Metal Contents in Honey Samples from Different Regions in Ankara

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ABSTRACT

In this study, metal concentrations (Cd, Co, Cu, Fe, Mn) of 100 different honey samples, collected from 5 different districts of Ankara (Kalecik, Kazan, Kızılcahamam, Central county, Ayas), were investigated. Honey samples were collected in July and August 2015. These metals are important in terms of public health and are researched primarily in such studies. Analyses were performed with ICP-OES instrument. The instrument was calibrated using the main stock solution which contained Cd, Co, Cu, Fe and Mn elements. Specimen collection was carried out in July-August when honey harvesting was most intensive. Cd, Co, Cu, Fe, and Mn metal analyses were performed on all honey samples. In general, evaluation based on Cd and Co values, results were determined as <LOD (limit of detection) in each district of Ankara. The highest results for Fe were found in Kızılcahamam. Respectively, the highest values for Cu, Fe, and Mn were found in the central district of Ankara, in Kızılcahamam and in Kazan. The contents of metals in honey samples were found to fall into the mean values for Cd and Co where <LOD and the mean values of Cu was 0.746±3.601 µg/g, Fe was 63.310±23.279 µg/g and Mn was 8.579±6.890 µg/g. According to our data, the levels of Cd and Co were found beloved the maximum residue level (MRL) of European Union (EU). Other metal levels were in the acceptable levels according to literature data. Honey samples were of good quality in terms of metal contents.

Keywords: Ankara; Honey; ICP- OES; Pollution; Metals.

INTRODUCTION

Honey, obtained by the honeybee (*Apis mellifera*) from nectar plants, with different composition according to floral sources, regional and climatic conditions is widely consumed for its nutritional value and various properties. It can be considered as a bioindicator of environmental pollution to assess the presence of metals, and pesticides. Honey is a significant source of vitamins and micro- and macro-elements essential for human health (1). It is used as food and an important ingredient in different kinds of manufactured foods (1, 2). Nowadays, its importance is not only nutritional, but it is also an indicator of environmental pollution (3, 4). Bees fly intensively in a radius of up to 3 km; for this reason, they and their products can serve as bio-indicators for the contamination of an area (5). A number of authors have utilized honey bees and/or their product for monitoring environmental pollution (4, 6, 7, 8, 9, 10, 11, 12). Air and water contain heavy metals from industry and traffic which can also contaminate the bee colonies and their products (13, 14). Due to the high transportation density, aluminum, calcium, copper, iron, lead, magnesium, silicon, zinc, barium, cadmium, chromium, nickel, palladium, platinum, and many other harmful metals are emitted as air pollutants (9).

Beekeeping is considered one of the most important agricultural activities around the world. Today the total number of bees in the world is estimated to be about 89 million, which produce about 1.5 million tons of honey (1). Turkey has an important place among the honey producer countries, it is placed 3rd for beehives and 2nd position among the honey producing countries in the world. In Turkey, there were

about 8 million beehives producing about 115,000 tons of honey in 2018 according to TUIK Reports (1, 15). Although all regions of Turkey are suitable for apiculture, the Aegean, Black Sea, and Mediterranean regions are considered to be the most important (15). Also Ankara, the capital city of Turkey, is considered most important for apiculture.

The aim of this study was to evaluate some metal (Cd, Co, Cu, Fe, Mn) contents of honey, collected from different regions (Kazan, Kızılcahamam, Kalecik, Ayas, Central) of Ankara, the capital city of Turkey.

MATERIALS AND METHODS

Samples

From five different regions of Ankara (Figure 1); Kalecik (20 samples), Kazan (20 samples), Kızılcahamam (20 samples), Central region (20 samples) and Ayas (20 samples); totalling one hundred natural liquid samples of honey (each 25 g) were collected from different beekeepers in July and August of 2015. Honey samples were taken into sample containers, the contact between air and honey were prevented by tightly securing the covers of the sample containers. The samples were stored in dark, below room temperature in 21-22 °C until the analyses were carried out.

Metal analysis

The process of extraction was carried out according to the method advised by AOAC (Association of Official Analysis Chemistry) (1984) (16), Stahr (1977) (17) and measurements were performed by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectroblue, Germany). According to this, 20 honey samples were taken from each sampling field and transferred into the sterilized tubes in order to prevent crystallization. The samples were placed into the water tank (NÜVE ST 30, Turkey) which was 70°C average for homogenization of the honey samples. Half a gram was taken from these samples, then 9 ml of (HNO_3) 65% nitric acid and 1 ml 30% hydrogen peroxide were added. The samples were burned in a microwave device (CEM MARS 6 System 240/50, USA). The working conditions of the microwave device are given in Table 1. The burned samples were subtilized with 5 ml of pure water. The blank (negative control; used for recovery studies) samples were prepared with the same process.

