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Assessment of trace element levels in Rhododendron honeys of Black Sea Region, Turkey

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Abstract

Rhododendron and multi-flower honeys obtained from Black Sea Region of Turkey (12 Rhododendron and 8 multi-flower honeys) were studied to determine the presence of the 14 trace elements such as Cu, Cd, Pb, Co, Cr, Ni, Al, Se, Zn, Mn, Fe, K, Ca and Mg. Trace element determination was performed by atomic absorption spectrometry (AAS) after microwave digestion. The results revealed that Rhododendron honeys exhibited higher concentrations of Cu, Co, Cr, Ni, Se, Zn, Ca and Mg but lower concentrations of Al, Mn, Fe and K than in the multi-flower honeys. Trace element levels in analyzed honey samples were generally lower than literature values.

Keywords: Rhododendron honey; Toxic honey; Trace element; Atomic absorption spectrometry; Turkey

1. Introduction

Rhododendron honey is produced by honeybees which collect nectar from the flowers of Rhododendron species. Rhododendron honey known as “mad honey” or “toxic honey” has been known since ancient times. In 400 B.C. Xenophon reported poisoning of Greek soldiers who had eaten honey made by bees from wild Rhododendrons [1]. Mad honey is produced from the nectar of *Rhododendron ponticum* growing on the mountains of the eastern Black Sea Region of Turkey and also in Japan, Nepal, Brazil and some parts of North America and Europe [2]. There are five species of rhododendron in Turkey, the most commonly found being *R. ponticum* and *R. flavum* [3]. The leaves, flowers, pollen and nectar of many *Rhododendron* species contain toxic diterpenoids (gryanotoxins), occurring only in Ericaceae plants [4]. Gryanotoxins are the main compound responsible for poisoning [5]. Toxic honey is used as an alternative medicine agent for the treatment of gastric pains, bowel disorders, and hypertension, and it is also believed to be a sexual stimulant in The Black Sea Region of Turkey [4]. Therefore, Rhododendron honey is marketed at a relatively high cost and is preferred for medical purposes by consumers in this region. As a foodstuff used for healing purposes, honey must be free of pollutants, such as heavy metals.

Honey is a useful bio-monitor for information related to the environment where the bees live. Since honey bees readily fly up to 4 km in all directions from their apiary and thus have access to an area of about 50 km^2 [6] and the bees come in contact not only with air but also with soil and water, the concentration of heavy metals in honey reflects their amount in the whole region [7]. Therefore, honey has been recognized as a biological indicator of environmental pollution [8]. But nevertheless determination of heavy metals in honey is of high interest mainly for quality control and nutritional aspect. High levels of metals are undesirable because of their known or supposed toxicity, so that, for instance, a limit of 1 mg kg^{-1} for lead is set in some countries [9]. Moreover, nectar of plants serves as potential source of exposure to metals such as lead, cadmium and copper which occur at various levels in the environment.

Trace element levels of honey from different regions of Turkey have been reported by many scientists [10–14]. Though there is no available data on the any unifloral honey produced in Turkey. Thus the work was initiated with the objectives to study the levels of the trace elements in the honeys from Black Sea

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Region of Turkey and to compile the trace elements data in an effort to characterize Rhododendron honeys.

2. Experimental

2.1. Samples

Honey samples from areas of Black Sea Region of Turkey where Rhododendrons grow naturally, obtained between the June and July in 2006, when the Rhododendrons were flowery. Other honey samples obtained from in the same area were multi-flower. Multi-flower honeys had not contained dominant and secondary pollen.

2.2. Analysis of sediment for the identification of honey samples

The 20 honey samples were classified according to their botanical origin using the method of Louveaux et al. [15]. Pollen grains were microscopically observed and compared with the reference slides for identification. Botanical classification was achieved when the pollen spectrum contained >45% of the corresponding dominant pollen. Pollen grains were identified and counted under the microscope.

