Citation: Portakal P, Çiltepe M. Investigation of heavy metal levels of beeswax in bee apiaries in Çankiri. U. Bee J. / U. Arı D. 24 (1): 26-37, DOI: 10.31467/uluaricilik.1386309

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INVESTIGATION OF HEAVY METAL LEVELS OF BEESWAX IN BEE APIARIES IN ÇANKIRI

Çankırı'da Arı İşletmelerindeki Peteklerin Ağır Metal Düzeylerinin Araştırılması

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Geliş Tarihi / Received: 05.11.2023 Kabul Tarihi / Accepted: 20.03.2024 DOI: 10.31467/uluaricilik.1386309

ABSTRACT

A total of 25 samples of beeswax (9 from the southern and 16 from the northern of Çankırı) were collected within the scope of this study. Heavy metal contents of these samples were analyzed by using inductively coupled plasma mass spectroscopy (ICP-MS). The mean iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), aluminum (Al), chromium (Cr), vanadium (V), cadmium (Cd), selenium (Se) levels in the samples were found to be 36.28 ppm, 1.69 ppm, 1.65 ppm, 1.14 ppm, 0.78 ppm, 0.68 ppm, 0.30 ppm, 0.13 ppm, 0.06 ppm, 0.01 ppm, respectively. Comparing the northern and southern regions, only Cd (1.10 ppm) was found to be higher in the southern region, whereas the other heavy metals were found to be higher in the northern region. No statistical difference was found between mean Fe, Pb, Zn, Al, Cr, V, and Se levels in the wax samples obtained from the northern and southern regions, whereas a statistically significant difference was found in Mn, Cu, and Cd concentrations. As a result, the heavy metal concentration in beeswax varied between the regions. Except for Cd element, all values were higher in the northern region of Çankırı and this region that is close to the highway with heavy traffic should be evaluated in terms of beekeeping.

Keywords: Beeswax, Heavy Metal, ICP-MS

ÖZ

Bu çalışma kapsamında Çankırı'nın güneyinden 9 ve kuzeyinden 16 olmak üzere toplam 25 örnek alınmıştır. Bu örneklerin ağır metal içerikleri indüktif eşleşmiş plazma kütle spektroskopisi (ICP-MS) kullanılarak analiz edilmiştir. Örneklerdeki ortalama demir (Fe), çinko (Zn), bakır (Cu), mangan (Mn), kurşun (Pb), alüminyum (Al), krom (Cr), vanadyum (V), kadmiyum (Cd), selenyum (Se) değerleri sırasıyla 36,28 ppm, 1,69 ppm, 1,65 ppm, 1,14 ppm, 0,78 ppm, 0,68 ppm, 0,30 ppm, 0,13 ppm, 0,06 ppm, 0,01 ppm'dir. Kuzey ve güney bölgeleri arasında sadece Cd (1.10 ppm) güney bölgesinde yüksek bulunurken, diğer ağır metaller kuzey bölgesinde yüksek bulunmuştur. Çalışmada, kuzey ve güney bölgelerinden alınan balmumu örneklerindeki Fe, Pb, Zn, Al, Cr, V ve Se ortalamaları arasında istatistiksel bir fark bulunmazken, Mn, Cu ve Cd konsantrasyonları arasında istatistiksel olarak anlamlı bir fark bulunmuştur. Sonuç olarak, arı ürünlerinden biri olan balmumundaki ağır metal konsantrasyonu bölgelere göre değişiklik göstermiştir. Cd elementi hariç tüm değerler Çankırı'nın kuzey bölgesinde daha yüksek olup, trafiğin yoğun olduğu karayoluna yakın olan bu bölgenin arıcılık açısından değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: Balmumu, Ağır Metal, ICP-MS

