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Mineral Composition and Heavy Metal Contents of Chestnut Honey Collected from Kastamonu Region

Kastamonu Bölgesinden Toplanan Kestane Balının Mineral Madde ve Ağır Metal İçerikleri

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Abstract

Chestnut honey, which is known for its apitherapic properties besides its nutritional attributes, is one of the main groups of monofloral honey types. Kastamonu is the most important province because where constitutes two-thirds of the chestnut forest flora of the Black Sea region. Kastamonu Chestnut Honey, produced in apiaries within the chestnut forests, has been registered as a geographical indication and designation of origin. The nutritional value of honey is related to its nutritional and chemical content. Kastamonu chestnut forests have a different distribution with their scattered structure from the coastline to 1000-1200 meters altitude. Therefore, this study was planned with the prediction that chestnut honey produced in different districts has a different composition. The aim of the study is to contribute to official regulations regarding the product, product standardization, and national branding studies by determining mineral and heavy metal contents based on the region where the chestnut honey is produced. As a result of the study, the main concentrations of the elements in the chestnut honey samples were affected by the geographical location, because the apiaries where honey samples were collected in chestnut forests in Kastamonu districts had different altitudes, climates and soil properties, and different secondary flora. The most abundant elements in the samples were potassium (1410.0-6581.5 ppm) sodium (0-204.4 ppm), calcium (125.3-287.5 ppm), and magnesium (24.73-61.88 ppm). Iron (1.32-9.54 ppm), zinc (1.34-20.84 ppm), and manganese (1.39-18.69 ppm) were also found in moderate and others in trace levels. Because manganese is found at moderate levels in all chestnut honey samples and it is found at higher levels than other honey types, it can be recommended that Mn, as fingerprints to detect imitation and adulteration of chestnut honey. While Cd, a heavy metal, was found in only one sample, Ni was not detected in any sample. Whereas Pb concentration varied between 1.84 ppm and 3.38 ppm for the samples, the contents of Al concentrations were quite high (2.94-13.94 ppm). Because there wasn't a maximum limit for heavy metal contents of honey types in the Turkish Food Codex Contaminants Regulation, no evaluation could have been made regarding the suitability of these heavy metal contents. It is thought that heavy metal contamination limits in honey types should be included in the regulations and especially secondary sources such as metallic containers used for storage during handling processes and harvesting of honey for heavy metal contamination should be reviewed.

Keywords: Chestnut honey, Mineral content, Heavy metal, Food security

Özet

Besleyici özelliklerinin yanı sıra apiterapik özellikleriyle de bilinen kestane balı, monofloral bal türlerinin ana gruplarından biridir. Kastamonu, Karadeniz bölgesinin kestane ormanı florasının üçte ikisini oluşturması nedeniyle en önemli kestane balı üretimi açısından önemli bir lokasyondur. Kestane ormanları içerisindeki arılıklarda üretilen Kastamonu Kestane Balı, coğrafi menşe işareti ile tescillenmiştir. Balın besin değeri, besinsel ve kimyasal içeriğiyle ilgilidir. Kastamonu kestane ormanları kıyı şeridinden 1000-1200 metre yüksekliğe kadar dağınık yapısıyla farklı bir dağılıma sahiptir. Bu nedenle farklı ilçelerde üretilen kestane balının farklı bileşime sahip olduğu öngörüsüyle bu çalışma planlanmıştır. Çalışmanın amacı kestane balının üretildiği bölgeye göre mineral ve ağır metal içeriklerini belirleyerek ürüne ilişkin resmi düzenlemelere, ürün standardizasyonuna ve ulusal markalaşma çalışmalarına katkı sağlamaktır. Çalışma sonucunda Kastamonu ilçelerindeki kestane ormanlarından bal örneklerinin toplandığı arılıkların farklı rakım, iklim ve toprak özelliklerine sahip olması nedeniyle kestane balı numunelerindeki elementlerin ana konsantrasyonlarının coğrafi konumdan etkilendiği ortaya çıkmıştır. Numunelerde en yüksek bulunan elementler potasyum (1410.0-6581.5 ppm), sodyum (0-204.4 ppm), kalsiyum (125.3-287.5 ppm) ve magnezyum (24.73-61.88 ppm) olarak belirlendi. Demir(1.32-9.54 ppm), çinko(1.34-20.84 ppm) ve manganez (1.39-18.69 ppm) orta düzeyde, diğerleri ise eser düzeylerde bulundu. Manganezin tüm kestane balı örneklerinde orta düzeyde ve diğer bal türlerine göre de daha yüksek düzeyde bulunması nedeniyle kestane balında taklit ve tağşişin tespit edilmesi amacıyla Mn'nin parmak izi olarak kullanılması önerilebilir. Ağır metallerden Cd yalnızca bir örnekte bulunurken, Ni hiç bir örnekte tespit edilmemiştir. Örneklerde Pb konsantrasyonu 1.84 ppm ile 3.38 ppm arasında değişirken Al konsantrasyonu oldukça yüksekti (2.94-13.94 ppm). Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde bal türlerinin ağır metal içerikleri için üst sınır bulunmadığından bu ağır metal içeriklerinin uygunluğu konusunda bir değerlendirme yapılamamıştır. Bal türlerinde ağır metal kontaminasyon limitlerinin mevzuata dahil edilmesi, özellikle ağır metal kontaminasyonunun bal hasadı ve depolama gibi proseslerde kullanılan metalik kaplar gibi ikincil kaynakların gözden geçirilmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Kestane balı, Mineral madde içeriği, Ağır metal, Gıda güvenliği

