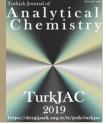
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# Comparison of some mineral and trace-heavy metal contents of blossom honey samples from Yamadağ and Battalgazi regions, Malatya, Türkiye

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# Abstract

Among bee products, honey is particularly valued for its nutritional profile, notably its content of essential macro- and microelements. The mineral composition of honey is shaped by the interplay of geographic origin, botanical source, and bee genotype. This study aimed to (i) characterize the trace element profiles of honey samples harvested from Yamadağ Mountain and the Battalgazi Plateau in Malatya Province, Türkiye, and (ii) assess the influence of two *Apis mellifera* genotypes; *A. m. caucasica* and *A. m. carnica*, on elemental composition.

Honey samples were digested and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for Fe, Cu, Zn, Se, Mn, Pb, Cb, Cr, Ni, As, Bi and Ag. Statistical analysis revealed that both geographic origin and bee genotype exert significant effects on mineral concentrations. Specifically, samples from Yamadağ Mountain exhibited lower total trace element levels compared to those from the Battalgazi Plateau, although concentrations of all measured heavy metals remained well below national and international safety thresholds. According to the obtained findings, the average values of trace elements iron, zinc, copper, selenium and manganese in Yamadağ Mountain honey samples were determined as 0.41 mg/kg, 0.62 mg/kg, 0.04 mg/kg, 0.17 mg/kg and 0.04 mg/kg, respectively. The average values of trace elements iron, zinc, copper and manganese in Battalgazi Plateau honey samples were found to be 7.55 mg/kg, 1.30 mg/kg, 0.18 mg/kg and 0.23 mg/kg, respectively. Selenium was not detected in Battalgazi Plateau honey samples. When the heavy metal contents of the honey samples were examined, silver was not detected in Yamadağ Mountain honey samples and silver, cadmium, arsenic and bismuth were not detected in Battalgazi Plateau honey samples. The average values of lead, cadmium, chromium, nickel, arsenic and bismuth in Yamadağ Mountain honey samples were found to be 0.10 mg/kg, 0.05 mg/kg, 0.46 mg/kg, 0.03 mg/kg, 0.12 mg/kg and 0.02 mg/kg, respectively. In Battalgazi honey samples were found to be 0.10 mg/kg, 0.05 mg/kg, 0.46 mg/kg, 0.21 mg/kg and 0.14 mg/kg, respectively.

These findings demonstrate that regional environmental factors and genetic variation within *Apis mellifera* colonies critically determine the mineral composition of honey. Accordingly, both geographic provenance and bee genotype should be considered in quality control protocols and in the development of region-specific standards for trace element content in honey.

Keywords: Honey, mineral composition, geographical variation, Malatya, Türkiye

# 1. Introduction

Minerals are essential inorganic nutrients required in small amounts for the maintenance of fundamental biochemical functions in the human body. In dietary supplements, they are commonly classified as macro minerals (e.g., calcium, magnesium, potassium) and trace minerals (e.g., iron, zinc, selenium), serving to correct deficiencies or support optimal health [1,2]. Minerals are essential inorganic nutrients required for human health and are naturally present in various foods. Dairy products serve as a primary source of calcium, while green leafy vegetables are rich in magnesium. Red

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meat and organ meats provide significant amounts of iron, whereas seafood is abundant in iodine and zinc. Nuts, legumes, and whole grains contribute essential minerals such as potassium (K), phosphorus (P), and selenium (Se). A well-balanced diet ensures adequate mineral intake, supporting vital biological processes and overall physiological functions [3].

Various organisms have been used as an alternative tool to detect pollution using low-cost conventional methods. In recent years, honey bees and their products (e.g., honey and beeswax) have been used to monitor

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pollutants, including heavy metals such as As, Pb, Cd, and Hg. Metals detected in bee products have been found to be correlated with atmospheric concentrations [4]. Honeybees produce honey by collecting nectar and other substances from plants contaminated by environmental pollution. Heavy metals are elements with high atomic mass and density that can exert toxic systems. effects on biological Environmental contaminants that contaminate honey; naturally present in soil, water, and air, these metals can also be introduced into the environment through anthropogenic activities such as industrial processes, mining, agriculture, and waste management. Lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are among the most concerning heavy metals, as they can cause severe health issues even at low concentrations through bioaccumulation and biomagnification. While some heavy metals, such as \*\*zinc (Zn), copper (Cu), and iron (Fe), are essential trace elements for living organisms, they can become toxic beyond certain thresholds. Therefore, monitoring heavy metal levels in food, water, and environmental samples is of critical importance. The elements found in honey can be beneficial or dangerous to health [5].