GENİŞLETİLMİŞ ÖZET

Amaç: Balmumu, arılar tarafından karınlarındaki bezlerden salgılanan bir maddedir. Arılar tarafından kovanlarında petek yapısını inşa etmek ve bal içeren hücreleri veya gelişmekte olan arıları mühürlemek için kullanılır. Doğal ve yenilenebilir bir kaynak olması açısından önemlidir ve kullanımı kültürel, tarihi ve çevresel öneme sahiptir. Geniş bir uygulama alanına sahiptir. Balmumunun lipit bazlı bileşimi, çevreden çok çeşitli maddeleri emmesini ve biriktirmesini sağlayarak onu kirlilik seviyelerini değerlendirmek için yararlı bir araç haline getirir. Balmumu petekleri, özellikle metallerin ve pestisitlerin varlığı olmak üzere çevre kirliliğinin biyoindikatörleri olarak kullanılmıştır. Araştırmacılar peteklerdeki bu kirleticilerin seviyelerini analiz ederek, arıların yiyecek aradıkları ve kovanlarını inşa ettikleri alanın çevresel kalitesi hakkında bilgi edinebilirler. Peteklerdeki kirletici seviyelerinin izlenmesi, belirli bir alandaki kirliliğin boyutu ve bunun ekosistem ve insan sağlığı üzerindeki potansiyel etkisinin yanı sıra arı popülasyonlarının sağlığı ve refahı hakkında da bilgi sağlayabilir. Endüstriyel alanlar ve araç trafiği nedeniyle havaya ve çevreye Al, Ca, Cu, Fe, Pb, Mg, Si, Zn, Ba, Cd, Ni, Pd, Pt gibi ağır metaller yayılabildiği gibi özellikle yoğun araç trafiği nedeniyle sularda arı ve arı ürünlerini kirletebilecek ağır metaller de bulunabilmektedir. Yapılan literatür taramasında Çankırı'da üretilen balmumunun ağır metal düzeyleri ile ilgili bir araştırmaya rastlanmamıştır. Bu çalışmanın amacı, 2022 Hava Kirliliği raporuna göre ulusal mevzuat limitlerinin altında olan Çankırı İlindeki 25 işletmeden alınan balmumlarındaki ağır metal seviyelerini belirlemektir (Şekil 1).

Gereç ve Yöntem: Arıların yaklaşık 3 km yarıçaplı bir alanda yoğun olarak uçtukları ve bu nedenle arıların ve arı ürünlerinin çevresel kirliliğin belirlenmesinde biyoindikatör olarak kullanılabileceği bildirilmiştir. Bu amaçla Çankırı Arı Yetiştiricileri Birliği'nin yardımıyla basit rastgele örnekleme yöntemiyle belirlenen 25 farklı arı işletmesinden balmumu örnekleri alınmıştır. Çankırı'nın güney ve kuzey kesimlerini temsilen alınan örneklerde işletmelerin konumları ve trafik yoğunluğunun en fazla olduğu D-765, D-180, E-80 karayollarına olan uzaklıkları dikkate alınmış ve analiz sonuçları buna göre değerlendirilmiştir (Tablo 1). Örnekler (ICP-MS) kullanılarak analiz edilmiştir.

Bulgular ve sonuç: Bu çalışmada analiz edilen balmumu örneklerinde bulunan ağır metallerin en düşük, en yüksek, ortalama miktarları ve standart sapmaları Tablo 4'te verilmiştir. Analiz edilen toplam 25 balmumu örneğinde en fazla bulunan ağır metallerin Fe, Pb, Mn, Zn, Cu, Al, Cr, Cd, V, Se olduğu tespit edilmiştir. İncelenen balmumundaki ağır metallerin bölgesel konsantrasyonlarını karşılaştırmak için bağımsız örneklem t-testi yapılmıştır. Çalışmada kuzey ve güney bölgelerdeki Mn, Cu ve Cd konsantrasyonları arasında istatistiksel olarak anlamlı bir fark bulunmuştur. İncelenen diğer tüm ağır metallerin konsantrasyonlarında istatistiksel olarak anlamlı bir farklılık görülmemiştir (Tablo 5). Kuzey ve güney bölgeleri arasında sadece Cd (1.10 ppm) güney bölgesinde yüksek bulunurken, diğer ağır metaller kuzey bölgesinde yüksek bulunmuştur. Sonuç olarak, arı ürünlerinden biri olan balmumundaki ağır metal konsantrasyonu bölgelere göre değişiklik göstermiştir. Cd elementi hariç tüm değerler Çankırı'nın kuzey bölgesinde daha yüksek olup, trafiğin yoğun olduğu karayoluna yakın olan bu bölgenin arıcılık açısından değerlendirilmesi gerekmektedir.