The main stock solution (1000 mg/L) was prepared

Table 1: The working conditions of ICP-OES device.

Parameters	Value
Plasma Power	1430 W
Pump Speed	30 rpm
Coolant Flow	13 L/min
Auxilory Flow	0,80 L/min
Nebulizer Flow	0,70 L/min
Number of replicates	3
Integration time (s)	3 s
Sample uptake rate (µL/min) (speed)	0.3 rps

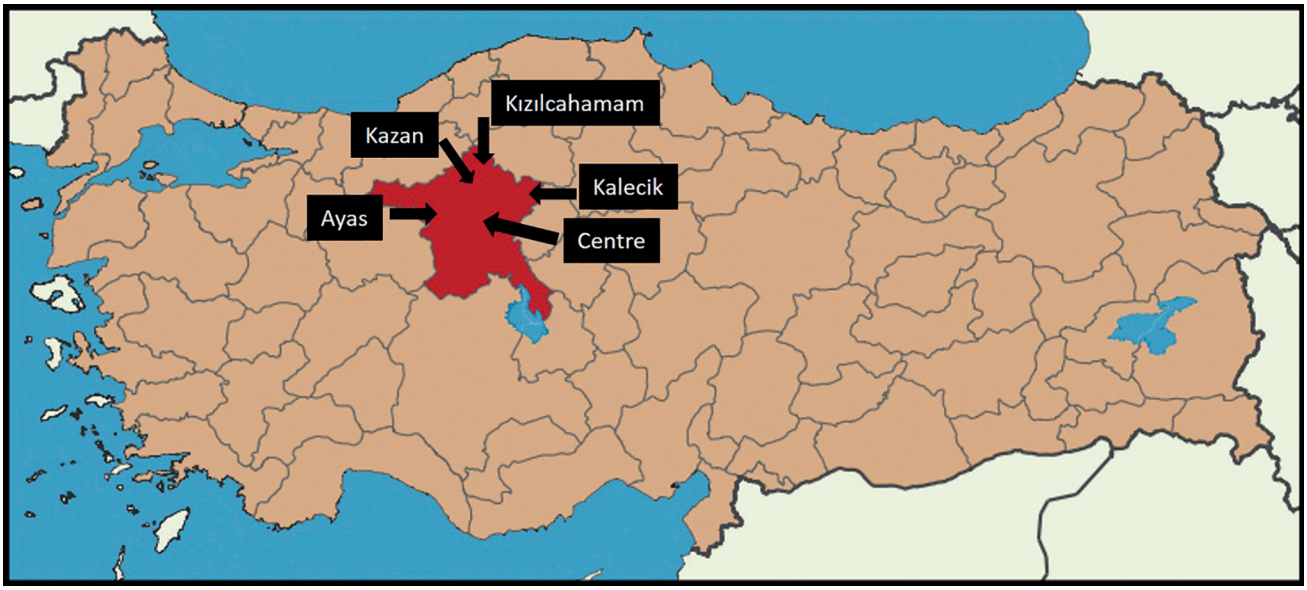

Figure 1: Samples collection areas; Kalecik, Kazan, Kızılcahamam, Ayas, and Central county.

from ICP Multi-Element standard Solution included Cd, Co, Cu, Fe and Mn which were diluted with citric acid. Calibration standards were prepared from this main stock solution as 0, 125, 250, 500, 1000, 2500, 5000 µg/L for Fe and for the others as 0, 10, 20, 50, 100, 200, 500, 1000 µg/L. The metal levels in honey were carried out by ICP-OES device in Kırıkkale University Centre for Scientific and Technological Researches and Applications. The device was calibrated with prepared calibration standards. The working conditions and results of the ICP-OES Device were given on Table 2.

According to the analyses carried out, the limits of detection for the elements Cd, Co, Cu, Fe and Mn were 0.0366 ppb; 0.289 ppb; 0.51 ppb, 0.65 ppb and 0.375 ppb, respectively.

Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA). When significant treatment effects were detected, Duncan's multiple range test was used to identify specific differences between the regional means at a probability level $p<0.05$.

RESULTS

Mean metal contents with their standard deviations and ranges are shown in Table 3. The mean and standard deviation of metal contents of 100 honey samples for Cu, Fe, and Mn were 0.746±3.601 µg/g, 63.310±23.279 µg/g, 8.579±6.890 µg/g, respectively. Cd and Co determined as <LOD levels for all samples. Iron, and manganese contents showed statistically significant differences ($p<0.05$) between regions of origin of the honey samples.

