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RESEARCH PAPER

PCA and LDA Assessment of the Heavy Metal Contamination in Honey Bees, Bee Pollen and Honey Produced in Urban Areas of Türkiye

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Abstract

Heavy metals are of great importance in terms of environmental pollution. Environmental pollution affects not only humans but also plants and animals. Living organisms with varying sensitivity to various pollutants can be used to assess environmental pollution. Honey bees have been used as bioindicators for this purpose because they operate in very large areas in their region. The aim of this study is to investigate heavy metal pollution in honey bees and bee products in 4 different urban regions of Konya using principal component analysis (PCA) and linear discriminant analysis. In this study, when the heavy metal contents of honey bees, bee pollen, and honey samples are compared, it is seen that bees are exposed to more heavy metal contamination. Honey bees don't reflect these amounts in the honey, and the heavy metal content in honey remains in much smaller amounts. When principal component analysis (PCA) is applied to these samples, the heavy metal variation of bees and bee pollen can be explained by three components, while the variation of the heavy metal contents of honey can be explained by four components. Linear discriminant analysis was performed to distinguish the geographic locations of the samples. According to the results of the LDA analysis for heavy metal content, the bees can be assigned to 80% and the pollen and honey to 100% of their correct geographical origin.

Introduction

Environmental issues are getting worse every day, along with population increases, urbanization, industrialization, and changes in consumer habits. One of the biggest problems of our time is environmental pollution, which affects daily life negatively by disrupting the ecological balance. Heavy metals are of great importance in environmental pollution.

In considering environmental problems, the term "heavy metal" has become widely used in recent years to refer to metals with a relatively high density that are toxic even at low concentrations. Although more than sixty elements can be given as examples of heavy metals, the most common and well-known ones are mercury, manganese, iron, cobalt, nickel, copper, zinc, cadmium, arsenic, chromium, lead, silver, and selenium. Some heavy metals, like nickel, copper, iron, zinc, and chromium, are essential to organisms (not having enough or having too much of them can cause disease), while others, like cadmium and lead, are toxic in trace amounts (Goretti et al., 2020; Keil et al., 2011).

Living organisms (bioindicators or biomonitors) with varied sensitivity to different contaminants can be used to assess the environmental contamination of an area. Harmful substances accumulate in the bodies of these creatures, and high mortality rates can be seen (Jaishankar et al., 2014). Honey bees that operate over a large area around the hive are a good biological indicator because they are directly exposed to the toxic conditions in that area and are sensitive to changes in the air, plants, water, and soil in their flight areas. Since the 1970s, they have been increasingly utilized to measure the environmental contamination caused by heavy metals (Celli & Maccagnani, 2003; Satta et al., 2012). Honey bees and bee products are important indicators of environmental contamination because of residues, according to a number of researchers (Porrini et al., 2003; Taha et al., 2017; Zhelyazkova, 2012).

Multivariate statistical analyses are extensively used in the data obtained in research on animal science. In multivariate statistical analysis, p variables or features related to n experimental units are examined. If the number of these variables (p) is high and most of them are related to each other, in other words, the most important technique that can be applied is PCA. Briefly, PCA is a method for expressing the structure explained by p variables with correlation with variables that have no correlation and are linear components of the original variables in numbers less than the number of original variables. The first purpose of PCA is to eliminate the inter-variable dependency structure, and the second is to reduce its size. Therefore, after the eigenvalues are found, it is necessary to decide on the number of significant eigenvalues. Many methods have been developed for this purpose, and the simplest and most widely used method is to decide on the number of components by adding λ values until they exceed 2/3 (67%) of the total variation. Another method is to decide the number of principal components according to the slope of the scree plot graph (Ozdamar, 2004).

Linear discriminant analysis is one of the multivariate statistical methods that aims to categorize individuals into groups they belong to with the least error. Discriminant functions obtained from discriminant analysis are derived from the linear components of the forecast variables under consideration. The most "effective" discriminant that emerged as a result of the analysis is that, with the help of the function, it is possible to predict which group a newly obtained observation will be included in. LDA is widely used in biology as well as in many fields of the social sciences. LDA is used to determine the geographic or botanical origin of plant or animal products (Bassbasi et al., 2014; Choi & Lee, 2012; Jöbstl et al., 2010).

