

Investigation of some metals in honey samples from West Mediterranean region of Turkey

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Abstract

Analysis of elements content in honey is important for honey quality and safety and for monitoring of environmental pollution. The levels of 22 elements, aluminum (Al), barium (Ba), calcium (Ca), cadmium (Cd), chromium (Cr), cobalt (Co), iron (Fe), copper (Cu), potassium (K), magnesium (Mg), manganese (Mn), nickel (Ni), sodium (Na), lead (Pb), strontium (Sr), silver (Ag), bismuth (Bi), gallium (Ga), indium (In), lithium (Li), thallium (Tl) and zinc (Zn), were determined in 70 samples obtained from beekeepers located in the West Mediterranean region of Turkey. Determination of elements content was carried out using ICP-OES. Chromium, Co, Cd, Ag, Bi, In and Tl were not detected in any of the tested honey samples. The most abundant metal was K which has an overall average of 764.26 mg kg⁻¹. Higher concentrations of Pb, Ni, Mg, Na, K and Mn were found in the samples obtained from Burdur compared to other provinces. The levels of Cu were statistically lower in Antalya in comparison to other regions. No significant differences were observed in Al, Zn, Fe, Sr, Ba, Ca and Ga levels between regions. The differences in the chemistry of honey samples collected from different regions may be due to geochemical soil composition and geographical differences. Their levels were below to the European limits and the honeys are safe for human consumption.

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Introduction

Honey produced by honeybees (*Apis mellifera*) has been used for different purposes for thousands of years. Uses of honey and its by-products such as propolis, wax pollens, etc. as food and medicine by mankind have resulted in nutritional benefit and therapeutic promise.¹ It contains sugars and other constituents like vitamins, volatile chemicals, phenolic acids, flavonoids, minerals, amino acids, organic acids, and aromatic substances. Certain compounds such as phenolic acids, vitamins, flavonoids, etc. show numerous biological effects including antioxidant, anti-inflammatory, and antimicrobial properties.² However, honey quality is important for the health of consumers. The presence of contaminants like micro-organisms, antibiotics, pesticides, heavy metals

reduces honey quality and safety and might cause significant health hazards.³ Heavy metals in honey is of great concern. Accumulation of metals in soil and plants due to environmental pollution or other anthropogenic sources of metals has a great influence on the mineral composition of honey.⁴ Honey also has been used for monitoring of environmental contaminants such as heavy metals, radioactivity, and pesticides.⁵⁻⁷ Honey prepared from nectar or flowers by bees flying up to 3 km away from the nest can be served as bioindicators for area contaminated with heavy metals.⁸⁻¹²

In honey, major elements such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), chlorine (Cl), and minor elements such as iron (Fe), iodine (I), fluorine (F), zinc (Zn), selenium (Se), copper (Cu), manganese (Mn), chromium (Cr), cobalt (Co),

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molybdenum (Mo), nickel (Ni) have important functions in human health.¹³ Sodium plays an important role in maintenance of blood pressure, kidney and muscle functions. Potassium, Ca and Mg also play essential functions in muscle contraction with Sodium. Also Ca and K work in nerve functions and heart action.¹⁴⁻¹⁶ Some heavy metals such as Co, Fe, Mn, Ni, Zn, and Cu, which are essential elements for the growth of organisms, exert biochemical and physiological functions in the body at normal levels. However, at higher concentrations, they show toxic effects including headaches, respiratory disorders, metabolic abnormalities, nausea, and vomiting.¹⁷ Unlike these metals, some heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd) and Cr are highly toxic for plants, humans, animals, and environments.^{18,19} According to the Agency for Toxic Substances and Disease Registry, the most hazardous heavy metals comprise Arsenic (As), Pb, and Cd. As and Cd poisonings are comparatively less common with honey, however, contamination with Pb is commonly reported.¹³ The bio-accumulation of heavy metals in body fed by plants, feeds and animal-origin foods with a high amount of heavy metals can cause tissue damage or even mortality.¹⁸ Soils naturally contain high amounts of heavy metals and areas such as mining, foundries, smelters and other metal-based industrial operations are points where environmental pollution is intensified.²⁰ Animals can be exposed to all these contaminants in these areas. The aim of this study was to determine the levels of 22 elements including aluminum (Al), barium (Ba), Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, strontium (Sr), silver (Ag), bismuth (Bi), gallium (Ga), indium (In), lithium (Li), thallium (Tl) and Zn in the honey samples collected from West Mediterranean region of Turkey (WMRT).

