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Trace Heavy Metal Levels in Microwave Digested Honey Samples from Middle Anatolia, Turkey

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ABSTRACT

The concentrations of trace heavy metals (Pb, Cd, Fe, Cu, Mn and Zn) in 15 different honey samples collected from 15 different farms in Middle Anatolia, Turkey, were determined by flame and graphite furnace atomic absorption spectrometry after microwave digestion. The accuracy of the method was corrected by standard reference material (NIST-SRM 1515 Apple leaves). The contents of trace metals in honey samples were found to be in the range of 1.0~5.2 μg/g, 0.25~1.10 μg/g, 0.18~1.21 μg/g, 1.1~24.2 μg/g, 17.6~32.1 μg/kg and 10.9~21.2 μg/kg for Fe, Cu, Mn, Zn, Pb and Cd, respectively. Results obtained are in agreement with data reported in the literature.

Key words: trace metals, honey, microwave digestion, atomic absorption spectrometry, middle Anatolia-Turkey

INTRODUCTION

Trace metals are important in daily diets, because of their essential nutritious value and possible harmful effects. Metals like iron, copper, zinc and manganese are essential metals since they play an important role in biological systems; whereas lead and cadmium, etc. are non-essential metals which can be toxic even in trace amounts[(1-5)]. The essential metals can also have harmful effects when their intakes exceed the recommended quantities significantly. Since food is one of the main source of heavy metal ions for human[(6-9)], the analysis of food samples for trace heavy metal contents have been continuously performed[(7-10)].

Honey is an important food for the human nutrition. Honey possesses valuable nourishing, healing and prophylactic properties[(11)] which result from its chemical composition. As food stuff used for healing purposes, honey must be free of objectionable contents. It should contain only small amounts of pollutants, such as trace metals. Analysis of honey for trace elements content is necessary in food quality control[(12)].

The climate and rich vegetation in Turkey provide a very suitable environment for apiculture which is in a state of expansion. Turkey is the third largest country with 3,686,000 hives in 1993, following Russia and USA[(11)]. The production of honey was 59.207 tons in 1995 and increased to 80.000 tons in 1997[(12,13)]. Recently, both international and Turkish studies have drawn attention to the occurrence of the metal contents of honey[(14-27)]. The middle Anatolia region of Turkey is ideal for apiculture due to its rainy season in spring and autumn. The common honeybee races are *Apis mellifera cacucasia*, *Apis mellifera anatilica* and their hybrid *Apis mellifera cacucasia gorb*. in middle Anatolia region. According to our survey, studies have not been carried out dealing with this subject matter in middle Anatolia-Turkey.

In this study, the contents of trace metals in honey samples collected from 15 farms in middle Anatolia, Turkey were determined by flame and graphite furnace atomic absorption spectrometry after microwave digestion.

MATERIALS AND METHODS

I. *Apparatus*

A Perkin Elmer AAnalyst 700 atomic absorption spectrometer (FAAS) equipped with HGA graphite furnace and a deuterium background corrector was used in the experiments. For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used. For graphite furnace measurements, argon was used as inert gas. The operating parameters for the working elements were set as recommended by the manufacturer (Table 1). Pyrolytic-coated graphite tubes (Perkin Elmer part no. B3 001264) with a platform were used. Samples were injected into the graphite furnace using a Perkin Elmer AS-800 auto sampler.

A Milestone Ethos D closed vessel microwave digestion system (maximum pressure 1450 psi, maximum temperature 300°C) was used. Teflon reaction vessels were used in all the digestion procedures. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion.