2.3. Apparatus

A PerkinElmer AAnalyst 700 atomic absorption spectrometer (FAAS) equipped with HGA graphite furnace and with 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. Zn, Mn, Fe, K, Ca and Mg were determined in flame AAS. The other elements were determined in graphite furnace AAS. For graphite furnace measurements, argon was used as inert gas. The operating parameters for the working elements were set as recommended by the manufacturer. Pyrolytic-coated graphite tubes (PerkinElmer part no. B3 001264) with a platform were used. Samples were injected into the graphite furnace using PerkinElmer AS-800 auto sampler. High purity argon was used for GFAAS.

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

2.4. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionised 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 dilute 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^{-1} of each element supplied from Sigma. Standard reference material (NIST-SRM 1515 Apple leaves) was used.

2.5. 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 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 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, 8 min for 550 W, vent: 8 min, respectively.

2.6. Statistical analysis

The whole data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated trace element concentrations of the samples. Student's *t*-test was employed to estimate the significance of values.

3. Results and discussion

The recovery values were nearly quantitative (≥95%) for microwave digestion method. The relative standard deviations were less than 10% for all investigated elements. *t*-Test was used to determine significant differences between mean values ($p < 0.05$). The accuracy of the method was evaluated by means of trace element determination in standard reference material (SRM). The results are given in Table 1. The achieved results were in good agreement with certified values.

The results of the microscopic analysis of the sediment from the honeys samples are summarized in Table 2. Percentages are always referred to pollen from nectar plants Rhododendron spp. Pollen was always predominant (50.81–75.14%) in Rhodo-

Table 1
Trace metal concentrations in certified reference material (NIST SRM 1515 Apple leaves), $N = 4$

Element	Certified value (μg g^{-1})	Our value (μg g^{-1})	Recovery (%)
Cu	5.64	5.60 ± 0.20[*]	99
Cd	0.013	0.014 ± 0.001	108
Pb	0.47	0.45 ± 0.03	96
Co	0.09	0.089 ± 0.005	99
Cr	(0.3)[a]	0.31 ± 0.02	103
Ni	0.91	0.88 ± 0.06	97
Al	286	280 ± 15	98
Se	0.05	0.048 ± 0.002	96
Zn	12.5	12.7 ± 0.8	102
Mn	54	53.2 ± 2.5	99
Fe	(83)	81.7 ± 4.4	98
K	16,100	15,800 ± 500	98
Ca	15,260	14,790 ± 350	97
Mg	2,710	2,650 ± 120	98

[a] The values in the parentheses are not certified.
[*] Mean expressed as 95% tolerance limit.

Table 2
Geographical and botanical origin of honey samples

Sample no.	Geographical origin	Botanical origin	Rhododendron pollen frequency (%)
1	Artvin	Rhododendron honey	52.25
2	Trabzon	Rhododendron honey	52.58
3	Artvin	Rhododendron honey	50.81
4	Hopa	Rhododendron honey	53.01
5	Hopa	Rhododendron honey	61.90
6	Trabzon	Rhododendron honey	65.35
7	Artvin	Rhododendron honey	63.29
8	Karaduz	Rhododendron honey	57.70
9	Artvin	Rhododendron honey	75.14
10	Trabzon	Rhododendron honey	53.38
11	Zonguldak	Rhododendron honey	74.37
12	Trabzon	Rhododendron honey	52.90
13	Ordu	Multi-flower honey	10.11
14	Ordu	Multi-flower honey	11.45
15	Giresun	Multi-flower honey	10.18
16	Giresun	Multi-flower honey	9.22
17	Rize	Multi-flower honey	5.13
18	Rize	Multi-flower honey	8.40
19	Rize	Multi-flower honey	13.25
20	Ordu	Multi-flower honey	9.8

dendron honeys according to the reported over representative presence of this pollen type.

Mean trace element contents are shown in Tables 3 and 4. In the analysis of trace element contents, 14 trace elements were identified and quantified: copper, cadmium, lead, cobalt, chromium, nickel, aluminum, selenium, zinc, manganese, iron, potassium, calcium and magnesium. Some literature values about the honey samples around the world were given in Table 5.