Honey is a natural bee product that serves as significant sources of essential minerals for human nutrition. Honey contains essential minerals such as sodium (Na), potassium, calcium (Ca), magnesium (Mg) and phosphorus, which support energy metabolism and are known as the macro elements of honey, as well as trace amounts of minerals such as iron, copper, zinc, and selenium [6,7]. These minerals play a crucial role in regulating cellular functions, enhancing immune system activity, and exerting antioxidant effects. As bioavailable and nutritionally valuable food sources, honey and pollen help address mineral deficiencies and positively impact. The mineral content of honey varies depending on geographical characteristics and floral composition. Among the richest honeys in terms of mineral content, chestnut honey stands out, while dark-colored forest honeys have been reported to contain higher ash content and, consequently, greater mineral concentrations [8,9].

This study aims to investigate the mineral composition of honey, considering the influence of geographical characteristics and floral composition. By analyzing the variations in mineral content among different honey types, particularly mountain and plateau honey samples, this research seeks to provide insights into their nutritional value and potential health benefits. Additionally, the study evaluates the bioavailability of essential minerals in honey, contributing to a better understanding of their role in human nutrition and dietary supplementation.

# 2. Materials and methods

# 2.1. Samples

Honey samples were obtained from two different locations in the Yamadağ Mountain and Battalgazi Plateau regions of Malatya province in the Eastern Anatolia Region of Türkiye, from two different Apis mellifera (A. m.) genotype bee races (Table 1). The Yamadağ Mountain massif, situated within the Sivas Province area and extending to the south and southeast, delineates the northern boundary of Malatya Province. Geologically, Yamadağ and its extensions exhibit a predominantly volcanic structure, attaining elevations above 1 500 m and forming a broad, high-relief plateau interspersed with distinct peaks [10]. The Battalgazi district spans a considerable altitudinal range and supports a rich assemblage of wild and cultivated flora, including solanaceous vegetables (tomato, pepper, cabbage), orchards (apricot, cherry, walnut, apple), vineyards, and ornamental nurseries [11].

The experimental trial was conducted at the Bee and Bee Products Development Application and Research Center apiary on the Battalgazi Campus of Malatya Turgut Özal University. In April, all colonies were equalized with respect to supplemental feeding and frame number. During the first week of June, forty colonies remained at the Battalgazi site, while a matched set of forty colonies was relocated to the Hekimhan– Yamadağ plateau. Twenty colonies were used in each group, and samples were harvested from these colonies. Honey was harvested in the second week of September; each honey frame was logged with hive identifier, bee genotype, and collection site, then filtered and transferred into amber glass jars for storage under dark, dry conditions until analysis.

# 2.2. Determination of metals by ICP-MS

Elemental analyses were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analyses were conducted with an Analytic Jena Plasma Quant MS instrument (Jena, Germany). Sample preparation involved acidic digestion using a microwave-assisted method (CEM - Mars 5, Matthews, North Carolina, USA), followed by measurement under the standard operating conditions of the instrument. Samples H1, H2, H3 and H4 were weighed in amounts of 0.4662 g, 0.7238 g, 0.6374 g and 0.5485 g, respectively, and placed in teflon tubes. The honey samples were digested in a microwave system using 2 mL of 37% hydrochloric acid (HCl) and 5 mL of 65% nitric acid (HNO<sub>3</sub>). Following digestion, the final volume was adjusted to 10 mL with ultrapure water prior to analysis. A multi-element analysis was performed, and internal standards were used to enhance measurement accuracy. Calibration curves were constructed using certified reference materials, and the accuracy of the analytical results was verified with quality control samples (Table 2) [12].

Table 1. Regions where honey samples were collected

Region	Honey Code	Race	Coordinates/ Altitude (meter)			
			(meter)			
Yamadağ	H1	A. m. caucasica	38° 54' 41" N 38° 7' 55"E/2306			
ramauag	H2	A. m. carnica				
Battalgazi	H3	A. m. caucasica	38°25'22"N 38°21'56"E/885			
	H4	A.m. carnica				

Prior to each analysis, the ICP-MS instrument was calibrated according to the protocol outlined in Table 3. Reported elemental concentrations have been adjusted to account for the applied dilution factors. After the solutions were prepared, the device was calibrated before reading the heavy metal content of the solubilized samples. The calibration graphs drawn for the elements were used as an indicator of the accuracy of the prepared standards [12].

#### 2.3. Statistics

All experiments were conducted in triplicate, and the results are expressed as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using SPSS software (version 22). Pearson's correlation coefficient was calculated to assess relationships between the variables. ANOVA was used for statistical analysis of the means of the elements Fe, Zn, Cu, Mn, Pb, Cr and Ni, and T-test was used for statistical analysis of the means of the elements Se, Cd, As, Bi and Ag. Differences were considered statistically significant when P < 0.05.

