Citation: Güldaş M. Investigation of honey types (Chaste Berry, Chestnut, Lavender, Jerusalem Thorn, Acacia and Sunflower) for specific macro and micro elements with heavy metal pollution. U. Arı D. / U. Bee J. 2023,23(1):23-36. DOI: 10.31467/uluaricilik.1191584 **ARASTIRMA MAKALESI / RESEARCH ARTICLE**

INVESTIGATION OF HONEY TYPES (CHASTE BERRY, CHESTNUT, LAVENDER, JERUSALEM THORN, ACACIA AND SUNFLOWER) FOR SPECIFIC MACRO AND MICRO ELEMENTS WITH HEAVY METAL POLLUTION

Bal Çeşitlerinin (Hayıt, Kestane, Lavanta, Karaçalı, Akasya ve Çiçek) Belirli Mikro ve Makro Elementler ile Ağır Metal Kirliliği Bakımından İncelenmesi

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ABSTRACT

In this research, heavy metal contents (AI, As, Pb and Cd) of 6 honey samples obtained from Marmara and Aegean regions of Turkiye (chaste berry, chestnut, jerusalem torn and sunflower kind of honeys) and 4 honey samples obtained from Bulgaria (lavender, acacia and sunflower kind of honeys) with micro and macro element contents including Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S and Ca were analyzed by ICP-OES (Inductively Coupled Optical Emission Spectrometer). It was found that the heavy metal contents (AI, As, Cd and Pb) in the investigated honey samples were below the toxic limit values specified by the World Health Organisation and the Turkish Food Codex. In general, the mineral contents of honey samples vary according to the regions where they were taken. Among the honey samples taken from different regions; the contents of Pb, AI, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P and S changed at 1% significance level, while Mn, Ni and Fe contents differ at 5% level of significance. It was determined that as the apiary locations from which honey samples were taken approached the urban areas, the Pb content increased statistically by 1%, while the As and Co content increased at the 5% level of significance.

Keywords: Honey, Heavy Metals, Micro Element, Macro Element, ICP-OES

ÖΖ

Araştırmada Türkiye Marmara ve Ege bölgelerinden temin edilen 6 bal örneği (hayıt, kestane, karaçalı ve çiçek balları) ile Bulgaristan'dan temin edilen 4 bal örneği (lavanta, akasya ve çiçek balları) ağır metal içerikleri (Al, As, Pb ve Cd) başta olmak üzere; Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S ve Ca gibi mikro ve makro element içerikleri ICP-OES (İndüktif Eşleşmiş Optik Emisyon Spektrometresi) ile analiz edilmiştir. İncelenen bal örneklerindeki ağır metal düzeylerinin (Al, As, Cd ve Pb) WHO ve Türk Gıda Kodeksi' nde belirtilen toksik limit değerlerinin altında olduğu tespit edilmiştir. Genel olarak, bal örneklerinin mineral içerikleri alındıkları bölgelere göre değişmektedir. Farklı bölgelerden alınan bal örnekleri arasında Pb, Al, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P ve S içerikleri %1 önem düzeyinde; Mn, Ni ve Fe içerikleri ise %5 önem düzeyinde farklılık göstermektedir. İstatistiksel analizler sonucunda; bal örneklerinin alındığı arılıklar yerleşim yerine yaklaştıkça Pb %1, As ve Co içerikleri ise %5 önem düzeyinde artış gösterdiği ortaya çıkmıştır.

Anahtar Kelimeler: Bal, Ağır Metaller, Mikro Element, Makro Element, Mineraller, ICP-OES

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GENİŞLETİLMİŞ ÖZET

Amaç: Bal, arı sütü, polen ve propolis gibi arı ürünlerinin sağlıklı yaşamı sürdürme ve hastalıklara karşı vücut bağışıklığını güçlendirme gibi insan sağlığı acısından önem tasıvan pek cok favdası vardır. Bu arı ürünleri içerdiği vitaminler, mineraller, enzimler, fenolik bileşenler ve diğer biyoaktif maddelerden dolayı antibakteriyel, antimikrobiyal, antiviral ve antiparaziter gibi birçok sağlık fonksiyonuna sahiptir. Demir, bakır, çinko, kalsiyum, sodyum ve potasyum gibi mineraller balda sıklıkla ve insan beslenmesi bulunan için önem taşımaktadır. Bu faydalı minerallerin yanı sıra bal ve diğer arı ürünleri şehir merkezleri, otoyollar ve endüstriyel kirlilik faktörler nedeniyle insan sağlığına zararlı bazı ağır metaller içerebilmektedir. Çünkü arı kovandaki bal ve diğer ürünlerini 3-4 km çapında bir çevresel alandan topladığından, kirlilik kaynaklarından bazı bulaşanları kovana taşıyabilmektedir. Bu nedenle ballar insan beslenmesine katkı veren besleyici mineraller açısından incelenmesi yanında, gıda güvenliği açısından bölgesel bazda çevre kirliliğinin bir vansıması olan ağır metal içerikleri bakımından da sık sık incelenmelidir. Türkiye ve Bulgaristan arıcılıkta önemli potansiyele sahip iki ülkedir. Her iki ülkenin coğrafi konumu, bitki çeşitliliği, ekolojisi, nektar kaynakları ve koloni varlığı bal üretimi için çok uygundur. Çalışmada, Türkiye'de Ege ve Marmara bölgelerinden 4 farklı bal çeşidine ait 6 ve Bulgaristan'dan temin edilen 3 farklı bal çeşidine ait 4 örnek, 16 farklı mineral (Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S ve Ca) ve 4 farklı ağır metal (Al, As, Cd ve Pb) incelenmiştir. Bal örneklerinin şehit merkezleri ile otoyolların neden olduğu ağır metal kirlilik düzeylerinden etkilenme durumları ortaya konulmaya çalışılmıştır. Temel analiz yöntemlerinin gelişmesine paralel olarak, son yıllarda besinlerin ağır metal analizlerinde daha güvenilir sonuçların elde edilmesi talep edilmektedir. ICP-OES analiz tekniği, temel araştırmalar için en hassas ve güvenilir bir mineral madde ve ağır metal analizi ölçüm yöntemi olarak kabul edilmektedir. Bu nedenle örneklerin mineral ve ağır metal içeriklerinin incelenmesinde, ICP-OES tekniği kullanılmıştır.