Aluminum (Al) ranges between 4.41 and 703 $\mu g\,kg^{-1}$ in honeys from Black Sea Region of Turkey. Multi-flower honeys contained higher level of aluminum than Rhododendron honeys (47.8–644 $\mu g\,kg^{-1}$). Honeys from Black Sea Region had higher Al content than that reported from different regions of Turkey [14]. This may be sourced from beekeeping equipments during processing of honeys as aluminum extractor and container. However, aluminum content in Chilean honey was found to be 0.04–22.06 $mg\,kg^{-1}$ higher than our study [16].

Copper, cadmium, lead, cobalt, chromium, nickel, aluminium and selenium in the Rhododendron honeys are ranging between 9.75 and 35.8, 0.28 and 2.37, 1.51 and 55.3, 1.44 and 28.5, 1.57

Table 3
Cu, Cd, Pb, Co, Cr, Ni, Al and Se contents in microwave digested honey samples ($\mu g\,kg^{-1}$), $N = 3$

Sample	Cu	Cd	Pb	Co	Cr	Ni	Al	Se
RH-1[a]	18.3 ± 1.3[*]	0.84 ± 0.07	5.30 ± 0.42	3.15 ± 0.32	3.53 ± 0.30	7.80 ± 0.60	307 ± 21.1	14.1 ± 1.1
RH-2	23.9 ± 2.1	1.11 ± 0.10	15.6 ± 1.12	8.13 ± 0.70	12.9 ± 0.11	45.8 ± 3.6	353 ± 21.2	141 ± 12.1
RH-3	15.4 ± 1.2	1.24 ± 0.10	16.0 ± 1.02	3.01 ± 0.20	2.97 ± 0.20	1.35 ± 0.10	235 ± 18.4	188 ± 12.8
RH-4	15.0 ± 1.2	1.19 ± 0.10	51.7 ± 4.21	1.44 ± 0.10	10.2 ± 0.90	2.20 ± 0.20	588 ± 38.5	62.5 ± 4.8
RH-5	10.5 ± 0.8	0.66 ± 0.05	7.21 ± 0.43	4.21 ± 0.32	4.03 ± 0.13	16.5 ± 1.2	334 ± 24.8	92.3 ± 8.7
RH-6	19.4 ± 1.7	0.65 ± 0.04	22.1 ± 1.32	4.99 ± 0.41	5.56 ± 0.42	56.4 ± 3.6	400 ± 28.2	91.2 ± 7.8
RH-7	11.4 ± 1.0	0.28 ± 0.02	2.83 ± 0.13	4.87 ± 0.42	2.48 ± 0.20	1.67 ± 0.10	118 ± 10.1	20.8 ± 2.0
RH-8	17.3 ± 1.4	1.51 ± 0.10	55.3 ± 4.73	28.5 ± 2.10	3.74 ± 0.30	26.2 ± 0.2	240 ± 21.2	176 ± 13.4
RH-9	18.9 ± 1.6	2.37 ± 0.20	32.0 ± 2.08	7.09 ± 0.60	3.23 ± 0.32	24.8 ± 1.1	644 ± 45.4	93.6 ± 7.6
RH-10	11.2 ± 0.5	1.56 ± 0.10	16.3 ± 1.46	5.95 ± 0.50	4.31 ± 0.40	14.3 ± 1.2	47.8 ± 3.9	102 ± 9.8
RH-11	35.8 ± 3.1	0.28 ± 0.02	1.51 ± 0.11	21.3 ± 1.20	1.57 ± 0.10	131 ± 12.1	349 ± 30.2	56.0 ± 4.9
RH-12	9.75 ± 0.7	0.41 ± 0.03	8.55 ± 0.57	2.28 ± 0.23	2.23 ± 0.20	7.64 ± 0.50	57.9 ± 4.4	323 ± 23.4
MH-13[b]	9.97 ± 0.7	0.38 ± 0.03	3.20 ± 0.23	5.14 ± 0.42	1.41 ± 0.10	1.21 ± 0.10	18.3 ± 1.7	114 ± 9.8
MH-14	15.2 ± 1.1	2.03 ± 0.20	17.1 ± 1.24	2.41 ± 0.20	2.67 ± 0.20	21.7 ± 1.7	262 ± 22.9	97.8 ± 7.8
MH-15	13.8 ± 1.2	0.29 ± 0.02	1.54 ± 0.11	2.60 ± 0.20	1.91 ± 0.11	8.26 ± 0.70	53.2 ± 4.5	159 ± 12.1
MH-16	23.0 ± 1.7	1.80 ± 0.10	5.74 ± 0.47	3.06 ± 0.21	1.51 ± 0.10	2.32 ± 0.22	154 ± 13.9	129 ± 10.1
MH-17	24.4 ± 1.9	1.06 ± 0.10	3.96 ± 0.26	2.77 ± 0.20	1.73 ± 0.10	12.7 ± 1.01	122 ± 11.2	146 ± 11.8
MH-18	23.3 ± 1.8	0.84 ± 0.06	36.7 ± 2.37	1.25 ± 0.10	1.68 ± 0.11	14.2 ± 1.2	703 ± 45.6	269 ± 21.1
MH-19	29.5 ± 2.1	0.57 ± 0.04	6.21 ± 0.29	2.26 ± 0.21	1.24 ± 0.10	41.1 ± 3.2	249 ± 17.4	94.2 ± 8.6
MH-20	14.5 ± 0.9	0.86 ± 0.07	20.4 ± 1.75	7.98 ± 0.60	1.92 ± 0.10	6.72 ± 0.50	4.41 ± 0.30	124 ± 10.1