In our previous study, Bayir and Aygun (2022), we attempted to determine whether there were any differences in bees and bee products among different regions. In this study, we aimed to investigate whether bees and bee products can be distinguished based on their source using PCA and discriminant analysis. Discriminant analysis can also be used to identify which region a subsequently obtained sample belongs to. Therefore, based on our study on bees or bee products and the data obtained from subsequent discriminant analysis studies, it is possible to estimate whether unknown bees and bee products originate from areas near roadways or from regions where factories are located.

Material and Methods

Sampling Sites

In 2018, the study was conducted in the Konya region of Türkiye. Konya is located between the north parallels of 36° 22′ and 39° 08′ and the east meridians of 31° 14′ and 34° 05′. In terms of surface area, Konya is the biggest city in Türkiye, with over 41 km^2. The climate in Konya is cold and semi-arid. Konya is the province in Türkiye with the least rainfall. Five honey bee colonies (20 in total) were placed in four different locations (L1 to L4), which were around urban areas (Figure 1).

L1: 38° 02' 05'' N, 32° 30' 10'' E, 1.180 m. 1.210 meters west of the highway, on the city's north side, and in the direction of its predominant wind. There are no industrial facilities, but a small area is where fruits and vegetables are farmed.

L2: 37° 55' 12'' N, 32° 26' 10'' E, 1.140 m. 4.300 meters distant from and north of the highway, on the city's northwest side, and in the direction of the city's predominant wind. There is no industrial facility nearby, but there are agricultural activities like fruit and vegetable farming in a limited region.

L3: 37° 51' 07'' N, 32° 33' 33'' E, 1.010 m. The main wind direction is from the industrial zones, which are southwest of the city and southeast of them (Plastic packaging industry, machinery industry, marble industry, furniture industry). This location is 1.800 meters south and 1.300 meters east of one highway, respectively. Agriculture, including the cultivation of grains, vegetables, and fruits, is practiced in the neighborhood.

L4: 37° 49' 12'' N, 32° 28' 45'' E, 1.027 m. The city side is where the wind is coming from on the city's southern side. It is located 3.400 meters west of one highway and 1.000 meters south of another. Agriculture, including the growing of fruits and vegetables, is widely practiced in the neighborhood.

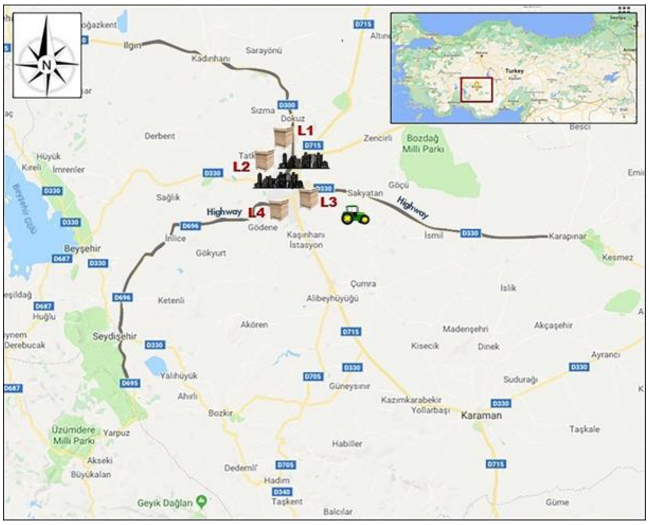

Figure 1: Locations of the honey bee colonies (sampling sites).

Colony Characteristics

Langstroth beehives with plastic bottoms and bee pollen traps were used in the study. Colonies were arranged with newly raised honeycomb and eight frame bees, and no additional feeding was given to the colonies. Honey bee, honey, and bee pollen samples were obtained from these 20 colonies, and the Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn contents of these samples were determined.

Collection and Conservation of Samples

Honey Bee: After August 15, each colony's entry hole was sealed off before midday (about 9:00–10:00), and 30 worker bees coming back from the field were apprehended at the hive entrance using plastic gloves. In glass jars, samples were kept at -18°C until analysis.

Bee pollen: Bee pollen was collected three times every 15 days in May and June, and the collected bee pollen was dried in a dark environment. 25 g (totaling 75 g) of bee pollen collected and dried from each colony at once was taken, mixed, put into glass jars, and stored at -18°C until analysis.

