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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ANALYSIS OF PESTICIDES, ANTIBIOTICS, AND HEAVY METAL LEVELS IN HONEY PRODUCED IN THE BAYBURT AND UPPER ÇORUH VALLEY REGIONS OF TÜRKİYE

Türkiye'nin Bayburt ve Yukarı Çoruh Vadisi Bölgelerinde Üretilen Ballarda Pestisit, Antibiyotik ve Ağır Metal Düzeylerinin Analizi

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ABSTRACT

This study investigates pesticide, antibiotic, and heavy metal levels in Bayburt and the Upper Çoruh Valley honey. Thirteen honey samples were collected from different apiaries managed by stationary and migratory beekeepers. These samples were analysed for heavy metals (Fe, Cu, Zn, Cd, Pb), antibiotics, pesticides, and chemical residues such as naphthalene. The results revealed that lead (Pb) levels exceeded international food safety standards in 2 honey samples, while pesticide residues were detected in 5 samples. Additionally, antibiotic residues were found in 6 samples, including sulfamethazine, tetracycline, and streptomycin. However, no naphthalene was detected in any of the samples. These findings highlight the importance of stricter regulations and monitoring systems to control chemical use in beekeeping practices. Enhancing awareness among beekeepers regarding the risks associated with pesticide and antibiotic use is crucial for improving honey quality and ensuring the health of beekeepers and consumers. The adoption of safer practices and adherence to guidelines are necessary to mitigate these health hazards.

Keywords: Beekeeping, Honey residues, Heavy metals, Pesticides

ÖZ

Bu çalışma, Bayburt ve Yukarı Çoruh Vadisi'nde üretilen ballarda pestisit, antibiyotik ve ağır metal seviyelerini araştırmaktadır. Sabit ve gezgin arıcıların işlettiği 13 farklı arılıktan toplanan bal örneklerinde ağır metaller (Fe, Cu, Zn, Cd, Pb), antibiyotikler, pestisitler ve naftalin gibi kimyasal kalıntılar analiz edilmiştir. Analiz sonuçlarına göre, 2 bal örneğinde kurşun (Pb) seviyeleri uluslararası gıda güvenliği standartlarının üzerinde bulunmuş, 5 örnekte ise pestisit kalıntısına rastlanmıştır. Ayrıca, 6 bal örneğinde sulfamethazin, tetracycline ve streptomycin gibi antibiyotik kalıntıları tespit edilmiştir; ancak hiçbir örnekte naftalin kalıntısına rastlanmamıştır. Bu bulgular, arıcılık faaliyetlerinde kimyasal kullanımının kontrol edilmesi ve sıkı denetimlerin uygulanmasının önemini vurgulamaktadır. Arıcıların pestisit ve antibiyotik kullanımı konusunda bilinçlendirilmesi, bal kalitesinin artırılması ve tüketici sağlığının korunması için kritik öneme sahiptir. Güvenli uygulamaların teşvik edilmesi ve standartlara uygunluk, bu risklerin azaltılmasına katkı sağlayacaktır.

Anahtar kelimeler: Arıcılık, Balda kalıntılar, Ağır metaller, Pestisitler

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışma, Bayburt ve Yukarı Çoruh Vadisi'nde faaliyet gösteren arıcıların ürettiği bal örneklerinde pestisit, antibiyotik ve ağır metal kalıntılarını belirlemeyi ve bu kalıntıların arıcılar ile tüketiciler üzerindeki potansiyel sağlık etkilerini değerlendirmeyi amaçlamaktadır. Özellikle sabit ve gezgin arıcıların işlettiği 13 farklı arılıktan toplanan bal örneklerinde yaygın olarak kullanılan kimyasalların kalıntı düzeyleri incelenmiş ve elde edilen bulgular ulusal ve uluslararası gıda güvenliği standartlarıyla karşılaştırılmıştır. Çalışma, kimyasal kalıntıların bal üretimi üzerindeki etkilerini ve bu kalıntıların potansiyel risklerini ortaya koymayı hedeflemektedir.

Gereç-Yöntem: Araştırma, kesitsel bir çalışma olarak planlanmış ve Ağustos-Eylül 2020 döneminde Bayburt ve Yukarı Çoruh Vadisi'nde faaliyet gösteren 13 arılıktan bal örnekleri toplanmıştır. Her arılıktan birer adet bal örneği alınarak, bu örneklerde ağır metaller (Fe, Cu, Zn, Cd, Pb), antibiyotikler (sulfamethazin, tetracycline ve streptomycin), pestisitler ve naftalin gibi kimyasal kalıntılar analiz edilmiştir. Ağır metal tayinleri ICP-AES cihazı ile pestisit ve antibiyotik kalıntı tayinleri ise HPLC-DAD ve floresan dedektörleri kullanılarak Bayburt Üniversitesi Merkezi Laboratuvarı'nda gerçekleştirilmiştir. Elde edilen sonuçlar, Türk Gıda Kodeksi ve Avrupa Birliği gıda standartları ile kıyaslanarak değerlendirilmiş ve her örneğin uygunluk durumu detaylı olarak analiz edilmiştir.

Bulgular: Analizler sonucunda, 13 bal örneğinden 2 tanesinde kurşun (Pb) seviyelerinin hem Türk Gıda Kodeksi hem de Avrupa Birliği standartlarının üzerinde olduğu belirlenmiştir. Kurşun kalıntıları, özellikle balın üretildiği bölgede bulunan çevresel faktörler ve arıların bu alanlarda temas ettiği kirleticiler nedeniyle yükselmiştir. Pestisit kalıntıları açısından, 5 bal örneğinde bu kalıntıların sınır değerlerinin aşıldığı tespit edilmiştir ve bu örnekler hem Türk Gıda Kodeksi hem de Avrupa Birliği standartlarına uygun bulunmamıştır. Antibiyotik kalıntılarında ise, 6 bal örneğinde sulfamethazin, tetracycline ve streptomycin kalıntılarına rastlanmıştır. Bu antibiyotik kalıntılarına sahip örneklerin 4'ü Türk Gıda Kodeksi'ne uygun bulunurken, 2'si kodekse uygunluk göstermemiştir. Avrupa Birliği standartlarına göre yapılan değerlendirmede ise, 8 bal örneğinin bu standartlara uymadığı, sadece 5 bal örneğinin uygunluk gösterdiği tespit edilmiştir. Buna ek olarak, analiz edilen bal örneklerinin hiçbirindenaftalin kalıntısına rastlanmamış olup, bu durum arıcıların bilinçlenme düzeyinin arttığını ve bu kimyasalın kullanımının azaldığını göstermektedir.

Sonuç: Çalışma sonuçları, Bayburt ve Yukarı Çoruh Vadisi'nde üretilen bal örneklerinde bazı ağır metal ve kimyasal kalıntıların bulunduğunu ve bunların bal kalitesi ile tüketici sağlığı üzerinde potansiyel riskler taşıdığını ortaya koymaktadır. Özellikle 2 bal örneğinde kurşun seviyelerinin yüksek olması ve 5 örnekte pestisit kalıntılarının sınırların üzerinde çıkması, arıcıların kimyasal kullanımı konusunda daha fazla bilinçlendirilmesi gerektiğini göstermektedir. Aynı şekilde, 6 örnekte tespit edilen antibiyotik kalıntıları, arıcıların ilaçlama uygulamalarında daha dikkatli ve kontrollü olmaları gerektiğini ortaya koymaktadır. Eğitim programlarının artırılması ve sıkı denetimlerin yapılması, arıcıların bilinç düzeyini yükselterek bal kalitesini artıracak ve tüketici sağlığını koruyacaktır. Sonuç olarak, bu çalışma, kimyasal kalıntıların kontrol altına alınması, güvenli arıcılık uygulamalarının teşvik edilmesi ve ulusal ve uluslararası standartlara uygun bal üretiminin sağlanması gerektiğini vurgulamaktadır. Özellikle bölgedeki arıcılık faaliyetlerinin sürdürülebilirliğini artırmak ve bal üretiminde kaliteyi korumak adına denetimlerin sıklaştırılması ve arıcılara yönelik eğitimlerin yaygınlaştırılması büyük önem arz etmektedir. Arıcıların pestisit ve antibiyotik kullanımı konusunda bilinçlendirilmesi, tüketici sağlığı ve ürün kalitesinin korunması açısından önemli rol oynayacaktır.