Abbreviations: TURKSTAT,Turkish Statistical Institute

1. INTRODUCTION

Turkey has a very rich genetic diversity in terms of secretion source trees such as pine and fir, which are considered important nectar sources by bees, and forest trees such as acacia, linden, maple and chestnut. This diversity has not been found even in countries with high honey yield (Kumova & Korkmaz, 2005). In addition, the climatic conditions and plant diversity of the seven regions of our country are different. Therefore, Turkey is very rich in bee genetic resources due to its diverse climate pattern. Scientific studies indicate that there are five different bee races (*Apis mellifera anatoliaca, Apis mellifera meda, Apis mellifera caucasica, Apis mellifera syriaca, Apis mellifera carnica*) in Turkey (Kandemir et al., 2006; Kukrer & Bilgin, 2020; Yıldız & Karabağ, 2022). Moreover, the local ecotypes registered by the Ministry of Agriculture and Forestry are the Caucasian and Anatolian Bee races, as well as the Efe, Gökçeada, Trakya, Hatay and Yığılca honey bee ecotypes (Anonymous, 2020). This situation increases the diversity of monofloral secretion honey produced in our country as well as polyfloral flower honey.

While the nutritional content, flavor and aroma of honey are generally determined by pollen and nectar sources in the environment, processing conditions, the equipment used from the hive to the consumer, and environmental factors are also effective in its physicochemical qualities. The nutritional, physicochemical, bioactive and apitherapeutic qualities of mono and polyfloral honey with various pollen contents collected from different locations also vary, and there are many studies in the literature on these issues (Cunningham et al., 2022; Habryka et al., 2021; Moldakhmetova et al., 2023). However, the number of studies on the mineral compositions and especially heavy metal contamination of honey is quite low (Bilandžić et al., 2019; Frazzoli et al., 2007; Güneş, 2021; Kanbur et al., 2021; Pavlin et al., 2023).

The mineral content of honey varies depending on the nectar yield of the plants and pollen composition. The main concentrations of elements in honey are also affected by the soil, air, water, etc. so the composition and content of elements in honey are affected by the geographical origin of the honey. The most abundant mineral in honey is potassium (45-85% of the total mineral content). Other major elements are sodium, calcium, and magnesium. Copper, iron, zinc, and manganese are found in moderate levels. Honey also contains lower levels (<1 µg/g) of trace elements (Kılıç Altun et al., 2017; Sager, 2017; Solayman et al., 2016). Since chestnut honey is rich in minerals, the composition of the honey varies according to its monofloral character, and the higher the chestnut nectar level of the honey, the higher its biological value (Rodriguez-Flores et al., Taş-Küçükaydın et al., 2023). The proximity of industrial zones, urbanization status, exhaust gases, asphalt resources in the environment (such as main roads close to the apiary), and the equipment used in the process of delivering honey to the consumer may also affect the variety and level of heavy metals that may contaminate honey (Bosancic et al., 2020; Manouchehri et al., 2021).