[a] RH: Rhododendron honey.
[b] MH: Multi-flower honey.
[*] Mean ± standard deviations.

Table 4
Zn, Mn, Fe, K, Ca and Mg contents in microwave digested honey samples ($\mu g\,g^{-1}$), $N = 3$

Sample	Zn	Mn	Fe	K	Ca	Mg
RH-1[a]	6.57 ± 0.61[*]	7.05 ± 0.60	12.8 ± 1.0	6029 ± 450	232 ± 22.1	35.8 ± 2.6
RH-2	1.29 ± 0.12	1.66 ± 0.10	2.55 ± 0.22	2386 ± 167	27.8 ± 2.1	30.3 ± 2.1
RH-3	1.22 ± 0.12	5.88 ± 0.50	1.44 ± 0.10	2765 ± 234	25.4 ± 2.2	41.6 ± 3.1
RH-4	1.26 ± 0.11	4.96 ± 0.43	1.15 ± 0.10	1859 ± 156	16.3 ± 1.4	34.4 ± 2.7
RH-5	0.72 ± 0.04	2.81 ± 0.22	1.87 ± 0.10	1809 ± 167	15.2 ± 1.2	40.0 ± 2.9
RH-6	0.82 ± 0.03	4.80 ± 0.41	1.39 ± 0.10	4540 ± 342	60.2 ± 4.5	67.5 ± 4.8
RH-7	0.47 ± 0.02	1.21 ± 0.10	1.12 ± 0.10	2092 ± 178	34.2 ± 2.1	42.3 ± 3.2
RH-8	3.15 ± 0.20	1.80 ± 0.10	3.79 ± 0.22	4344 ± 345	31.9 ± 3.0	39.1 ± 2.4
RH-9	4.32 ± 0.30	1.47 ± 0.10	2.37 ± 0.21	699 ± 56.9	22.7 ± 2.1	25.5 ± 2.1
RH-10	1.28 ± 0.10	2.09 ± 0.21	2.16 ± 0.11	1613 ± 145	3.28 ± 0.9	32.1 ± 2.5
RH-11	0.94 ± 0.07	33.0 ± 2.12	1.85 ± 0.12	5430 ± 454	86.1 ± 7.6	42.4 ± 3.4
RH-12	1.02 ± 0.10	3.04 ± 0.21	2.28 ± 0.20	1623 ± 134	47.7 ± 3.8	34.2 ± 2.4
MH-13[b]	0.65 ± 0.03	1.11 ± 0.12	2.82 ± 0.20	3773 ± 323	8.04 ± 0.7	23.5 ± 2.1
MH-14	6.27 ± 0.50	2.31 ± 0.10	1.61 ± 0.10	2393 ± 178	31.5 ± 2.9	24.2 ± 1.8
MH-15	0.71 ± 0.05	7.71 ± 0.70	1.55 ± 0.10	2465 ± 189	44.9 ± 3.9	27.1 ± 2.1
MH-16	0.76 ± 0.06	19.6 ± 1.40	1.90 ± 0.11	3140 ± 250	168 ± 14.8	48.7 ± 3.7
MH-17	0.55 ± 0.04	61.0 ± 3.72	2.17 ± 0.21	4580 ± 387	176 ± 14.9	57.2 ± 4.5
MH-18	0.84 ± 0.06	74.2 ± 4.30	2.10 ± 0.22	7030 ± 568	197 ± 16.4	65.8 ± 5.3
MH-19	1.01 ± 0.10	19.8 ± 1.20	1.91 ± 0.11	7340 ± 678	71.9 ± 5.6	54.1 ± 3.8
MH-20	3.20 ± 0.21	3.22 ± 0.20	12.9 ± 1.10	350 ± 29.7	15.6 ± 1.2	21.9 ± 2.0