INTRODUCTION

Beekeeping is an activity that combines the use of plant resources, bees, and labour to produce products such as honey, royal jelly, bee venom, pollen, and propolis, which humans have utilised for nutrition, health protection, and treatment purposes since ancient times. In addition to these products, beekeeping also includes activities such as the production of queen bees, swarms, and package bees, which are significant sources of income. The vital role of bees in pollination is also of great importance for the agricultural sector. Beekeeping is the most nature-dependent livestock activity because the life cycle of honeybees and the raw material sources of the products obtained are directly tied to nature (Fıratlı et al. 2000).

Beekeeping is a globally widespread agricultural activity, with approximately 100.996 hives and 1.830,768 tons of honey produced, according to the 2022 FAO (Food and Agriculture Organization) statistics. The reasons for choosing beekeeping include low capital requirements, high return rates, low costs, relatively low labour needs, long shelf life of products, and opportunity for a hobby and additional income. Additionally, the fact that beekeeping does not require land makes it an attractive option for landless farmers (Gösterit and Gürel 2004).

Türkiye has highly favourable natural conditions for beekeeping. Utilising these natural advantages more consciously can contribute to the increased production of honey, an excellent food in every aspect, and other bee products (Gürcan and Soysal 2005).The antibiotics such as oxytetracycline and tylosin, widely used in beekeeping to manage bacterial brood diseases, have been shown to leave harmful residues in honey, raising health concerns for both beekeepers and consumers. For instance, residues of tylosin in honey have been detected at levels that can persist for months, potentially leading to antimicrobial resistance and contamination risks (Caldow et al. 2005).

Oxytetracycline, another commonly used antibiotic, has been found to degrade slowly in honey and brood nest areas, leading to long-term residue persistence, which could disrupt microbial flora in humans and potentially contribute to resistance development (Matsuka and Nakamura 1990).Similarly, pesticides such as amitraz and fluvalinate, used to control Varroa mites, can also leave residues in honey. Amitraz has been shown to have toxic effects on hormonal systems in humans when consumed over time (Kochansky 2004). Fluvalinate residues, detected in honey, have been associated with risks of chronic diseases due to their neurotoxic properties (Gilliam and Argauer 1981).

Pesticide residues are a significant factor in honey contamination. They occur through the direct contact of bees with pesticides or the indirect transfer of pesticides applied to plants in agricultural areas into the hives. It is known that even at low doses, pesticides can have harmful effects, accumulate in fat tissues, and cause carcinogenic and organ damage (Aygün 2020). In contrast, some types can damage nerve cells and cause cognitive disorders. This situation necessitates the conscious use of pesticides. Honeybees, as indicators of environmental pollution, are essential in detecting pesticide residues, as these residues can lead to harmful residues in bee products when medicines used against the varroa parasite are applied (Çakar 2019). Commonly used licensed drugs in Türkiye include active ingredients such as flumethrin, amitraz, and malathion. Chemical methods are often preferred for treating honeybee diseases, and antibiotics are widely used. Some antibiotics, such as chloramphenicol, have been banned in many countries (Sunay 2006).

Honey contamination with PAHs (Polycyclic Aromatic Hydrocarbons) can result from using naphthalene and sources like industrial facilities, while heavy metal contamination arises due to industrial pollution and improper beekeeping practices. Bees and bee products are considered effective bioindicators for detecting environmental pollution (Bogdanov et al. 2003, Gül et al. 2005, Lambert 2012, Morzycka 2002). The purpose of this study is to determine the pesticide, antibiotic heavy metal analyses of 13 different honey samples produced in Bayburt and Upper Coruh Valley Regions of Türkiye and to study the honeys contents comprehensively. It is thought that determining the contents of the honeys in these regions will be a precursor for future studies.

MATERIALS AND METHODS

Study Region

This study, planned as a cross-sectional research, involved the collection of thirteen honey samples from stationary and migratory beekeepers operating in the Çoruh Valley and Bayburt province. The aim was to detect commonly used chemicals and pesticides in the sector. The samples were collected from thirteen different apiaries in the same region, and the analysis for heavy metals (Fe, Cu, Zn, Cd, and Pb), as well as chemical residues (antibiotics, pesticides, and naphthalene residue tests), was conducted through a service obtained from the Central Laboratory of Bayburt University. The objective was to assess the potential health effects of these chemical residues, whether they enter the food chain affecting all consumers or pose risks to the beekeepers themselves, and to propose measures to mitigate these risks. Approximately 550-600 beekeepers are active in the region. Information regarding the locations where the honey samples were collected is presented in Figure 1 and Table 1.

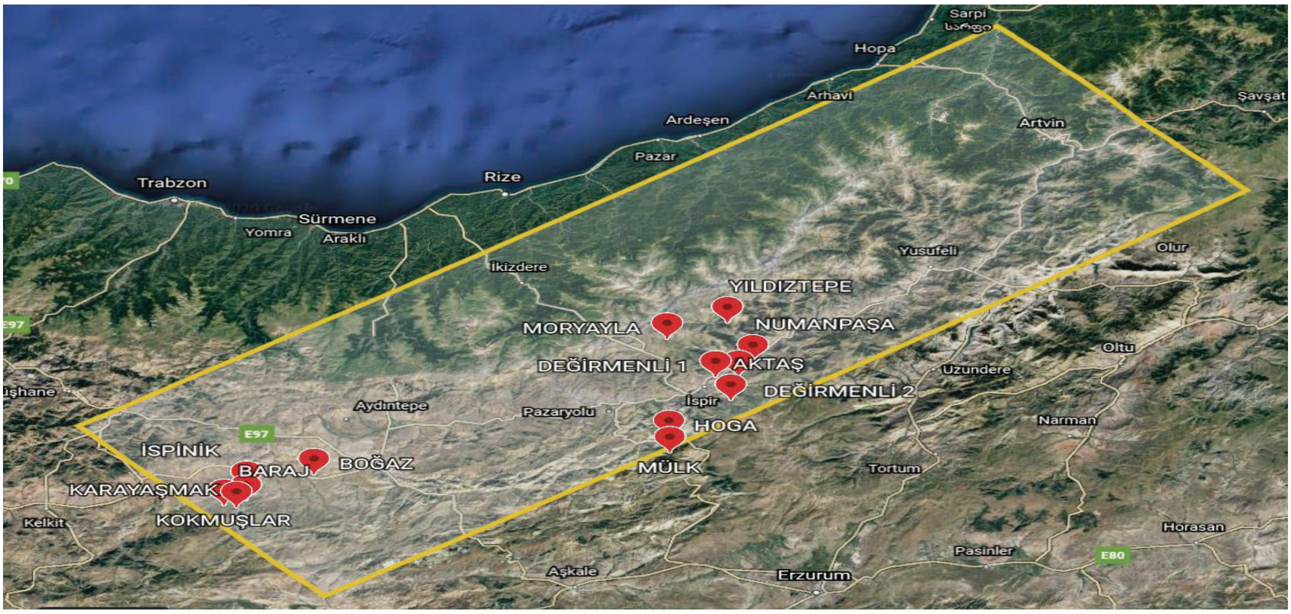

Figure-1. Regional distribution of examined apiaries

Table-1. Location information of examined apiaries

Apiary number	Region of the apiary	Coordinates	Date of examination
1	Mülk	40° 17’ 28° N - 40° 55’ 14° E	August - September 2020
2	Yıldıztepe	40° 39’ 20° N - 41° 03’ 46° E	August - September 2020
3	Değirmenli 1	40° 30’ 12° N - 41° 01’ 45° E	August - September 2020
4	Değirmenli 2	40° 30’ 18° N - 41° 05’ 01° E	August - September 2020
5	Moryayla	40° 36’ 36° N - 40° 54’ 49° E	August - September 2020
6	Aktaş	40° 26’ 17° N - 41° 03’ 56° E	August - September 2020
7	Numanpaşa	40° 32’ 56° N - 41° 07’ 05° E	August - September 2020
8	Karayaşmak	40° 09’ 41° N - 39° 54’ 47° E	August - September 2020
9	Kokmuşlar	40° 11’ 22° N - 39° 50’ 23° E	August - September 2020
10	Baraj	40° 07’ 53° N - 39° 53’ 23° E	August - September 2020
11	Boğaz	40° 13’ 42° N - 40° 04’ 31° E	August - September 2020
12	İspinlik	40° 11’ 19° N - 39° 54’ 44° E	August - September 2020
13	Hoga	40° 20’ 12° N - 40° 55’ 07° E	August - September 2020