Chestnut honey, which has distinctive features in a certain geography, is one of the main groups of monofloral honey types. It can be distinguished more easily from other kinds of honey, especially due to its taste, color and aroma (Hendek Ertop & Atasoy, 2018). It is known for apitherapy properties besides the nutritional attributes such as high antioxidant activity, B and C vitamin contents. In the Black Sea region, Kastamonu is the most important province for chestnut honey production because of that Kastamonu province constitutes two-thirds of the chestnut forest flora of the Black Sea region.

Chestnut forests in Kastamonu, where apiaries where chestnut honey are produced, have a different distribution with their scattered structure from the coastline to 1000-1200 meters altitude. As well as the climatic characteristics of these locations, their secondary flora, which is a source of pollen and nectar except chestnuts, also differs. Therefore, this study was planned with the prediction that chestnut honey produced in different districts has a different composition. Some mineral and heavy metal contents of chestnut honey obtained from 15 different locations in Kastamonu province were examined. It aims to contribute to official regulations regarding the product, product standardization, and national branding studies by determining mineral and heavy metal contents based on the region where the honey is produced.

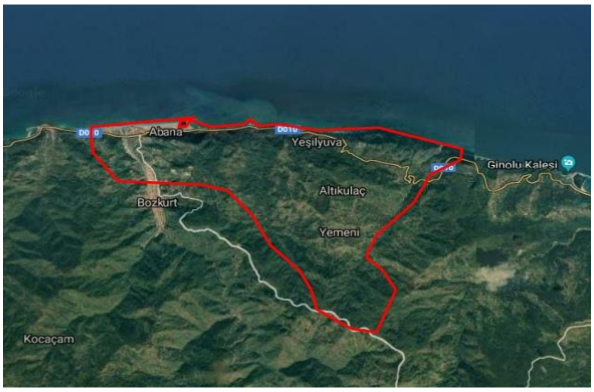
2. MATERIALS and METHODS

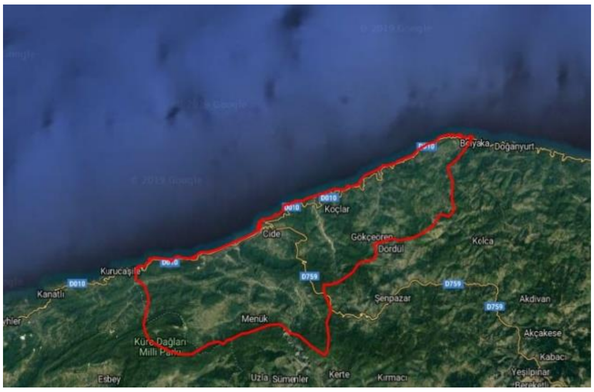
2.1. Material

The chestnut honey samples used in the study were collected in 15 production locations in Abana, Bozkurt, Doğanyurt, Cide, Küre, and İnebolu Districts, which are stated in Table 1. These Districts are located in the area of İnebolu and Küre Forest Enterprise Directorate. 50% of Turkey's chestnut production is provided by the chestnut forests located in the İnebolu Forest Enterprise area (Anonymous, 2018). The research area is based on the Greenwich prime meridian; It is located between 32°04'55"-34°13'02" eastern longitudes and 41°04'07"-41°57'08" northern latitudes. In this area, chestnut forests have a scattered distribution, starting from the coastline and reaching an altitude of 1600 m. As the material supply area, 15 different chestnut honey production locations in Abana, Bozkurt, Doğanyurt, Cide, Küre and İnebolu Districts, which are located in the İnebolu and Küre Forest Enterprise Directorate area and have dense chestnut forests, were determined. The districts where the samples were obtained were identified as CBC (Geographical Information). Map images taken from the database are shown in Figure 1.

Table 1. Code and locations collected of the honey samples

Code	Location	Village
A	Abana	Akçam
B1	Bozkurt1	Darsu
B2	Bozkurt2	Işığan
B3	Bozkurt3	Günvakti
C1	Cide1	Kovanören
C2	Cide2	Başköy
C3	Cide3	Ağaçbükü
C4	Cide4	Kayaardı
D1	Doğanyurt1	Yukarı Mescit
D2	Doğanyurt2	Gözalan
İ1	İnebolu1	Yuvacık
İ2	İnebolu2	Gökbel
K1	Küre1	İkizciler
K2	Küre2	Uzunöz
K3	Küre3	Başören