 Table 2. The properties of elemental analysis by inductively Coupled

 Plasma Mass Spectrometry (ICP-MS)

Parameter		Value
Flow Parameters (L/min)		
	Plasma Flow	9.0
	Auxiliary Flow	1.65
	Sheath Gas	0.00
	Nebulizer Flow	1.10
Torch Alignment (mm)		
	Sampling Depth	7.0
Other		
	RF Power (kW)	1.40
	Pump Rate (rpm)	12
	Stabilization delay (s)	10
Ion Optics (volts)		
	First Extraction Lens	-46
	Second Extraction Lens	-161
	Third Extraction Lens	-246
	Corner Lens	-194
	Mirror Lens Left	23
	Mirror Lens Right	32
	Mirror Lens Bottom	39
	Entrance Lens	-5
	Entrance Plate	-68
	Fringe Bias	-4.6
	Pole bias	0.0
iCRC (mL/min)		
	Skimmer Gas Source	H2
	Skimmer Flow	60
Nitrox		
	Flow (ml/min)	0.0

#### 3. Results and discussions

LOD (limit of detection) can indicate the presence of a substance but cannot determine its exact amount. LOQ (limit of quantification) is the lowest level at which the amount of a substance can be measured accurately and reliably. LOD and LOQ values were determined for Fe, Zn, Cu, Se, Mn, Pb, Cd, Cr, Ni, As, Bi and Ag [12]. The calibration range, isotope of element, blank equivalent concentration, calibration equation, correlation coefficient, limit of detection, and limit of quantification of the calibration curves for determination of the element using inductively coupled plasma mass spectrometry are given in Table 3. Calibration curves showed very strong linearity. Values show that the device is quite sensitive. The correlation coefficients of all the calibration curves were equal to or greater than 0.9983 (R<sup>2</sup>≥0.9983). These correlation coefficients showed that there was a strong positive correlation and linear fit for the absorbance change with concentration. The calibration equation formulas of the elements are given in Table 3.

c/s: Signal ratio obtained from the device. It expresses how the measured concentration changes depending on the ratio of the internal standard.

conc: Analyt contration

I/S: Internal standard ratio

LOD: The lowest concentration that the device can reliably detect.

LOQ: The lowest concentration at which the instrument can reliably quantify.

BEC: The equivalent concentration of the signal given when the blank sample is measured (Table 3).

Heavy metal concentrations in honey must remain below thresholds that could compromise human health. Accordingly, honey products within the scope of this standard shall conform to the maximum heavy metal limits established by the Codex Alimentarius Commission [13]. Under Article 8 "Contaminants" of the Honey Communique (22 April 2020, No. 31107), the provisions of the Turkish Food Codex Contaminants (Official No. 28157, Regulation Gazette 29 December 2011) are applied to covered products. Although the "Communique on Maximum Limits of Contaminants in Foodstuffs" [14] does not specify heavy metal limits for honey, metal residues in honey are monitored via the National Residue Control Plan [15]. Heavy metals, defined by a physical density exceeding 5 g cm<sup>-3</sup>, comprise over 60 elements, notably lead, cadmium, chromium, iron, cobalt, copper, nickel, mercury, and zinc [12,16]. While the human body tolerates certain levels of dietary heavy metals, concentrations above specific thresholds induce toxicity.

