 μg/cm^3 were analyzed. It is evident from Fig. 1 that cadmium absorbance is in linear relationship with the potassium content in the sample:

$$\gamma_{Cd}^{corrected}/\gamma_{Cd}^{found} = 0.000972\ \gamma_K + 0.890786$$

It follows that the correct value of Cd concentration can be calculated using:

$$\gamma_{Cd}^{corrected} = (0.000972\ \gamma_K + 0.890786)\ \gamma_{Cd}^{found}$$

Using this procedure, Cd was determined in thirty different samples of various species of genus *Thymus* in different areas in the Republic of Macedonia. The results of this examination are given in Table 4. Cadmium was found in the range from 0.08 to 0.42 μg/g, with the exception of one sample containing 1.20 μg/g.

The results obtained by the proposed procedure are similar to those obtained by ICP–AES method (Table 4).

There are different approaches to the process of mineralization of plant samples, though dry [22-24] and wet ashing [25-27] are generally applied. Dry ashing techniques have been used in this work to decompose plant samples, but these have limitations since they involve strict temperature control as Cd is volatile at temperatures greater than 450°C. Wet mineralization using different acid mixtures [25-27] is better than dry mineralization, which requires longer time to carry out and risks loss of the element being determined. To avoid contamination of the treated solution, using wet ashing, mineralization with HNO_3 is applied in our investigations, getting dry and completely dissolving in HNO_3 (4% w/w) residue.

Matrix interferences of some elements (Na, K, Mg, Al, etc.), or salts with higher concentration (Al^{3+}, Ca^{2+}, SO_4^{2-}, F^-, etc.) on the cadmium absorbance during the plant material analyses, have been investigated [28-30] and no significant interferences were found. In our investigation, potassium was found to have an influence on cadmium absorbance in the examined samples. For that reason, a correction factor for determination of the real value of Cd was established.

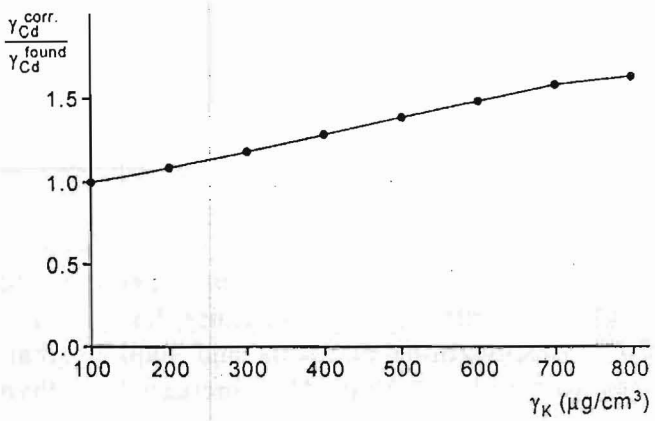

Fig. 1. Dependence of the ratio of corrected and found concentration of Cd on the potassium concentration

Table 4. The content of Cd in species of *Thymus* genus determined by ETAAS (n = 5) and by ICP-AES

№	Samples	AAS		ICP-AES
		Cd (μg/g)	RSD (%)	Cd (μg/g)
	Thymus tosevii Velen. Ssp. *tosevii* var. *tosevii*			
1	v. Nikolic, 1994	0.20	0.06	0.18
2	v. Laki, 1994	0.10	8.68	0.12
3	Vitacevo, 1995	0.30	5.99	0.33
4	v. Tajmiste, 1995	0.15	0.19	–
5	v. Majdan, 1995	0.15	3.46	0.15
6	v. Rajko Zinzifov, 1995	1.20	0.48	–
	Thymus tosevii Velen. ssp. *tosevii* var. *longifrons* Ronn.			
7	Karadzica, 1994	0.32	8.08	0.31
	Thymus tosevii Velen. ssp. *substriatus* (Borb) Matevski			
8	v. Nikolic, 1994	0.08	8.11	0.078
9	Vitacevo, 1994	0.19	2.26	0.21
10	Vitacevo 1995	0.17	0.92	–
	Thymus tosevii Velen. ssp *tosevii* var. *degenii* (H. Br.)			
11	Ronn			
12	Preslap, 1994	0.39	1.24	0.36
13	Lazaropole, 1995	0.21	1.94	–
	Vrben, 1995	0.23	1.80	0.25
	Thymus alsharensis Ronn.			
14	v. Majdan, 1994	0.22	3.93	–
15	v. Majdan, 1995	0.13	6.19	–
16	v. Majdan, 1995	0.15	8.60	–
	Thymus longidens Velen. Var. *lanicaulis* Ronn.			
17	v. Sonje, 1994	0.33	1.52	0.29
18	v. Banjani, 1994	0.21	2.34	0.21
19	v. Banjani, 1995	0.26	6.82	–
	Thymus longidens Velen. *var. dessareticus* Ronn.			
20	Karadzica, 1994	0.32	1.54	0.30
21	Karadzica, 1995	0.18	8.24	–
	Thymus ciliatopubescens			
22	v. Sonje, 1994	0.42	2.11	–
	Thymus macedonicus (Degen et Urumov) Ronn.			
23	v. Laki (in bloom), 1994	0.10	7.20	–
24	v. Laki (before bloom), 1994	0.16	2.64	–
25	v. Laki (in bloom), 1995	0.09	6.50	–
	Thymus moesiacus Velen.			
26	Popova Sapka, 1994	0.12	5.89	–
27	Bistra, 1994	0.34	1.48	–
28	Popova Sapka, 1995	0.10	5.71	–
29	Bistra, 1995	0.28	4.56	–
	Thymus albanus H. Braun			
30	Popova Sapka, 1995	0.33	7.85	–

v. – village

The presence of Cd in of genus *Thymus* species indicates some contamination. The concentration of Cd in uncontaminated plants and food stuffs ranges from 0.01 to 1.0 μg/g [17]. Increased concentration might originate from the air and earth contamination [9]. It is of great importance that Cd concentration should be checked since thyme is used in folk medicine, pharmacy and as a

spice. The content of Cd in all the samples we examined was within these limits. The only exception was *Thymus tosevii ssp. tosevii* collected in 1995 in the area Rajko Zinzifov near Veles (where the lead and zinc smelting plant is situated) with the Cd concentration of 1.20 μg/g.

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