II. *Reagents*

All reagents were of analytical reagent grade unless

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Table 1. Instrumental analytical conditions of element analyses

FAAS					
Element	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Fe	2.0	17.0	248.3	0.2	30
Zn	2.0	17.0	213.9	0.7	15
GFAAS					
Instrumental conditions	Pb	Cd	Mn	Cu	
Wavelength (nm)	283.3	228.8	279.5	324.8	
Slit width (nm)	0.7	0.7	0.2	0.7	
Lamp current (mA)	30	4	20	15	
Argon flow (mL/min)	250	250	250	250	
Sample volume (μL)	20	20	20	20	
Modifier (μL)	5	10	5	5	
	Heating program temperature (°C)				
Drying 1	100 (5, 20)[a]	100 (5, 20)	100 (5, 20)	100 (5, 20)	
Drying 2	140 (15, 15)	140 (15, 15)	140 (15, 15)	140 (15, 15)	
Ashing	700 (10, 20)	850 (10, 20)	1200 (10, 20)	1000 (10, 20)	
Atomization	1800 (0, 5)	1650 (0, 5)	2300 (0, 5)	2300 (0, 5)	
Cleaning	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)	2600 (1, 3)	

[a](ramp time, hold time (sec)).

otherwise stated. Double distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. HNO_3 and H_2O_2 were of suprapure quality (E. Merck, Darmstadt). All the plastic and glassware were cleaned by soaking in diluted HNO_3 (1+9) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element supplied by Sigma.

III. *Sampling*

Fifteen different honey samples were collected from 15 different farms in villages around middle Anatolia, Turkey in September~October 2004. One sample was taken in each sampling point. The samples were preserved in covered plastic containers and kept at 4~5°C until analysis.

IV. *Microwave Digestion*

Microwave digestion procedure was applied for honey samples. One gram of each sample was digested with 6 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) in a microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W and 8 min for 550 W, vent: 8 min, respectively.

V. *Analytical Procedure*

Detection limit is defined as the concentration corresponding to three times the standard deviation of 10 blanks. Detection limit values of elements as milligram per liter in flame AAS were found to be 0.003 for Zn and 0.007 for Fe. Pb, Cd, Cu and Mn were below detection limit of flame AAS. Lead, cadmium, copper and mangane were determined using graphite furnace AAS. During analyses, internal argon flow rate through the graphite tube was 250 mL/min; gas flow was interrupted during atomization. Sample volume, ramp and hold times for the drying, ashing, atomization and cleaning temperatures were optimized before analysis to obtain the maximum absorbance and the minimum background.

Matrix modifiers were added 200 μg $NH_4H_2PO_4$ for Pb, 15 μg Pd + 10 μg $Mg(NO_3)_2$ for Cd and 50 μg $Mg(NO_3)_2$ for Mn and Cu. Most of the matrix was removed before the atomization step and less interference occurred during atomization. Each graphite furnace atomic absorption spectroscopic analysis calls for 20 μL of solution, 5~10 μL of the matrix modifier was added if necessary. The signals were measured as peak area. The absolute sensitivity is defined by the mass of an element, which gives a peak absorbance of 0.0044; it was found 10 pg for Pb, 0.5 pg for Cd, 2.0 pg for Mn and 4.0 pg for Cu.

RESULTS AND DISCUSSION

The accuracy of the microwave digestion method was checked by standard reference material (NIST-SRM 1515 Apple leaves). The results are given in Table 2. There are a good harmony between the certified and our values for analyte ions. Also recovery tests for the analyte ions were also performed by a microwave digested honey sample. The results for this work are summarized in Table 3. It can be seen that the recovery of investigated trace metals by the microwave digestion method was quantitative.

According to light of these results, the concentration

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Table 2. Trace metal contents of apple leaves (NIST SRM 1515) reference material as μg/g, n = 4

Element	Certified value	Our value[a]	Recovery (%)
Cu	5.64	5.47 ± 0.21	97
Mn	54	51.3 ± 3.4	95
Zn	12.5	12.3 ± 0.7	98
Fe	(83)[b]	79.7 ± 4.9	96
Pb	0.47	0.45 ± 0.03	96
Cd	0.013	0.012 ± 0.001	95

[a]Data presented as mean ± standard deviation.
[b]The value in the parenthesis is not certified.