-10-50-100-200 -10-50-100-200 0.5-1-5-10-20	57 64 65	5.94 2.85	c/s = (788.3 + 334.4 + 132.4*conc)*[I/S Ratio] c/s = (282.4 + 232.9 + 99.41*conc)*[I/S Ratio]	0.9993	1.55	5.15
0.5-1-5-10-20		2.85	$a/a = (282.4 \pm 222.0 \pm 0.0.41*com a)*[I/S Patio]$			
	65		$C/S = (202.4 \pm 202.7 \pm 99.41 \text{ COUC}) [1/5 \text{ Kallo}]$	0.9991	1.57	5.23
10 50 100 200		0.13	c/s = (21.7 + 2.742 + 167.2*conc)*[I/S Ratio]	0.9999	0.14	0.45
-10-50-100-200	78	0.65	c/s = (5.1 + 6.431 + 7.917*conc)*[I/S Ratio]	0.9983	3.05	10.18
0.5-1-5-10-20	55	0.45	c/s = (536.7 - 18.37 + 1214*conc)*[I/S Ratio]	0.9996	0.05	0.17
0.5-1-5-10-20	207	0.21	c/s = (7.0 + 5.277 + 32.68*conc)*[I/S Ratio]	0.9998	0.49	1.62
0,5-1-5-10-20	112	0.12	c/s = (8.2 - 0.365 + 67.24*conc)*[I/S Ratio]	0.9999	0.05	0.16
0.5-1-5-10-20	52	0.08	c/s = (48.3 - 3.845 + 612.4*conc)*[I/S Ratio]	0.9995	0.06	0.21
0.5-1-5-10-20	58	-0.44	c/s = (-66.0 - 22.49 + 150.2*conc)*[I/S Ratio]	0.9997	0.55	1.83
-10-50-100-200	75	-5.46	c/s = (-83.2 - 29.59 + 15.57*conc)*[I/S Ratio]	0.9991	4.62	15.41
0.5-1-5-10-20	209	0.07	c/s = (8.7 + 0.043 + 121.4*conc)*[I/S Ratio]	0.9988	0.1008	0.3359
0.5-1-5-10-20	107	0.21	c/s = (80.0 + 8.323 + 391.9*conc)*[I/S Ratio]	0.9996	0.1158	0.3861
	0.5-1-5-10-20 0.5-1-5-10-20 0.5-1-5-10-20 0.5-1-5-10-20 0.5-1-5-10-20 10-50-100-200 0.5-1-5-10-20 0.5-1-5-10-20	0.5-1-5-10-20         55           0.5-1-5-10-20         207           0.5-1-5-10-20         112           0.5-1-5-10-20         52           0.5-1-5-10-20         58           :10-50-100-200         75           0.5-1-5-10-20         209           0.5-1-5-10-20         107	$\begin{array}{cccccccc} 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 55 & 0.45 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 207 & 0.21 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 112 & 0.12 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 52 & 0.08 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 58 & -0.44 \\ \text{-}10\text{-}50\text{-}100\text{-}200 & 75 & -5.46 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 209 & 0.07 \\ 0.5\text{-}1\text{-}5\text{-}10\text{-}20 & 107 & 0.21 \\ \end{array}$	0.5-1-5-10-20550.45 $c/s = (536.7 - 18.37 + 1214*conc)*[I/S Ratio]$ 0.5-1-5-10-202070.21 $c/s = (7.0 + 5.277 + 32.68*conc)*[I/S Ratio]$ 0.5-1-5-10-201120.12 $c/s = (8.2 - 0.365 + 67.24*conc)*[I/S Ratio]$ 0.5-1-5-10-20520.08 $c/s = (48.3 - 3.845 + 612.4*conc)*[I/S Ratio]$ 0.5-1-5-10-2058 $-0.44$ $c/s = (-66.0 - 22.49 + 150.2*conc)*[I/S Ratio]$ 10-50-100-20075 $-5.46$ $c/s = (-83.2 - 29.59 + 15.57*conc)*[I/S Ratio]$ 0.5-1-5-10-202090.07 $c/s = (8.7 + 0.043 + 121.4*conc)*[I/S Ratio]$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

 $\mathbf{R}^2$ : Determination coefficient; BEC: Blank equivalent concentration; Mass: Isotope of element; LOD: Limit of detection; LOQ: Limit of quantification

Maximum daily intake levels for cadmium, lead, zinc, and copper are 60 µg, 210 µg, 12–15 mg, and 30 mg, respectively [12], whereas the maximum permissible concentrations in food for tin and mercury are 50 mg kg<sup>-1</sup> and 1 mg kg<sup>-1</sup>, respectively [14]. The U.S. Environmental Protection Agency has set a soil arsenic limit of 75 mg kg<sup>-1</sup>, and both WHO and USEPA have reduced the permissible arsenic concentration in water from 50 µg L<sup>-1</sup> to 10 µg L<sup>-1</sup>, a standard likewise adopted by the Turkish Standards Institute for drinking water [16].

In this study, some trace element and some heavy metal contents of honey from Yamadağ and Batttalgazi regions, which are at two different altitudes with rich floral characteristics in terms of honey production, were compared. The values found are summarized in Table 4 and Table 5.

Among the five trace elements analyzed using ICP-MS (Fe, Cu, Zn, Se, and Mn), iron (Fe) was found to be the most abundant. Se, Fe, Mn, Cu, Zn and Ni at recommended doses have antioxidant properties, enzymatic activities and the ability to contribute to general human development. In contrast, Pb, As, Cr and Cd have no known benefits and may be toxic in relatively small amounts [5]. The average iron concentration was determined as 0.41 µg/g in Yamadağ honey and 7.55 mg/kg in Battalgazi honey. The approximately 20-fold higher iron content in Battalgazi honey compared to Yamadağ honey is particularly noteworthy.