Gereç ve Yöntem: Bu çalışmada Türkiye'nin Marmara Bölgesinde Bursa-Mudanya'nın 4 köyü ile Bursa-Karacabey'in Malkara Köyü kırsal alanından 1 ve Ege Bölgesi'ndeki İzmir Bergama'dan 1 olmak üzere ülke genelinde 6 örnek incelenmiştir. Bulgaristan'ın ise Oblast ili Şumnu, Benkovski, Kayaloba ve Dobromirtsi şehirleri kırsal alanlarından 4 bal örneği temin edilmiş ve incelenmiştir (Tablo 1). Bu bölgelerin bal üretimi için önemli olduğu bilinmektedir. Çalışma boyunca ağır metaller de dahil olmak üzere 20 mineral analiz edilmiştir. Analiz, Bursa Uludağ Üniversitesi Ziraat Fakültesi Toprak Bilimi Bölümü ve Bitki Besleme Araştırma Laboratuvarı'nda ICP-OES cihazı kullanılarak gerceklestirilmistir. Bal örnekleri asitte mikrodalga kullanılarak yakılmış ve seyreltme işleminden sonra analiz edilmiştir. Kalibrasyon testleri sonrasında ICP-OES cihazı analize uygun hale getirilmiş ve numuneler standart bir eğri kullanılarak hesaplanmıştır.

Sonuçlar ve Tartışma: Türkiye'den alınan bal örnekleri içerisinde en yüksek Al (4,04±0,20 ppm) ve As (0,39± içerikleri T5 ve T2 (3,60±0,24 ppm) bal örneklerinde ölçülmüştür. Bulgaristan'da ise en yüksek Al içeriğine Kayaloba' dan alınan çiçek balında (B3; 2,06±0,18 ppm), As içeriğine ise B4 örneğinde rastlanılmıştır. Türkiye bal örnekleri içerisinde en yüksek kurşun (Pb) içeriğine İzmir Bergama'dan alınan bal örneğinde (2,79±0,16 ppm) rastlanırken, Bulgaristan bal örnekleri içinde Pb kalıntı miktarı en yüksek B1 örneğinde (0,84±0,01 ppm) tespit edilmiştir. Kadmiyum içeriği en yüksek Türkive bal örneği olarak T5 örneği (0.36±0.00 ppm) ve en yüksek Bulgaristan bal örneği olarak B3 (0,13±0,16 ppm) bulunmuştur. Analizler sonucunda tüm bal örneklerinde element içeriklerinin kabul edilebilir düzeyde olduğu, toksik ağır metal (As, Al, Pb ve Cd) içeriklerinin toksik düzeyin altında olduğu belirlenmiştir. Özkul ve diğerleri (2018) de belirlediği sehir merkezi ve verleşim alanlarına gibi, yaklaşıldıkça bal örneklerindeki kirlenme oranının (Pb ve As içerikleri) arttığı ve bu artışların istatistiksel olarak %1 ve %5 düzeylerinde önem taşıdığı tespit edilmiştir. Araştırma sonunda en yüksek kurşun içeriğine sahip olan hayıt balının bile günlük tüketilebilecek maksimum bal miktarı ile orantılandığında FDA'nın güvenli limit değeri altında kaldığı belirlenmiştir.

Diğer yandan balların besin değeri ve mineral zenginliği bakımından değerlendirildiğinde (Tablo 2); tüm bal örneklerinin kemik sağlığı açısından önemli bir mineral olan Ca içeriklerinin yüksek olduğu ve 67,44±2,21 ile 198,70±1,44 ppm arsında değiştiği bulunmuştur. Balların kansızlık açısından önemli bir element olan Fe içeriği bakımından da zengin bir kaynak olduğu ve bu element içeriğinin ballarda (B1) 29,13±0,36 ppm'e varan düzeylere kadar çıkabildiği belirlenmiştir. Yine bir koenzim faktörü olarak vücutta görev yapan Zn; Türkiye ballarında (T5)

2,94±0,02, Bulgaristan ballarında (B3) ise 3,79±0,01 ppm düzeylerinde ölçülmüş olup balın zengin bir mineral madde kaynağı olarak beslenmede rol oynadığı ortaya konulmuştur.

INTRODUCTION

In recent years, the impact of rapid industrialization on nature in terms of environmental pollution increased significantly in our country parallel to the World. Industrial plants in business enterprises such as paint, automotive and plastic cannot completely prevent toxic and harmful chemicals from production, although waste treatment units are mandatory, toxic substances continue to spread to the environment through water and air (Tchounwou et al. 2012). Bees are accepted as indicator creatures due to their interaction with nature in maintaining the balance in nature. They are living organisms that are indispensable in ensuring the sustainability of plant production by pollination (Sıralı and Cinbirtoğlu 2018). Environmental problems such as heavy metal pollution in nature pose a risk not only to human health and nutrition but also to bee life and health, elevating the risk of pollution of the products such as honey, pollen and royal jelly produced by bees.

Beekeeping is a socio-economic activity that uses plant resources, bees, technical knowledge, and labor to produce various bee products. (Burucu ve Gülse Bal 2018). The orientation towards providing the nutrients and energy elements needed by the body from natural sources with the understanding of healthy life has led to the development of beekeeping activities and to a significant increase in the value of these products. Bee products such as honey, royal jelly, pollen and propolis have great benefits in terms of maintaining a healthy life, preventing certain diseases and strengthening the immune system. These products have antibacterial, antimicrobial, antiviral and antiparasitic health functions due to their antioxidant elements such as vitamins, minerals, enzymes, phenolic components and their bioactive substances. In addition to all benefits, beekeeping is important in these agricultural activities due to the reasons such as earning income in a short time, it can be done with little capital and it does not require a lot of land area (Tepge 2021).

Turkiye has significant potential in beekeeping and is very favorable for honey production due to its geographical location, plant diversity, ecology, nectar reserves and the existence of colonies or hives (Borum 2014). Approximately 5.9% of the honey obtained in the world in 2019 was produced in Turkiye. This brings Turkiye to second place in the world in honey production (Güler 2021). Projection studies show that honey production will increase even more in Turkiye. According to the data of 2021, while around 97 thousand tons of honey was produced in Turkiye (TUIK 2021), honey production is expected to be between 121 thousand and 125 thousand tons in 2023 (Burucu ve Gülse Bal 2017).

