RESEARCH ARTICLE

Heavy metal in honey bees, honey, and pollen produced in rural and urban areas of Konya province in Turkey

Huseyin Bayir[1] · Ali Aygun[1]

Abstract

We investigated the levels of heavy metals in honey bee, honey, and pollen samples obtained from different locations in Konya City in Turkey. Five honey bee colonies were placed in eight different locations, four of them around urban areas and four in rural areas, in the province of Konya City in Turkey. Heavy metal (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) levels were determined in honey bee, honey, and pollen samples taken from these colonies, with a comparison between samples from urban and rural areas. The values of Cd in honey samples and those of Cd in pollen samples did not differ significantly among the locations. All heavy metal values of honey bee samples were lower in rural areas than in urban areas. Significant statistical differences were determined for Cr, Cu, Fe, Mn, Ni, Pb, and Zn values of honey and pollen samples among the locations. Heavy metal values of honey and pollen samples obtained from different locations were in agreement with the International Food Standard values.

Keywords Honey bee colonies · Pollution · Cadmium · Lead

Introduction

Beekeeping is sometimes practiced in places close to or within residential and industrial areas. Apiaries that are not suitable for bee colonies and mistakes made in production deteriorate the natural characteristics and quality of bee products.

Environmental problems that arise with population growth, urbanization, industrialization, and changing consumption habits are becoming increasingly common. For example, heavy metals released into the air in the form of dust and ash from various sources accumulate on plants (Taha et al. 2017) and cause the pollution of soil and water resources by precipitation or by sedimentation. Additionally, heavy metals in wastewater, agricultural chemicals, and fertilizers can also affect animals and humans via accumulation on the food chain (Yılmaz 1996; Duruibe et al. 2007; Türközü and Şanlıer 2014; Squadrone et al. 2020).

Heavy metals enter the bodies of animals and humans via ingestion (food and water), respiration, and the skin; they can be classified as essential and non-essential (Özbolat and Abdullah 2016). For example, some elements such as copper, zinc, iron, manganese, and selenium are essential for body development and proper functioning. On the other hand, non-essential elements, such as lead, cadmium, and mercury, can cause various disorders by accumulating in the body and affecting the biological structure, even at low concentrations (Tuzen et al. 2007; Uluozlu et al. 2007).

To determine the environmental pollution of a region, living organisms (bioindicators or biomonitors) that show different sensitivities to various pollutants can be used (Yılmaz 1996). In this sense, honey bees and bee products can contain residues of pollutants, making them important indicators of environmental pollution (Porrini et al. 2003; Bogdanov 2008; Ahmida et al. 2012; Zhelyazkova 2012; Taha et al. 2017).

Honey bees can easily live in different environmental conditions, can be kept easily, have a short life cycle, and a high reproductive rate, and as a colony, they can be transported to any place. They move around the apiary within an area of approximately 7 km^2 in search of food, and samples for analysis can be taken during flight activity (Leita et al. 1996; Conti and Botrè 2001; Perugini et al. 2011).

Bees and bee products can be contaminated with pollutants from different sources, and the contamination may differ according to the environmental conditions of the beekeeping area

(Hennessy et al. 2010; Costa-Silva et al. 2011; Pohl et al. 2011). Demirezen and Aksoy (2005) and Bogdanov (2008) reported that heavy metal accumulation is higher in bee products obtained from industrial regions and areas with heavy vehicle traffic, especially those close to large settlements and garbage incinerators.

In this context, this study compared the heavy metal contents of honey bees, honey, and pollen obtained from places close to residential and industrial areas with those obtained from rural areas and determined whether honey and pollen taken from these areas pose a problem in terms of food safety.

Materials and methods

Sampling sites (locations)

The study was carried out in the Konya region, Turkey, in 2018. Konya is located between the north parallels of 36°
22′ and 39° 08′ and the east meridians of 31° 14′ and 34° 05′. Konya is the largest city in Turkey in terms of surface area, with approximately 41 km^2. There are numerous lakes in the area, notably Beyşehir Lake, which is the second largest lake in Turkey. Konya is Turkey's 7th most populated city, with a population of almost 2 million people. Konya has a cold semi-arid climate. Konya is the province with the least rainfall in Turkey. Five honey bee colonies (40 in total) were placed in eight different multifloral locations, four of which were around the urban areas (L1 to L4) and four in the rural areas (L5 to L8) (Fig. 1).

The locations had the following characteristics:

L1: 38°02′05″ N, 32°30′10″ E, 1180 m. On the northern side of the city and in the prevailing wind direction of the city, 1210 m away from and west of the highway. Vegetables and fruits are grown in a limited area surrounding it, and there are no industrial facilities.