INTRODUCTION

Beeswax is a substance secreted by bees from glands located in their abdomen. It is used by bees to build honeycomb structures in their hives and to seal cells containing honey or rearing broods (Samarghandian et al. 2017). It consists of high fatty acid esters, hydrocarbons, alcohols, and proteins and contains trace amounts of minor components such as pigments, vitamins, and minerals (Fratini, et al. 2016). It is important because it is a natural and renewable resource and its use has cultural, historical, and environmental importance. It has a wide range of applications. It is mainly used in the beekeeping industry to make the foundation for combs, as well as in lip balms, lotions, creams, and other cosmetic products thanks to its moisturizing and protective qualities. It has been used historically in some traditional medicines and ointments. Beeswax can be used to protect wood since it provides a natural and non-toxic alternative to commercial wood finishes. Sometimes it is used in food preparation, such as in the process of making cheese, as a coating on certain types of products, and even in the production of mead (honey wine). Beeswax coatings serve as an eco-friendly alternative to plastic coatings, used to cover and store food items (Chaireh et al. 2020, Metin and Koçyiğit 2022, Topal et al. 2020).

Beeswax is a valuable instrument for determining pollution levels because of its lipid-based nature, which enables it to absorb and accumulate a wide range of pollutants from the environment (Valdovinos-Flores et al. 2017). Beeswax combs have been employed as bioindicators of environmental pollution, particularly indicating the presence of metals and pesticides. Examining the combs containing these pollutants, scientists can learn more about the state of the environment, where bees are feeding and establishing their colonies. Monitoring the concentrations of pollutants in combs can also reveal information on the level of pollution in a given area and its possible effects on human health, ecosystem, and bee populations' well-being (Gajger et al. 2019).

Heavy metals are elements that have a high atomic weight and density, and they can be toxic to both humans and the environment. Even though they are naturally present in the Earth's crust, human activities such as industrial processes, mining, and agriculture can release these elements into the air, water, and soil, which causes pollution (Sankhla et al. 2016). There are hazardous consequences of heavy metal exposure for both consumers (humans) and bees. In humans, many heavy metals, such as lead, cadmium, and mercury may accumulate in the kidneys and can cause damage to these vital organs, potentially leading to chronic kidney diseases (Johri et al. 2010, Mishra et al. 2022). Exposure to high levels of lead can result in lead poisoning. This can lead to various health issues, including neurological problems, cognitive deficits (especially in children), and high blood pressure. Some heavy metals, such as arsenic, cadmium, and nickel, have been associated with an increased risk of cancer in humans (Türker 2023). Ingestion or exposure to certain heavy metals can cause gastrointestinal problems such as nausea, vomiting, diarrhea, and abdominal pain (Aalami et al. 2022). Certain heavy metals, such as lead and cadmium, have the potential to affect reproductive health and fertility in both men and women by disrupting hormone regulation and causing damage to the reproductive organs (Emek 2023). Bees, which serve as important pollinators, play a fundamental role in ecosystems and agriculture. Exposure to heavy metals might alter bee behavior by affecting foraging patterns, navigational abilities, and communication within the hive. In addition, heavy metals can impair the immune system of bees, which makes them more susceptible to disease and infection. As in humans, it can disrupt the reproductive systems of bees and affect their ability to reproduce and maintain healthy populations. It can lead to deformations, decreases in growth rates, and even death in bee broods during larval development, as well as shortening the life span of adult bees. These effects disrupt the normal development of the colony and can weaken the colony's overall health and reduce pollination efficiency (Hassona and El-Wahed 2023).

Beeswax can vary between the regions, where bees live, depending on vegetation, climate, and other environmental factors. Clean and safely produced beeswax has a wide range of uses in various industries and is important for health. Manufacturers should produce quality and reliable products by considering these factors (Valdovinos-Flores, et al. 2017). Due to industrial areas and heavy vehicle traffic, heavy metals such as Al, Ca, Cu, Fe, Pb, Mg, Si, Zn, Ba, Cd, Ni, Pd, and Pt can spread into the air, water, and the environment and contaminate bees and bee products (Ernest et al. 2018). In this context, it was reported that bees and bee products can be used as bioindicators to determine environmental pollution (Sitarz-Palczak et al. 2015, Yarsan et al.2007).

No study on the heavy metal content of beeswax produced in Çankırı was found in the existing literature. Therefore, the present study aims to evaluate the levels of heavy metals in beeswax samples obtained from 25 apiaries in Çankırı province, a region that is currently below the national legal limits as reported in the 2022 Air Pollution Report (Figure 1).