Honey: The honey of each colony in different locations was harvested separately between July 15 and July 20 without using a smoker. Approximately 500 g of honey was collected from each colony and stored in glass jars at room temperature and in the dark until analysis.

Preparation of Samples and Heavy Metal Analysis

To create a homogenous sample, dried bee pollen that had been kept at room temperature was ground (Kacar & İnal, 2008). A total of 2 g of ground bee pollen samples and preserved bee samples were collected and dried in a 70°C oven until they attained a consistent weight before being utilized in the study.

For the heavy metal analysis, 0.2 g of bee pollen, honey, and bee (whole bee) samples were weighed into heat-resistant teflon containers from all sites. 5 mL of concentrated HNO_3 (Nitric Acid) and 2 mL of H_2O_2 (Hydrogen Peroxide) (30% w/v) were added to the weighed samples and the samples were thawed in a microwave device (Cem MARSXpress) at high temperature (210 °C) and pressure (200 PSI). To ensure the reliability of the analysis, 1 control (blank) and 1 certified reference material (Peach Leaves, NIST, SRM 1547) were added to the 40-cell microwave set. The volumes of the thawed samples were made up to 20 ml with deionized water and filtered with blue banded filter paper. The heavy metal contents of the samples (total Pb, Cd, Cr, Zn, Cu, Ni, Mn, and Fe) were determined by an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry, Varian-Vista Model, Axial) device (USDA, 2004).

Statistical Analysis

ANOVA was applied to determine whether there is a statistically significant difference between heavy metals measured in honey bees, bee pollen, and honey samples. The heavy metal contents were subjected to Duncan Test, one of multiple comparison tests. Data measured from bees, honey, and bee pollen was evaluated with PCA and LDA. The number of variables was reduced by creating groups with the PCA method. After PCA analysis, geographical origins of bee honey and bee pollen were tried to be determined by LDA. Statistical analyzes were made using SPSS 19 statistical program.

Results

Table 1 shows the ANOVA results to check whether there is a statistically significant difference between bees, honey, and bee pollen in terms of heavy metal content. As a result of the analysis, it was observed that the bees contain more heavy metals than the bee products (P < 0.01). While there is no significant difference in Cr between bee and bee pollen, honey contains the least Cr (0.053 mg/kg) (P < 0.01). In all of the other heavy metals used in the study, the highest heavy metal content was found in bees and the lowest in honey, and these differences are statistically significant in all of them (P < 0.001). In this study, bees had the highest Cd (0.0189 mg/kg) and Pb (0.335 mg/kg) content compared to honey and bee pollen.

Table 1. Descriptive statistics and ANOVA results for heavy metals ($\bar{X} \pm S_x$)

Heavy metals (mg/kg)	Honey bee	Bee pollen	Honey
Cd	0.0189 ± 0.0005^a	0.0105 ± 0.0005^b	0.0065 ± 0.0003^c
Cr	0.084 ± 0.0039^a	0.075 ± 0.0029^b	0.053 ± 0.0031^b
Cu	15.06 ± 0.56^a	6.23 ± 0.22^b	0.77 ± 0.034^c
Fe	98.38 ± 1.548^a	86.61 ± 3.637^b	9.53 ± 0.847^c
Mn	30.66 ± 1.297^a	15.31 ± 0.881^b	0.98 ± 0.065^c
Ni	0.396 ± 0.0112^a	0.353 ± 0.0144^b	0.203 ± 0.0074^c
Pb	0.335 ± 0.0096^a	0.146 ± 0.0079^b	0.113 ± 0.0046^c
Zn	41.42 ± 1.347^a	18.41 ± 0.525^b	1.48 ± 0.054^c

Means with different superscript letters are significantly different (P < 0.01).

Assessment of Honey Bee Data by PCA and LDA

In this study, the principal component was determined as much as the number of eigenvalues greater than one, which is the generally accepted method. Accordingly, three principal components are suitable for the bee, and 32% of the total variance in heavy metals in bees is explained by the first PC and 68% by the first three PCs (Table 2). PC1 consists of Cr, Cu, and Fe metals and it can be said that the majority of heavy metal variance in bees can be explained by these three metals. PC2 is composed of Mn and Zn metals.