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

L2: 37°55′12″ N, 32°26′10″ E, 1140 m. On the northwestern side of the city and in the prevailing wind direction of the city, 4300 m away from and north of the highway. There is agricultural activity, such as growing fruit and vegetables, in a small area around it, but there is no industrial facility.

L3: 37°51′07″ N, 32°33′33″ E, 1010 m. Southeast of the city, southwest of the industrial regions; the prevailing wind direction is from the industrial regions (plastic packaging industry, machinery industry, marble industry, furniture industry). This site is 1800 m away from and south of one highway and 1300 m away from and east of another highway. In the surrounding area, agricultural activities such as grain, vegetable, and fruit farming are cultivated.

L4: 37°49′12″ N, 32°28′45″ E, 1027 m. On the southern side of the city, the prevailing wind direction is from the city side. It is 1000 m away from and south of one highway and 3400 m away from and west of another highway. In the surrounding area, agricultural activities such as fruit and vegetable cultivation are practiced extensively.

L5: 37°28′16″ N, 31°48′56″ E, 1131 m. This site is 4600 m away from and northwest of the highway. There is only one aluminum-processing plant at a distance of 3750 m in the southeast. Fruit and vegetable cultivation takes place in a small area surrounding it.

L6: 37°42′05″ N, 33°31′20″ E, 1003 m. This site is 1400 m away from and south of the highway. There are no factories in the area. Sunflowers and corn are grown in a small area around it.

L7: 38°05′07″ N, 32°16′45″ E, 1630 m. In the vicinity of this site, there is very little agricultural activity (grain), and there are no highways, industrial facilities, etc., nearby.

L8: 36°58′38″ N, 32°22′51″ E, 1775 m. In the vicinity of this site, there is very little agricultural activity (fruit), and there are no highways, industrial facilities, etc., nearby.

The prevailing wind direction in Konya is north. In the months of May to August 2018, which were the sampling months, the area experienced 67% northerly winds (39% north, 13% north east, and 15% northwest).

Beehive and colony characteristics

We used Langstroth type beehives with a plastic bottom and pollen trap. The colonies were arranged with a newly raised honeycomb, eight frame hives, and 1-year-old queen bee colonies were used without additional feeding.

Collection and conservation of samples

Pollen: Pollen samples were collected from each colony three times every 15 days in May and June. These samples, taken from each colony, were dried under suitable conditions. 25 g (a total of 75 g) of dried pollen samples from each colony were mixed. It was then put into glass jars and kept at − 18 °C until it was analyzed.

Honey: The honey of each colony was harvested separately between July 15 and August 15 without using a smoker. Approximately 500 g of honey from each colony was placed in glass jars, and the honey was kept at room temperature and in the dark until analysis.

Honey bee: After August 15, the entrance hole of each colony was closed before noon (around 09:00–10:00), and 30 worker bees returning from the field were caught at the hive entrance using plastic gloves. Samples were placed in glass jars and stored at − 18 °C until analysis.

Preparation of samples and heavy metal analysis

Dried pollen was ground to obtain a homogeneous sample (Kacar and İnal 2008). Approximately 2 g of ground pollen samples and bee samples kept at − 18 °C were taken and dried in an oven at 70 °C until constant weight before being used in the analysis.

In the analysis of heavy metals, 0.2 g of honey, pollen, and bee (whole bee) samples obtained from all locations were weighed into heat-resistant Teflon containers. Subsequently, 5 ml of concentrated HNO_3 and 2 ml of H_2O_2 (30% w/v) were added to the weighed samples, and the samples were thawed in a microwave device under high temperature (210 °C) and pressure (200 PSI). To ensure the reliability of the analysis, one control (blank) and one certified reference sample (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, filtered with blue-banded filter paper, and stored in the refrigerator at + 4 °C until readings were taken.

The heavy metal contents of the samples (total Pb, Cd, Cr, Zn, Cu, Ni, Mn, and Fe) were determined using an ICP-AES (inductively coupled plasma atomic emission spectrometry, Varian-Vista Model, Axial) (USDA 2004). Elemental amounts of the samples are given in µg/kg for Cd and Cr and in mg/kg for the other elements.

Statistical Analysis

Heavy metal amounts were determined in five honey bee, honey, and pollen samples taken from each of the eight different locations. The study was carried out in a randomized plot design. The data were subjected to one-way analysis of variance, and Tukey's test was used to determine the differences among groups. Data are presented as mean and standard error.