Figure-1 Pollution Level in Provinces in 2021 (Dirim 2023)

MATERIALS AND METHODS

It was reported that bees fly intensively in an area of approximately 3 km radius and therefore bees and bee products can be used as bioindicators to determine environmental contamination (Aygün 2020, Sitarz-Palczak et al. 2015). For this purpose, beeswax samples were obtained from 25 different bee apiaries determined by using the simple random sampling method with the help of Çankırı Beekeepers Association. In the samples representing the southern and northern parts of Çankırı, the locations of the apiaries and their distances to the D-765, D-180, and E-80 highways, where the traffic density is very high, were taken into consideration while taking the samples and the analysis results were evaluated accordingly (Table 1).

Sample Collection

Honeybee wax samples were collected from 25 different beekeeping apiaries in Çankırı province (Figure 2). Nine of the beekeeping apiaries were selected from the southern part of Çankırı and 16 apiaries were selected from the northern part of Çankırı, with large forest areas. Five of the apiaries in the southern region were located on the Çankırı-Ankara highway, where the level of traffic density is high, and the others were in rural areas where the level of traffic density is low. While seven of the apiaries in the northern region were located near the Çankırı-Istanbul highway where traffic is heavy, the rest were in areas with less traffic. Wax samples made by the bees themselves were collected in the first weeks of May and June 2023 and then stored in a dark and cool place until analysis.

Microwave Oven Dissolution for ICP-MS analysis: In sample preparation for ICP-MS method, approximately 1 gram of the samples were weighed, transferred to Teflon containers of the microwave oven, and then added with 10ml of 65% nitric acid (Merck). For the blank, 10ml of 65% nitric acid was added to an empty Teflon container. The Teflon containers were tightly closed and placed in a CEM brand MARS6 ONE TOUCH (USA) model microwave disintegrator oven. The maximum temperature was increased to 200 ℃ within 15 minutes and kept at this level for 15 minutes. In total, the samples were kept in the closed system for 30 minutes and the dissolution process was carried out. After the microwave oven temperature cooled down to ambient conditions, the solution in the Teflon containers was washed thoroughly with ultrapure water and the Teflon containers were taken into 50 ml balloon jugs.

Table 1. Locations where samples were obtained

Regions	Samp.No	Location	Distance to Highway (km)
Northern Çankırı Region	1	Ömerli	0.8 (E-80)
	2	Ilgaz-a	1.8 (E-80)
	3	Ilgaz-b	1.8 (E-80)
	4	Ilgaz-c	1.8 (E-80)
	5	Ilgaz-d	1.8 (E-80)
	6	Gaziler Köyü	1.5 (E-80)
	7	Cendere	2 (E-80)
	8	Yazı Köyü	3.3 (E-80)
	9	Kızılıprık	4.5 (E-80)
	10	Süleyman Hacılar-a	4.6 (E-80)
	11	Süleyman Hacılar-b	4.6 (E-80)
	12	Ödemiş	5.3 (E-80)
	13	Güney Köyü-a	7 (E-80)
	14	Güney Köyü-b	7 (E-80)
	15	Yeşildumlupınar-a	8.2 (E-80)
	16	Yeşildumlupınar-b	8.2 (E-80)
Southern Çankırı Region	17	Tüney-a	0.7 (D-765)
	18	Tüney-b	0.7 (D-765)
	19	Tüney-c	0.7 (D-765)
	20	Çankırı/Merkez-a	0.2 (D-765)
	21	Çankırı/Merkez-b	0.2 (D-765)
	22	Gölezkayı	6.7 (D-765)
	23	Eldivan	9.4 (D-765)
	24	Kırovacık-a	11.7 (D-180)20 (D-765)
	25	Kırovacık-b	11.7 (D-180)20 (D-765)