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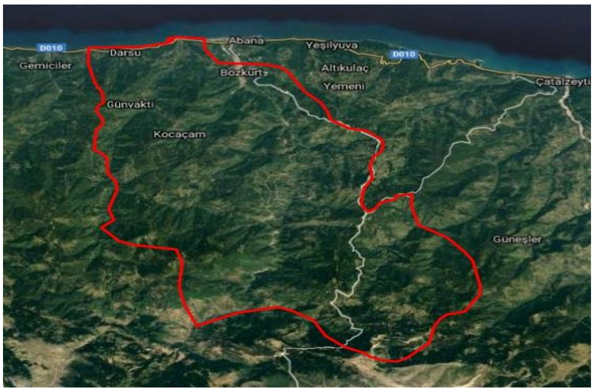

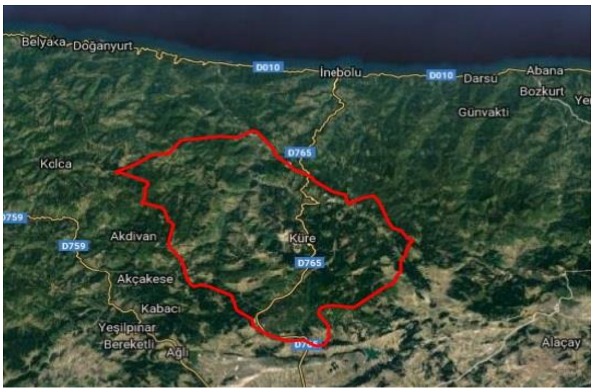

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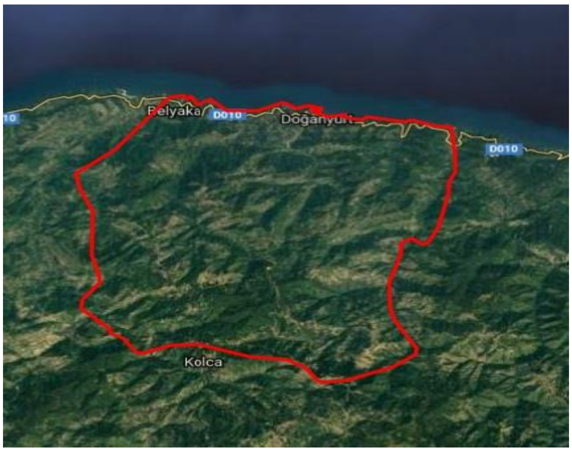

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Figure 1. Geographical regions where chestnut honey samples were obtained (a: Abana; b: Bozkurt, c: Cide, d: Küre, e: İnebolu, f: Doğanyurt)

2.2. Mineral Content

The mineral content was measured by using the microwave (CEM MARS6, USA) nitric acid digestion procedure followed by the inductively coupled plasma-optical emission spectrometry (ICP-OES) (SpectroBlue, Germany) method.

The sample (approximately 1 mL) was transferred directly to PTFE flasks after adding 10 mL of HNO_3 (67% v/v) and 1 mL H_2O_2 (30% v/v) and then subjected to the following digestion program: the temperature was raised to 200 °C (15 min) and kept at 200 °C for 15 min. After cooling at room temperature, sample solutions were transferred into 50 mL polyethylene flasks and the volume was completed with ultrapure water. The digested samples were filtered through microfilters and analyzed with ICP-OES (Al Khalifa & Ahmad, 2010). Multi-element standard stock solution (Merck, Germany) provided for ICP-OES was used in the preparation of calibration standards. The measurements were carried out in triplicate, and the dilution factor was taken into consideration in the calculations.

2.3. Statistical Analysis

Statistical evaluation of the analysis results was made using the SPSS 17.0.1 software (SPSS Inc., Chicago, Illinois, US).

The difference between the data averages subjected to analysis of variance (ANOVA) was determined by performing the Tukey multiple comparison test at the $p<0.05$ significance level.

3. RESULTS and DISCUSSION

Within the scope of the study, Sodium (Na), calcium (Ca), potassium (K) and magnesium (Mg) concentrations as the major elements of honey samples obtained from 15 different locations were determined and the average values on a location basis (Table 2).