Due to the rapid increase in the world population, industrialization and agricultural production activities recently, heavy metal pollution in soil and nature has reached serious levels (Mikhailenko et al. 2020). Soil is an important medium in which plants grow and acts as filtration for pollutants. Since there is an exchange of substances and energy between the three main ecosystems in nature (water, air and soil), the pollutant is able to transport from one ecosystem to another (Figure 1). As a result of the accumulation of heavy metals in the soil, these can be transported to humans through these plants and can play a role as significant risk factors responsible for some chronic diseases (Kara and Kara 2018). The increase in the heavy metal concentration in Turkey's natural resources with each passing day keeps this issue on the agenda and leads to an increase in the number of studies on this subject (Sönmez and Kılıç 2021). In a study conducted in the province of Istanbul, soil samples were examined from 40 different locations and the highest pollution values were determined in the unwashed leaf samples taken from the roadside as 14.90 ± 2.96 $\mu q/q$ for Pb, 0.65 ± 0.13 $\mu q/q$ for Cd, 19.94 ± 1.17 μ g/g for Cu and 42.53 ± 3.08 μ g/g for Zn (Ozturk et al. 2017). It has been determined that there is a linear relationship between heavy metal accumulation, traffic density and proximity to the roadside. In a study carried out in the Altıntaş plain, located in the south of Kütahya, a region where agricultural activities are intense, the heavy metal contents were analyzed by ICP-MS by sampling from 15 points of agricultural lands. (Özkul 2018). As a result of the analysis of soil samples taken from the study area, while the pollution was moderate for Cu, Pb, Sb and Zn heavy metals, Hg, As and Ni were found as the heavy metals that cause the most pollution, respectively. In another study, the heavy metal contents of the soils in the playgrounds in the city center of Kütahya have investigated, and found

significant soil contamination in terms of heavy metal contents of As, Cd, Hg, Ni, Pb, and Zn (Özkul 2019). Among these, it has been observed that the contamination rate of heavy metals, especially As and Cd, has reached serious levels. Pollution caused by heavy metals is easily able to transport between the ecosystems through the food chain. In this respect, heavy metals can be transmitted to humans through many pathways including food production, drinking water, inhalation and skin contact (Figure 1). Pollution sources occurring in the ecosystem causes serious negative effects if it is taken into the metabolism of the living organisms (Vareda et al. 2016). Heavy metals mixed with the soil can be transported to the human body either directly through plants or indirectly through animal meats and products that consume plants (Kara and Kara 2018).

Figure 1: Pollution caused by heavy metals.

Şekil 1: Ağır metallerin sebep olduğu kirlilik.



In Turkiye, which has a wide and diverse flora potential and geography where flowering continues at all times of the year; beekeeping is an agricultural business area that can be done almost anywhere from sea level to high plateaus (Tepge 2021). The high quality of honey in our country is based on the abundance of nectar-producing plant varieties in different climatic conditions and different periods. For these reasons, an important part of the honey produced from these regions is a mixture, that is, multi-floral. In addition, herbal kinds of honey specific to a local plant flora, which are unique to certain regions in Turkiye, can also be produced. **Chasteberry honey**, one of these kind of honey, is one of the only plant kind of honey that is produced intensively in the Aegean and Marmara Regions and consumed in common. Chasteberry (Vitex agnuscastus) is an herb, also known as priest pepper or five-finger plant. The chasteberry plant is a good source of nectar for honey bees and has an estrogenic effect and hormone-balancing characteristics (Uçak Koç et al. 2017).

Jerusalem thorn honey is produced mostly in Marmara and Thrace regions in Turkiye (Malkoç et al. 2019). *Paliurus spina-christi*, known as Jerusalem thorn, is a shrub plant belonging to the *Miller Rhamnaceae* family. The flowers of the Jerusalem thorn plant turn yellow in May and July depending on the weather conditions; medium sweet and slightly bitter honeys that can crystallize very quickly are obtained. Jerusalem thorn herb has traditionally been used for the treatment of diuretic, antirheumatic, hypocholesterolemic, and chronic obstructive pulmonary disease (Şen 2018, Zor et al. 2017).

Lavender honey is among the most admired highquality honeys for its pleasant aroma and taste. Lavender is a popular aromatic Mediterranean plant belonging to the *Lamiaceae* family (Castro-Vázquez et al. 2014).

Chestnut honey is honey obtained from the extracts collected by the bees from the flowers of the chestnut trees in a certain period. Since chestnut trees (*Castanea sativa*) begin to bloom in June, this honey made by bees that work intensively during this period is produced less frequently in our country, so its economic value is higher than the many other honey varieties (Alkan 2020).

Acacia honey (*Robinia pseudocacia*), with its light yellow appearance, delicate scent and floral aroma, is one of the most popular honey varieties available in the European market (Oroian et al. 2015, Schievano et al. 2019). In order for a honey sample to be classified as real acacia honey, it must contain at least 45% granular acacia pollen as specified in the regulation on honey quality (Soares et al. 2017).

Sunflower honey (*Helianthus annuus*) is one of the most produced monofloral (single flower) honey varieties in Turkiye and it is the most exported honey in the country after pine honey. It is generally used by beekeepers as winter food or bee feed. Sunflower

blooms in July and is harvested in August. Sunflower honey is honey having golden yellow in color, has a unique taste, and crystallizes very quickly. The quick crystallization of this honey is due to its high glucose and pollen content. It looks like a yellow candle when it crystallizes (Sen 2019).

Chasteberry, chestnut and Jerusalem thorn are plants preferred by bees, which naturally reproduce in forest areas far from city centers for honey production. Lavender, on the other hand, is an aromatic plant that is extensively planted as an important raw material for the perfume and cosmetics industry, generally in the fields relatively close to cities and industrial pollution sources compared to other forest plants examined in the research. These plants, which exist both in forest areas and in areas close to cities, are among the plants used extensively by honey bees (Apis mellifica) to create unmatured liquid honey. Residue levels in the honey produced by bees collecting liquid honey extracts and pollen from these plants may vary depending on the distance from the environmental pollution sources of the plants where the bee collects the nectar and the area where the beehive is located.