Results and discussion

Heavy metal values in honey samples

Heavy metal (Cd, Cr, Fe, and Zn) mean values and standard errors of honey samples are shown in Table 1. In honey samples, significant statistical differences were found among the locations in terms of Cr, Fe, and Zn, except for Cd ($P < 0.05$).

The Cd value of honey samples ranged from 4.301 to 6.898 µg/kg, and the difference among locations was statistically insignificant. Bosancic et al. (2020) stated that no statistically significant differences were found between conventional and organic honey production systems. On the other hand, Arslan and Arıkan (2013) found the highest amount of Cd in honey samples taken from stations close to the highway. Adugna et al. (2020) found the Cd content of honey samples from different regions in the range of 17–35 µg/kg, which was higher compared with our results.

The Cr, Fe, and Zn values of honey samples were highest in the urban areas (L3, 67.01 µg/kg; L4, 14.500 mg/kg; L4, 1.635 mg/kg, respectively) and lowest in the rural areas (L8, 27.70 µg/kg; L6, 6.266 mg/kg; L6, 1.039 mg/kg, respectively).

Leblebici (2006) reported that the Cr content of honey samples taken from sites close to residential areas was higher than that from rural areas ($P < 0.05$). Gurel et al. (1998) found significant differences among honey samples obtained from different locations in terms of Fe concentration ($P < 0.01$). Leblebici (2006) found the Fe content of honey samples taken from locations close to the city center was higher than that of locations far from the city center ($P < 0.05$). Taha et al. (2017) found the Zn content of honey samples taken from colonies placed at different distances from a cement factory was higher in samples close to the factory ($P < 0.05$). The Zn values (1.039–1.635 mg/kg) obtained in our study were lower than the value (4.814 mg kg) determined by Aliu et al. (2020) in honey.

The values for Cu, Mn, Ni, and Pb found in honey are presented in Figs. 2 and 3. The Cu, Mn, Ni, and Pb values of honey samples were highest in the urban areas (L2, 0.950 mg/kg; L1, 1,270 mg/kg; L3, 0.216 mg/kg; and L3, 0.118 mg/kg, respectively) and lowest in the rural areas (L7, 0.395 mg/kg; L6, 0.520 mg/kg; L8, 0.106 mg/kg; and L8, 0.073 mg/kg, respectively).

Demirezen and Aksoy (2005) found that the Cu content of honey samples taken from sites close to urban areas was higher than those from rural areas ($P < 0.01$). On the other hand, Silici et al. (2016) stated that there was no statistical difference among the Cu values of honey samples taken from different distances to a thermal power plant. Arslan and Arıkan (2013) did not detect a significant difference among the Mn values of honey samples obtained from colonies placed at different distances from the highway. Taha et al. (2017) found that the Ni content in honey samples taken from colonies placed at different distances from the cement factory was higher in samples close to the factory ($P < 0.05$). Demirezen and Aksoy (2005) stated that the Ni content of honey samples taken from sites close to residential areas was higher than those from rural areas ($P < 0.01$).

The Pb content of honey samples ranged between 0.073 and 0.118 mg/kg, and the differences among the locations were statistically significant ($P < 0.05$). Similarly, Roman (2010) determined that the Pb content of honey samples obtained from settlements was higher than the values obtained from agriculture and forest areas ($P < 0.05$). The Pb value (0.073–0.118 mg/kg) obtained in our study was similar to the value (0.020–0.098 mg/kg) determined by Purcarea et al. (2017) in Polish honey. In our study, the Cd and Pb values of honey samples were within the International Food Standard values (Alimentarius 2015).

Heavy metal values in pollen samples

The mean values and standard errors of Cd, Cr, Cu, and Fe in pollen samples are given in Table 2. In pollen samples, the

Table 1 Heavy metal content in honey samples

Areas	Locations	Cd (µg/kg)	Cr (µg/kg)	Fe (mg/kg)	Zn (mg/kg)
Urban	L1	6.509 ± 0.642	43.63 ± 3.634bc	6.880 ± 0.711b	1.289 ± 0.071ab
	L2	5.849 ± 0.853	42.77 ± 1.679bc	6.753 ± 0.580b	1.378 ± 0.069ab
	L3	6.625 ± 0.701	67.01 ± 6.194a	10.000 ± 1.076ab	1.606 ± 0.114a
	L4	6.898 ± 0.447	60.40 ± 4.943ab	14.500 ± 1.577a	1.635 ± 0.116a
Rural	L5	5.803 ± 0.574	39.13 ± 1.864c	7.915 ± 0.922b	1.159 ± 0.054b
	L6	4.861 ± 0.645	42.57 ± 4.007bc	6.266 ± 0.514b	1.039 ± 0.070b
	L7	4.301 ± 0.682	36.85 ± 3.217c	6.744 ± 0.656b	1.148 ± 0.075b
	L8	4.816 ± 0.339	27.70 ± 2.187c	6.326 ± 0.819b	1.158 ± 0.025b
P value		0.059	0.000	0.000	0.000

a–c: Different letters in the same column indicate significant differences ($P < 0.05$)