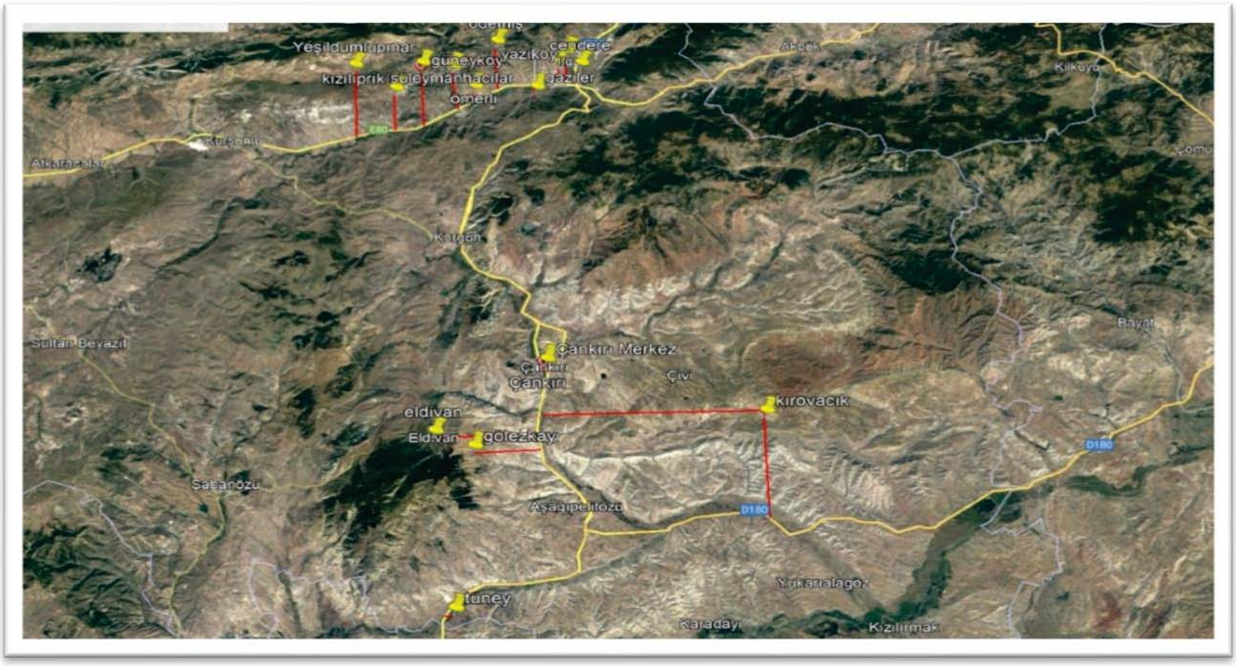

Figure-2 Southern and Northern Parts of Çankırı (Google Earth pro)

Table 2. Calibration standards

Analytes	Std1 (ppb)	Std2 (ppb)	Std3 (ppb)	Std4 (ppb)	Std5 (ppb)	Std6 (ppb)	Internal standards
Al, Cd, Cr, Fe, Mn, Pb, Se, V, Zn	0.5	1	5	25	50	100	^{89}Y
Fe	12.5	25	125	625	1250	2500	

ICP-MS Method for elemental analysis: ICP-MS calibration solutions were prepared by diluting commercially available multi-element standards with 1% (suprapure nitric acid ultrapure water) and the calibration graph was prepared by preparing the concentrations specified in Table 2. ICP-MS NexION 2000 (PerkinElmer Inc., USA) with a quartz nebulizer, cyclonic spray chamber, and an integrated auto-sampler was used for elemental analysis of the samples. Using 18.2 MΩ ultrapure water from the SartoriusTM Wall Mounted Arium pro-Ultrapure Water System, a washing solution containing 1% suprapure nitric acid-ultrapure water was prepared at the concentrations indicated in Table 2. In addition, ICP-MS calibration was performed before each measurement. ^{89}Y internal standard at a concentration of 100 ppb was used for control of elemental analyses.

By using a peristaltic pump, the samples were sent into the cyclonic spray chamber with a flow of argon gas. A high percentage of helium gas was used in addition to argon gas to avoid interferences. Syngistix for ICP-MS software version 2.2 was used to control the instrument, including setup, interferences, data acquisition, and data analysis (Table 3).

Table 3. Operating conditions of the ICP-MS

Parameter	Explanation/ Value
Nebulizer	MEINHARD® plusGlassType C
Spray Chamber	Glass cyclonic (baffled), 4 ºC
One-Piece Torch	w/2.5 mm i.d.
Nebulizer Flow	Optimized for<2% oxides
RF power	1600 W
Cones	Ni
Replicates	3
Dwell time	50 ms
Aerosol Dilution	Set to 2.5x
Sample Delivery Rate	350 µl/min
Rinse time	45 second
Nebulizer gas flow rate	0.93 L/min
Deflector voltage	-12 V
Analog stage voltage	-1750 V
Pulse stage voltage	1100V
Discriminator threshold	26
Sample Tubing (Orange-Yellow)	Flared PVC PumpTubes 0.51 mm/0.89 mm
Internal Standard Tubing (Orange-red)	Flared PVC PumpTubes 0.19 mm/0.91 mm
Peristaltic pump speed	35 rpm
Alternating current (AC) rod offset	-4

RESULTS

The lowest, highest, average amounts, and standard deviations of the heavy metals found in the beeswax samples analyzed in this study are given in Table 4. It was determined that the most abundant heavy metals in a total of 25 wax samples analyzed were Fe, Pb, Mn, Zn, Cu, Al, Cr, Cd, V, and Se.