Table 2. PCA analysis results for heavy metals measured in honey bees

Heavy metals	PC1	PC2	PC3
Cd	0.355	0.346	**-0.383**
Cr	**0.485**	0.067	0.325
Cu	**0.324**	-0.307	-0.072
Fe	**0.448**	-0.277	-0.221
Mn	-0.052	**0.607**	-0.270
Ni	0.455	0.025	**0.470**
Pb	0.246	-0.195	**-0.623**
Zn	0.250	**0.545**	0.107
Eigenvalue	2.5424	1.8834	1.0102
Proportion	0.318	0.235	0.126
Cumulative	0.318	0.553	0.680

As a result of the discriminant analysis performed to determine the geographical origin of bees in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 12.576, 2.442 and 0.350, respectively. The large eigenvalue indicates that most of the variance in the dependent variable is explained by that function. Here, the first function with the 12.576 eigenvalue explains most of the variance in the dependent variable. Wilks' lambda values are 0.016 (p = 0.00), 0.215 (p = 0.131), and 0.741 (p = 0.690) respectively. Wilks' lambda indicates the significance of the discriminant function. The smaller the Wilks' lambda value, the higher the discrimination power of the model (Caravaca et al., 2009; Stella, 2019). Since the significance of the first Wilks' lambda value is less than 0.05, the first separation function can separate the groups in a meaningful way. Function 1 explains 81.8% of the total variance, function 2 explains 15.9% and function 3 explains 2.3%. It can be said that the first function is more effective since it explains the majority of the variance. As a result of the classification, the bees in the L1, L2, L3 regions were classified 100% correctly, while the bees in the L4 region were classified correctly at 80%.

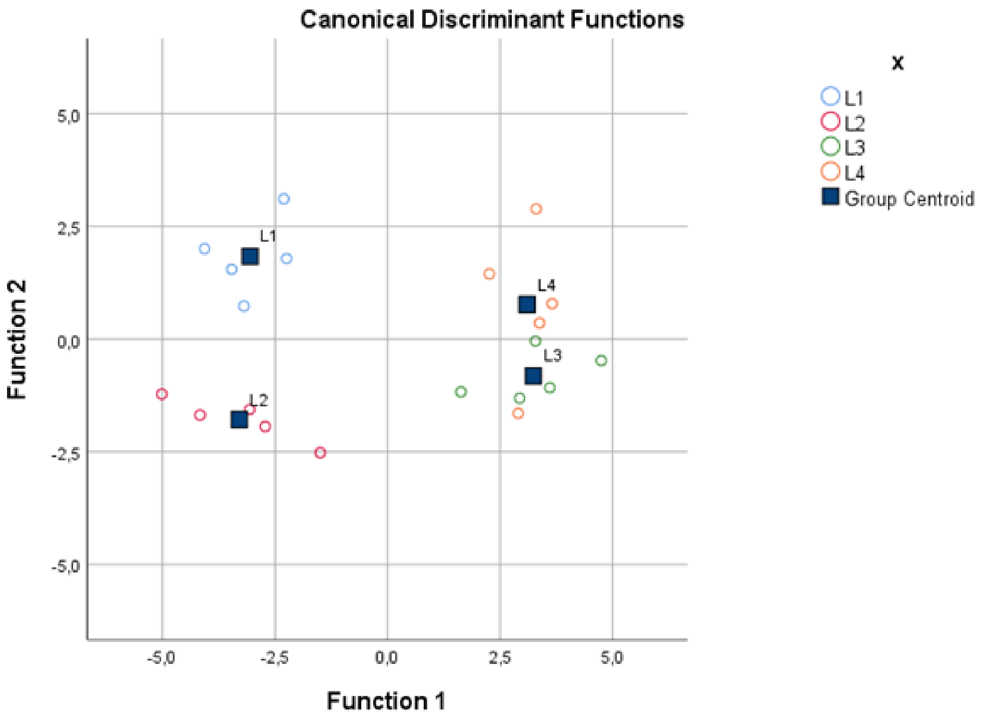

Figure 2: Canonical discriminant score plot according to the geographical origin of honey bees

Figure 2 visually shows that bees are classified according to their geographic origin. The metal with the highest coefficient in the first function is Cr (1.320), in the second function Mn (1.015) and in the third function Zn (0.662) and other coefficients are given in Table 3.