[a] RH: Rhododendron honey.
[b] MH: Multi-flower honey.
[*] Mean ± standard deviations.

and 12.9, 1.35 and 131, 47.8 and 644, 14.1 and 323 $\mu g\,kg^{-1}$, respectively. The levels of these elements in the Rhododendron honeys are higher amounts than multi-flower honeys in this region. The mean copper level in the Rhododendron and multi-flower honeys from Black Sea Region are lower than that of reported by Tuzen and Soylak [17], Erbilir and Erdogrul [13], Tuzen et al. [14] for another regions of Turkey. Average values for copper were also lower than those found in the literature for honey samples from India [18], Chile [16], Spain [19] and Greece [7]. The cadmium levels were between 0.38 and 2.03 $\mu g\,kg^{-1}$ for multi-flower honeys from Black Sea Region. The cadmium levels of the honey samples are lower than that reported by Erbilir and Erdogrul [13] for honey samples from Kahramanmaras region and by Tuzen [11] from Tokat region in Turkey. Similarly, the mean value of cadmium content was lower than in previous reports for honeys from Poland [8], Greece [7] and Chile regions [16]. The minimum and maximum lead levels observed were 1.51–55.3 $\mu g\,kg^{-1}$ in Rhododendron honeys and 1.54–36.7 $\mu g\,kg^{-1}$ for multi-flower honeys. Lead values in the literature have been reported as 5.23–9.82 $\mu g\,kg^{-1}$ for honey samples from Middle Anatolia [17] and 30.3–58.6 $\mu g\,kg^{-1}$ from Tokat region [11]. Lead content is lower than that reported by Przybylowski and Wilczynska [8], Rashed and Soltan [20], and Fredes and Montenegro [16]. The average recommended daily intake in foods is reported to be 12–15 $mg\,day^{-1}$ for zinc, 30 $mg\,day^{-1}$ for copper and 60 $mg\,day^{-1}$ for cadmium [21,22].

The lower and higher cobalt concentrations were found as 1.44–28.5 and 1.25–7.98 $\mu g\,kg^{-1}$ for Rhododendron and multi-flower honeys, respectively. The mean of cobalt levels of the Black Sea Region honeys are lower than that reported by Ioannidou et al. [7], Fredes and Montenegro [16], and Rashed and Soltan [20].