The Çoruh Valley is known for its rich biodiversity due to its natural features. The valley is located in the Caucasus Ecological Region, one of the world's 200 most ecologically significant areas identified by the WWF (World Wildlife Fund), and it is one of nine essential plant areas on the Turkish side of this region. The basin, with an area of 19.748 km², lies between 39° 40’ and 42° 35’ longitude and 39° 52’ and 41° 32’ latitude, bordered by the Eastern Black Sea Mountains to the north, the Giresun Mountains to the west, Otlukbeli, Dumlu, Kargapazarı, Güllü, and Allahüekber Mountains to the south, and the Yanlızçam Mountains and Georgia to the east. As one moves inland from the Black Sea coast, the climate transitions from temperate to continental. The mountains surrounding the Çoruh River rise to 3.000 meters within 15 km, while the valley floor descends to 75 meters near the Georgian border (Erdoğan et al. 2014, Erdoğan and Erdoğan 2014). Bayburt, located within the study area, is situated in the Eastern Black Sea region of the Black Sea Region, between 40° 37’ north latitude and 40° 45’ east longitude, 39° 52’ south latitude and 39° 37’ west longitude. Bayburt, located along the Çoruh

River at an elevation of 1.550 meters above sea level, covers an area of 3.739 km². Geographically, it consists of a basin extending between mountain ranges to the north and south, and its topography includes mountains, plains, and valleys (Birinci 2013). Bayburt is a newly developing region in terms of beekeeping, with 72.266 hives recorded in 2018 and 408 tons of honey produced in the same year, according to Turkish Statistical Institute (TÜİK) data. Recently, migratory beekeeping activities have increased significantly in the region (Koday and Karadağ 2020).

Residue Analysis Method in Honey

Element Analysis Method: During element analysis, approximately 1 g of each honey sample was weighed into Teflon containers, and 10 mL of nitric acid was added. After tightly sealing the containers, they were placed in a microwave digestion unit. The honey samples were then digested using microwave radiation and cooled. The extracts obtained were filtered through blue band filter paper into 25 mL volumetric flasks and diluted to 25 mL with ultra-pure water (Demirezen and Aksoy 2005). An ICP-AES (Varian Model-Liberty Series II) device was used to determine metal concentrations. The results were calculated as mg/kg based on wet weight by measuring each element individually. When selecting each component of the honey samples, calibration curves obtained using ICP standards of known concentration (High-Purity standards) were utilised (Gül 2008).

Analysis Method for Medications Used by Beekeepers for Bee Diseases: In the honey samples collected in this study, residue analysis was conducted for sulfonamide, tetracycline, and streptomycin, medications commonly used by beekeepers for bee diseases. Screening analysis was performed using the Charm II 6600/7600 system, and the residue quantities were determined using HPLC-DAD (Diode Array Detector) and fluorescence detectors along with columns. To identify positive honey samples, all honey samples were first subjected to screening analysis using the Charm II device, and standard solutions were processed through the Charm II to establish control points. The presence of these medication residues in the honey samples was determined based on the defined control point. Honey samples found to contain residues were prepared for HPLC analysis. Further analysis in HPLC was performed to quantify the residue levels, considering the retention times and peak areas of standard solutions (Gül 2008).

Pesticide Residue Analysis Method: In the honey samples collected during the study, residues of organophosphate pesticides such as amitraz and coumaphos, which are used by some beekeepers against bee diseases, were analysed. To prepare the standard solutions, 10 mg of amitraz and coumaphos standards were separately dissolved in hexane in 10 mL volumetric flasks, resulting in a primary stock solution of 1,000 µl/mL (mg/kg). A separate 1/1 dilution (1 µL/mL) was then prepared from the primary stock solution. From the prepared primary stock solution, 100 µL was taken and diluted with acetonitrile in a 10 mL volumetric flask to obtain an intermediate stock solution of 10 µL/mL (1 mg/kg). Sequential dilutions of 5, 10, 25, 50, 100, and 200 µL/L (ppb) were prepared from the intermediate stock solution, and the peak areas were determined using a GC-MS (HP 6890 Series / 5972 A- GC-MS (Gas Chromatography-Mass Spectrometry) (Gül 2008).

Naphthalene Analysis Method: Naphthalene analysis in the honey samples collected during the study was performed using GC-MS (HP 6890 Series / 5972 A- GC-MS (Gas Chromatography-Mass Spectrometry) and a headspace sampler. For the analysis, 5 g of honey from each sample was weighed into 15 mL headspace vials and kept at 90 °C for 45 minutes. After heating, the samples were immediately placed into the headspace sampler for analysis (Gül 2008).

RESULTS

Heavy Metal Analysis Values in Honey Samples

The heavy metal residue analysis results for the 13 honey samples collected from the study area are presented in Table 2.

Table-2. Heavy metal analysis values in honey samples

Samp. No	Region	Cd (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
1	Mülk	*N.D.	1.69±0.01	0.16±0.07	0.21±0.03	0.06±0.00
2	Yıldıztepe	*N.D.	1.77±0.01	0.17±0.02	0.23±0.04	0.04±0.00
3	Değirmenli 1	*N.D.	1.79±0.02	0.15±0.03	0.23±0.05	0.1±0.00
4	Değirmenli 2	*N.D.	1.33±0.01	0.15±0.01	0.20±0.01	0.6±0.01
5	Moryayla	*N.D.	1.25±0.03	0.16±0.03	0.19±0.07	0.16±0.01
6	Aktaş	*N.D.	1.41±0.04	0.14±0.01	0.22±0.01	0.13±0.08
7	Numanpaşa	*N.D.	1.07±0.01	0.10±0.01	0.17±0.09	0.12±0.07
8	Karayaşmak	*N.D.	0.95±0.01	0.09±0.00	0.19±0.01	0.11±0.00
9	Kokmuşlar	*N.D.	1.19±0.02	0.09±0.00	0.21±0.07	0.14±0.02
10	Baraj	*N.D.	1.86±0.03	0.17±0.06	0.23±0.01	2.01±0.01
11	Boğaz	*N.D.	1.95±0.02	0.19±0.01	0.25±0.06	2.0±0.03
12	İspinlik	*N.D.	1.97±0.03	0.16±0.07	0.25±0.01	1.94±0.09
13	Hoga	*N.D.	1.46±0.05	0.16±0.08	0.22±0.01	0.13±0.06

*N.D.: Not Detected

Residue Analysis Results in Honey Samples

The results of the residue analysis for medications used against bee diseases and pests in the 13 honey samples collected from the study area are presented in Table 3.

Table-3. Residue analysis values for abtibitics and organophosphate ınsecticides used against bee diseases and pests in honey samples

Samp. No	Region	Naphthalene (mg/kg)	Sulfamethazin (mg/kg)	Tetracycline (mg/kg)	Streptomycine (mg/kg)	Coumaphos (mg/kg)	Amitraz (mg/kg)
1	Mülk	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
2	Yıldıztepe	*N.D.	*N.D.	*N.D.	0.021	*N.D.	*N.D.
3	Değirmenli 1	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
4	Değirmenli 2	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
5	Moryayla	*N.D.	*N.D.	0.001	*N.D.	*N.D.	*N.D.
6	Aktaş	*N.D.	*N.D.	*N.D.	0.002	*N.D.	*N.D.
7	Numanpaşa	*N.D.	*N.D.	*N.D.	*N.D.	0.002±0.000	0.044±0.000
8	Karayaşmakk	*N.D.	0.007±0.000	*N.D.	*N.D.	*N.D.	*N.D.
9	Kokmuşlar	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	0.007
10	Baraj	*N.D.	0.001±0.000	*N.D.	*N.D.	*N.D.	0.006
11	Boğaz	*N.D.	*N.D.	0.001±0.000	*N.D.	0.06±0.001	0.004±0.000
12	İspinlik	*N.D.	0.010	*N.D.	*N.D.	*N.D.	0.035±0.000
13	Hoga	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	0.004±0.000

*N.D.: Not Detected

DISCUSSION

Evaluation of Heavy Metal Analysis

In this study, 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region were analysed for heavy metals, including Iron (Fe), Copper (Cu), Cadmium (Cd), Zinc (Zn), and Lead (Pb). The data obtained from the analysis are presented in Table 2.