Materials and Methods

Samples. A total number of 70 honey samples (1000 g of each) were collected from individual beekeepers in WMRT (Burdur province, n = 20; Antalya province n = 28; Isparta province, n = 22) from May to April 2018 (Fig. 1). The samples were stored in clean and closed glass jars and kept in the dark at 4 °C until used for analysis.

Determination of heavy metals. The extraction was performed using a method described by Yücel and Sultanoğlu²¹ and the measurements of metals were performed by inductively coupled plasma optical emission spectroscopy (ICP-OES; Spectroblue, Kleve, Germany) after microwave-assisted acid digestion. For analysis, 0.50 g of a sample was placed in the vessel and 9.00 mL of 65.00% nitric acid (HNO₃, Merck Millipore, Darmstadt, Germany) and 1.00 mL of 30.00% hydrogen peroxide (H₂O₂, Merck Millipore) were added to the vessels. The samples were burned with a microwave device (MARS 6 iWave System 240/50; CEM Corporation, Matthews, USA).

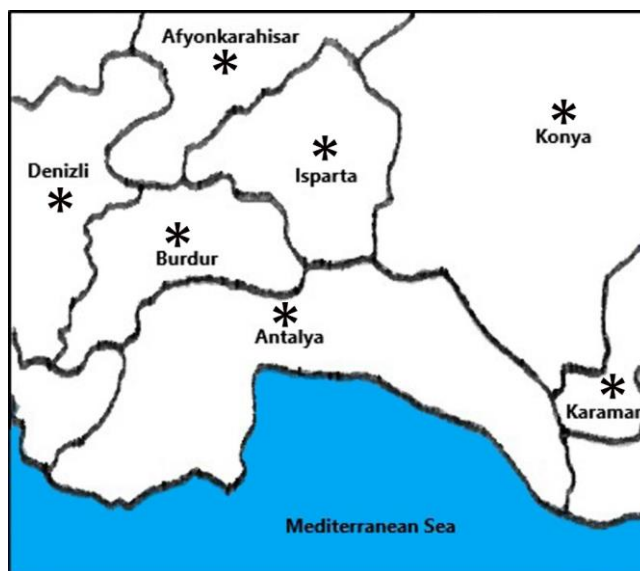


Fig. 1. Honey sampling regions (asterisks).

A blank sample was prepared in the same manner as the samples. It was also subjected to the same procedure. The working conditions of the microwave device were: 70 °C, 400 W for 5 min; 100 °C, 800 W for 5 min; 150 °C, 800 W for 10 min; 200 °C, 800 W for 10 min and cooling for 10 min. The burned samples were diluted with 20.00 mL of ultrapure water (Merck Millipore). This solution was used for the analysis of some metals.

The main stock solution (1000 mg L⁻¹) was prepared from ICP multi-element standard solution IV (Merck Millipore) containing Cu, Ag, Bi, Ga, In, Li, Tl, Zn, Al, Ba, Ca, Cd, Co, Cr, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, that were diluted by nitric acid. Calibration standards were prepared from this stock solution (0, 500, 1000, 2000, 5000, 10000 µg L⁻¹ for Ba, Fe, Mg, Na, Ni and 0, 100, 200, 500 µg L⁻¹ for others). The method was validated by parameters of recovery and limit of detection. Recovery experiment was performed by known spiking honey samples with 20 ppm of the stock solution. The metal levels in the samples were measured at Kırıkkale University Scientific and Technological Research Application and Research Center. The device was calibrated with the calibration standards prepared. The operating conditions of the ICP-OES device were plasma power of 1430 W, coolant flow of 13.00 L min⁻¹, nebulizer flow of 0.75 L min⁻¹, pump speed of 30 rpm and auxiliary flow of 0.80 L min⁻¹. Detection limits of working elements are given in Table 1.