Table 3. Recovery of trace metals from spiked Yozgat Yudan honey using present method, n = 3

Element	Added[a]	Found[a]	Recovery (%)
Fe	—	2.1 ± 0.1	—
	1.0	3.0 ± 0.1	97
	2.0	3.9 ± 0.2	95
	4.0	5.8 ± 0.3	95
Mn	—	0.52 ± 0.05	—
	0.25	0.74 ± 0.06	96
	0.50	0.98 ± 0.07	96
	1.00	1.45 ± 0.08	95
Zn	—	1.3 ± 0.1	—
	0.5	1.8 ± 0.1	100
	1.0	2.2 ± 0.1	96
	2.0	3.2 ± 0.2	97
Cu	—	0.25 ± 0.02	—
	0.10	0.34 ± 0.03	97
	0.25	0.48 ± 0.02	96
	0.50	0.73 ± 0.05	97
Cd	—	11.8 ± 0.9	—
	5.0	16.0 ± 0.7	95
	10.0	21.1 ± 0.8	97
	20.0	30.2 ± 1.2	95
Pb	—	25.4 ± 2.1	—
	10.0	34.8 ± 2.6	98
	25.0	48.5 ± 3.2	96
	50.0	72.6 ± 4.3	96

[a]Pb and Cd (μg/L); others (μg/mL).

of analytes in the honey samples from middle Anatolia have been analyzed by atomic absorption spectrometry after microwave digestion. The results, which were repeated four times, are given in Table 4. All metal concentrations were determined on a wet weight basis. According to these data, zinc has the highest concentration and followed by iron, manganese, copper, lead and cadmium. Metal concentrations in the honey samples analyzed were not different from values reported earlier in Turkey[17-19].

The lower and higher iron concentrations were found as 1.0 μg/g in honey sample from Yozgat Sorgun Kulhuyuk and 5.2 μg/g in honey sample from Yozgat Kale Koyu, respectively. Iron values in honey samples have been reported in the range of 0.40~52.51 μg/g[14], 3.45~8.94 μg/g[17], 0.97~1.91 μg/g[28], respectively. Data reported for iron of honey samples from Canary Islands[14] are generally higher than those reported for middle Anatolia. On the other hand, the levels of iron in hones from Black sea region-Turkey[17] and from Saudi Arabia[28] are generally at the same level of our samples.

The lower manganese level was found 0.18 μg/g in honey sample from Yozgat Gevrek. The higher manganese level was found as 1.21 μg/g in honey sample from Yozgat Kale Koyu. The reported some manganese values in the literature for honey were 0.32~1.70 μg/g[17], 0.9~10.2 μg/g[29], respectively. Manganese values found in the present study are in agreement with the manganese levels of honey samples from Black sea region-Turkey[17] and Ireland[29].

Zinc is also an important element for human. Zinc values in honey samples have been reported in the range of 0.18~19.1 μg/g[14], 1.15~4.95 μg/g[17], 1.6~22.5 μg/g[29], respectively. The lower zinc content was found 1.1 μg/g in Yozgat Sorgun honey (Table 4). The higher zinc content was found 24.2 μg/g in honey sample from Yozgat Recepli. The range of zinc for middle Anatolia region is similar to the range of zinc for honey samples from Canary Islands[14]. The zinc levels of our samples are higher than that reported by Tuzen[11,14] for honey samples from the Black sea region