Concentrations in honey for the other trace elements ranged from 1.57 to 12.9, 1.33 to 56.4, 14.1 to 323 $\mu g\,kg^{-1}$ in Rhododendron honeys, 1.24 to 2.67, 1.21 to 41.1, 26.4 to 94.2 $\mu g\,g^{-1}$ in multi-flower honeys for chromium, nickel and selenium, respectively. The chromium levels of our samples are lower than that reported by Przybylowski and Wilczynska [8] for honey samples from Poland and by Tuzen et al. [14] for honeys from different regions of Turkey. Contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can generate high Cr content, due to corrosive effect of honey acidity [8]. Some scientists were not detected Ni in their honey samples from Kahramanmaras city in Turkey and Greece [7,13]. Nickel content in honeys from different region was found to be 2.6–29.9 $\mu g\,kg^{-1}$ [14]. The selenium levels of our samples were lower than that of the reported by Tuzen et al. [14].

It can bee seen that Rhododendron honeys generally have higher trace element contents than those reported from the multi-flower honey samples in the other regions of Turkey. However, concentrations of analyzed trace element generally lower than previously reported studies in other countries.

The results showed that Zn, Ca and Mg were the most abundant of the major elements in Rhododendron honeys with average concentrations of Zn, 0.47–6.57 $\mu g\,g^{-1}$; Ca 3.28–232 $\mu g\,g^{-1}$ and Mg 25.5–67.5 $\mu g\,g^{-1}$. The other elements as Mn, Fe and K were presented in lower concentrations than multi-flower honeys obtained from in the same region. The average value for zinc was almost the same as found Poland Przybylowski and Wilczynska [8]. The Zn level was slightly higher than those found in the literature for Tokat region [11], Chilean [16] and Lazio region honeys [23]. However, zinc levels of Rhododendron honeys was lower than Moracco [24], Indian [18] and Poland honeys [8]. Nanda et al. [18] revealed that Trifolium honey was the best source of calcium with an average

Table 5
Literature and our values of trace elements in honey samples

Elements	Literature values			Our values	
	mg kg^{-1}	μg g^{-1}	μg kg^{-1}	μg g^{-1}	μg kg^{-1}
Cu	0.06–4.32 [16], 0.25–1.30 [11], 0.8–2.4 [19], 0.0–0.09 [13], 1.74–2.9 [18], <1 [24], 0.14–0.48 [7], 0.30–1.45 [12]	1–1.75 [20], 0.31 [23], 0.25–1.10 [17], 0.23–2.41 [14]	–	–	9.75–35.8
Cd	0.31–0.34 [13], 0.01–0.05 [16], 0.078–0.222 [7], 0.008–0.027 [8]	0.01–0.5 [20]	0.9–17.9 [14], 5.45–10.76 [12], 10.9–21.2 [17], 5.23–9.82 [11]	–	0.28–2.37
Pb	0.01–0.11 [16], 0.025–0.071 [8]	4.2–9.3 [20]	8.4–105.8 [14], 17.6–32.1 [17], 39.30–64.50 [12], 30.3–58.6 [11]	–	1.51–55.3
Cr	0.03–1.98 [16]	–	2.4–37.9 [14]	–	1.24–12.9
Ni	0.01–1.48 [16], did not found [7,13]	1.25–4.1 [20]	2.6–29.9 [14]	–	1.21–131
Zn	0.8–2.4 [19], 1.15–4.95 [11], 4.17–22.3 [8], 2.5–16.77 [18], 0.01–4.93 [16], <1 [24], 1.50–5.30 [12]	3.14 [23], 1.1–24.2 [17], 7.0–9.3 [20]	1.1–12.7 [14]	0.47–6.57	–
Co	0.3–1.45 [12], 0.01–0.62 [16], 0.010–0.087 [7], 0.010–0.131 [7]	1.75–3.2 [20]	–	–	1.25–28.5
Al	0.04–22.06 [16], 163.3–304.31 [18]	–	83–325 [14]	–	4.41–703
Fe	0.04–1.21 [13], 8.86–13.25 [18], 1.33–34.97 [24], 3.45–8.94 [11], 0.1–7.66 [16], 3.20–7.63 [12]	58–3690 [20], 4.51 [23], 1.8–10.2 [14], 1.0–5.2 [17]	–	1.12–12.9	–
Mg	2.03–111.36 [13], 17–52 [11], 14–48 [12]	102–1325 [20], 37 [23]	–	21.9–67.5	–
K	270–1800 [11], 13.98–2388 [24], 489.52–932.56 [18], 400–1500 [12]	213–15.500 [20], 472 [23]	–	350–7340	–
Mn	0.0–0.09 [13], 0.32–0.70 [11], 0.01–6.97 [16], 0.11–0.20 [7], 0.38–0.85 [12]	3 [23], 0.32–4.56 [14], 0.5–5.7 [20], 0.18–1.21 [17]	–	1.11–61.0	–
Ca	50–250 [17], 32.6–84.63 [18], 50–300 [12]	47.7 [23]	–	3.28–232	–
Se	–	–	38–113 [14]	–	14.1–323