Honey, which is loved and consumed by all age groups, is a functional food that is easy to digest and has a curative or protective effect against some diseases. Honey contains elements such as potassium, sodium, phosphorus, magnesium, sulfur, manganese, chlorine and iron, which are important for human health and nutrition. According to the Turkish Food Codex Honey Communiqué, honey; is defined as a natural bee product created by the collection of plant nectars, the secretions of living parts of plants, or the secretions of plant-sucking insects living on the living parts of plants by honey bees (TGK 2020). For this reason, honey is one of the natural food products whose source is nature and therefore can be affected by environmental pollution sources such as heavy metal pollution in nature.

In the composition of honey; there are main nutritional components such as sugars (31% glucose, 38% fructose), vitamins (ascorbic acid, etc.), moisture (10-20%), elements (potassium, sodium, calcium, magnesium, phosphorus and organic acids (gluconic acid, acetic acid, etc.). The amounts of major and trace elements in honey vary according to the element contents of the sources (soil and vegetation) from which the bee collects the nectar. The elements are generally transported to the honey through the nectars and nectars collected by the bees from the plants through the roots of the flowering plants (Lanjwani and Channa 2019). Apart from environmental pollution, the amount of heavy metals and minerals varies according to the geographical and flora characteristics of honey. Heavy metals are elements with a density higher than 5 g/cm3 in terms of physical properties in three or higher periods in the periodic table. More than 60 metals are in this group, including lead, cadmium, chromium, iron, cobalt, copper, nickel, mercury and zinc. The most common heavy metals determined in polluted soils are Cr, Pb, Cu, Cd, Zn, Hg and As, respectively (Khalid et al. 2017).

In many studies on the element contents in honey, macro elements such as Na, K, Cl, Ca, P, S and Mg, and micro elements such as Al, Mn, Pb, Cd, Cu, Tl, Co, Zn, Rb, Ni, Ba, Bi, Pt, Be, V, Pd, Te, Fe, Mo, Hf, Sb, Sn, U, La, Sm, I, Tb, Th, Dy, Sd, Nd, Pr, Lu, Gd, Yb, Er, Ho, Cr, B, As, Br, Ce, Cd, Se, Sr and Hg have been investigated in detail (Lanjwani and Channa 2019). However, since environmental pollution is a rapidly increasing problem both in our country parallel to the World, it is necessary to frequently examine and evaluate foods such as honey, which is a product obtained from nature, in terms of pesticide and heavy metals which have serious toxic effect on human health.

Bees take heavy metals from plants and water sources on the soil surface while collecting nectar. These heavy metals are transmitted to plants from the soil, pesticides, chemicals and industrial wastes in the region. Although the typical food collection area of the honey bee varies according to the distance of the food and water sources around the hive; most of the time it is collected within a radius of 600-800 of an area. However, they can often fly distances of 2 km to gather food and can even follow a flight route of up to 5 km. It is stated that in cases of food and water deprivation, bees can fly up to a maximum distance of 13 km in order to find food sources and under experimental conditions (Pahl et al. 2011). Metals can contaminate honey by a mechanism defined as migration, not only from the environment and nature but also from the metal surfaces, metal-based containers and cauldrons and equipment used in the production steps (Özcan and AL Juhaimi 2012). Environmental pollution, on the other hand, is a global problem of today, and its effects are increasing day by day and reaching a dangerous dimension. Among the main factors

polluting nature, heavy metals, various pesticides, organic compounds, radioactive substances and hydrocarbon combustion products at first (Doelsch et al. 2006). Due to food safety concerns, studies on the determination of heavy metal content in honey have increased in recent years. It is stated that if these toxic substances are taken by the organism above a certain level, they can harm the metabolism and the body (Bengü and Kutlu 2020).

There is no separate classification regarding the heavy metal limit values in honey. There are regulations published by the World Health Organization (WHO 2007), European Union (EC 2006), The Food and Drug Administration (FDA 2021) regarding heavy metal residue limits in foods. In Turkiye, heavy metal limit values are considered as toxic residue values in foods like in other countries, and there is no separate legal classification for heavy metal contents only in honey (Resmi Gazete 2011).

In this study, 16 different micro and macro elements and 4 heavy metals were tried to be measured in the honey samples (chasteberry, chestnut, jerusalemthorn, and sunflower honeys) obtained from two regions of Turkiye and the honeys (lavender, acacia, and sunflower honeys) from four settlements of Bulgaria. With statistical methods, it has been tried to reveal the level of being affected by environmental pollution according to the distance of the honey to the pollution sources that cause environmental pollution such as city centers and highways.

MATERIALS AND METHODS

Collection of Samples

In the study, 10 honey samples produced in Turkiye and Bulgaria were used. For this purpose, 6 honey samples belonging to the Marmara and Aegean regions of Turkiye and 4 honey samples belonging to 4 settlements of Bulgaria were examined in terms of heavy metals (4) and micro and macro elements (16) (Table 1 and Figure 2). Honey samples without honeycombs (500 g) were brought to the laboratory in clean glass jars, labeled and stored at 4-8 °C until analysis.

Figure 2. Honey sampling locations on the map

Şekil 2. Bal örneklerinin alındıkları bölgeler



Table 1. Geographical coordinates and regions for honey sampling

Tablo 1. Bal örneklerinin alındıkları bölgeler ve coğrafi koordinatları

Sample Code	Honey Variety	Geographical Position	Coordinates
T1	Chasteberry	Turkiye/Izmir/Bergama	39.29774,27.28536
T2	Chestnut	Turkiye/Bursa/Malkara/Karacabey	40.31248,28.34772
Т3	Jerusalemthorn	Turkiye/Bursa/Mudanya/Sogutpinar	40.46111,28.62627
T4	Jerusalemthorn	Turkiye/Bursa/ Mudanya/Mesudiye	40.57991,28.58261
T5	Jerusalemthorn	Turkiye/Bursa/ Mudanya/Camlık	40.45518,28.56538
Т6	Sunflower	Turkiye/Bursa/Mudanya/Evciler	40.55469,28.49159
B1	Lavander	Bulgaria/Sumnu	43.45616,26.90330
B2	Acacia	Bulgaria/Benkovski	41.60622,25.22514
B3	Sunflower	Bulgaria/Kayaloba	41.52410,25.17348
B4	Sunflower	Bulgaria/Dobromirtsi	41.46227,25.15832

Sample Preparation and ICP-OES Method

Approximately 1 g of honey samples were weighed and burned with 4 ml of 37% HCl (Honeywell, Germany) and 3 ml of 65% HNO₃ (Merck, Darmstadt, Germany) using a Multiwave 5000 (Anton Paar, Austria) microwave device. The samples were completed with ultrapure water to a final volume of 50 ml. The samples were burned using HNO₃ and H₂O₂ in a Berghof MWS-2 model microwave oven. In the extracts obtained, 4 heavy metal types (Al, As, Pb and Cd) and micro and macro elements (Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S and Ca) were determined with Perkin Elmer OPTIMA 2100DV model ICP OES apparatus. Measurement conditions applied in the device are given in Table 2.