Statistical analysis. Statistical calculations were performed using SPSS (version 15.0; IBM, Chicago, USA). Data were expressed as mean ± standard deviation. One-way ANOVA was used to determine the significant differences among provinces and the significance of the differences among the groups was determined by Duncan's test. The probability level of $p < 0.05$ was considered statistically significant.

Table 1. Validation data obtained for metals in honey (n ≥ 4).

Elements	Detection limits (ppb)	Recovery (%)	Correlation Coefficient (r ²)
Aluminum	0.57	85.22	0.9998
Barium	0.19	90.01	0.9999
Calcium	0.52	124.70	0.9990
Cadmium	0.20	81.99	0.9999
Cobalt	0.17	106.80	0.9999
Chromium	0.66	115.90	0.9998
Copper	0.56	105.16	0.9999
Iron	4.08	105.20	0.9999
Potassium	2.89	76.28	0.9992
Magnesium	0.15	97.41	0.9999
Manganese	0.09	89.07	0.9999
Sodium	2.66	121.28	0.9992
Nickel	0.68	92.40	0.9998
Lead	0.99	82.14	0.9999
Strontium	0.56	94.06	0.9998
Zinc	0.08	81.06	0.9999
Silver	2.00	87.95	0.9999
Bismuth	0.05	111.93	0.9999
Gallium	3.89	95.01	0.9999
Indium	9.16	80.23	0.9997
Lithium	0.11	101.97	0.9997
Thallium	1.11	88.81	0.9999

Results

The analysis of the recovery rate was achieved with spiked honey samples for the minerals in order to assure the reliability of the results. There was a good accuracy with recovery ranging from 76.28% to 124.70% for the 22 elements (Table 1). The detection limits obtained were adequately low to monitor elements safely in consideration of the maximum metal

levels established in honey by the European Commission Regulation. The metal concentrations (mg kg⁻¹) found in the honey samples collected from three provinces (Burdur, Isparta and Antalya) are given in Table 2. Chromium, Co, Cd, Ag, Bi, In and Tl were not detected in any of the tested honey samples (not shown in Table 2). In all the honey samples, the most abundant metal was the K with an overall average of 764.26 mg kg⁻¹. Higher concentrations of Pb, Ni, Mg, Na, K and Mn in the samples obtained from Burdur compared to other provinces were found ($p < 0.05$). The copper levels were statistically lower in Antalya in comparison to other regions. The lithium levels were statistically higher in honey samples collected from Isparta compared to others. In contrast, no significant differences were observed in Al, Zn, Fe, Sr, Ba, Ca and Ga levels among regions ($p > 0.05$). The content of the elements in honey samples from all three regions was determined in the following descending order: K, Na, Ca, Mg, Fe, Al, B, Zn, Pb, Cu, Sr, Li, Mn, Ga, Ni and Ba.

Discussion

In the current study, the most abundant macro-elements were K, Ca, Mg and Ca in all honey samples. Potassium was the dominant metal and ranged from 146.72 mg kg⁻¹ to 3202.00 mg kg⁻¹ in our honey samples. The contents of Ca, Mg and Na in honey samples were in the range of 79.72-123.88 mg kg⁻¹, 9.28 - 117.16 mg kg⁻¹ and 70.64 - 613.20 mg kg⁻¹, respectively. The levels of Mg, Na and K in honey samples collected from Burdur province were found to be higher than the others (Antalya

Table 2. Metal concentrations (mean and range) in the honeys. Data are presented as mean, maximum level (max), minimum level (min), standard deviation (SD).