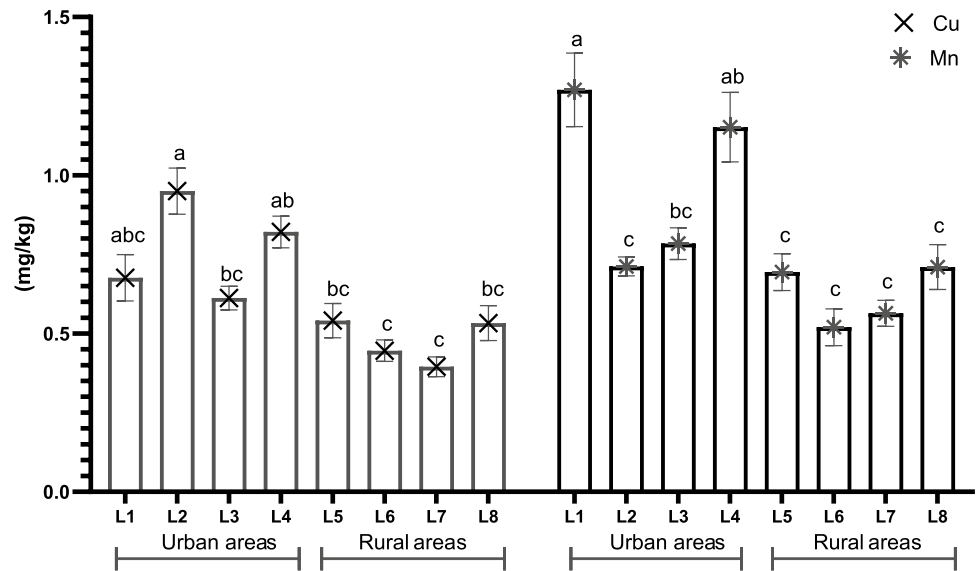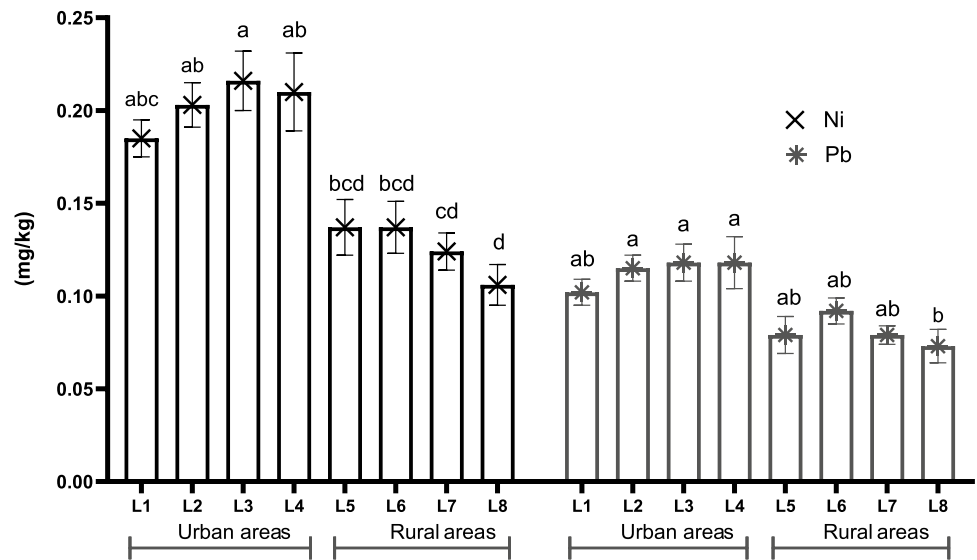**Fig. 2** The Cu and Mn content of honey produced in different locations

Fig. 3 The Ni and Pb content of honey produced in different locations

differences among the locations were statistically significant in terms of Cr, Cu, and Fe, except for Cd ($P < 0.05$).

The Cd content of the pollen samples ranged between 6.535 and 11.304 µg/kg, and the difference among the locations was statistically insignificant. Similarly, Arslan and Arıkan (2013) stated that there was no significant difference among the Cd values of pollen samples taken from colonies placed at different distances from the highway. The Cd value (6.535–11.304 µg/kg) obtained in our study was lower than the value (26.1–92.0 µg/kg) determined by Formicki et al. (2013) in Poland pollen.