Table 2. The Measurement Conditions Of ICP-OES

Tablo 2. ICP OES ölçüm koşulları

Reding Time	5 (s)					
Rf Power	1.20 (kW)					
Stabilization Time	15 (s)					

Chemical Analysis

During heavy metal and other element analyses, appropriate calibration standards were used at periodic intervals to maintain measurement accuracy between samples and to minimize productivity loss. For this purpose, the accuracy of the ICP-OES data was checked using blank reagents and calibration standards prepared with standard solutions (Agilent Technologies Co.). Calibrations were generally applied in the range of 0-0.1 ppm for micro elements and 5-80 ppm for macro elements.

Statistical Analysis

Whether the heavy metal contents of honey samples showed normal distribution was analyzed by Kolmogorov-Smirnov test. As a result of the analysis; It was determined that Pb, Al, Cr, Cu, Ba, Zn, B, Ca, Fe, K, Na and S elements showed normal distribution, while As, Cd, Co, Mn, Ni, Sr, Mg and P elements did not show normal distribution. Whether there is a difference between the mean element levels of honeys was analyzed by t-test.

At the same time, whether there is a relationship between heavy metal levels of honey and their distance from settlements and highways was examined with the help of Pearson correlation tests for elements with normal distribution and Spearman correlation tests for elements that do not show normal distribution.

RESULTS

The contents of the heavy metals with micro and macro elements determined in the honey samples are given in Table 3.

Toxic Heavy Metal Contents (Pb, Cd, Al and As) of the Honey Samples

Balların Toksik Ağır Metal (Pb, Cd, Al ve As) İçerikleri

Toxic heavy metal (Pb, Cd, Al and As) contents of the honeys are given in Table 3.

Lead (Pb) is an element with atomic number 82 in the periodic table and has very important in terms of its toxicity and food safety (Bengü and Kutlu, 2020). In the analyzes made, the highest lead content was found in chasteberry honey (T1), which is one of the test samples, with a level of 2.79 ppm. The lead content in other honey samples varies between 0.03-1.70 ppm (Table 3). The sources of lead pollution in nature are usually lead-containing industrial wastes and gasoline-powered car emissions. The maximum amount of lead that can be taken from food is stated by the US Food and Drug Administration (FDA) as 12.5 micrograms per day (FDA 2022). Considering the average daily honey consumption in Turkiye, this limit corresponds to 4.1 ppm lead content that can be taken with honey. When evaluated in terms of honey samples examined in our research; even the chasteberry honey, which has the highest lead content, has a content far below the lead level that can be safely consumed daily.

Cadmium (Cd) is a soft metal known for its bluish color and is a metal that can slowly oxidize due to moisture in the air. The cadmium mineral with atomic number 48 is less than 0.01% in the earth's crust (Bengü and Kutlu 2020). Long-term intake of cadmium causes accumulation in the human organism, especially in the liver and kidney. When cadmium accumulates in the body and its level in the renal cortex reaches 0.2-0.3 mg/g, it causes damage to the tubules. The tolerable weekly dose for an adult (70 kg) is considered to be 0.49 mg of cadmium (Wang et al. 2012). The amount of cadmium in all honey varieties examined in the study varied between 0.01-0.36 ppm (mg/kg), and no significant Cd presence that could cause toxicity in the human organism was found.

Alüminyum (AI) is the third most common element in the world (Rafati Rahimzadeh et al. 2022) and 50-150 mg of aluminum is found in the human body. It is stated that the level of aluminum is higher in aging organisms. The daily intake of aluminum to the body is at the level of 2-10 mg, and the dose that can be absorbed by the gastrointestinal tract is very small, that is, in trace amounts (Wang et al. 2012). It is stated that as a result of environmental pollution, aluminum can pose a great threat to humans, animals and plants. Aluminum, which accumulates in an overdose in the body, can cause damage by increasing oxidative stress in the brain, liver and kidney. This damage can occur in the form of disruption of the mechanism of action of some enzymes, protein synthesis, utilization of nucleic acids and dysfunctioning of cell membrane permeability in the body. It is stated that they can affect triglyceride levels in plasma and fat metabolism in the body (Bengü and Kutlu 2020, Rafati Rahimzadeh et al. 2022). Al was found at the highest level of 4.04 ppm in jerusalemthorn honey (T5) and 3.60 ppm in chestnut honey (T2) in the honey samples examined, and it ranged between 0.06-1.83 ppm in other honey samples.