Cd and Co mean values were determined as <LOD in all samples. The mean value of Cu was found to be 0.746 µg/g; the lowest Cu value was <LOD in Kalecik and Ayas, the highest Cu value was determined as 25.27 µg/g in the Central county of Ankara. The lowest Cu mean value was found in Kalecik and Ayas with the <LOD, the highest Cu mean value was found in the Central district with 2.656 µg/g.

The mean value of Fe was found 63.310 µg/g; the lowest Fe value was determined in Ayas as of 25.12 µg/g and the highest Fe value was determined in Kızılcahamam as 125.94 µg/g. The lowest Fe mean value was found in Ayas with 25.12 µg/g and the highest Fe average value was found in Kızılcahamam district with 94.567 µg/g.

Table 2: ICP-OES instrument conditions and results.

Display Name	BEC (ppb)	LOD (ppb)	LOQ	Std. Error (ppb)	Corr. Coef	Range (ppb)	Recovery (%)
Cd 214.438	5.5	0.036	0,118	5.12	0.99991	0.036 - 1.2e+003 ppb	81.2
Co 228.616	15.0	0.289	0,953	4.71	0.99992	0,289 - 1.2e+003 ppb	86.5
Cu 324.754	11.8	0.510	1,683	5.44	0.99990	0.510 - 1.2e+003 ppb	84.0
Fe 259.941	11.5	0.650	2,145	11.00	0.99998	0.650 - 6e+003 ppb	96.4
Mn 257.611	2.5	0.375	1,2375	4.77	0.99992	0.375 - 1.2e+003 ppb	95.2

Table 3: Metal contents in honey samples according to regions (as µg/g; mean±standart deviation and range).

Region	Cd	Co	Cu	Fe	Mn
Kalecik ***(n=20)***	<LOD	<LOD	<LOD	45,476±11,903[a] (29,38-67,60)	1,873±0,667[a] (0,90-3,00)
Kazan ***(n=20)***	<LOD	<LOD	0,641±2,707 (0-12,13)	76,062±13,847[b] (62,07-114,26)	17,1705±0,57[b] (16,19-18,24)
Kızılcahamam ***(n=20)***	<LOD	<LOD	0,435±1,076 (0-3,86)	94,567±10,054[c] (80,97-125,94)	15,051±1,005[c] (13,37-16,64)
Central ***(n=20)***	<LOD	<LOD	2,656±7,345 (0-25,27)	63,773±7,765[d] (52,47-75,81)	8,459±2,247[d] (5,86-12,13)
Ayas ***(n=20)***	<LOD	<LOD	<LOD	36,671±6,876[e] (25,12-51,88)	0,342±0,146[e] (0,17-0,77)
Total ***(n=100)***	<LOD	<LOD	0,746±3,601 (0-25,27)	63,310±23,279 (25,12-125,94)	8,579±6,890 (0,17-18,24)

[a b c d e]: Means within in the same columns with different letters are statistically significant ($p<0.05$).

The mean value of Mn was found 8.579 µg/g; the lowest Mn value was determined in Ayas as of 0.17 µg/g and the highest Mn value was determined in Kazan as of 18.24 µg/g. The lowest average value for Mn was found in Ayas district with 0.342 µg/g and the highest Mn average value was found in Kazan district with 17.1705 µg/g.

DISCUSSION

The increase in industrial activities and the human population has caused environmental pollution. The problems due to environmental and food pollution pose a threat to public health. There can be elemental residues posing a potentially toxic level in nutrients which are essential for human and living beings. Throughout their lives, bees are constantly interacting with the environment. Thereby bees can be affected by these harmful materials. Consequently, the pollutants can be found in honey and other bee products.

There are limited studies for metal contents of honey, especially in Turkey. Also, these studies were related to the local regions and areas. Yılmaz and Yavuz (18) investigated Na, K, Ca, Mg, Cu, Fe, Mn, Zn, and Co levels for honey from different parts of south-eastern Anatolia. The mean values for Cu, Fe, Mn, Zn, and Co were 1.8 mg/kg, 6.6 mg/kg, 1.0 mg/kg, 2.7 mg/kg, and 1.0 mg/kg, respectively. The values for Fe (63,310±23,279) and Mn (8,579±6,890) of our study results were higher than south-eastern Anatolia honey; but Cu results (0,746±3,601) of our study were lower than south-eastern Anatolia honey. Tuzen (10) determined some trace metal (Pb, Cd, Fe, Cu, Mn, and Zn) contents in honey samples collected from Tokat city (Central Anatolia region) in Turkey, during the years 2000 and 2001. The author observed that Cu was 0.62 µg/g, Fe was 5.22 µg/g, Mn was 0.49 µg/g, and Zn content was 3.45 µg/g.