The Cr, Cu, and Fe values of pollen samples were highest in the urban areas (L3, 79.36 µg/kg; L4, 7.275 mg/kg; and L4, 96.95 mg/kg, respectively) and lowest in the rural areas (L8, 46.10 µg/kg; L6, 3.915 mg/kg; and L7, 61.40 mg/kg, respectively).

Conti and Botrè (2001) found higher Cr content in pollen samples obtained from inner-city locations compared to urban locations ($P < 0.01$). Taha et al. (2017) found that the Cu content of pollen samples taken from colonies at different distances from a cement factory was higher in samples close to the factory ($P < 0.05$). Taha (2015) found significant statistical differences among pollen obtained from different plants in terms of Fe content ($P < 0.05$). Arslan and Arıkan (2013) did not find a significant statistical difference among the Fe values of pollen samples obtained from colonies placed at different distances from the highway.

Table 2 Heavy metal content in pollen samples

Areas	Locations	Cd (µg/kg)	Cr (µg/kg)	Cu (mg/kg)	Fe (mg/kg)
Urban	L1	10.426 ± 0.981	$69.71 \pm 3.475ab$	$5.600 \pm 0.378abc$	$73.18 \pm 5.362ab$
	L2	9.848 ± 1.514	$75.51 \pm 5.626ab$	$5.685 \pm 0.223ab$	$80.99 \pm 6.902ab$
	L3	11.304 ± 1.331	$79.36 \pm 6.060a$	$6.355 \pm 0.288ab$	$95.32 \pm 8.379ab$
	L4	10.567 ± 0.712	$75.76 \pm 8.980ab$	$7.275 \pm 0.473a$	$96.95 \pm 4.494a$
Rural	L5	9.343 ± 0.694	$64.96 \pm 4.808ab$	$5.430 \pm 0.298bc$	$89.93 \pm 6.951ab$
	L6	9.328 ± 1.127	$58.08 \pm 3.582ab$	$3.915 \pm 0.252c$	$72.70 \pm 5.600ab$
	L7	6.535 ± 0.771	$56.47 \pm 7.630ab$	$5.260 \pm 0.318bc$	$61.40 \pm 5.642b$
	L8	8.983 ± 1.023	$46.10 \pm 3.855b$	$5.405 \pm 0.227bc$	$71.45 \pm 6.923ab$
P value		0.111	0.003	0.000	0.003

a–c: Different letters in the same column indicate significant differences ($P < 0.05$)

The values for Mn, Zn, Ni, and Pb found in pollen are shown in Figs. 4 and 5. The Mn, Zn, Ni, and Pb values of pollen samples were highest in the urban areas (L1, 19.149 mg/kg; L3, 20.27 mg/kg; L4, 0.384 mg/kg; and L4, 0.180 mg/kg, respectively) and lowest in the rural areas (L6, 5.356 mg/kg; L7, 10.25 mg/kg; L7, 0.218 mg/kg; and L8, 0.059 mg/kg, respectively).

Fakhimzadeh and Lodenius (2000) found no significant statistical difference among industrial, urban, and rural areas in terms of Mn values in pollen samples. The Zn content of the pollen samples was highest in the urban location (L3), at 20.27 mg/kg and lowest in the rural location (L7), at 10.25 mg/kg ($P < 0.05$). On the other hand, Arslan and Arıkan (2013) did not detect significant statistical differences among the Zn values of pollen samples obtained from colonies placed at different distances from the highway. The Zn value (10.25–20.27 mg/kg) obtained in our study

was lower than the value (75.2–159.3 µg/g) determined by Formicki et al. (2013) from Poland pollen. Altunatmaz et al. (2017) stated that mineral levels are related to plant type rather than to the soil and geographical situation. Taha et al. (2017) found that the Ni content in the pollen samples taken from the colonies placed at different distances to a cement factory was higher in the samples close to the factory ($P < 0.05$). Conti and Botrè (2001) found higher Pb contents in pollen samples taken from the colonies in the city center compared to those from colonies around the city ($P < 0.01$).

Heavy metal results in honey bee samples

Heavy metals (Cd, Cr, Cu, and Fe; mean values and standard errors) of honey bee samples are summarized in Table 3.