Table 4. Heavy metal levels of 25 beeswax samples analyzed

Regions	Samp. No	Elements									
		Al (ppm)	V (ppm)	Cr (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Se (ppm)	Cd (ppm)	Pb (ppm)
Northern Çankırı Region	1	0.59	0.10	0.19	1.13	24.41	2.29	3.21	0.03	0.03	0.49
	2	0.45	0.66	0.06	0.38	15.34	1.00	1.26	0.01	0.01	0.07
	3	0.86	0.13	0.28	1.89	61.70	1.62	2.18	0.17	0.03	0.51
	4	0.67	0.11	0.15	1.32	33.17	1.28	1.58	0.03	0.02	0.40
	5	0.90	0.15	0.25	1.52	53.87	1.46	1.60	0.02	0.06	1.13
	6	0.61	0.09	0.14	1.02	20.45	1.21	1.34	0.03	0.01	0.40
	7	0.53	0.08	0.17	0.51	16.97	1.30	1.34	0.00	0.02	0.37
	8	1.71	0.36	0.49	4.27	178.07	1.38	1.42	0.02	0.01	0.58
	9	0.64	0.10	0.17	1.29	28.30	2.34	1.56	0.01	0.02	0.42
	10	0.64	0.10	0.21	1.34	34.16	2.27	1.53	0.01	0.03	0.50
	11	0.58	0.09	0.15	0.89	20.39	1.87	4.11	0.02	0.02	1.11
	12	0.58	0.08	1.58	0.60	27.59	1.05	1.34	0.01	0.03	7.13
	13	0.76	0.13	0.24	1.48	48.24	2.18	1.45	0.05	0.02	0.44
	14	0.76	0.12	0.22	1.39	40.73	2.29	1.60	0.01	0.03	0.47
	15	0.53	0.09	0.17	1.48	26.32	2.83	1.48	TE	0.04	0.54
	16	0.60	0.11	0.21	1.48	30.98	3.63	1.46	0.01	0.03	0.45
Southern Çankırı Region	17	0.55	0.08	0.14	0.63	15.03	1.13	1.44	0.01	0.03	0.45
	18	0.63	0.10	0.22	0.82	38.26	1.32	1.42	TE	0.02	0.44
	19	0.55	0.08	0.14	0.48	15.09	1.04	1.36	0.01	0.02	0.40
	20	0.72	0.13	0.24	0.78	23.05	1.78	1.99	TE	0.03	0.52
	21	0.66	0.09	0.19	0.73	21.50	1.17	1.51	TE	0.04	0.99
	22	0.57	0.08	0.15	0.79	20.15	1.19	1.46	TE	0.02	0.40
	23	0.51	0.10	0.81	0.51	15.12	1.13	1.29	0.01	0.02	0.41
	24	0.86	0.18	0.79	1.31	81.33	1.44	1.75	TE	1.10	0.62
	25	0.57	0.11	0.20	0.63	16.94	1.14	1.64	0.01	0.04	0.45
	Min.	0.45	0.08	0.06	0,38	15.03	1.00	1.26	0.00	0.01	0.07
	Max.	1.71	0.66	1.58	4.27	178.07	3.63	4.11	0.17	1.10	7.13
	Av±St-De	0.68 ± 0.24	0.13 ± 0.12	0.30 ± 0.32	1.14 ± 0.77	36.28 ± 33.76	1.65 ± 0.65	1.69 ± 0.64	0.01 ± 0.33	0.06 ± 0.21	0.78 ± 1.34

An independent sample t-test was performed to compare the regional concentrations of heavy metals in the investigated beeswax. The study found a statistically significant difference between Mn, Cu, and Cd concentrations in northern and southern locations. All the other heavy metals studied exhibited no statistically significant differences in concentrations (Table 5). Comparing the northern and southern regions, only Cd (1.10 ppm) was found to be higher in the southern region, whereas the other heavy metals were found to be higher in the northern region. As a result, the heavy metal concentration in beeswax, which is one of the bee products, varied between the regions. Except for Cd element, all values were higher in the northern region of Çankırı and this region, which is close to the highway with heavy traffic, should be evaluated in terms of beekeeping.