Table 3. The Contents of Heavy Metals, Micro and Macro Elements in the Honey Samples (ppm)

	MINERALS																				
		AI	As	Pb	Cd	C o	Cr	Cu	Mn	Ni	Ва	Sr	Zn	в	Ca	Fe	к	Mg	Na	Ρ	s
	Т 1	LOD 2	LOD	2,79 ±0,1 6	0,02 ±0,0 3	L O D	0,10 ±0,0 0	0,20 ±0,0 0	0,27 ±0,0 3	0,07 ±0,0 0	LOD	0,12 ±0,0 1	0,44 ±0,0 6	6,69 ±2,3 6	81,87 ±4,13	0,40± 0,05	386,04 ±2,20	15,84 ±0,53	77,27 ±3,24	236,11 ±19,33	21,46 ±0,92
	Т 2	3,60 ±0,2 4	0,08 ±0,0 0	1,70 ±0,5	0,01 ±0,0 0	L O D	0,12 ±0,0 1	0,59 ±0,0 3	2,22 ±0,1 0	0,11 ±0,0 1	0,18 ±0,0 4	0,21 ±0,0 2	1,17 ±0,0 8	5,16 ±1,0 4	131,2 7±3,3 0	8,42± 0,22	2697,7 5±0,02	41,00 ±0,75	93,23 ±1,74	294,96 ±9,26	56,86 ±0,30
	Т 3	0,18 ±0,0 2	0,06 ±0,0 0	1,67 ±0,1 3	0,02 ±0,0 0	L O D	0,09 ±0,0 0	0,32 ±0,0 3	0,44 ±0,0 1	0,15 ±0,0 1	0,05 ±0,0 1	0,15 ±0,0 1	0,76 ±0,1 1	3,38 ±0,1 1	67,44 ±2,21	0,70± 0,19	2610,0 3±82,9 7	26,40 ±0,23	76,15 ±2,62	221,88 ±27,00	43,76 ±2,19
	Т 4	1,62 ±0,0 8	0,10 ± 0,08	0,61 ± 0,04	0,03 ± 0,01	0, 02 ± 0, 02	0,04 ± 0,01	0,35 ± 0,02	0,64 ± 0,00	0,9 ± 0,01	0,18 ± 0,02	0,22 ± 0,00	1,35 ± 0,01	3,26 ±0,5 5	105,2 5 ± 0,31	1,43± 0,31	1102,8 9 ± 5,79	26,93 ± 0,1	54,24 ± 0,29	205,77 ±6,88	32,66 ±1,19
MPLES ¹	Т 5	4,04 ±0,2 0	0,39 ± 0,07	0,23 ± 0,12	0,36 ± 0,01	0, 02 ± 0, 00	0,04 ± 0,01	0,86 ± 0,16	0,50 ± 0,00	0,28 ± 0,05	0,24 ± 0,01	0,63 ± 0,01	2,94 ± 0,02	3,29 ±0,2 5	192,0 8 ± 0,57	1,18± 0,16	1196,0 9 ± 4,24	68 ±0,18	111,4 6 ± 0,32	2,30±0 ,14	27,95 ±1,43
SAI	Т 6	1,29 ± 0,07	0,11 ± 0,01	0,44 ± 0,13	0,07 ± 0,00	0, 06 ± 0, 00	0,04 ± 0,00	0,29 ± 0,01	0,38 ± 0,00	0,14 ± 0,02	0,16 5 ± 0,02	0,12 ± 0,00	1,30 ± 0,02	4,42 ±0,1 9	76,6± 0,37	1,51± 0,39	203,33 ± 1,08	24,89 ±2,37	57,19 ±3,61	205,49 ±3,06	41,86 ±1,50
	В 1	0,06 ±0,0 2	0,08 ±0,0 1	0,84 ±0,0 1	0,02 ±0,0 0	L O D	0,10 ±0,0 0	0,22 ±0,0 1	0,48 ±0,0 2	0,04 ±0,0 0	0,01 ±0,0 1	0,15 ±0,0 3	1,69 ±0,1 6	8,01 ±0,9 9	132,8 6±3,5 4	29,13 ±0,36	966,10 ±14,87	22,33 ±0,48	63,30 ±2,60	205,93 ±10,87	23,90 ±1,02
	В 2	1,83 ± 0,09	0,12 ± 0,02	0,41 ± 0,14	0,02 ± 0,00	0, 02 ± 0, 00	0,04 ± 0,01	0,46 ± 0,05	0,36 ± 0,07	0,15 ± 0,03	0,18 ± 0,00	0,18 ± 0,00	2,52 ± 0,01	5,61 ±0,9 5	131,5 4 ± 0,19	10,76 ±0,64	589,04 ± 1,20	27,07 ± 0,07	45,56 ± 0,07	148,82 ±28,49	26,04 ±2,54
	В 3	2,06 ± 0,18	0,14 ± 0,02	0,45 ± 0,09	0,13 ± 0,16	0, 37 ± 0, 00	0,11 ± 0,02	0,75 ± 0,01	0,32 ± 0,01	0,12 ± 0,04	0,35 ± 0,02	0,18 ± 0,00	3,80 ± 0,01	4,98 ±0,8 7	198,7 ± 1,43	13,46 ±0,92	594,7 ± 2,24	42,11 ± 0,32	64,46 ± 0,28	210,07 ±7,21	21,75 ±1,53
	B 4	0,81 ± 0,08	0,15 ± 0,03	0,03 ±0,0 9	0,05 ± 0,00	0, 03 1 ± 0, 01	0,10 ± 0,01	0,17 ± 0,04	0,13 ± 0,00	0,13 ± 0,00	0,17 ± 0,01	0,21 ± 0,00	1,39 ± 0,01	5,81 ±0,8 6	70,2 ± 0,57	11,02 ±0,33	299,25 ± 1,51	21,68 ± 0,11	41,48 ± 0,15	243,58 ±33,29	33,78 ±2,11
¹ T _{1.2}	345	e or B12	34: The	sample	s from	Turkiv	e as the	T" an	the sa	mples f	rom Bul	daria as	the "B	were c	oded						

Çizelge 3. Bal Örneklerindeki Ağır Metal, Mikro ve Makro E	Element İçerikleri (ppm)
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²LOD: Under limit of detection

Arsenic (As) is one of the important risk factors for public health due to its high toxicity level. It can be transmitted to individuals as a result of the direct consumption of food and water contaminated with arsenic or within the scope of the situations covered by the definition of occupational disease in the workplace (Gupta et al. 2017). Arsenic causes neurodegenerative diseases in the body, especially on the nervous system (Mitra et al. 2022). The World Health Organization (WHO) has determined the level of 10 µg/kg as the safe limit for arsenic (Singh et al. 2017). In the study, arsenic was not detected in chasteberry honey (T1), but it was found to be between 0.06-0.39 ppm and at trace level in other honeys. According to these results, it was observed that the honey analyzed in the study was within the safe limits determined by the World Health Organization (WHO) in terms of arsenic content, which has a serious toxic effect on humans.