content of 84.63 mg kg^{-1}, while the citrus honey had the lowest value for calcium. Calcium content of Rhododendron honey was found to be higher than that reported by Nanda et al. [18] for Trifolium, *Helianthus anuus*, *Eucaylptus lanceolatus*, *Brassica campestris* honeys. Rhododendron honey contained higher level of magnesium than honeys from Lazio region [23] and Tokat city in Turkey [11]. However, honeys analyzed contained lower level Mg than reported in literature [13,20]. The presence of these elements in Rhododendron honey in high concentrations was due to botanical origin, namely elemental nectar composition. Similarly, Rashed and Soltan [20] revealed that honeys contained different element concentrations depended highly on the type of flowers (sesame, clover and orange) utilized by bee. For instance, in their study clover honey contained higher levels of Cd, Cu, Ni, Sr and Zn and orange honey contained higher level of iodine than in the other honeys. Also, these concentrations correlated with flower elemental concentrations [25]. El-Sherbiny and Rizk [26] stated that cotton honey contained higher element concentration than clover honey.

In this work, Mn, Fe and K elements were found higher concentrations in Rhododendron honeys. The minimum and maximum Mn levels observed were 1.11–74.2 μg g^{-1}. Manganese content is higher than that previously reported both Turkish honeys [11,13,14] and Greece and Lazio honeys [7,23]. Iron concentrations in honey were ranged from 1.55 to 12.9 μg g^{-1}. The Fe content is higher than that reported Turkish honeys [13,14] and Poland and Italy honeys [8,23] but lower than that reported by Terrab et al. [24] and Nanda et al. [18] for Moracco and Indian honeys. Mean values of potassium content varied between 2393 and 7340 μg g^{-1} in multi-flower honeys from Black Sea Region of Turkey. The potassium level is higher than previously reported [11,12,23–26]. Rodriguez-Otero et al. [27] reported potassium was the most abundant element with an average content of 1500 mg kg^{-1} in Galicia honey.

As a result, trace element levels of Rhododendron honeys almost were higher than multi-flower honey obtained in the same region. Rodriguez-Otero et al. [27] stated that the mineral content and trace element in honey samples could give an indication of the geographical origin of honey. But it is not adequate explanation for differentiation of trace element content of our honeys obtained from in the same area. Differences in trace and mineral content are as owing to the different botanical origins of honeys. However, other factors such as geographical conditions are also expected to affect the mineral content.

Therefore, it is important to take in account not only the botanical origin of honey but also the floral density, season of the year and rainfall among others. Moreover, type and quality of equipment used to store honey after harvesting, processing and/or preparation of honey can generate high Cr content. Likewise, storing honey in galvanized or aluminum containers can be source of Zn and Al contamination. In addition element content of individual nectar can contribute to this abundance.

4. Conclusion

Trace elements play important negative and positive roles in human life [28–31]. Honey is an important source of trace elements for human. Many factors are effective in element contents of honey as geographical and botanical origin, soil, atmosphere, beekeeping equipments, element content of nectar, season of the year, and rainfall among the others. Analyzed Rhododendron honeys contained higher quantities of Cu, Co, Cr, Ni, Se, Zn, Ca and Mg levels than multi-flower honeys. Therefore, this composition can characterize the Rhododendron honey. Rhododendron honey samples should be analyzed more often in Black Sea Region of Turkey with respect to toxic metals.

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