Region	No.	Statistics	Analyzed metals (mg kg ⁻¹)														
			Cu	Ni	Pb	Al	Zn	Fe	Sr	Ba	Ca	Mg	Na	K	Mn	Ga	Li
Isparta	22	Mean	0.42 ^a	0.025 ^b	0.72 ^b	1.86	1.10	3.10	0.34	0.04	62.02	21.89 ^b	108.98 ^b	408.72 ^b	0.16 ^b	0.16	0.35 ^a
		Min	0.16	<LOD	0.08	1.16	0.32	1.36	0.24	<LOD	41.16	11.12	75.64	162.60	<LOD	0.12	0.28
		Max	0.84	0.52	0.88	3.96	3.24	6.24	0.76	0.12	89.88	47.76	306.56	1482.48	0.76	0.24	0.68
		SD	0.19	0.11	0.16	0.77	0.81	1.25	0.12	0.03	14.51	10.43	63.16	292.83	0.19	0.03	0.10
Antalya	28	Mean	0.24 ^b	0.028 ^b	0.79 ^b	2.64	1.10	3.62	0.36	0.03	68.72	30.01 ^b	96.79 ^b	557.59 ^b	0.19 ^b	0.21	0.28 ^b
		Min	0.04	<LOD	0.10	1.12	0.44	0.20	0.20	<LOD	39.72	10.12	70.64	153.16	<LOD	0.12	0.24
		Max	0.48	0.56	1.24	25.80	6.00	16.36	0.80	0.12	123.88	87.56	198.08	1822.60	0.76	1.60	0.40
		SD	0.13	0.10	0.18	4.58	1.04	2.97	0.16	0.03	19.27	16.68	25.07	407.26	0.18	0.27	0.04
Burdur	20	Mean	0.54 ^a	0.11 ^a	0.90 ^a	2.59	1.19	4.29	0.30	0.02	58.81	55.85 ^a	196.95 ^a	1444.71 ^a	0.46 ^a	0.14	0.30 ^b
		Min	0.12	<LOD	0.72	1.00	0.44	1.80	0.20	<LOD	42.20	9.28	74.60	146.72	<LOD	<LOD	0.28
		Max	1.52	0.36	1.12	7.64	4.40	12.60	0.52	0.04	91.84	117.16	613.20	3202.00	1.12	0.20	0.52
		SD	0.34	0.10	0.12	1.50	0.86	2.36	0.08	0.02	16.86	28.05	150.95	792.23	0.26	0.05	0.05
All samples	70	Mean	0.38	0.05	0.80	2.38	1.13	3.65	0.33	0.03	63.78	34.84	129.24	764.26	0.25	0.17	0.31
		Min	0.04	<LOD	0.08	1.00	0.32	0.20	0.20	<LOD	79.72	9.28	70.64	146.72	<LOD	<LOD	0.24
		Max	1.52	0.56	1.24	25.80	6.00	16.36	0.80	0.12	123.88	117.16	613.20	3202.00	1.12	1.60	0.68
		SD	0.25	0.10	0.17	3.02	0.91	2.38	0.13	0.03	17.49	23.44	98.09	675.03	0.24	0.17	0.07
China ⁴⁰		-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	
EC ^{6,41} /FAO/WHO ⁴²		-	0.50	1.00	-	10.00	-	-	-	-	-	-	-	-	-	-	

Cu: Copper; Ni: Nickel; Pb: Lead; Al: Aluminum; Zn: Zinc; Fe: Iron; St: Strontium; Ba: Barium; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Mn: Manganese; Ga: Gallium; and Li: Lithium. LOD = Limits of detection.