The Cd content of honey bee samples was highest in the urban location (L4), at 20.78 µg/kg, and lowest at the rural

Fig. 4 The Mn and Zn content of pollen produced in different locations
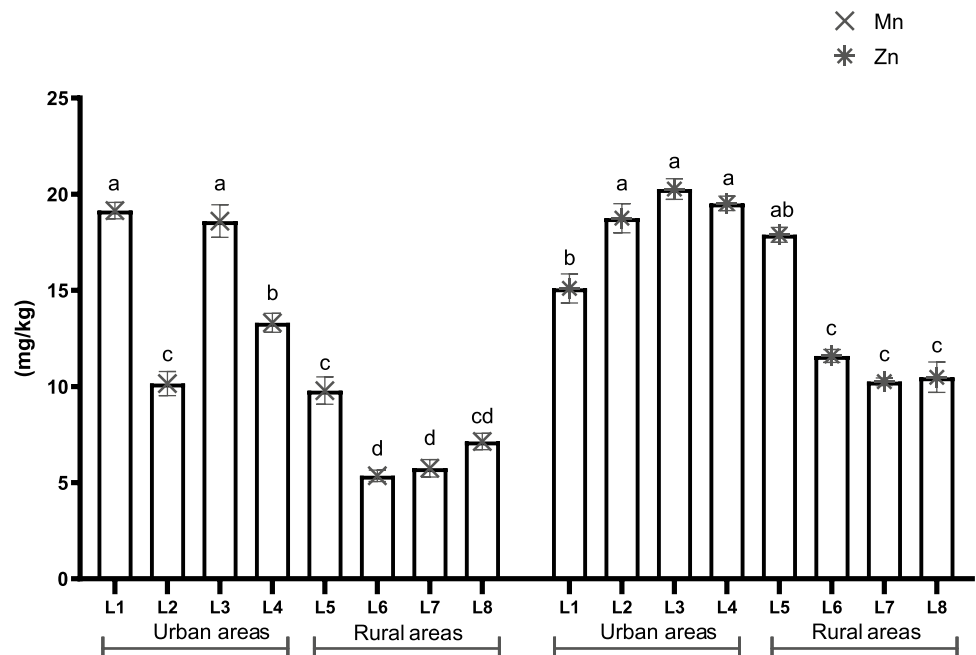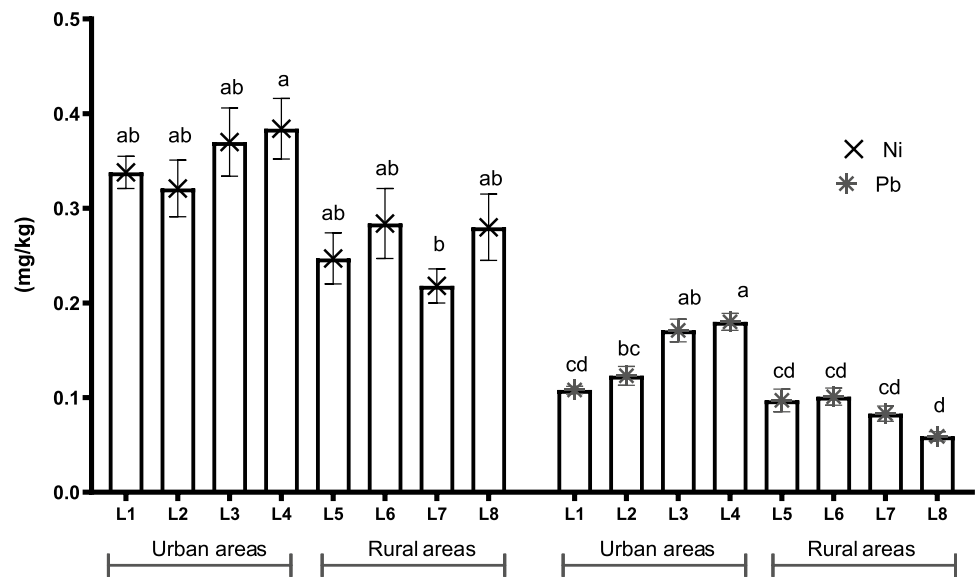
Fig. 5 The Ni and Pb content of pollen produced in different locations

location (L8), at 9.52 µg/kg ($P < 0.05$). Similar results were found by Conti and Botrè (2001), where the Cd content of bee samples obtained from the inner-city location was higher than that from the surrounding locations ($P < 0.01$). Similarly, Fakhimzadeh and Lodenius (2000) found a higher Cd content in bee samples taken from urban and industrial areas compared to samples from rural areas ($P < 0.05$). The Cd values (9.52–20.78 µg/kg) obtained in our study were lower than the range (0.03–0.30 mg/kg) determined by Goretti et al. (2020).