The analysis revealed that the lowest iron (Fe) concentration was found in sample 8, at 0.95 mg/kg, while the highest concentration was observed in sample 12, at 1.97 mg/kg. Türkiye, there is no specific standard for the amount of iron that may be present in honey; however, as shown in Table 4, all honey samples were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum iron (Fe) concentration range of 1.5-15 mg/kg in food products (see Figure 2).

Table-4. Maximum permitted heavy metal levels in food according to fao-who codex alimentarius (WHO and FAO 1972)

Heavy metals	Maximum permissible levels in food (mg/kg)
Cadmium (Cd)	Not Allowed
Lead (Pb)	0.1-2.0
Copper (Cu)	0.1-5.0
Iron (Fe)	1.5-15
Zinc (Zn)	5
Fe + Cu + Zn	20

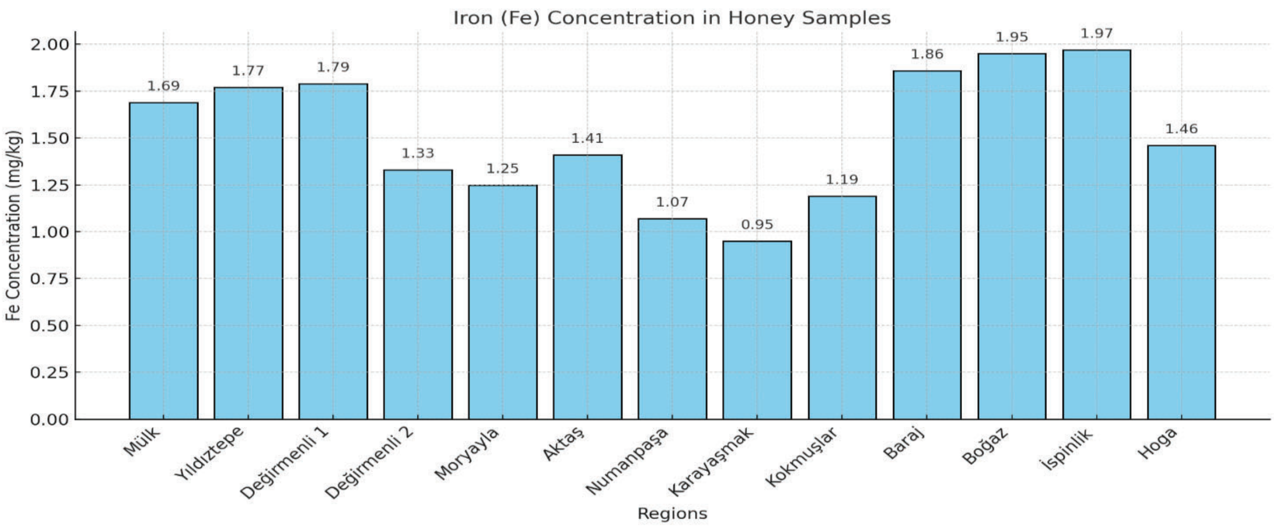

Figure-2. Iron (Fe) concentration in analyzed honey samples (mg/kg)

The lowest copper (Cu) concentration was found in samples 8 and 9 0.09 mg/kg, while the highest concentration was observed in samples 11 0.19 mg/kg. Türkiye has no specific standard for the amount of copper in honey. However, as shown in Table 4, all honey samples complied with the FAO-WHO Codex Alimentarius standards, which permit a maximum copper (Cu) concentration range of 0.1-5.0 mg/kg in food products (see Figure 3).

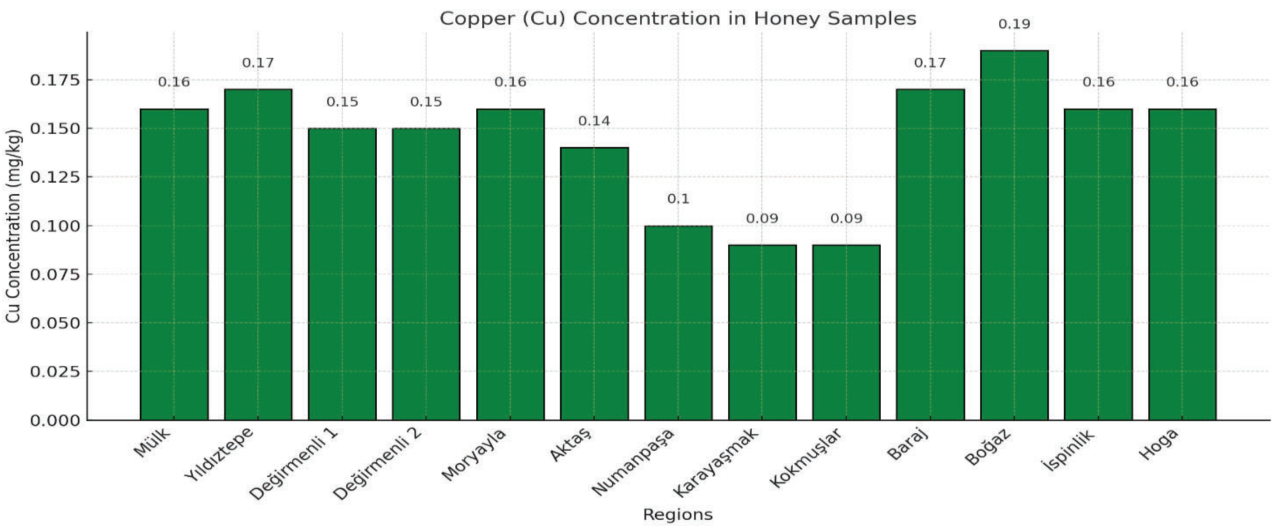

Figure-3. Copper (Cu) concentration in analyzed honey samples (mg/kg)

In the study, the lowest zinc (Zn) concentration was found in sample 7 0.17 mg/kg, while the highest concentration was observed in samples 11 and 12 0.25 mg/kg. In Türkiye, there is no specific standard for the amount of zinc that may be present in honey, as with other heavy metals. However, as shown in Table 4, all honey samples were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum zinc (Zn) concentration of 5 mg/kg in food products (see Figure 4).