^{ab} Mean within in the same columns with different letters are significant ($p < 0.05$).

and Isparta; $p < 0.05$). Other researchers have confirmed that these metals (especially K) were the most abundant in honey and our results were in agreement with data reported in the literature.²¹⁻²⁵

The mean of Cu concentrations in all honey samples from WMRT was $0.38 \pm 0.38 \text{ mg kg}^{-1}$. Although minimum Cu was found as 0.24 mg kg^{-1} in honey from Antalya, maximum Cu was found as 0.54 mg kg^{-1} in the honey from Burdur. The honeys from WMRT showed higher Cu content than those reported from different regions of Turkey^{8,25,26} and showed lower Cu content than honey samples from south-eastern Turkey ($1.80 \mu\text{g g}^{-1}$), Bursa province of Turkey ($2.41 \mu\text{g g}^{-1}$) and Tokat province of Turkey (range, $0.3\text{-}1.45 \text{ mg kg}^{-1}$).^{27,28} Average values for Cu were slightly higher than those reported from Lazio region in Italy²⁹ and Podkarpacie region in Poland.²³

In the current study, the levels of Pb in honey (ranged from 0.08 to 1.24 mg kg^{-1}) was exactly higher than those reported by other researches carried out in different regions of Turkey. The contents of Pb in honey were in the range of $17.60 - 32.10 \mu\text{g kg}^{-1}$, $1.51 - 55.30 \mu\text{g kg}^{-1}$ and $30.30 - 58.60 \mu\text{g kg}^{-1}$ in Middle Anatolia, in Black Sea region and in Tokat region, respectively.^{25,30,31} In another study, the levels of Pb in the honey samples collected from different regions of Turkey ranged from 8.40 to $105.80 \mu\text{g kg}^{-1}$.²⁸ However, Sireli *et al.* have reported that the content of Pb in different honey brands ($n = 15$) ranged from 0.69 to 1.10 mg kg^{-1} .²⁶ In Ardabil province of Iran, the mean of Pb level was 0.50 mg kg^{-1} (range, $0.11 - 1.620 \text{ mg kg}^{-1}$).³² The results of this study were similar to those of Sireli *et al.* and Aghamirlou *et al.*^{26,32} These were not completely consistent with our results may be due to differences in the plant origin.

The average concentration of the Al in the samples collected from three regions was 2.38 mg kg^{-1} (in range of $1.00 - 25.80 \text{ mg kg}^{-1}$). The honeys from WMRT had higher Al content than those reported from different regions of Turkey (in range of $4.41 - 4570 \mu\text{g kg}^{-1}$).^{25,26,28} However, Yarsan *et al.* reported that Al was the most abundant metal (range $7.21 - 19.12 \text{ mg kg}^{-1}$) in honey samples in Turkey.⁸ The content of Al in these honey samples was found to be higher than Al content of all our honey samples.⁸ This may be caused by the aluminum equipment used during the processing of honey.

Although zinc is an essential mineral for the body, it can cause toxic at high concentrations.³³ The content of Zn in honey collected from WMRT was in the range of $0.32 - 6.00 \text{ mg kg}^{-1}$ (mean $1.13 \pm 0.91 \text{ mg kg}^{-1}$). Aghamirlou *et al.* reported that Zn was the most abundant metal and ranged from 122.86 to $6638.81 \mu\text{g kg}^{-1}$ (mean value $1481.64 \mu\text{g kg}^{-1}$) in honey samples.³² The zinc level was higher than those found in honey samples from Thrace region of Turkey (mean $1.98 \mu\text{g kg}^{-1}$),¹⁰ from Chile ($0.01 - 4.93 \text{ mg kg}^{-1}$),⁹ some provinces of Turkey (mean $49.9 \pm 49.7 \mu\text{g kg}^{-1}$),²⁴ from southeastern China (mean $1329.5 \mu\text{g kg}^{-1}$)³⁴

and from Turkey ($1.10 - 12.70 \text{ mg kg}^{-1}$).²⁸ The Zn content was lower than those founded from Orumieh city of Iran (mean $19.90 \pm 15.07 \text{ mg kg}^{-1}$)¹² and from Black Sea region of Turkey ($0.47 - 6.57 \text{ mg kg}^{-1}$).²⁵