Table 3. Standardized canonical discriminant function coefficients for honey bees

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.658	-0.470	-0.696
Cr	1.320	-0.003	-0.130
Cu	0.810	-0.097	0.506
Fe	-0.458	-0.176	-0.277
Mn	0.134	1.015	-0.155
Ni	-0.019	0.087	0.090
Pb	0.304	-0.062	0.489
Zn	0.359	0.194	0.662

Assessment of Bee Pollen Data by PCA and LDA

If we look at the number of eigenvalues greater than one, bee pollen is explained by three principal components. The first PC explains 36.7%, the second PC explain 55.3% and the three PC explain 68.1% of the total variance (Table 4). Cu, Fe, Pb, and Zn constitute the first principal component, while Cd, Cr, and Ni constitute the second PC and other metals constitute the third PC. The Principal Component Analysis (PCA) results obtained from bee pollen samples are clearly different from those obtained from bee samples. When the Table 4 is reviewed, Mn, unlike other elements, is found in the third component.

Table 4. PCA analysis results for heavy metals measured in bee pollen

Heavy metals	PC1	PC2	PC3
Cd	0.197	**0.448**	0.005
Cr	0.388	**0.441**	0.214
Cu	**0.334**	-0.302	-0.233
Fe	**0.450**	0.046	-0.100
Mn	-0.108	0.291	-**0.886**
Ni	0.366	**0.392**	0.129
Pb	**0.463**	-0.275	-0.288
Zn	**0.370**	-0.444	0.079
Eigenvalue	2.9359	1.4885	1.0246
Proportion	0.367	0.186	0.128
Cumulative	0.367	0.553	0.681

As a result of the discriminant analysis performed to determine the geographical origin of bee pollens in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 15.185, 8.082, and 0.972, respectively. Here, the first two function with the 15.185 and 8.082 eigenvalues explains most of the variance in the dependent variable. Wilks' lambda values are 0.003 (p = 0.00), 0.056 (p = 0.01), and 0.507 (p = 0.18), respectively. Since the significances of the first two Wilks' lambda value is less than 0.05, the first two separation functions can separate the groups in a meaningful way. Function 1 explains 62.6% of the total variance, function 2 explains 33.3% and function 3 explains 4.0%. It can be said that the first two functions are more effective since it explains most of the variance. As a result of classification, bee pollen in all regions is classified 100% correctly.

Figure 3 visually shows that bee pollens are classified according to their geographic origin. The metal with the highest coefficient in the first function is Mn (1.255), in the second function Pb (0.741) and in the third function Zn (0.943) and other coefficients are given in the Table 5.

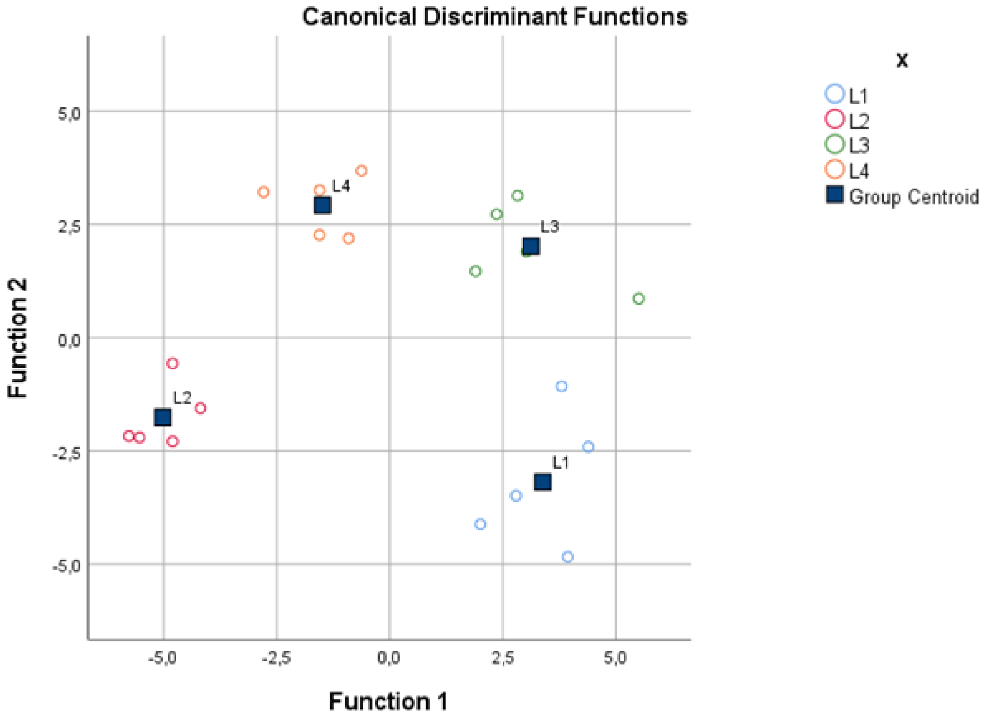

Figure 3. Canonical discriminant score plot according to the geographical origin of bee pollens