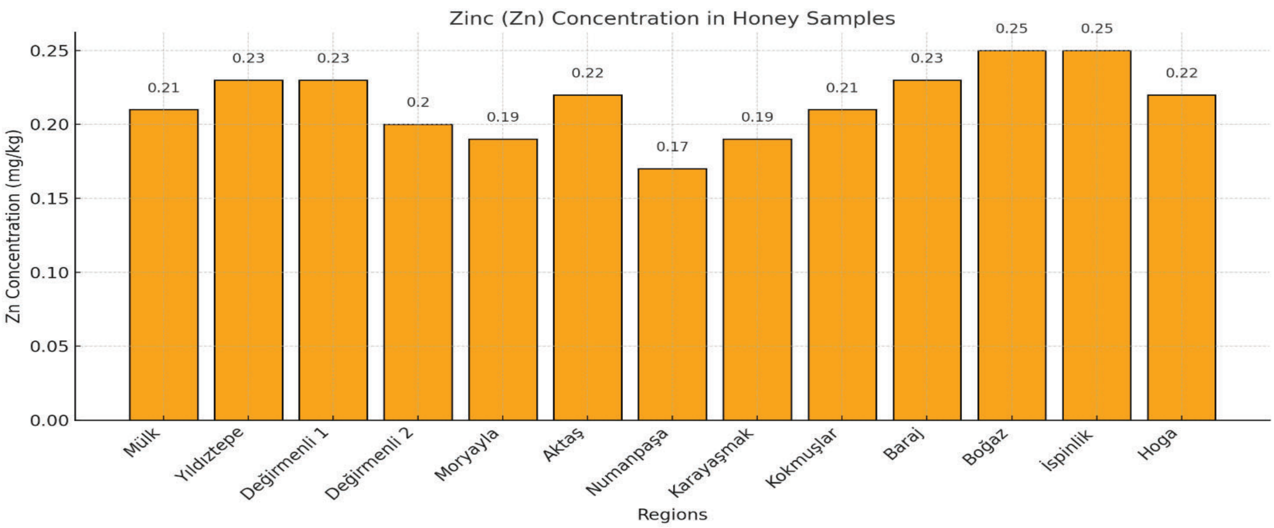

Figure-4. Zinc (Zn) concentration in analyzed honey samples (mg/kg)

Zinc and iron are essential elements for humans, animals, and plants. According to the National Academy of Science (NAS) and other sources, the recommended daily intake for an adult (aged 19-70) is 40 mg of zinc and 45 mg of iron. Considering the amounts that people can consume through their daily diet, the results of this study indicate that the levels of Zn and Fe found are significantly below these limits. Therefore, all honey samples analyzed in terms of these metals are considered safe (Demirezen and Aksoy 2005). In this study, cadmium (Cd) was not detected in any of the honey samples analyzed. In Türkiye, there is no specific standard for the amount of cadmium that may be present in honey; however, as shown in Table 4, all samples were found to comply with the FAO-WHO Codex Alimentarius standards, which state that cadmium (Cd) should not be present ('not detectable'). Various studies conducted in different regions of Türkiye have identified a wide range of Zn, Fe, and Cu levels in honey samples. For instance, in 25 honey samples collected from six different regions, Zn, Fe, and Cu levels were found to range between 1.1-12.7 mg/kg, 1.8-10.2 mg/kg, and 0.23-2.41 mg/kg, respectively (Tüzen et al. 2007). In 60 honey samples from Central Anatolia, Zn and Cu levels were determined to be 1.1-24.2 mg/kg and 0.25-1.10 mg/kg, respectively, which were higher than the results reported in the current study (Arslanbaş 2010). In 20 honey samples collected from the Black Sea Region, Zn, Fe, and Cu levels were reported as 0.47-6.57 mg/kg, 1.12-12.9 mg/kg, and 0.009-0.035 mg/kg, respectively, which were observed to be similar to the results of the present study (Silici et al. 2008). In samples obtained from provinces in Eastern Anatolia, the Fe, Zn, and Cu levels were measured as 9.799±5.615 mg/kg, 3.705±1.708 mg/kg, and 2.635±1.198 mg/kg, respectively, which were found to be lower than those in the current study (Güleç 2007). In 45 honey samples collected across Türkiye, Fe levels were determined to range between 3.71-5.43 mg/kg, while Zn levels were found to range between 6.24-11.53 mg/kg (Yarsan et al. 2007).

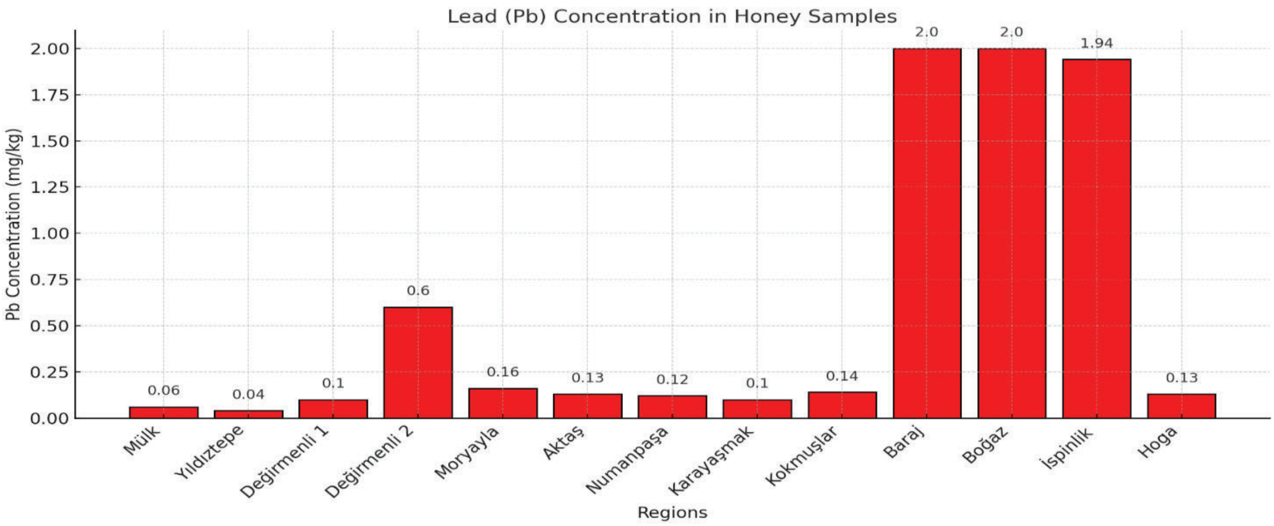

Figure-5. Lead (Pb) concentration in analyzed honey samples (mg/kg)

The lowest lead (Pb) concentration was found in sample 2 0.04 mg/kg, while the highest concentration was observed in sample 10 2.01 mg/kg. In Türkiye, there is no specific standard for the amount of lead that may be present in honey. However, as shown in Table 4, all honey samples, except for sample 10, were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum lead (Pb) concentration of '0.1-2.0' mg/kg. Sample 11 was found to be at the limit value of 2.0 mg/kg (see Figure 5). The Upper Çoruh and Bayburt regions, where the honey samples were collected, are generally not significant industrial areas in Türkiye. Therefore, the heavy metal content in the collected honey samples was not found to be high. Except for the lead (Pb) level in sample 10, all samples were within the limit values specified in the FAO-WHO Codex Alimentarius. The honey sample with a lead (Pb) level above the limit was collected from an apiary located approximately 300 meters from the Bayburt Demirözü Irrigation Pond. This area has recently become a tourist destination with many visitors, leading to heavy vehicle traffic, especially in the summer months. It is considered that the bees and vegetation in this area may have been exposed to exhaust gases, resulting in contamination of bee products. Therefore, the honey and other bee products produced within 5 km of this area are not considered safe in terms of carcinogenic residue risk and are believed to potentially pose a health risk to the public. It is recommended to support tourism activities in this area and conduct beekeeping activities at least 5 km away from this region. Various studies conducted in Türkiye and other countries have compared Pb and Cd levels in honey samples, revealing a wide range of variability. In a study conducted in Türkiye by Tüzen et al. (2007), Pb and Cd levels were found to range between 0.0084-0.106 mg/kg and 0.0009-0.0179 mg/kg, respectively (Tüzen et al. 2007). In Poland, Pb and Cd levels in honey samples were reported as 0.025-0.071 mg/kg and 0.008-0.027 mg/kg, respectively, which were lower than the Pb levels but higher than the Cd levels in the current study (Przybyloeski and Wilczynska 2001). In 200 flower honey samples collected from Eastern Anatolia, average Pb and Cd levels were determined to be 0.131±0.081 mg/kg and 0.006±0.007 mg/kg, respectively, with Pb levels higher and Cd levels lower compared to the current study (Güleç 2007). Honey samples from the vicinity of Mount Erciyes in Kayseri showed Pb and Cd levels of 0.1-0.85 mg/kg and 0.11-0.18 mg/kg, respectively, both of which were lower than the results of the current study (Demirezen and Aksoy 2005). In honey samples from Kahramanmaraş, the average Cd level was reported as 0.32 mg/kg, which was lower than the current study's findings (Erbilir and Erdoğrul 2005). In France, honey samples available for sale were found to have Pb and Cd levels of 0.28-1.08 mg/kg and 0.08-0.25 mg/kg, respectively, with Pb levels higher and Cd levels lower than those of the current study (Devillers et al. 2002). In samples from Tenerife Island, Spain, Pb and Cd levels were measured as 0.03733 mg/kg and 0.00438 mg/kg, respectively, both of which were lower than the results of the current study (Frias et al. 2008).