In our study, the metal contents for the Ankara region were 0.746 µg/g, 63.310 µg/g, 8.579 µg/g for Cu, Fe, and Mn, respectively. These results were different from Tuzen's (10) results except in the case of copper. Iron and Mn results in our study were found to be higher than Tuzen's (10) results. It is thought that these changes were caused by the fact that the honey samples were collected from different areas. Different kinds of honey are produced in different regions of Turkey peculiar to their plant flora. The element content of honey also depends on the soil which shelters the plants with the nectar, the season the plant was grown, the climate, season and environmental pollution (10, 18). The importance of determining the elemental contents of honey has been gaining importance. Thereby, it has been thought that bees and bee products can be important indicators for observation of environmental pollution (3, 9).

Sevimli *et al.* (4) studied the metal contents in honey. Samples were collected from different cities of Turkey which included Malatya, Bolu, Firuzkoy, Odemis, and Finike. Fe, Zn, Cr, and Co contents ranged as 2.1–67.0, 0.009–6.0, 0.043–1.07, and 0.019–0.073 mg/kg, respectively. In our study, Fe contents were determined as 63.310±23.279 µg/g; these findings similar to Sevimli *et al*. (4).

Silici *et al.* (19) investigated the presence of the 14 trace elements such as Cu, Cd, Pb, Co, Cr, Ni, Al, Se, Zn, Mn, Fe, K, Ca and Mg for Rhododendron and multi-flower honey obtained from Black Sea Region of Turkey. The results revealed that Rhododendron honey exhibited higher concentrations of Cu, Co, Cr, Ni, Se, Zn, Ca and Mg but lower concentrations of Al, Mn, Fe, and K than in multi-flower honey. Also the metal contents for honey samples were determined as follows; Cd; 14±1 ηg/g, Co; 89±5 ηg/g, Cu; 5.60±0.20 µg/g, Fe; 81.7±4.4 µg/g, Mn; 53.2±2.5 µg/g. Our study results different from these concentrations; Cu (0.746 µg/g), Mn (8.579 µg/g), and Fe (63.310 µg/g) contents were lower than these findings.

Yarsan *et al*. (20) investigated metal contents in honey from six different regions of Turkey: Black Sea region, Central Anatolia region, Mediterranean region, Aegean region, South-eastern Anatolia region and Eastern Anatolia region during the years 2002 and 2003. The mean and standard deviation of the ash metal contents of 45 honey samples for Al, Co, Cu, Fe, Mn, Ni, and Zn were 12.30±3.86 µg/g, 0.02±0.01 µg/g, 0.20±0.18 µg/g, 0.87±0.47 µg/g, 4.75±2.44 µg/g, 0.49±0.47 µg/g, 0.67±0.65 µg/g, and 7.92±3.0 µg/g respectively. The mean values of Cu and Fe were also lower than our study. In our study, the mean value of Mn was higher than this study.

In another study (21) the metal contents in honey samples, collected from some of the cities of East-Anatolian region in Turkey were investigated. In this study, the concentrations of metals (Pb, Cd, Cu, Fe, Mn, Zn) in 200 different honey samples collected from some of the cities of East-Anatolian Region in Turkey (Muş, Ağrı, Bitlis, and Erzurum) in July and August in 2004 (25 samples per month) were examined. Although Pb, Cd, Cu, Fe, Mn, and Zn were found

in all of the samples, no Cd was found in one sample from Erzurum and in two samples from Muş. The content of Pb and Cd did not differ significantly in the mean values of July and August. There was an increase in the Cu, Fe and Zn contents in August and a decrease in the Mn content according to July. The contents of metals in honey samples were found to be in the mean of Pb; 0.131±0.081 µg/g, Cd; 0.006±0.007 µg/g, Cu; 2.635±1.198 µg/g, Fe; 9.799±5.615 µg/g, Mn; 2.592±1.318 µg/g, Zn; 3.705±1.708 µg/g. In this study, the mean values of Cd and Co were similar with the mean values of Fe and Mn lower than our study.

There are some differences and similarities among the results. This observation can be related to various factors, such as industry, mining, emission of automobile exhaust gases and different botanical origins. However, other factors, such as geographical conditions, are also expected to affect the mineral content. Also, honey that comes into contact with metal containers or equipment during storage, processing, or shipping may have elevated levels of some metals, such as iron. The sources of some elements (Fe, Ni, Cd) were considered to be the steel or galvanized containers used in processing or storage (12, 22, 23).

Mineral contents determined in the honey of different regions of Ankara, Turkey showed differences. Mineral contents of honey were highly dependent on the type of flower from which bees take nectar (8, 11, 23). Extensive research is required to establish physicochemical properties and mineral content variations according to the geographical area. The determination of reliable levels are considered very useful for both the constant monitoring and the prevention of future problems due to the emission of heavy metals in the environment.

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