Table 5. Regions differences in heavy metal concentration

Elements	Regions/N	Mean ± Standard deviation	t	p
Al	1/16	0.71±0.29	0.87	0.39
	2/9	0.62±0.10		
V	1/16	0.15±0.14	0.99	0.33
	2/9	0.10±0.03		
Cr	1/16	0.29±0.35	-0.20	0.84
	2/9	0.32±0.27		
Mn	1/16	1.37±0.87	2.10	0.04
	2/9	0.74±0.24		
Fe	1/16	41.29±38.77	0.98	0.33
	2/9	27.38±21.49		
Cu	1/16	1.87±0.72	3.13	0.00
	2/9	1.26±0.22		
Zn	1/16	1.77±0.78	0.89	0.38
	2/9	1.54±0.21		
Se	1/16	0.02±0.40	1.64	0.11
	2/9	0.00±0.00		
Cd	1/16	0.02±0.01	-1.01	0.34
	2/9	0.14±0.35		
Pb	1/16	0.93±1.67	0.74	0.46
	2/ 9	0.52±0.18		

P > 0.05 (no significant difference) N: number of samples

DISCUSSION

Beeswax is a substance produced by bees and used by honeybees to rear their young members, store their pollen and nectar, make honey, and sometimes even make the honeycomb cells used to ferment pollen. The environmental conditions in which bees live, especially heavy metal pollution in soil, vegetation, and water sources, can affect the quality of wax, which in turn can have negative effects on the health of bee colonies (Gajger et al. 2019). However, more studies should be carried out on this subject and the possible effects on bee colonies, the ecosystem, and human health should be better understood.

This study aims to evaluate the heavy metal content of beeswax at different locations. The heavy metal composition of beeswax depends on several variables, including the geography and proximity of the location of beekeeping apiaries to large settlements, waste incineration facilities, heavy vehicle traffic, and large industrial facilities (Arslan and Arıkan 2013, Gajger et al. 2019) The lowest Fe content in the wax samples analyzed in this study was found to be 15.03 ppm and the highest one to be 178.07 ppm. In a study carried out by Gajger et al. (2019), Fe content in beeswax was determined to range between 56.470 and 285 ppm. In other studies carried out in different regions, Fe content was reported in the ranges of 2.068-5.041 ppm (Hassona and El-Wahed 2023), 1.080-334 µg/g (Formicki et al. 2013) and 5.972-18.516 mg/g (Aljedani 2020). The difference in Fe concentrations may vary depending on the geographical location of bee farms, soil composition, water sources, and other environmental pollution factors. Disruption of iron metabolism can lead to various health problems. Although it leads to common diseases such as anemia when the amount of Fe is low, it can also cause iron accumulation in the body and diseases such as hemochromatosis (Gürsel et al. 2015).

Hassona and Wahed (2023) determined the amount of Pb in beeswax collected in Behaira province of Egypt in the range of 0.040-0.185 ppm for a 5-year period between 2018 and 2022.

As a result of this study, it was observed that the concentration of heavy metals increased as the wax combs aged. In a study carried out by Bakırcı in Aydın province, the average level of Pb in beeswax was determined to be 201.63 µg/kg (Bakirci 2019). In a study carried out by Zafeiraki et al. (2022) in Slovakia, the maximum concentration of Pb was found to be 3.193 mg/kg and the lowest one to be 59 µg/kg. However, in a study carried out by Gajger et al. (Gajger et al. 2019), the range was found to be 1.230-5.430 ppm was determined. In the present study, the lowest Pb value was 0.07 ppm and the highest was 7.13 ppm. Exposure to lead occurs through environmental and industrial means. Daily lead intake in humans varies between 20 and 400 mg. The committee of experts established in cooperation with the Food and Agriculture Organization of the United Nations and the World Health Organization set the provisional tolerable weekly intake (PTWI: Provisional Tolerable Weekly Intake) for lead at 3000 mg. However, half of this amount has been accepted as the safe limit for children (Özbolat and Tuli 2016). Di et al. (2016) examined the effects of Cd, Cu, and Pb on *A. mellifera* larvae and foragers and revealed that each heavy metal individually slowed larval development and caused a decrease in prepupal and pupal weights. Heavy metals accumulated in beeswax have negative effects not only on human health but also on bees. Therefore, since lead accumulation varies depending on the region where beeswax is produced, it is thought that beekeeping regions should be kept under control.