Residue Analysis Evaluation

In the study, residue analysis was conducted for substances categorized as residues in the 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region. These substances included residues from medications used by beekeepers to combat bee diseases and pests (sulfamethazine, tetracycline, and streptomycin), pesticide residues (amitraz and coumaphos), and naphthalene residues. The data obtained from these analyses are presented in Table 3. Standards for the levels of these substances in honey, as established by Türkiye and the European Union for medications used against bee diseases and pests, are shown in Table 5 (Sunay 2006).

Table-5. Permitted medication levels for bee diseases and pests according to the Turkish food codex honey communiqué

Medicine used for bee diseases and pests	Turkish food codex drug tolerance level (mg/kg)	European Union drug tolerance level (mg/kg)
Amitraz	0.02	0.02
Coumaphos	0.01	0.01
Streptomycine	0.02	Not found
Sulfonamid Group	0.01	Not found
Tetracycline Group	0.01	Not found

Evaluation of Medication Analyses Used for Bee Diseases and Pest Control

Evaluation of Sulfa Group Antibiotic Analyses

In this study, the amount of sulfamethazine, an antibiotic used by beekeepers for bee diseases and pests, was detected in samples 8., 10., and 12. at concentrations of 0.0071 mg/kg, 0.0098 mg/kg, and 0.102 mg/kg, respectively. Accordingly, samples 8. and 10. were found to comply with the limit value of '0.01 mg/kg' specified in the Turkish Food Codex Medication Tolerance Level, while sample 12. was found to exceed both the limit value specified in the Turkish Food Codex and the limit value of 'not detectable' specified by the EU Medication Tolerance Level (see Table 5 and Figure 6). In our study, only one of the three honey samples containing detectable levels of sulfa group antibiotics was considered unsafe in terms of sulfa group antibiotic content. Currently, the most common honeybee diseases faced by beekeepers are brood diseases and nosema disease. To protect or treat bee colonies from these diseases, chemical methods are often used. Antibiotics such as streptomycin, tetracyclines, or sulfonamides are commonly applied, especially against brood diseases. Frequent and unconscious use of antibiotics can increase the resistance of bacteria causing these diseases, resulting in negative effects for both honeybees and bee products. It is considered that beekeepers need education on the correct dosage and conscious application of medications for bee diseases (Söğüt et al. 2019). In honey samples collected from 22 different regions of Türkiye in 2006, the presence of antibiotics from the sulfa, tetra, and strepto groups was investigated, and among sulfa antibiotics, only sulfadimidine was detected. Analysis revealed that 10% of the 1,714 samples contained this antibiotic, and 5% of the 91 samples showed streptomycin residues above 0.0177 mg/kg (Sunay 2006). In a study conducted on 536 honey samples collected between 2007 and 2009, sulfa group antibiotic analysis identified average residue levels of 0.102 mg/kg sulfanilamide, 0.597 mg/kg sulfamethazine, and lower levels of other sulfa antibiotics (Erdoğdu et al. 2011). The levels of sulfa antibiotics detected in this study were found to be lower than those reported in previous studies.

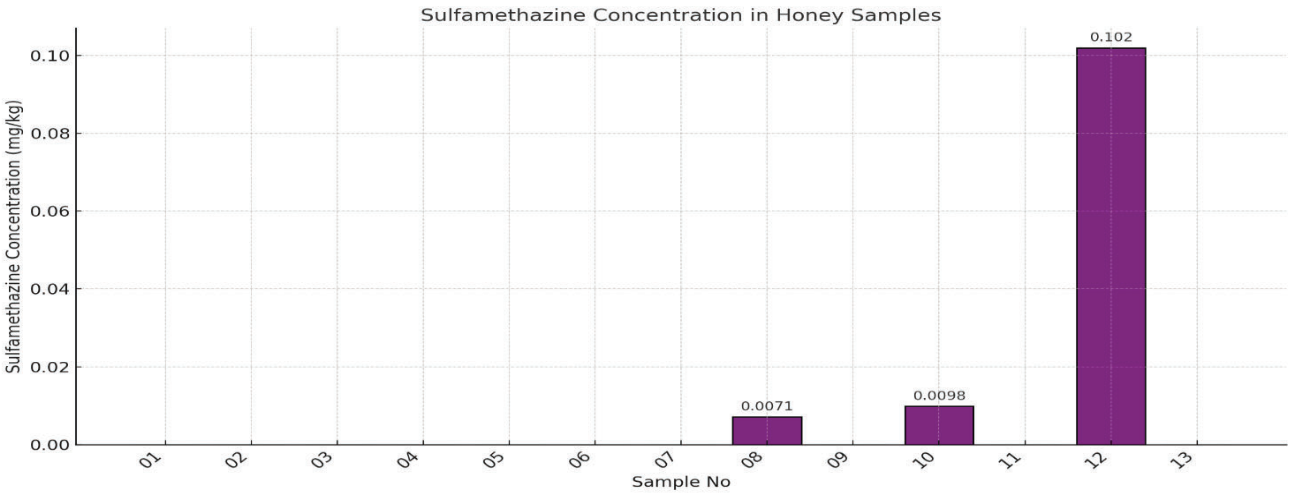

Figure-6. Sulfamethazine concentration in analyzed honey samples (mg/kg)

Evaluation of Tetra Group Antibiotic Analyses

In this study, tetracycline, an antibiotic used by beekeepers for bee diseases, was detected in samples 5 and 11 at concentrations of 0.0014 mg/kg and 0.0011 mg/kg, respectively. Both samples complied with the Turkish Food Codex limit of 0.01 mg/kg but did not meet the EU standard, which requires no detectable residue. Overall, all 13 samples were deemed safe regarding tetracycline levels (see Table 5 and Figure 7). In a study conducted in Greece, drug residues were detected in 29% of honey samples, with 20.3% attributed to tetracyclines and their derivatives. Residue levels ranged between 0.018-0.055 mg/kg, with some samples reaching up to 0.1 mg/kg (Saridaki et al. 2006). In Poland, an analysis of 178 honey samples revealed insecticide residues ranging from 0-0.06 mg/kg, attributed to environmental contamination (Wilczynska and Przybylowski 2007). In another study on honey produced in and imported to Belgium, 0.015 mg/kg streptomycin, 0.01 mg/kg sulfamethazine, 0.01 mg/kg penicillin, and 0.0001 mg/kg chloramphenicol were detected, with residues predominantly found in imported honey samples (Reybroec 2003). The levels of tetracycline group antibiotics detected in this study were found to be lower than those reported in the three aforementioned studies.

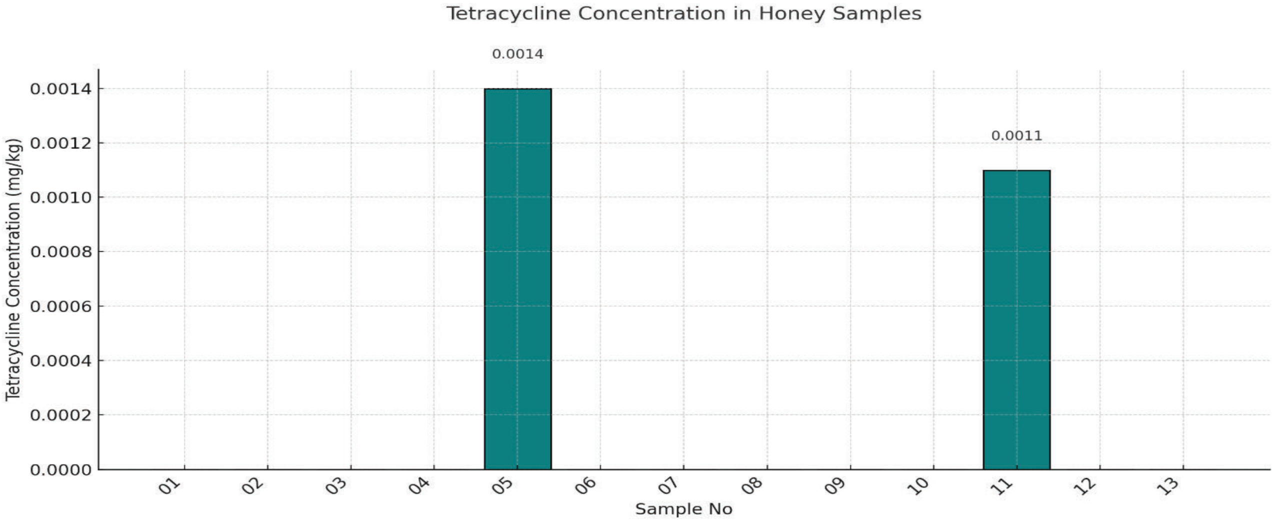

Figure-7. Tetracycline concentration in analyzed honey samples (mg/kg)