Table 5. Standardized canonical discriminant function coefficients for bee pollens

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.087	0.186	-0.226
Cr	-0.268	-0.757	0.767
Cu	0.065	0.504	-0.455
Fe	-0.065	0.698	-0.050
Mn	1.255	0.035	0.188
Ni	0.871	0.461	-0.263
Pb	-0.105	0.741	-0.473
Zn	0.090	0.427	0.943

Assessment of Honey Data by PCA and LDA

The first principal component and the first four principal components together account for 27% and 74% of the total variance in honey, respectively (Table 6). Cr, Fe, and Zn are in the first basic component; Ni, Cu, Mn, Cd, and Pb are included in other components. Compared with the correlation and PCA results in bees and bee pollen, quite different results were obtained in honey. These results show that honey does not reflect the heavy metal content of the region very well.

Table 6. PCA analysis results for heavy metals measured in honey

Heavy metals	PC1	PC2	PC3	PC4
Cd	0.167	0.486	-0.286	**0.575**
Cr	**0.546**	-0.096	-0.124	-0.150
Cu	-0.303	-0.342	-0.329	-0.357
Fe	**0.518**	-0.180	-0.022	0.232
Mn	0.062	0.308	**0.597**	-0.196
Ni	-0.142	**-0.586**	-0.044	0.531
Pb	0.260	0.147	**-0.592**	-0.358
Zn	**0.471**	-0.382	0.293	-0.131
Eigenvalue	2.1324	1.372	1.3487	1.0566
Proportion	0.267	0.172	0.169	0.132
Cumulative	0.267	0.438	0.607	0.739

As a result of the discriminant analysis performed to determine the geographical origin of honey in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 11.293, 4.917, and 2.164, respectively. Wilks' lambda values are 0.004 (p = 0.00), 0.053 (p = 0.01), and 0.316 (p = 0.02) respectively. Since the significance of three Wilks' lambda values is less than 0.05, three separation functions can separate the groups in a meaningful way. Function 1 explains 61.5% of the total variance, Function 2 explains 26.8%, and Function 3 explains 11.8%. As a result of the classification, the honey in all regions is classified 100% correctly.

Figure 4 visually shows that the bee pollen is located according to its geographic origin. The metal with the highest coefficient in the first function is Cr (1.134), in the second function, Mn (1.165) and in the third function Cu, (1.042) and other coefficients are given in Table 7.