The Cr content of honey bee samples was highest, at 99.24 µg/kg, in the urban location (L3) and lowest, at 58.24 µg/kg, in the rural site (L7) ($P < 0.05$). Gutiérrez et al. (2015) found the highest Cr content in honey bee samples taken from different locations, including urban, industrial, agricultural, and forested areas ($P < 0.01$).

In this study, the Cu content of honey bee samples was highest in the urban area (L3), with 17.18 mg/kg, and lowest in the rural area (L6), with 12.18 mg/kg ($P < 0.05$). Roman (2010) found that the Cu content of honey bee samples

obtained from residential areas was higher than those obtained from agricultural and forest areas ($P < 0.05$). Silici et al. (2016), on the other hand, did not detect a statistical difference among the Cu values of honey bee samples taken at different distances from a thermal power plant.

The Fe content of honey bee samples was highest at the urban area (L3), with 101.81 mg/kg, and lowest at the rural area (L6), with 82.46 mg/kg ($P < 0.05$). Nisbet et al. (2013) reported that the Fe content of honey bee samples taken from locations with different environmental and flora characteristics varied from region to region ($P < 0.05$). Taha et al. (2017) found that the Fe contents in honey bee samples taken from colonies placed at different distances to a cement factory were higher in samples close to the factory ($P < 0.05$).

The values for Mn, Zn, Ni, and Pb found in honey bee samples are shown in Figs. 6 and 7. The Mn contents of honey bee samples were highest in the urban location (L1), with 35.55 mg/kg, and lowest in the rural location (L6), with 15.63 mg/kg ($P < 0.05$). On the other hand, Silici

Table 3 Heavy metal content in honey bee samples

Areas	Locations	Cd (µg/kg)	Cr (µg/kg)	Cu (mg/kg)	Fe (mg/kg)
Urban	L1	17.48 ± 0.744ab	66.30 ± 2.138b	13.42 ± 1.059ab	91.59 ± 1.579ab
	L2	18.50 ± 1.067a	69.79 ± 5.443b	14.77 ± 1.067ab	100.39 ± 3.757a
	L3	19.20 ± 1.058a	99.24 ± 3.247a	17.18 ± 0.911a	101.81 ± 2.102a
	L4	20.78 ± 0.916a	98.54 ± 3.613a	14.90 ± 1.128ab	99.72 ± 3.318a
Rural	L5	12.98 ± 0.623bc	68.42 ± 3.421b	13.93 ± 0.795ab	96.34 ± 3.287ab
	L6	13.25 ± 0.706bc	64.20 ± 3.452b	12.18 ± 1.132b	82.46 ± 4.651b
	L7	12.41 ± 0.786c	58.24 ± 3.511b	13.16 ± 0.710ab	85.43 ± 2.737ab
	L8	9.52 ± 0.623c	58.69 ± 4.119b	13.01 ± 1.181ab	85.31 ± 1.494ab
P value		0.000	0.000	0.047	0.000

a–c: Different letters in the same column indicate significant differences ($P < 0.05$)

et al. (2016) did not detect a statistical difference among the Mn values of honey bee samples taken at different distances to a thermal power plant.

The Ni values of honey bee samples were highest in the urban location (L3), with 0.432 mg/kg, and the lowest in the rural location (L7), with 0.201 mg/kg ($P < 0.05$). Nisbet et al. (2013) found a statistical difference among the Ni contents of honey bee samples obtained from locations with different environmental and floral characteristics ($P < 0.05$).

The Pb contents of honey bee samples were highest in the urban location (L3), with 0.358 mg/kg, and lowest in the rural location (L8), with 0.192 mg/kg ($P < 0.05$). Roman (2010) found higher Pb levels in honey bee samples in residential areas compared to agricultural and forest areas ($P < 0.05$).

The Pb values (0.192–0.358 mg/kg) obtained in our study were lower than those (4–27 µg/g) determined by Leita et al. (1996). On the other hand, our values were similar to those (0.14–0.52 mg/kg) determined by Perugini et al. (2011).

The Zn contents of honey bee samples were highest in the urban location (L4), with 44.58 mg/kg, and lowest in the rural location (L8), with 29.93 mg/kg ($P < 0.05$). Nisbet et al. (2013) reported that the Zn values of honey bee samples taken from locations with different environmental and floral characteristics varied from region to region ($P < 0.05$), In a similar study, Taha et al. (2017), analyzing honey bee samples from colonies placed at different distances to a cement factory, found higher Zn values in the samples closer to the factory ($P < 0.05$).