Evaluation of Strepto Group Antibiotic Analyses: In this study, streptomycin was detected in samples 2 and 6 at 0.00211 mg/kg and 0.00212 mg/kg, respectively. While both samples met the Turkish Food Codex limit of 0.02 mg/kg, they did not comply with the EU standard of 'not detectable' (see Table 5 and Figure 8). Nevertheless, all 13 samples were deemed safe regarding strepto group antibiotics. In a study conducted on 180 honey samples collected from the central and district areas of Ardahan province, streptomycin residues were detected in 37% of the samples, while sulfonamide residues were found in 52% of the samples. (Özkan et al. 2015). The levels of streptomycin group antibiotics detected in this study were found to be lower than the values reported in the previously mentioned studies.

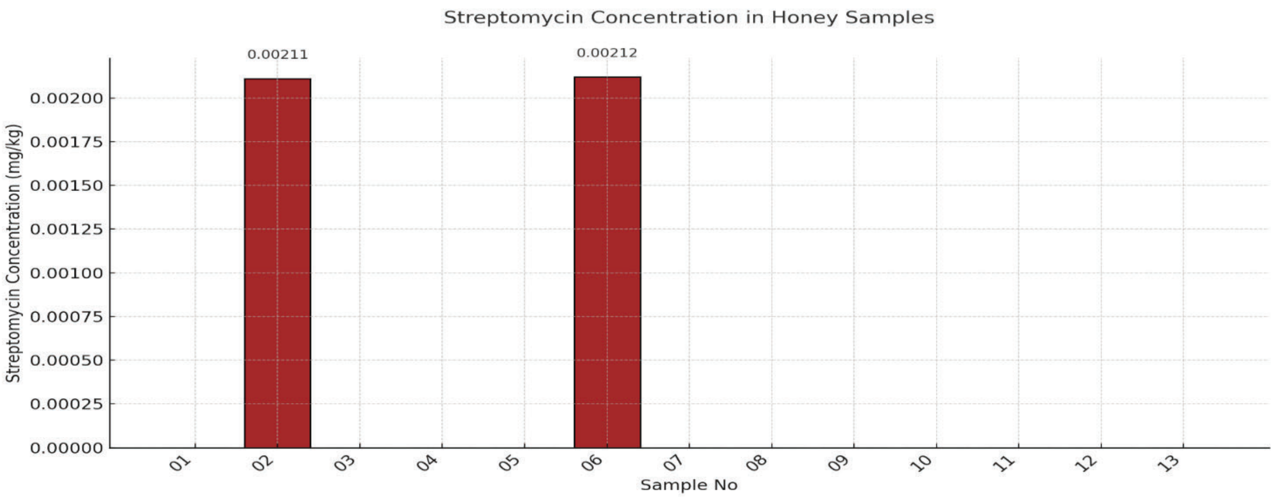

Figure-8. Streptomycin concentration in analyzed honey samples (mg/kg)

Evaluation of Pesticide Analyses

Evaluation of Coumaphos Analyses: Coumaphos, amitraz, and malathion are chemical pesticides widely utilized in beekeeping to control Varroa destructor, a parasitic mite that poses a severe threat to honeybee health and colony survival. While these substances are effective in controlling the mites, their use raises concerns due to the potential for residues accumulating in bee hives. These residues can contaminate hive products such as honey, wax, and propolis, potentially affecting food safety and the health of bees and humans. Residue persistence may also interfere with the natural behavior of bees and the long-term sustainability of beekeeping practices. Thus, while these chemicals are valuable tools in mite management, their use requires careful regulation and adherence to safe application practices to mitigate environmental and health risks. In this study, the amount of coumaphos, a pesticide, was detected in samples 7 and 11 at concentrations of 0.0002 mg/kg and 0.06 mg/kg, respectively. Sample 7 complied with both the Turkish Food Codex and EU Medication Tolerance Levels, which set the limit at 0.01 mg/kg. However, sample 11 exceeded the limit set by both codices (see Table 5 and Figure 9).

One sample was found unsafe due to coumaphos content. Some honey samples had pesticide residues exceeding limits set by the Turkish Food Codex Honey Communiqué. The likely cause is the low education level of beekeepers in the sampled regions, leading to excessive and uncontrolled pesticide use and environmental contamination. Varroa mite populations rise in summer and peak in fall; honey samples were collected in August and September. Coumaphos, commonly used in Türkiye for varroa control, should be applied at proper doses in early spring and late fall, when hive brood activity is low, to minimize residue risk. In a study investigating coumaphos residues in honey and comb samples from different regions of Türkiye and Israel, it was found that coumaphos levels in 49 out of 55 honey samples from Türkiye averaged 0.0308 mg/kg, and in all 10 comb samples, the average was 0.0213 mg/kg. In Israel, 33 out of 38 honey samples contained an average of 0.0461 mg/kg coumaphos, while 60 out of 67 comb samples had an average of 0.0030 mg/kg (Barel et al. 2011).

In Spain, coumaphos residues ranging from 0.001-0.053 mg/kg were detected in 32 out of 221 honey samples (Garcia et al. 1996). The coumaphos levels detected in this study were lower than those reported in the aforementioned studies.

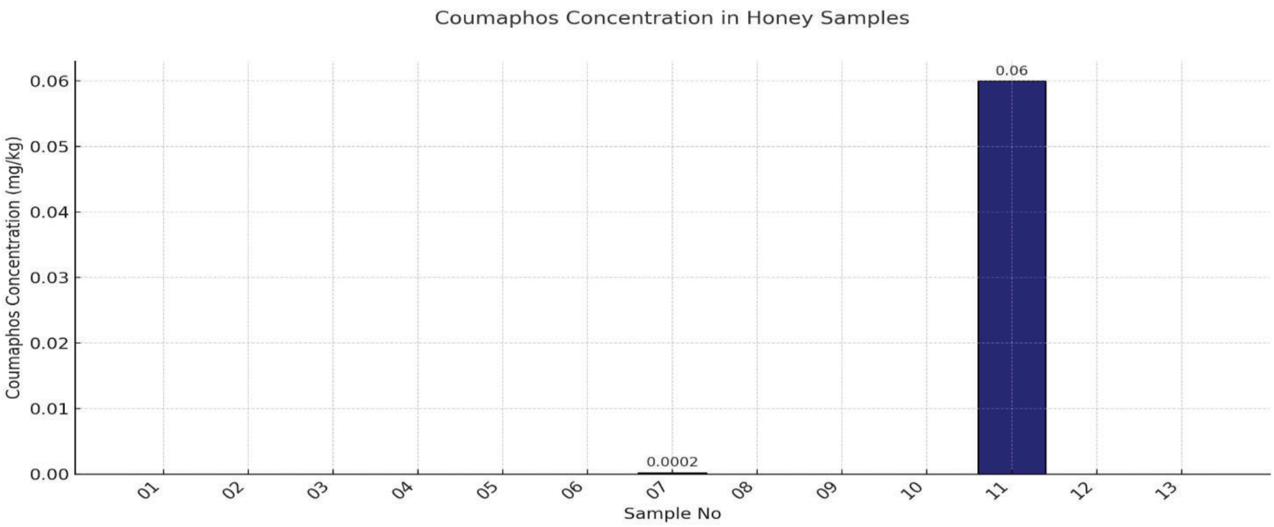

Figure-9. Coumaphos concentration in analyzed honey samples (mg/kg)

Evaluation of Amitraz Analyses: Amitraz, a commonly used pesticide for controlling the varroa mite due to its low cost, is typically applied in the evening when bees return to the hive, at temperatures between 15-20 ºC, over 4 consecutive days. When properly applied, it has shown effective results (Daş and Aksoy). In this study, amitraz was detected in samples 7, 9, 10, 11, 12, and 13 at concentrations of 0.0436 mg/kg, 0.0069 mg/kg, 0.0057 mg/kg, 0.0041 mg/kg, 0.0352 mg/kg, and 0.0044 mg/kg, respectively. Samples 9, 10, 11, and 13 complied with both the Turkish Food Codex and EU standards, which set the limit at 0.02 mg/kg. However, samples 7 and 12 exceeded these limits.