Contents of Macro and Micro Element (Na, K, Ca, Fe, Cu, Zn, Mn, B, Mg, P, Cr, S, Ba, Ni, Co and Sr) in the Honeys

The macro and micro element contents of honeys (Na, K, Ca, Fe, Cu, Zn, Mn, B, Mg, P, Cr, S, Ba, Ni, Co and Sr) are given in Table 3.

Sodium (Na) was found to be between 41.48-111.46 ppm in the honeys examined and the highest was found in jerusalemthorn honey (T5). When the amount of honey consumed daily and the amount of Na that can be taken into the body are compared, it does not pose any risk. Sodium is a component in the body that regulates the osmotic pressure of the extracellular fluid. It also activates some enzymes such as amylase. The absorption of sodium begins 3 to 6 minutes after ingestion, is guite rapid and is completed within 3 hours. The average daily sodium intake for the body with food is 2.5 g for women and 3.3 g for men. The average requirement for adults is between 1.3-1.6 g/day, which is equivalent to 3.3-4.0 g/day of table salt (NaCI) intake. Too little or too much Na intake into the body can cause serious disorders such as hypertension (Wang et al. 2012).

Potassium (K) is an alkaline and soft metal, compared to the other elements examined, it is an element found in the highest amount in honey. The potassium levels in the honey samples varies between 203.33-2697.75 ppm. Potassium is the basic cationic element in the cell fluid and has an important function in maintaining the acid-base balance. Potassium is an important component of the glycogenesis energy cycle and the regulation of

intracellular osmotic pressure in the cell (Bengü and Kutlu 2020). It is an important nutritional element in terms of cell membrane permeability and cofactor functions for some enzymes. The daily amount of potassium taken into the body by diet is 2-5.9 g. The minimum daily requirement is estimated to be around 780-800 mg (Wang et al. 2012). Therefore, when honey samples are considered in terms of nutrition, they can be recommended as one of the important sources in providing the potassium that the body needs for individuals other than diabetics.

Calcium (Ca) is a metallic element of the alkaline earth group. As a result of elemental analysis, when all honey samples are taken into account in the research, it has been determined that it varies between 67.44-198.70 ppm. Calcium is another essential element for healthy nutrition, which plays a role in the regulation of the nervous system and the realization of muscle functions, and is also found as a building block in teeth and bones. In addition to the conversion mechanism of prothrombin to thrombin in blood coagulation; it is a vital element for muscle contraction, nerve conduction and membrane permeability (Bengü and Kutlu 2020).

Iron (Fe) is the most abundant element in the earth's crust, and its total amount in the human body is 4-5 g. Most of the iron in the body is found in the structure of hemoglobin in the blood and myoglobin in the muscle tissue. Iron, which is also found in the structure of enzymes such as hydroxylase, peroxidase, and catalase, is an essential element in terms of maintaining the normal function of the body. For this reason, it is one of the important blood markers sought in determining whether the body maintains a healthy life. Although the iron requirement of the individual varies according to age and gender, it varies between 1.5-2.2 mg/day (Wang et al. 2012). In the study, the highest iron contents were determined as lavender honey (B1) 29.13 ppm and then sunflower honey (B3) 13.46 ppm. It is thought that, among the honeys examined in the research, especially lavender and jerusalem thorn honeys can be used as an important food in the diets of individuals suffering from iron anemia and without a history of diabetes.

Copper (Cu) is an important element that plays a role in iron absorption (Chandra 1990) and its daily requirement is 1-1.5 mg (Wang et al. 2012). The copper content of the honey samples examined in the research ranged between 0.17-0.86 ppm. Although the Cu content of honey is not very high, it

is stated that when taken together with other nutrients in the diet, honey may be one of the food sources that can play a role in meeting the daily Cu requirement.

Zinc (Zn) is an element found in the structure of plant and animal foods and in all living cells. There is a total of 2-4 g of zinc in an adult human body, and the daily requirement of the body is 5-10 mg. This amount can be easily met daily with the foods included in the diet in individuals with a normal diet. Zinc is a cofactor component that plays a key role in maintaining the function of many enzymes such as malate dehydrogenase, alcohol dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase and carboxypeptidase (Wang et al. 2012). Zinc (Zn) was found between 0.44-3.80 ppm in research honeys and is one of the important natural food sources in providing the body's daily zinc requirement in the daily diet.

Manganese (Mn) was found to be the highest in chestnut honey (T2) at 2.22 ppm among the honey types used in the study. It was determined between 0.13-0.64 ppm in the other three honey types (chasteberry, jerusalemthorn and lavender). Manganese is one of the important enzyme cofactors like zinc; it is an essential element for the functioning of decarboxylase, hydrolase and transferase enzymes (Bengü and Kutlu 2020). The amount of manganese in the body is about 10-40 mg in total. The daily manganese requirement of the body is a very small amount, such as 2-5 mg. This requirement can be easily met up to 48 mg per day, depending on the manganese content of the foods consumed in the daily diet. It is stated that even if manganese is taken in excess of the body's requirement, it does not have a toxic effect (Wang et al. 2012).

Boron (B) is an element found between 3.26-8.01 ppm in honey varieties evaluated in the research. Turkiye is a country known for its rich boron reserves (Elevli and Laratte 2022). As can be seen from the boron content of local country honeys such as chasteberry, chestnut and jerusalem thorn honey, which are especially studied, this rich element source is; it is estimated that it is carried to the hive by bees through plants grown in the soil and running water sources. It is stated that the daily boron requirement for the body is 1-2 mg. In addition, it is thought that boron is an element that interacts with calcium, magnesium and vitamin D and has an effect on bone formation (Wang et al. 2012).

Magnesium (Mg), considering all the examined honey varieties, is an element that is detected between 15.84 and 41.00 ppm and can be taken into the body as 300-500 mg in daily nutrition. Magnesium is one of the important enzyme activators like zinc and manganese. It has a function in the stabilization of cell and plasma membranes and nucleic acids. The lack of magnesium in the body can pave the way for important problems in metabolism (Wang et al. 2012).

Phosphorus (P) is an element with a daily requirement of about 0.8-1.2 g. It was found that the phosphorus content of the honey varieties used in the research varied between 205.93 and 294.96 ppm, and chestnut honey had the highest phosphorus content. Phosphorus is present in the structure of bones and teeth, DNA and RNA in the body. It is effective in the bipolarity of membrane lipids and lipoproteins in the bloodstream. Phosphorus also; it is an element involved in many metabolic processes such as energy production (ATP) and conversion in the cell, buffering of blood, regulation of gene transcription, enzyme activation (cofactor), renal system excretion function, immune system activity and signal transmission (Calvo and Lamberg-Allardt 2015).