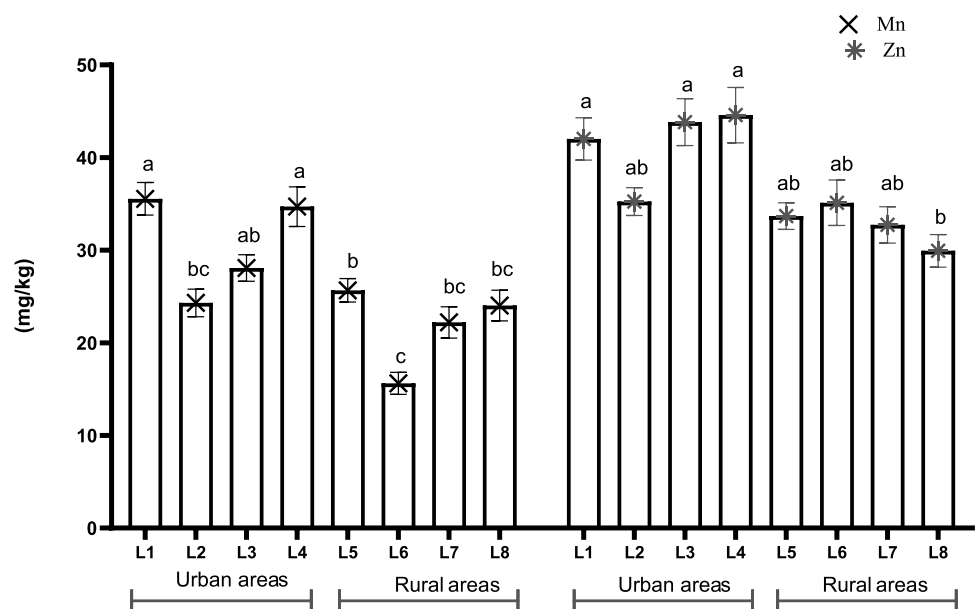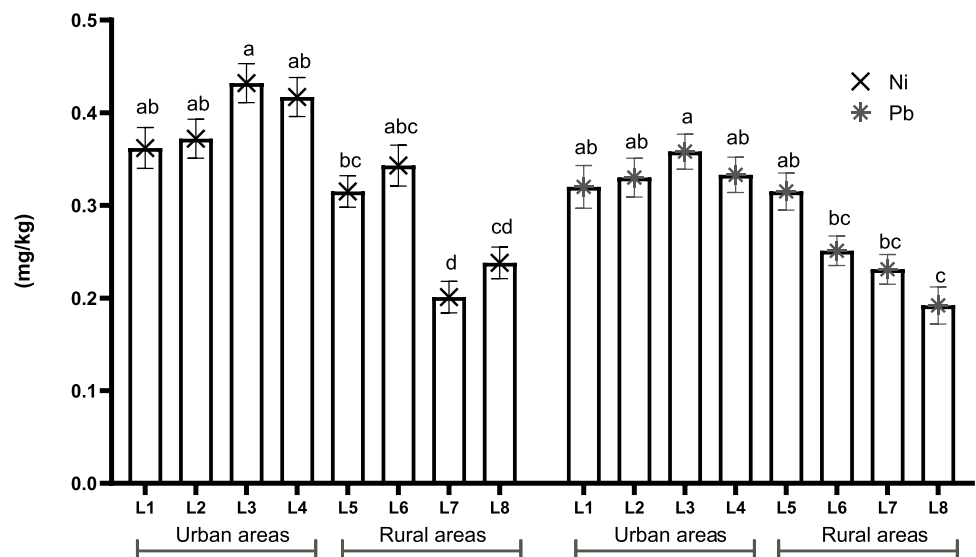**Fig. 6** The Mn and Zn content of honey bees in different locations

Fig. 7 The Ni and Pb content of honey bees in different locations

Conclusions

There were no statistical differences among the locations in terms of Cd in honey samples. However, the Cr, Cu, Fe, Mn, Ni, Pb, and Zn values were lower in samples from rural areas.

Regarding the pollen samples, we observed significant differences among the locations in terms of Cr, Cu, Fe, Mn, Ni, Pb, and Zn, with values being lower in rural areas than in urban areas. The Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn values of honey bee samples were also lower in rural areas than in urban areas.

The Cd and Pb values of honey and pollen samples obtained in our study were found to be lower than the maximum levels prescribed by international food standards for the food grade category (Alimentarius, 2015). To obtain healthy bees and bee products, it is recommended that the colonies be placed away from residential areas, industrial areas, highways, polluted water sources, and areas where bee products are likely to be subjected to contamination.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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