Bees are exposed to chemicals in a variety of ways: by inhaling metals while flying and collecting them bound to particulate matter or soil particles on their hairy bodies; by ingesting metals from the water they carry; and by collecting pollen and nectar from plants that may be rich in bioavailable metals found in soil, water, and air, thus adding them to bee products. These interactions affect the quality of what honeybees produce, making honey a potentially valuable indicator of environmental pollution [4]. Honey serves as a bioindicator of environmental contamination, with its mineral profile reflecting not only the regional flora but also local soil, water, and climatic conditions. In Malatya Province, post-earthquake demolition, debris-removal operations, and reconstruction efforts have markedly increased atmospheric particulate levels, particularly in urban and district centers relative to highland plateaus. This anthropogenic pollution likely accounts for the elevated trace-element concentrations observed in Battalgazi honey versus Yamadağ Mountain samples. Statistical analysis demonstrated a strong positive correlation between manganese and iron concentrations ( $R^2 = 0.993$ , p< 0.01), indicative of co-accumulation or common environmental sources. In contrast, iron and arsenic exhibited a pronounced negative correlation ( $R^2 = -0.992$ , p < 0.01) (Table 6), consistent with the known adsorption of ionic arsenic species onto iron and aluminum oxides in acidic to neutral soils, which reduces arsenic bioavailability to plants [16].

<b>Table 4</b> . Trace elements of the honey samples (mg/kg)
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Region	Honey Code	Fe	Zn	Cu	Se	Mn	
	H1	0.32	1.10	0.05	0.34	0.05	
Yamadağ	H2 0.49		0.13	0.03	ND	0.03	
	Mean±SD	0.41±0.12	0.62±0.69	0.04±0.01	0.17±0.24	$0.04 \pm 0.01$	
	H3	7.19	1.28	0.21	ND	0.23	
Battalgazi	H4	7.91	1.31	0.15	ND	0.23	
	Mean±SD	7.55±0.51	1.30±0.02	0.18±0.04	ND	0.23±0.00	
Sig.		0.003*	0.296	0.047*		0.003*	

ND: Not detected; Mean±SD: Arithmetic mean and standard deviation; \*: P<0.05

Region	Honey Code	Pb	Cd	Cr	Ni	As	Bi	Ag
Yamadağ	H1	0.17	0.10	0.84	0.04	0.13	0.03	ND
	H2	0.03	ND	0.07	0.01	0.11	ND	ND
	Mean±SD	0.10±0.10	0.05±0.07	$0.46 \pm .54$	0.03±0.02	0.12±0.01	0.02±0.02	ND
Battalgazi	H3	0.08	ND	0.16	0.13	ND	ND	ND
0	H4	0.13	ND	0.25	0.14	ND	ND	ND
	Mean±SD	0.11±0.04	ND	0.21±0.06	0.14±0.01	ND	ND	ND
Sig.		0.952		0.585	0.020*			

Table 5. Heavy metal amount of honey samples (mg/kg)

ND: Not detected; Mean±SD: Arithmetic mean and standard deviation; \*: P<0.05

Similarly, the average zinc concentration in honey from the Yamadağ region was found to be 0.62 mg/kg, while in honey from the Battalgazi region, it was 1.30 mg/kg. This indicates that the zinc content in Battalgazi honey is approximately twice as high as that in Yamadağ honey. No significant differences were observed between different bee species in this context.

The amount of zinc in chestnut honey was reported to be 1.45 mg/kg [17]. According to Bengü and Kutlu [18], zinc and iron values are 5.00-12.0 mg/100g and 10.0-27.0 mg/100g, respectively. In honey samples from the Yamadağ region, the average copper concentration was found to be 0.04 mg/kg, whereas in the Battalgazi region, it was 0.18 mg/kg. When compared to existing literature, the copper levels in honeys from both regions are considerably low. In a study [18], the elements in honey samples were determined as chromium 2.51 ppb, copper 0.98 ppb, iron 28.84 ppb, manganese 3.93 ppb, nickel 2.02 ppb and zinc 9.71 ppb. In a study conducted on chestnut honey from the Kastamonu region, it was reported that the amount of iron mineral varied between 1.32 and 9.75 mg/kg [9]. In the same study, the average copper amount was found to be 0.07 mg/kg. The amount of copper in New Zealand Manuka honey was reported as 0.35 mg/kg [1] and in pine honey as 0.84 [19]. Pipoyan et al. [20] reported that copper concentrations in several samples of honey they examined were above the maximum allowable level. The concentrations of copper ranged from 9.00E-02 to 1.86E+00 mg/kg.

The amount of selenium element, known as an essential element and having an important role in antioxidant activity, was found to be 0.34 mg/kg. In a study conducted on many honey samples collected from different regions of Anatolia, it was reported that the amount of selenium varied between 0.42 and 19.9 mg (Kg) [21]. *A. m.* genotype from Yamadağ region but below the detection limits in *A. m. carnica* genotype. In the honeys of Battalgazi region, it was determined that the detection limits were at six, that is, at a very low level. In their study, Tuzen et al. [22] reported the amount of selenium as 38 to 112 mg/kg, while it was reported as 0.04 mg/kg in pine honey [19].