Table 2. Content of major elements of the samples

Sample code	Na (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)
A	56.03^{f}	173.7^{f}	**1410.0^{l}**	41.75^{d}
B1	nd	141.5^{h}	3041.4^{k}	**24.73^{j}**
B2	84.30^{d}	225.7^{c}	4172.5^{g}	46.31^{b}
B3	115.00^{b}	180.7^{f}	3405.7^{j}	29.12^{i}
C1	32.33^{h}	198.2^{e}	4000.9^{h}	32.32^{h}
C2	13.13^{i}	236.0^{b}	4778.4^{e}	35.14^{g}
C3	84.50^{d}	225.0^{c}	4846.2^{de}	37.23^{ef}
C4	58.73^{ef}	213.8^{d}	5821.6^{b}	43.74^{c}
D1	**204.4^{a}**	**125.3^{i}**	3368.5^{j}	28.89^{i}
D2	98.00^{c}	197.2^{e}	4726.0^{ef}	36.68^{f}
İ1	61.50^{e}	**287.5^{a}**	4935.3^{d}	**61.88^{a}**
İ2	4.70^{j}	142.7^{h}	4607.9^{f}	34.82^{g}
K1	**3.90^{j}**	145.6^{h}	5664.8^{c}	38.52^{e}
K2	39.83^{g}	182.3^{f}	**6581.5^{a}**	42.44^{cd}
K3	61.03^{ef}	155.7^{g}	3568.6^{i}	31.53^{h}

*nd: Non determined

** a–l: Means with different superscripts in the same column are significantly different ($p < 0.05$).

The difference between the major element contents of chestnut honey samples between the locations was found to be statistically significant (p<0.05). It is clear that K is the most abundant mineral in all honey samples, followed by Ca, Na, and Mg as the second, third and fourth major minerals, respectively. In line with most of the previous studies (Chua et al., 2012; Pisani et al., 2008, Vanhanen et al., 2011), the mineral of potassium covered about 70% of the total elements of the honey samples, ranging from 81.93% to 96.08% in this study. According to the results, the main concentrations of the elements in the chestnut honey samples were affected by the geographical location. The reason for this was that although the apiaries where honey samples were collected were in chestnut forests, Kastamonu districts had different altitudes, climates and soil properties, and different secondary flora.

Besides the major minerals, some elements such as Mn, Zn and Fe were also detected in honey samples. These elements were found in moderate levels (Table 3).

Table 3. Content of other elements found in moderate levels.in the samples

Sample code	Mn (ppm)	Zn (ppm)	Fe (ppm)
A	**1.39**^{m}	3.63^{k}	**9.54**^{a}
B1	10.13^{g}	**1.34**^{l}	**1.32**^{j}
B2	17.12^{b}	5.10^{j}	1.72^{i}
B3	11.25^{f}	7.45^{gh}	2.98^{gh}
C1	5.52^{l}	8.69^{f}	3.33^{f}
C2	6.89^{j}	14.70^{d}	4.10^{e}
C3	7.08^{ij}	5.98^{i}	4.71^{d}
C4	13.47^{d}	7.06^{h}	4.02^{e}
D1	6.37^{k}	16.35^{c}	8.39^{b}
D2	9.84^{gh}	6.72^{hi}	2.77^{h}
İ1	9.54^{h}	12.82^{e}	4.27^{e}
İ2	12.62^{e}	7.36^{h}	3.00^{gh}
K1	14.72^{c}	8.21^{fg}	4.76^{d}
K2	**18.69**^{a}	**20.84**^{a}	3.23^{fg}
K3	7.39^{i}	18.49^{b}	6.89^{c}

*nd: Non determined

** a–m: Means with different superscripts in the same column are significantly different ($p < 0.05$).

The difference between the other major element contents of chestnut honey samples according to locations was found to be statistically significant ($p<0.05$). In a conducted study by Küçük et al. (2007), when the comparison of mineral contents of chestnut, rhododendron, and multi-floral honey samples was carried out, the Mn concentration of the chestnut honey (9.69 ppm) was found quite high than the others (2.14 and 0.59 ppm, respectively). In another study (Kolaylı et al., 2008), similarly the Mn concentration of the chestnut honey (17.20 ppm) was found quite high than the Anzer honey (2.30 ppm) and Bayburt honey (1.20 ppm). This situation can be interpreted as Mn content being a good fingerprint in detecting imitation and adulteration of chestnut honey. Fe concentration varied between 1,32 ppm- 9,54 ppm, and Zn concentration varied between 1,34 ppm and 20,84 ppm in this study. This level is high in the findings of several researchers (Grembecka & Szefer, 2013; Kolaylı et al., 2008), who reported the amount of Zn and Fe content in chestnut honey.