Of the six honey samples where amitraz was detected, two were considered unsafe regarding amitraz content. (see Table 5 and Figure 10). Between 1986 and 1990 in Germany, amitraz residues exceeding 0.05 mg/kg were detected in 8.5% of 330 honey samples (Hammerling et al. 1991). In studies conducted in Spain, residues of amitraz, bromoprophylate, coumaphos, and fluvalinate ranged from 0.001-0.04 mg/kg, with amitraz levels reported as high as 0.033-1.82 mg/kg in some samples (Garcia et al. 1995). In a study conducted in Türkiye, amitraz residues ranging from 0.0013-0.0334 mg/kg were detected in 25 of 135 honey samples (Bilgili and Selçukoğlu 2022). The levels of amitraz identified in this study were found to be lower than those reported in the aforementioned studies.

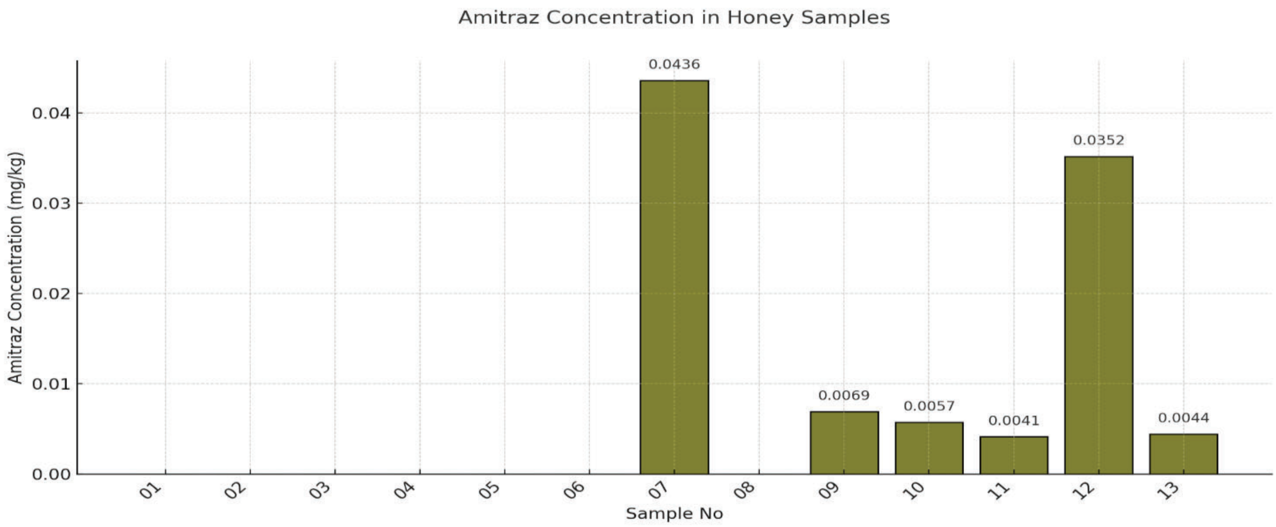

Figure-10. Amitraz concentration in analyzed honey samples (mg/kg)

Evaluation of PAH Analyses

Evaluation of Naphthalene Analyses: Beekeepers typically use naphthalene in the fall, after honey extraction, to combat wax moths in improperly stored combs. When naphthalene-treated combs are returned to the colony, the residues can transfer into the honey (Johnson et al. 2010).

In this study, none of the 13 honey samples collected showed any detectable naphthalene residues. It is believed that the lack of naphthalene residues in the honey is due to the increased information campaigns for beekeepers in recent years about the harmful effects of naphthalene. This outcome indicates the effectiveness of recent training efforts warning beekeepers against the use of naphthalene. Therefore, the honey samples collected from the study area are considered safe in terms of naphthalene residues. In a three-year study conducted in Greece to detect naphthalene residues in honey, 115 commercial honey samples and 1,060 beehive honey samples were analyzed. In the first year, higher levels of naphthalene were detected in commercial honey compared to honey obtained directly from beekeepers. A decrease in naphthalene levels was observed over the subsequent two years (Tananaki et al. 2006).

In Romania, honey samples collected from eight regions revealed naphthalene levels ranging from 0.17-0.665 mg/kg in areas near urban settlements, and from 0.027-0.068 mg/kg in areas near rural regions (Dobrinas et al. 2008). In Türkiye, as part of the "National Residue Monitoring Project" conducted by the Ministry of Agriculture and Rural Affairs' Directorate General for Protection and Control in 2002, naphthalene residues were detected in 22% of the 118 analyzed honey samples (Daş 2004). In contrast, no naphthalene residues were detected in any of the 13 honey samples collected within the scope of the present study.

Conclusion: "In the study, heavy metal analysis was conducted on 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region, focusing on Iron (Fe), Copper (Cu), Cadmium (Cd), Zinc (Zn), and Lead (Pb). Except for Cd, all other elements were detected in the samples; however, only 2 samples did not comply with the maximum allowable heavy metal levels set by the FAO-WHO Codex Alimentarius for food. It was also determined that there is no established standard for heavy metal residues in food in Türkiye. Regarding the analysis of residues from medications (sulfamethazine, tetracycline, and streptomycin), pesticides (amitraz and coumaphos), and naphthalene used by beekeepers to prevent and control bee diseases and pests, sulfamethazine residues were detected in 3 sample, tetracycline in 2 sample, streptomycin in 2 sample, coumaphos in 2 sample, and amitraz in 6 sample.

No naphthalene residues were detected in any of the samples. Based on the analyses conducted, it was determined that out of the 13 honey samples collected from the region, 2 samples (samples 11 and 12) exceeded the limit values specified in the Turkish Food Codex Honey Communiqué. Additionally, 8 samples (samples 2, 5, 6, 7, 8, 10, 11, and 12) did not comply with the values specified in the European Union Standard and Codex Standards. However, except for samples 11 and 12, all other samples were found to comply with the Turkish Food Codex Honey Communiqué. Overall, only two samples (11 and 12) did not comply with either codex.

As a general observation from the results obtained in the study, it was noted that some beekeepers in the region, although not in large numbers, were using medications for bee diseases and pests, as well as some pesticides, either unconsciously or illegally. This poses health risks for bees, beekeepers, and consumers. However, the absence of naphthalene and similar PAHs, which are major issues in honey exports, indicates that there is increased awareness among beekeepers on this matter. To address the problem of the use of unlicensed antibiotics, particularly against brood diseases, and other medications not licensed for beekeeping that are intended for poultry, small livestock, and cattle, control mechanisms need to be more effectively enforced. Harmonization efforts between EU and Turkish regulations should be accelerated, and beekeepers should be trained on beekeeping practices, the prevention and control of bee diseases and pests, and how to produce high-quality bee products.

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Author Contribution: Mustafa Özdemir: investigation, analysis, writing–original draft, writing–review & editing; Osman Yıldızlar: research planning, writing–review & editing.

Data Availability: Data are available in the manuscript.

Declaration of interest: The authors declare that there is no conflict of interest.

Ethics: Ethics committee approval is not required for this study.

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