Table 4. Trace metal concentrations[a] in analyzed honey samples, n = 4

Honey samples	Fe	Mn	Zn	Cu	Cd	Pb
Yozgat Yudan	2.1 ± 0.1[b]	0.52 ± 0.05	1.3 ± 0.1	0.25 ± 0.02	11.8 ± 0.9	25.4 ± 2.1
Yozgat Recepli	4.9 ± 0.3	0.34 ± 0.02	24.2 ± 1.9	0.28 ± 0.02	14.1 ± 1.1	19.5 ± 1.3
Yozgat Gelingullu Koyu	2.4 ± 0.2	0.26 ± 0.03	1.2 ± 0.1	0.30 ± 0.03	17.3 ± 1.4	30.1 ± 2.5
Yozgat Sorgun Yazitaş	4.6 ± 0.4	0.25 ± 0.02	1.2 ± 0.1	0.27 ± 0.02	20.1 ± 1.7	25.2 ± 2.2
Yozgat Bacili Koyu	4.9 ± 2.8	0.43 ± 0.05	1.2 ± 0.1	0.32 ± 0.03	12.4 ± 1.1	27.7 ± 2.5
Yozgat Sorgun Dişli	1.8 ± 0.1	0.25 ± 0.02	9.3 ± 0.7	0.33 ± 0.02	10.9 ± 0.9	23.4 ± 2.2
Yozgat Sorgun	3.9 ± 0.3	0.25 ± 0.03	1.1 ± 0.1	0.26 ± 0.02	14.5 ± 1.2	19.5 ± 1.4
Yozgat Çandir	1.1 ± 0.1	0.78 ± 0.05	6.7 ± 0.5	0.36 ± 0.03	17.3 ± 1.5	30.5 ± 2.6
Yozgat Kale Koyu	5.2 ± 0.5	1.21 ± 0.10	4.2 ± 0.3	0.54 ± 0.05	21.2 ± 1.8	32.1 ± 2.7
Yozgat Sorgun Kulhuyuk	1.0 ± 0.1	0.25 ± 0.02	23.9 ± 2.2	0.30 ± 0.03	12.5 ± 1.1	26.7 ± 2.1
Sivas Zara	4.3 ± 0.3	0.26 ± 0.03	2.2 ± 0.2	0.33 ± 0.03	17.3 ± 1.4	17.6 ± 0.9
Yozgat Alci Koyu	1.8 ± 0.2	0.24 ± 0.02	12.9 ± 1.1	0.27 ± 0.02	15.6 ± 1.2	28.2 ± 2.4
Yozgat	3.9 ± 0.4	0.86 ± 0.07	9.7 ± 0.8	1.10 ± 0.10	19.8 ± 1.4	30.1 ± 2.3
Yozgat Sorgun Sahmuratli	1.1 ± 0.1	0.25 ± 0.03	1.8 ± 0.1	0.40 ± 0.04	20.6 ± 1.6	19.6 ± 1.4
Yozgat Gevrek	4.9 ± 0.4	0.18 ± 0.02	1.7 ± 0.1	0.45 ± 0.04	14.9 ± 1.1	23.5 ± 2.1

[a]Pb and Cd (μg/kg); others (μg/g).
[b]Data presented as mean ± standard deviation.

of Turkey[11,14].

The minimum and maximum copper levels observed were 0.25 μg/g in honey sample from Yozgat Yudan and 1.10 μg/g in Yozgat, respectively. Copper values in the literature have been reported as 0.25~1.30 μg/g[17] for honey samples from Black sea-Turkey, 1.8 μg/g[18] for samples from south-eastern Anatolia of Turkey, 0.31 μg/g[30] for Lazio region (central Italy) honeys, respectively. The values for the copper contents of our samples were generally are at the same level of the literature values[17,18,28,30].

The honey samples contained insignificant amounts of lead and cadmium. Therefore, it is appropriate to analyze concentrations of these metals in honeys to test the contamination of the environment by trace heavy metals[15]. The lower lead content was found as 17.6 μg/kg in Sivas Zara honey. The higher lead content was found as 32.1 μg/kg in honey sample from Yozgat Kale Koyu. Lead contents of some honey samples around the world have been reported as 48 μg/kg[15], 30.3~58.6 μg/kg[17], 3.3~45.0 μg/kg[31] and 30~240 μg/kg[28], respectively. Lead data of honey samples from Saudi Arabia is much higher than that of our samples.

The lower and higher cadmium concentrations were found as 10.9 μg/kg in Yozgat Sorgun Dişli honey and 21.2 μg/kg in Yozgat Kale Koyu honey, respectively. Cadmium contents of honey samples in the literature have been reported as 15 μg/kg[15], 5.23~9.82 μg/kg[17], 8 μg/kg[28], <2~63.0 μg/kg[31], respectively. The level of cadmium of our samples was higher than some of the previous data[15,17,28,32].

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