Manganese (Mn), which is a grayish metal in its pure form, is a hard and brittle substance. Manganese is found in nature in the form of an oxide and has physical properties similar to iron. Manganese is naturally found in soil, water, and rocks, but it is not found as a base metal in nature. Ocean events that mobilize the earth's crust, earthquakes, volcanic events, fires and vegetation are also natural sources of manganese in the atmosphere [12,23]. The average manganese concentrations in honey samples from both regions were approximately 0.04 mg/kg, with no significant differences observed between the regions or among different genotypes. The results showed a strong positive correlation between manganese and nickel (R<sup>2</sup>= 0.991, p≤0.01) (Table 6). In fact, in a study [24] it was observed that the adsorption for manganese (II) and nickel (II) increased as the pH increased. The results showed a strong negative correlation between manganese and arsenic (R2= -0.982, p≤0.01) (Table 6). These manganese levels are relatively low compared to findings from other studies. For instance, research on honey samples from Türkiye reported manganese concentrations ranging from 0.096 to 29.496  $\mu$ g/g, while another study found levels between  $0.25 \pm 0.24$  mg/kg. These variations in Mn content are likely influenced by environmental factors, floral sources, and regional characteristics. In the study conducted on chestnut honey, Mn amounts were found to be 1.39 to 18.69 mg/kg [9], while it was reported as 28 mg/kg in Manuka honey [1], 1.1 to 4.2 mg/kg in Manuka honey [25] and 2.8 mg/kg in pine honey [19].

Heavy metals, the elements characterized by high atomic weight and density, are among the most environmental pollutants persistent and pose toxicological risks even at trace concentrations. These contaminants originate from both natural and anthropogenic sources, leading to deposition in air, soil, and water [5]. Plant uptake of heavy metals from contaminated soils results in bioaccumulation within floral tissues, which foraging honeybees (Apis mellifera) then transport to the hive. During nectar processing, bees incorporate these metals into honey, creating a route of dietary exposure for humans. Chronic consumption of heavy metal-contaminated honey can lead to progressive accumulation in human organs and tissues [18].

Unlike degradable organic pollutants, heavy metals remain chemically persistent and can cycle indefinitely

through environmental compartments. Principal soil contaminants include cationic species such as mercury (Hg), cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), zinc (Zn), chromium (Cr), and manganese (Mn), as well as oxyanionic forms of arsenic (As), molybdenum (Mo), and selenium (Se) [16]. Empirical studies have documented considerable variability in trace-metal concentrations across honey types. For instance, Rhododendron honeys analyzed by Silici et al. [26] contained Cu (9.75-35.8 µg/kg), Cd (0.28-2.37 µg/kg), Pb (1.51–55.3 µg/kg), Co (1.44–28.5 µg/kg), Cr (1.57– 12.9 µg/kg), Ni (1.35–131 µg/kg), Al (47.8–644 µg/kg), and Se (14.1-323 µg/kg), exceeding levels typically observed in regional multifloral honeys. Meister et al. reported Cr (0.02–0.03 mg/kg), [25] Cu (0.13 -0.30 mg/kg), Fe (0.72–1.20 mg/kg), and Zn (0.32– 0.47 mg/kg) in Manuka honey. Aygün [27] detected Al, As, Cd, and Pb at 435.9, 4.8, 337.9, and 409.9 µg/kg, respectively, in Turkish honey samples, whereas Sobhanardakani and Kianpour [28] found mean Cd, Cr, Ni, and Zn levels of 63.18, 58.05, 56.15, and 684.43 µg/kg. Ligor et al. [29] identified As up to 0.49 µg/kg in polyfloral and linden honeys, Ni in excess of 400 µg/kg in samples from Lesser Poland, the highest Cr content  $(3.76 \ \mu g/kg)$  in buckwheat honey, and Mo  $(5.94 \ \mu g/kg)$  in dandelion honey.

Iron stands out among trace elements for its vital roles in living organisms: it is an essential component of hemoglobin, myoglobin, cytochromes, peroxidases, catalases, ferritin, and transferrin, and it participates in the biosynthesis of large biomolecules [30]. In contrast, nickel and certain nickel compounds are classified as carcinogenic; dietary Ni accumulates over time in pulmonary, gastrointestinal, and dermal tissues. contributing to chronic pulmonary fibrosis, cardiovascular disorders, and renal toxicity [12].