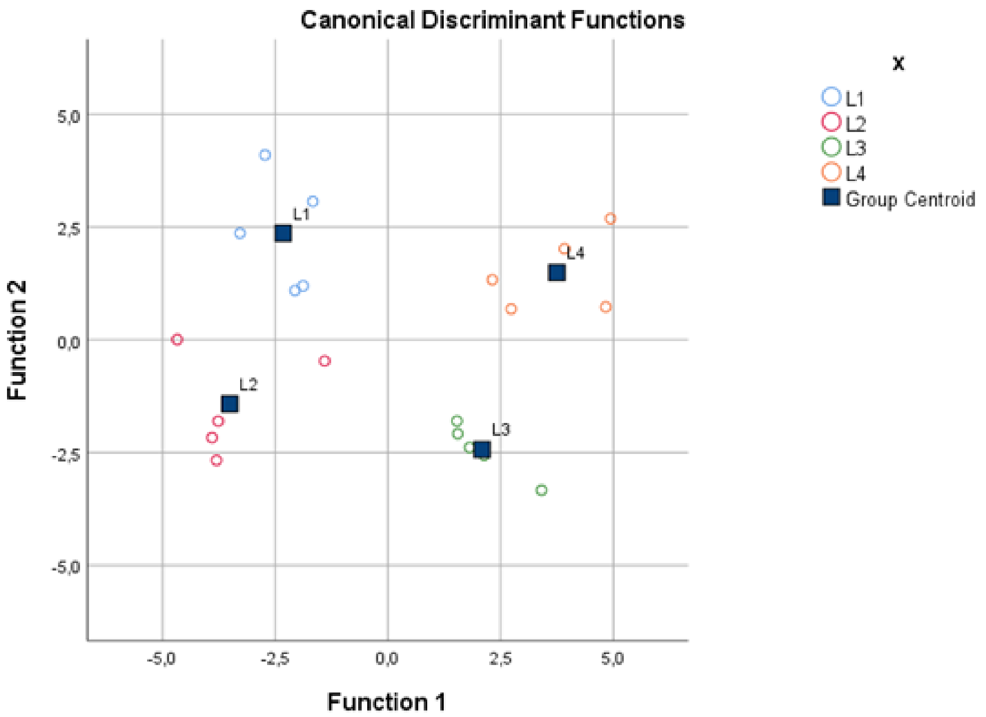

Figure 4. Canonical discriminant score plot according to the geographical origin of honey

Table 7. Standardized canonical discriminant function coefficients for honeys

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.566	0.057	0.057
Cr	1.134	-0.299	-0.412
Cu	-0.102	0.214	1.042
Fe	0.882	0.610	0.475
Mn	0.500	1.165	-0.170
Ni	1.234	-0.404	-0.243
Pb	0.158	-0.395	0.129
Zn	0.359	-0.781	0.333

Discussion

Heavy metal accumulation in bees is higher than in bee products, according to studies (Al Naggar et al., 2018; Ćirić et al., 2021; Formicki et al., 2013; Sari et al., 2020). Leblebici and Aksoy (2008) reported that honey bees do not fully reflect the metal pollution they receive from plants and that it acts as a kind of natural filter. In other words, honey bees claim that it is a better bioindicator of heavy metal pollution than honey. The results obtained in this study were in agreement with those in the literature (Conti et al., 2022; Conti & Botrè, 2001; Silici et al., 2016). Cadmium and lead are highly toxic heavy metals. They are more common in industrial areas. In particular, quite a lot of Pb is emitted from automobile exhaust. For this reason, it is defined as the most common air pollutant. The mixing of air and water in these regions endangers the lives of both animals and plants. Long-term exposure, even in very low amounts, adversely affects many systems in the body. In some studies, it was determined that the Cd and Pb contents were higher in the samples taken from the city (Conti & Botrè, 2001).

In this study, bees had the highest Cd (0.0189 mg/kg) and Pb (0.335 mg/kg) content compared to honey and bee pollen. The lowest value is in honey in our study. It was determined that the Cd and Pb values of bee pollen and honey samples were within the international food standard values (Alimentarius, 2015). While Cd and Pb were found in small amounts in honey in many studies (Ahmida et al., 2012; Bayir & Aygun, 2022; Fredes & Montenegro, 2006; Manu Kumar et al., 2013; Pisani et al., 2008; Roman, 2010), some studies found no Cd in honey (Boussaid et al., 2018; El-Haskoury et al., 2018; Silici et al., 2016; Taha et al., 2017). The difference in Cd content in the studies is thought to be related to the proximity of the region where the samples were taken to the highway. The reason why Cd and Pb were found in honey in this study, albeit in small amounts, is that the samples were taken from the city center.

According to a study by Zarić et al. (2017), PCA can identify the sources of heavy metals in honey bees. Heavy metal studies in bees have also shown that PCA can successfully reduce the size of heavy metals (Di Fiore et al., 2022). According to the PCA results obtained in this study, the elements in the first two components are generally elements of natural origin. Pb and Cd, which have a higher load in the 3rd component, are found in the atmosphere, especially in areas with intense traffic and mining activities (Mohammed et al., 2011). Since the area where the samples were taken is around the city center, it is thought that these traffic-related elements are found in bee samples.

Chromium, nickel, zinc, copper, iron and manganese are abundant in nature and are essential elements for the human body. Their release to the environment is due to fossil fuels (Ni), the steel industry (Mn, Cr), smelting (Cu, Zn), and other industry activities. In cases of excessive intake, it has negative effects on many organs. In this study, the Cr content in bees and bee pollen was not statistically significant. The Cr content in bees (0.0835 mg/kg) and bee pollen (0.75 mg/kg) is higher than honey (0.053) ($P < 0.01$). Other heavy metals were found more in bees. This study's detection of the mentioned heavy metals was comparable to those of earlier studies (Boussaid et al., 2018; Conti & Botrè, 2001; Manu Kumar et al., 2013; Satta et al., 2012).