Chromium (Cr) was found to vary between 0.09-0.12 ppm in honey samples. Chromium, as an enzyme activator (phosphoglucomutase enzyme), is an element that has an effect on increasing the activity of insulin and regulating blood glucose levels. In case of chromium deficiency in the body, glucose tolerance decreases and therefore the risk of cardiovascular disease increases. It is stated that feeding with 25 ppm chromate in experimental mice does not cause any toxic effect (Wang et al. 2012). Considering the maximum amount of honey that can be consumed daily, it is seen that the chromium content in research honeys has a content well below the level that can be toxic.

Sulfur (S) is an element in the macro elements group found in foods. Sulfur is found in the composition of many metabolites that are important for the maintenance of cell structure and the maintenance of biological activities (Bohrer and Takahashi 2016). After calcium and phosphorus, it is the third mineral substance in the body, constituting approximately 0.3% of the human body (Hewlings and Douglas, 2019). It was determined that the sulfur content of the research honeys varied between

21.46-56-86 ppm and was found mostly in chestnut honey.

Barium (Ba) is an element found in the skeletal system (0.5-10 μ g/g), teeth (0.1-3 μ g/g), heart, lung, kidney and liver in the human body. As a result of excessive Ba intake into the body, especially; health problems such as heart and/or kidney failure, pulmonary edema, respiratory paralysis and bleeding in the stomach and intestinal tract occur. As an orally adult reference dose for barium, 0.2 mg/kg/day has been determined by the U.S. Environmental Protection Agency (Kravchenko et al. 2014). In the study, although barium was not detected in chasteberry honey; the Ba level in chestnut, jerusalemthorn and lavender honeys is between 0.01-0.18 ppm.

In the study, **nickel (Ni)** was found to be 0.07, 0.11, 0.15 and 0.04 ppm at trace levels in chasteberry, chestnut, jerusalem thorn and lavender honeys, respectively. Nickel is an element that is a cofactor for many enzymes and plays a role in increasing insulin activity. Nickel requirement is estimated to be 35-500 μ g per day; The amount of Ni that can be taken with the daily diet is at the level of 150-700 μ g (Wang et al. 2012).

Strontium (Sr) is a trace element whose function in the human body is not fully understood. In some animal experiments, strontium is thought to be effective in physiological processes such as muscle contraction, blood clotting, and secretion of certain hormones. It is stated that the daily adult dose is about 4 mg (Kołodziejska et al. 2021). In our study, 0.12-0.21 ppm of strontium was found in the honey samples examined (chasteberry, chestnut, jerusalemthorn and lavender), and these levels are far below the daily intake dose.

DISCUSSION

In our study, when the daily honey consumption of people is evaluated in grams of portion, it is seen that the possible heavy metal doses that can be taken into the body with these portions are far below the toxic values. While there are studies on honey that overlap with the results of this research, there are also research results that do not overlap and indicate that heavy metal pollution increases as you get closer to settlements. For example, in 11 different honey samples taken from Bingöl and its surroundings, 18 mineral substances were analyzed (Bengü and Kutlu, 2020) and honey samples were found to be reliable in terms of heavy metal contents, in parallel with this study. But there are also research findings to the contrary. For example, Demirezen et al. (2005) determined that the heavy metal contents of honey samples taken from places close to the residential area were generally higher.

Although the honey used in our study was generally supplied from the Marmara and Aegean regions. where industrialization is intense, the amount of Al contained in some honey samples was even lower than the amounts contained in the honey obtained from Bingöl, which is a city far from industrial and production facilities that may cause environmental pollution. For example, Cd, Zn and Ni concentrations in honey samples taken from Kayseri Ercives Mountain and its surroundings were determined to be in the range of 0.11-0.18 ppm, 2.2-11 ppm and 0.2-0.8 ppm, respectively (Demirezen and Aksoy, 2005). According to the analysis results, the contents of Cd (0.01-0.02 ppm), Zn (0.44-1.69 ppm) and Ni (0.04-0.15 ppm) in the present study were lower than the honey samples investigated in Kayseri. It is estimated that the difference in the honeys of these two regions, which are not close to the industrial zones, may be due to the metal content and elemental composition in the soil and other natural resources, rather than the pollution caused by the industrial facilities and city centers in nature.

When honey samples were compared in terms of heavy metals and elements examined, the statistical significance level was found 1% between honey samples and their Pb, Al, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P and S contents. A significant difference of 5% was found in terms of Mn, Ni and Fe contents. No statistical difference was found between Cd and Co levels.

Based on the coordinates of the apiaries where honey samples were collected, the distance to the highway and the nearest settlement was determined in kilometers. Considering the relationship between the distances of the apiaries from the highway or settlement centers with the contents of elements detected in honey; it has been determined that there is an inversely proportional relationship between the amounts of Pb, As and Co and the distance to the settlements. In other words, Pb, As and Co contents increase as the apiaries from which honey samples are taken get closer to the settlement. The said correlation value is significant at the level of 1% for Pb and 5% for As and Co.

There are many studies on heavy metal and trace element contents in honey produced in Turkiye. However. environmental pollution changes depending on many factors such as geography, climate and pollution sources, it is important for data security to repeat the effects of environmental pollution on people and food as often and in as many different and many examples as possible. Heavy metals, which are an indicator of environmental pollution, seriously pollute the soil, water and air. The increase in the heavy metal level in the soil also increases the heavy metal accumulation in the plants. The bees, which obtain most of their food sources from plants, and their water needs from water sources in nature, carry nectars such as sap, nectar and pollen they collect from plants and heavy metals to the hive. Therefore, heavy metal and element contents in the composition of bee products such as honey, pollen, royal jelly and propolis may vary depending on the heavy metal and element content in environmental sources such as soil, water and air.

Preserving the naturalness of honey produced and consumed in different regions of our country is very important for our health. Honey, which is an important food for human health in nutrition, can be exposed to many sources of contamination during the processes after it is produced.

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