Minor elements Co, Cu, Ga, Cr and Ag concentrations of the honey samples obtained from different locations were examined. Cu, Ga and Cr were found in trace levels. Co and Ag were not determined in any location (Table 4).

Table 4. Content of minor elements of the samples

Sample code	Co (ppb)	Cu (ppb)	Ga (ppb)	Cr (ppb)	Ag (ppb)
A	nd*	**11832.30^{a}**	4820.50^{fg}	33.50^{cd}	nd
B1	nd	1360.20^{d}	**1696.70^{i}**	nd	nd
B2	nd	**508.20^{f}**	3186.80^{hi}	30.60^{d}	nd
B3	nd	612.90^{f}	**4179.60^{gh}**	33.53^{cd}	nd
C1	nd	7874.70^{b}	6505.10^{de}	nd	nd
C2	nd	1317.00^{d}	6028.10^{ef}	30.80^{d}	nd
C3	nd	4194.00^{c}	6546.10^{cde}	44.20^{ab}	nd
C4	nd	3819.70^{c}	6750.80^{bcde}	41.10^{abc}	nd
D1	nd	nd	8832.70^{a}	33.70^{cd}	nd
D2	nd	nd	8183.7^{ab}	nd	nd
İ1	nd	nd	**9185.00^{a}**	nd	nd
İ2	nd	nd	8060.9^{abc}	nd	nd
K1	nd	nd	8337.30^{a}	33.90^{cd}	nd
K2	nd	nd	8980.60^{a}	35.30^{bcd}	nd
K3	nd	nd	7657.70^{abcd}	50.80^{a}	nd

*nd: Non determined

** a–h: Means with different superscripts in the same column are significantly different ($p < 0.05$).

Copper in the chestnut honey was detected in the A, B and C coded samples. It means Cu was determined in the close locations of the same districts. This can be probably due to the use of pesticides in the farmlands, which are located outside the forest area. Although a trace amount of copper is essential for the production of melanin which is responsible for pigmentation in the skin, and for the formation of hemoglobin, the tolerable daily intake of copper is limited to 3 mg/60 kg adult body weight (Joint FAO/WHO, 1999).

The heavy metals (Cd, Pb, Ni and Al) concentrations of the honey samples obtained from different locations were determined (Table 5).

Table 5. Content of heavy metals of the samples

Sample code	Cd (ppb)	Pb (ppb)	Ni (ppb)	Al (ppb)
A	nd*	nd	nd	8230.20[e]
B1	nd	nd	nd	**2938.90[l]**
B2	nd	nd	nd	5451.60[ij]
B3	nd	940.30[e]	nd	4731.90[k]
C1	**11.10**	1007.40[e]	nd	8178.50[e]
C2	nd	1365.10[cde]	nd	7148.70[fg]
C3	nd	1298.60[de]	nd	7585.70[f]
C4	nd	nd	nd	6476.40[h]
D1	nd	1714.70[cd]	nd	8949.60[d]
D2	nd	1203.30[e]	nd	6987.30[gh]
İ1	nd	1829.10[c]	nd	**13944.30[a]**
İ2	nd	**835.90[f]**	nd	4913.80[jk]
K1	nd	1355.60[cde]	nd	5623.40[i]
K2	nd	2396.80[b]	nd	9901.30[c]
K3	nd	**3378.40[a]**	nd	13021.90[b]

*nd: Non determined

** a–l: Means with different superscripts in the same column are significantly different ($p < 0.05$).

In this study, while the Cd element was detected in only one sample (C1), the Ni element was not detected in any chestnut honey sample. Because there wasn't a maximum limit for both of them in the Turkish Food Codex Contaminants Regulation (2011), no evaluation could have been made regarding the suitability of these heavy metal contents. Indeed there is no limit regarding the heavy metal contents value determined for honey types in the current Turkish Food Codex Contaminants Regulation (2011). The Contaminants Regulation was revised and published as a draft text in 2022. According to the Draft Regulation, the maximum Pb limits were indicated as 0.15 ppm for floral honey and 0.1 ppm for secretion honey starting from the date of 31.12.2023. Pb concentration varied between 1.84 ppm and 3.38 ppm for the samples. According to the Pb content results obtained in this study, while all honey samples are in compliance with the current regulation, only 4 chestnut honey samples will be in compliance with the Contaminants Regulation after December 31, 2023.