The geographic origins of bees, mostly in terms of morphological characters, were determined by discriminant analysis. (Guler & Bek, 2002; Meixner et al., 2011; Ozbakir & Firatli, 2013; Sıralı et al., 2003; Strange et al., 2008). However, a discriminant analysis study with heavy metal content in bees could not be found. As can be seen from the results of this study, geographical origins can be separated from LDA with 80% accuracy in terms of metal content.

Many discriminant analysis studies have been conducted to determine the geographic origin of bee pollen. As a result of the discriminant analysis for some toxic fatty acids (Ares et al., 2020), physicochemical properties (Pascoal et al., 2022), phenolic profiles (Kaškonienė et al., 2015), or organic matter content (Sattler et al., 2015) in the bee pollen content, geographical data were obtained. It has been determined that the origins can be separated successfully (Ares et al., 2022a; Ares et al., 2022b). According to Lilek et al. (2022), as a result of PCA and Da analysis for the 11 metal contents of bee pollen samples taken from 4 different regions of Slovenia, bee pollen samples could be successfully separated by LDA according to their geographical origins.

Considering the result of PCA, honey and bee pollen gave closer results in terms of the relationships between heavy metals, while completely different results were obtained in honey. Heavy metal pollution in the environment affects honey differently than bees in this regard. It is common practice to utilize PCA to identify potential sources of heavy metals in environmental samples (Bazeyad et al., 2019). Many studies have been conducted on the determination of the source of heavy metal contamination in soil or water with PCA (Li et al., 2006; Yang et al., 2013; Zhiyuan et al., 2011). There are very few PCA application studies on the heavy metal content of bees and their products, which are very sensitive to environmental pollutants and have bioindicator properties. Ciric et al. (2021), demonstrated that heavy metals can be explained by two components and that PCA analysis can be successfully applied to bees and bee products because of a PCA study on bees and bee products.

The physicochemical properties (Adgaba et al., 2017; Fechner et al., 2016; Karabagias et al., 2014; Serrano et al., 2004), carbohydrate profile (Nozal et al., 2005), and voltage compounds (Stanimirova et al., 2010) of honey were analyzed using LDA. It was observed that they can be successfully separated according to their geographical origins. Yayinie and Atlabachew (2022), determined 14 different metal contents of the honey they collected from seven different regions, and they saw that 93.33% correct discrimination was made as a result of PCA and LDA analysis. Fernández-Torres et al. (2005), analyzed the mineral content of honeys obtained from 4 different regions of Spain. As a result of the discriminant analysis, they concluded that the honey can be separated according to their geographical origins.

Oroian and Ropciuc (2017), determined the presence of 10 different metal contents in bee pollen samples collected from three different regions of Romania. The researchers performed a PCA-LDA analysis to assess the separation of samples based on their botanical and geographical origins. They found that the samples were successfully classified based on their botanical origin, with an accuracy of 80.8%. However, the samples could not be accurately classified based on their geographical origin, with only 21.2% accuracy.

Conclusion

In this study, it is seen that heavy metals are more common in bees than bee pollen and honey, and the heavy metal contents of bee pollen and honey samples from the same hives vary considerably. Based on these findings, it is evident that bees play a crucial role in indicating the presence of heavy metal contamination in the environment, making them the most effective bioindicators. It is seen that bees, bee pollen, and honey form different components in terms of heavy metal content because of PCA regarding the heavy metal content of the samples. In addition, when investigating the source of heavy metal contamination, it is observed that bees give better results in the evaluation of bees and bee products with PCA. The use of PCA-LDA to analyze the heavy metal levels of bee, bee pollen, and honey samples revealed that the samples could be effectively differentiated based on their respective collection sites.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors state that there are no conflicts of interest.

Author Contributions

Conceptualization, H.B. and A.A.; methodology, H.B. and A.A.; validation, H.B. and A.A; formal analysis, H.B., A.A. and F.İ.; investigation, H.B. and A.A.; data curation, H.B., A.A. and F.İ.; writing-original draft preparation, H.B. and A.A.; writing-review and editing, H.B., A.A. and F.İ.; supervision, H.B., A.A. and F.İ.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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