Bakırcı (2019) determined the Mn concentration in beeswax to be 361.30-10359.37 µg/kg in his study carried out in Aydın province in 2019. In other studies on beeswax, Mn values were determined between 0.182 and 41.904 mg/kg (Zafeiraki et al. 2022), 16.630 and 32.870 ppm (Gajger et al. 2019), 22.200 and 450 µg/g (Formicki et al. 2013), and 0.365, 0.501, 1.311, and 0.414 mg/g (Aljedani 2020). In the wax samples analyzed in this study, Mn was found to range between 0.38 and 4.27 and was observed to be at lower levels when compared to the results reported in other studies (Bakirci 2019).

Zn was determined to range between 1.26 and 4.11 ppm in the wax samples analyzed in this study, when compared to 1 to 81,200 µg/g (Formicki et al. 2013), 5,707 µg/g (Ullah et al. 2022), 19,699, 6,272 and 0,776 mg/g (Aljedani 2020) in other studies.

Cu was detected within the range of 1.00-3.63 ppm in the wax samples analyzed in this study. In a study in other studies by Gajger et al. (2019) in areas where copper preparations are widely used for plant protection, Cu concentration was found to be between 12.8 and 40.93 ppm, suggesting that environmental contamination is important.

The lowest Al content in the wax samples analyzed in this study was 0.45 ppm and the highest one was 1.71 ppm. The World Health Organization (WHO) and other health organizations do not recommend a specific upper limit for daily aluminum intake, because aluminum is an element commonly found in our bodies and is naturally present in many foods. However, excessive intake of aluminum can have adverse health effects, which is of particular concern for some groups. Also, some studies suggested that aluminum may be associated with an increased risk of Alzheimer's disease, but there is no conclusive evidence on this subject and the issue is still under investigation (Öztürk and Karan 2009, Pastacı, et al. 2010).

The presence of Cr accumulated in beeswax is considered an indicator of environmental pollution (Ullah et al. 2022). In the present study, Cr concentration was found to be 0.06-1.58 ppm. In previous studies, Cr content was reported to range between 41,030 and 56,280 ppm (Gajger et al. 2019), 2,016, 2,300, and 3,920 mg/g (Aljedani 2020), 82 982 µg/kg (34), 432 µg/kg (Bommuraj et al. 2019), 131,200, 85,770 and 247,600 µg/kg (Ćirić et al. 2021), and between 1,008 and 2,728 ppm (Hassona and El-Wahed 2023). It is thought that the difference between the results reported in these studies is related with environmental pollution.

In a study in other studies by Van der Steen et al. (2015) on the determination of Cd, Pb, and V heavy metal concentrations in honeybees in the Netherlands, it was concluded that honeybees may be useful as an alternative to mechanical devices in monitoring air pollution. In the present study, V concentration in samples was found to be 0.08-0.66 ppm.

The concentration of Se in the wax samples analyzed in this study was found to be 0.00-0.17 ppm. Hladun et al. (2012) concluded in their study that, if honeybees are fed with nectar containing Se, then there may be a decrease in population due to toxicity. Therefore, excessive selenium accumulation will negatively affect bee health.

Monitoring heavy metal concentrations contributes to environmental protection efforts and helps protect ecosystem health and human health. The differences in the concentrations of heavy metals in bee products may be associated with many factors.

The main reasons for these differences are soil conditions, vegetation, and weather conditions in the regions where bees collect nectar and pollen, as well as pesticides, beekeeping medicines, and various chemicals used in agricultural areas. Besides that, factors such as intensive industrial activities and traffic can affect the concentrations of heavy metals.

Heavy metals can negatively affect the larval development of bees and the health of adult bees, causing bee colonies to be weak and productivity to decrease. This can be a major economic problem for beekeepers. In addition, heavy metal residues in bee products raise food safety concerns and may pose a health risk to consumers. Efforts to reduce heavy metal exposure include stricter industrial regulations, proper waste disposal, and the adoption of sustainable agricultural practices. Monitoring and reducing heavy metal contamination are important steps both for protecting human health and the environment.

Author Contributions: PP, MÇ conceived and planned the experiments. PP and MÇ carried out the experiments. PP and MÇ planned and carried out the simulations. PP and MÇ contributed to sample preparation. PP and MÇ contributed to the interpretation of the results. PP took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of Interest: The authors declared that there is no conflict of interest.

Data Availability Statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement: This study does not present any ethical concerns.

Financial support: This research has been supported within the content of the project no 2209 by Tübitak.

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