In the context of food biochemistry, iron, copper, zinc, and manganese are essential trace elements vital for various metabolic processes. However, at elevated concentrations, these elements can exhibit toxic effects and are thus categorized as heavy metals. Analyses of honey samples from Malatya's Yamadağ and Battalgazi regions have determined that the levels of these trace elements are well below toxic thresholds, indicating no significant health risk associated with their consumption. The results showed a strong positive correlation between selenium and cadmium (R<sup>2</sup>= 1.000, p<0.01), and selenium and bismuth (R<sup>2</sup>= 1.000, p<0.01) (Table 6). The positive increase relationships between them are thought to be due to factors related to agricultural fertilizers, agricultural pesticides and environmental pollution [5].

In the study, concentrations of seven different heavy metals, lead, cadmium, chromium, nickel, arsenic, and

silver, were measured in honey samples. The levels of these metals were found to be below the safe consumption limits. The results showed a strong positive correlation between bismuth and cadmium (R<sup>2</sup>= 1.000, p<0.01), and a strong negative correlation between nickel and arsenic (R<sup>2</sup>= -0.951, p<0.05) (Table 6). In a study [4], a moderate correlation was found between nickel and cadmium in honey samples (r = 0.41, p > 0.001). In addition to environmental effects, weak but statistically significant correlations were reported between nickel and arsenic (r = 0.18, p = 0.003), cadmium and lead (r = 0.18, p = 0.003), and chromium and nickel (r= 0.14, p = 0.02). It was emphasized that the lack of significance or weak correlations between metal concentrations was due to the fact that the sources and magnitudes of metal pollution varied greatly between sample locations. In another study [20], concentrations of lead, cadmium, arsenic, and nickel ranged from 2.30E-03 to 4.50E-02 mg/kg, from 6.00E-04 to 3.10E-03 mg/kg, from 5.00E-03 to 4.80E-02 mg/kg, and from 2.40E-01 to 8.49E-01 mg/kg respectively. In a study, the amount of lead in some honey samples was found to be 0.08-0.44 mg/kg and the amount of selenium was found to be 2.20 to 12.13 mg/kg [5]. In addition to the botanical origin of honey, the density of flowers, the production period, and the amount of rainfall affect the composition of honey. In addition, the containers in which honey is stored and packaged after harvest can cause an increase in the amount of chromium in honey. Similarly, storing honey in galvanized or aluminum containers can cause zinc and aluminum contamination. On the other hand, the elements found in nectar also cause an increase in the element content of honey [26].

In a study [31], the amounts of mineral elements in *Ziziphus* spp. honey varied according to the honey bee species. The highest amounts of iron (33.54 mg/kg) and manganese (0.61 mg/kg) were found in honey produced by *A. florea*. The highest amounts of Mg (145.35 mg/kg), zinc (13.37 mg/kg) and copper (0.58 mg/kg) were obtained from honey harvested from *A. m. jemenitica* colonies. Highly significant positive correlations were found between all mineral elements determined in *Ziziphus* spp. honey produced by *A. florea* and *A. mellifera*.

Chromium is important for animals and humans. There are human and animal studies showing that chromium deficiency negatively affects lipid metabolism and causes atherosclerosis [12]. Manouchehri et al. [32] summarized studies reporting heavy metal levels in honey samples examined in different countries. In the studies conducted, it was emphasized that the rate of contamination of honey with heavy metals is directly related to the number of industrial centers and the pollution rate in the region. It was determined that the amounts of heavy metals (especially cadmium and mercury) in the examined honey samples were above the permitted rate.

Table 6. Pearson correlation matrix on the honey samples (n=4)

		Fe	Zn	Cu	Se	Mn	Pb	Cd	Cr	Ni	As	Bi
Б.	Pearson Correlation	1	0.692	0.928	-0.590	0.993**	0.055	-0.590	-0.421	0.979*	-0.992**	-0.590
Fe	Sig. (2-tailed)		0.308	0.072	0.410	0.007	0.945	0.410	0.579	0.021	0.008	0.410
7	Pearson Correlation		1	0.732	0.173	0.755	0.709	0.173	0.352	0.825	-0.616	0.173
Zn	Sig. (2-tailed)			0.268	0.827	0.245	0.291	0.827	0.648	0.175	0.384	0.827
C···	Pearson Correlation			1	-0.471	0.957*	0.039	-0.471	-0.339	0.933	-0.935	-0.471
Cu	Sig. (2-tailed)				0.529	0.043	0.961	0.529	0.661	0.067	0.065	0.529
c	Pearson Correlation				1	-0.515	0.741	1.000**	$0.977^{*}$	-0.411	0.669	1.000**
Se	Sig. (2-tailed)					0.485	0.259	0.000	0.023	0.589	0.331	0.000
	Pearson Correlation					1	0.117	-0.515	-0.347	0.991**	-0.982*	-0.515
Mn	Sig. (2-tailed)						0.883	0.485	0.653	0.009	0.018	0.485
וח	Pearson Correlation						1	0.741	0.866	0.245	0.063	0.741
Pb	Sig. (2-tailed)							0.259	0.134	0.755	0.937	0.259
01	Pearson Correlation							1	0.977*	-0.411	0.669	1.000**
Cd	Sig. (2-tailed)								0.023	0.589	0.331	0.000
C	Pearson Correlation								1	-0.229	0.518	$0.977^{*}$
Cr	Sig. (2-tailed)									0.771	0.482	0.023
<b>N</b> T <sup>1</sup>	Pearson Correlation									1	-0.951*	-0.411
Ni	Sig. (2-tailed)										0.049	0.589
	Pearson Correlation										1	0.669
As	Sig. (2-tailed)											0.331
р.	Pearson Correlation											1
Bi	Sig. (2-tailed)											