The change of Al concentration in chestnut honey samples, due to location was evaluated. As a result of the study, it was determined that the contents of Al concentrations were quite high, and there are great differences in terms of Al concentration between locations (between 1.32 ppm-13.94 ppm). Some portion of Al content might be attributed to secondary sources such as metallic containers used for storage during handling processes and harvesting of honey (Pisani et al., 2008). This metal contamination is usually accompanied by Cr contamination from the same sources (Chua et al, 2012).

The total multi-elemental content of the chestnut honey samples was presented using a column chart as in Figure 2. The total mineral contents of all samples varied widely between 1720.9 ppm and 6910.1 ppm. The locations with the lowest total mineral content were districts A (Abana) and B (Bozkurt). Locations in K (Küre) and C (Cide) districts generally presented high mineral substance content.

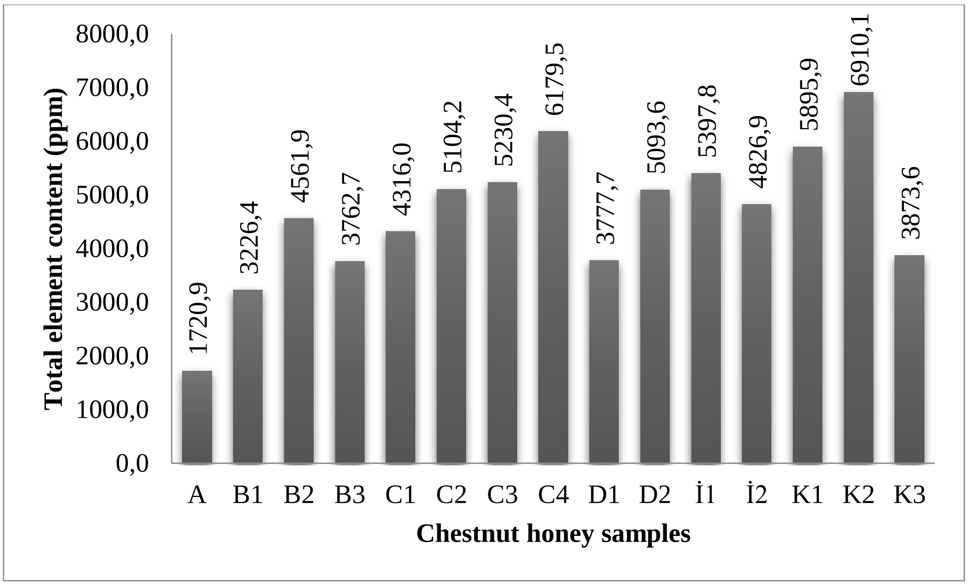

Figure 2. Total element contents of the chestnut honey samples (ppm)

4. CONCLUSION

In this study, 15 chestnut honey, which is widely produced in Kastamonu, samples were collected from Abana, Bozkurt, Doğanyurt, Cide, Küre, and İnebolu districts, and their mineral composition and heavy metal contents were examined. The mineral composition of honey varied widely depending on district and location. It was determined that especially Mg and K concentrations of chestnut honey were significantly high. Mn, Fe and Zn concentrations of chestnut honey examined in the study were found to be higher than other honey types. For this reason, it can be recommended to use some minor elements, especially Mn, as fingerprints to detect imitation and adulteration of chestnut honey. As a result of the study, it was determined that the location-based changes in heavy metals were statistically significant as a result of variance analysis. However, heavy metal findings could not be compared since there are no limit values for heavy metal residues in honey in the Contaminants Regulation in force based on the Turkish Food Codex. More studies are needed on heavy metal contaminants in other bee products, especially honey. Nowadays, as clean agricultural areas decrease and urbanization increases, the possibility of contaminating heavy metals such as arsenic and lead into the hive is increasing in the location where the bees are located. Furthermore, in this study, the strained honey samples collected from different locations were used that were prepared from natural comb honey by the producers. It is thought that the reason for the high lead and aluminum levels in honey samples is due to the equipment used in the strained honey production process. In addition, aluminum content may be contaminated from different environmental sources. According to the studies, heavy metal contents of some species in the flora affect the honey contents (for example, the density of *Mentha* species is increased in the amount of Cr). And this effect varies according to honey types (secretion, monofloral, multifloral, etc.). These interactions should be re-evaluated with more comprehensive studies. Additionally, the limit values for other heavy metal contaminants in the Turkish Food Codex, Draft Contaminants Regulation should be re-evaluated.

DECLARATIONS

The authors have no conflicts of interest to declare.

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