In the present study, the average concentration of Fe in all honey samples was $3.65 \pm 2.38 \text{ mg kg}^{-1}$ (range $0.20 - 16.36 \text{ mg kg}^{-1}$). Osman *et al.* have reported that Fe content was the second most abundant with a range of $0.31 - 3.19 \text{ mg kg}^{-1}$ in Saudi Arabian honey.³⁵ Iron levels were found to be higher than those reported from south and east region of Turkey (mean 268 to $1036 \mu\text{g kg}^{-1}$),²⁴ from Orumieh of Iran (mean $0.70 \pm 0.20 \text{ mg kg}^{-1}$, range $0.37 - 1.98 \text{ mg kg}^{-1}$).¹² The Fe content found in the current study was in agreement with the data reported in the study carried out in Kahramanmaraş province of Turkey (mean 0.36 mg kg^{-1}).³⁶ Iron content in Lazio region of Italy (mean $4.51 \pm 0.39 \text{ mg kg}^{-1}$)²⁹ and in Black Sea Region of Turkey (1.55 to 12.9 mg kg^{-1}) was found to be higher than that of our study.

Although Cd concentrations ranged from 0.38 to $17.9 \mu\text{g kg}^{-1}$ in different regions of Turkey,^{25,28,36} Cd was not detected in this study. Strontium, Li, Mn, Ga, and Ba were the lowest metals in our study. The mean of Ni level in honey from WMRT was $0.05 \pm 0.10 \text{ mg kg}^{-1}$. The content of Ni in Burdur Province was more than other regions (Antalya and Isparta) which may be related to soil. The content of Ni in the present study was lower than others reported in ($651.78 \mu\text{g kg}^{-1}$) Iranian honeys³² and Hatay province of Turkey (mean 0.35 mg kg^{-1}).²¹ The contents of Ba ($0.03 \pm 0.03 \text{ mg kg}^{-1}$) and Sr ($0.33 \pm 0.13 \text{ mg kg}^{-1}$) in our honeys were similar to the values found by Fernandez-Torres *et al.*³⁷ and Yücel and Sultanoğlu²¹ (Ba $0.05\text{-}0.68 \text{ mg kg}^{-1}$, Sr $0.12\text{-}2.46 \text{ mg kg}^{-1}$) and Chuzinska and Baralkiewicz.³⁸ Manganese content (mean $0.25 \pm 0.24 \text{ mg kg}^{-1}$) in our honey were similar to the studies.^{8,21} However, Mn level in honey samples from Poland (mean 4.96 mg kg^{-1})³⁸ was found to be higher than our study. The average concentration of Ga ($0.17 \pm 0.17 \text{ mg kg}^{-1}$) in our honey was higher than the value (mean $5.6 \pm 1.5 \mu\text{g kg}^{-1}$) of the study carried out by Döker *et al.* in Çankırı province of Turkey.³⁹ Although it was reported that Li was not detected in Çankırı province, Li was found in our samples (average value of $0.31 \pm 0.07 \text{ mg kg}^{-1}$).³⁹

The most important heavy metals include Pb, Cd, Hg, Cr, Cu, Mn, Ni, Zn, and Ag which are toxic at low concentrations. Although there is no specific maximum residue limit (MRL) levels for these elements, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) have established acceptable levels in some heavy metals for some foods (shown in Table 2). Also, there is no specific legislation on metal contents in honey in Turkey. However, maximum allowable contaminant levels in foods and honey (GB2762-2017) in China has been determined.⁴⁰ The honeys from WMRT exhibited high levels of Pb, Al, Cu, Zn, Mg and Fe in heavy

metals. However, the results for toxic and trace elements in tested honey samples showed that the metal levels were known to be within the acceptable limits of European Union⁴¹ and WHO and the levels are in safety levels for human consumption. In the current study, the metals levels varied among different regions owing to some variables. The difference in metal levels depends on both natural and anthropogenic factors like geographical and botanical origin, soil, industrial activities, atmosphere, traffic, mining, beekeeping equipments, element content of plant nectar, agriculture application, season of the year. Also, honey is a good indicator for monitoring of environmental pollution with metals and useful for the prevention of future problems.

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Conflict of interest

The authors have no conflicts of interest.

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