Correlation is significant at the 0.01 level (2-tailed). \*. Correlation is significant at the 0.05 level (2-tailed).

Another study reported that honey samples from Türkiye, Argentina, Nigeria and Pakistan were contaminated with heavy metals such as cadmium and arsenic. It was stated that this pollution may be due to the presence of industrial areas in the region. The results of studies conducted in Croatia and Kosovo showed that the lead content in honey samples was higher than the amount reported in other European countries, and it was emphasized that this situation was alarming.

It was stated that these findings indicate that honey bee colonies should be placed in areas away from roads and railways. In the study conducted in Nigeria, it was found that the amount of iron, copper, manganese, and zinc in honey samples was higher than the maximum permissible concentration and average concentration determined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). It was stated that the rate of heavy metals in industrial cities was higher than in rural areas.

According to the Turkish Food Codex Honey Communiqué, honey must not contain heavy metals in amounts that could pose a health risk (TSE) [15]. Therefore, the heavy metal concentrations in the analyzed honey samples are within acceptable levels as per current legal regulations and health standards.

The mineral content of honey varies not only with the flora of the region where it is produced but also with the conditions of the soil, water, and air. In areas with abundant vegetation, the mineral content of honey is typically higher. The concentrations of heavy metals in honey serve as significant indicators of environmental pollution, reflecting the levels of contaminants present in the surrounding soil, water, and air. Honeybees, through their foraging activities, accumulate these pollutants from various environmental sources, including industrial emissions, agricultural and runoff, atmospheric deposition [4,32]. Consequently, the heavy metal content in honey mirrors the extent of environmental contamination in the area where it is produced. It is thought that the fact that Yamadağ honey samples have higher heavy metal content than the honey samples of Battalgazi district is due to Yamadağ being a volcanic mountain.

Studies have demonstrated that honey can effectively reflect the levels of heavy metals in the environment. For instance, research conducted in the Black Sea region of Türkiye analyzed honey samples from various locations to determine heavy metal concentrations. The findings indicated that the levels of heavy metals in honey varied depending on the proximity to pollution sources, such as industrial areas and urban centers. This variability underscores the role of honey as a bioindicator of environmental pollution [33].

Furthermore, the mineral content of honey is influenced by the flora of the region where it is produced, as well as the conditions of the soil, water, and air. In areas with abundant vegetation, the mineral content of honey is typically higher [26].

Therefore, analyzing the heavy metal content in honey provides valuable insights into the environmental quality of the region, highlighting the interconnectedness of air, water, and soil pollution [4].

#### 4. Conclusion

In this study, some trace element and some heavy metal contents of honey from Yamadağ and Batttalgazi regions, which are at two different altitudes with rich floral characteristics in terms of honey production were compared. The approximately 20-fold higher iron content in Battalgazi honey compared to Yamadağ honey is particularly noteworthy. Similarly, the average zinc concentration in honey from the Yamadağ Mountain was found to be 0.62 mg/kg, while in honey from the Battalgazi Plateau, it was 1.30 mg/kg. In honey samples from the Yamadağ Mountain, the average copper concentration was found to be 0.04 mg/kg, whereas in the Battalgazi Plateau, it was 0.18 mg/kg. A. m. genotype from Yamadağ Mountain but below the detection limits in A. m. carnica genotype. Analyses of honey samples from Malatya's Yamadağ and Battalgazi regions have determined that the levels of these trace elements are well below toxic thresholds, indicating no significant health risk associated with their consumption.

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## **Declaration of competing interest**

The authors declare no competing interest.

#### **Ethical approval**

This study did not involve animal or human subjects.

# Author contributions

Semiramis Karlıdağ: Samples production, formol analyses, statistical analysis; Sevgi Kolaylı: Conception, writing.

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