Concentration levels and an assessment of human health risk of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in honey and pollen

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Abstract
Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) accumulate in the food chain due to their physical and chemical properties and adversely affect human health. For this reason, this study aimed to determine the PAH and PCB concentration levels in pollen and honey samples in urban and semi-urban areas and to evaluate the risk of cancer that may occur by ingestion in Bursa, Turkey. The average total concentrations of 14 PAH (\(\sum_{14}\text{PAH}\)) compounds in pollen and honey samples were found to be 304.3 ± 192.3 ng/g (average ± standard deviation) and 650.2 ± 118.1 ng/g for the urban area, and 329.6 ± 160.6 ng/g and 464.3 ± 66.4 ng/g for the semi-urban area, respectively. Similarly, \(\sum_{14}\text{PCB}\) concentrations in pollen and honey samples were found to be 8.7 ± 3.6 ng/g and 13.0 ± 4.8 ng/g for the urban area and 7.7 ± 2.2 ng/g and 17.4 ± 4.0 ng/g for the semi-urban area, respectively. It was determined that the pollen and honey samples in both sampling areas were affected by local PCB sources. The Pearson correlation coefficient (PCC) method determined the relationship between pollen and honey samples. According to the PCC values obtained, it was observed that pollen and honey in both sampling regions exhibited a significant relationship with each other. Finally, while there was no cancer risk for PCBs due to ingestion of honey and pollen in both sampling areas, acceptable cancer risk has been calculated for PAHs.

Keywords PAHs · PCBs · Pollen · Honey · Risk assessment

Introduction
Food safety is a crucial issue for food health and economics in developed and developing countries (Bolek 2020). Also, the main objective of food safety includes protecting the health of consumers and their confidence in basic foods (Schröder et al. 2016; Bonerba et al. 2021). In parallel with the increase in population, the demand for basic food products is increasing day by day (Ruby et al. 2019). However, rapidly increasing industrialization causes deterioration in the environment and ecosystem, leading to pollution of basic food products (Altunay et al. 2019). It is essential to monitor pollutants in basic food products such as honey and pollen to prevent health risks that may occur due to human consumption of food (Panseri et al. 2020).

Studies on polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are increasing each day due to their permanence, biological accumulation, toxicity, long-distance atmospheric transport, and adverse effects on human health (Fu et al. 2018; Qiu et al. 2019). PAHs and PCBs are highly persistent in the environment because of their high bioaccumulative and lipophilic properties (Medehouenou et al. 2011). They accumulate in the food chain due to their physical and chemical properties and affect human health negatively (Mamontova et al. 2017). Although most POPs such as PBCs were banned in the 1970s and the 2000s, they are still found at a significant level in food today (Suarez-Lopez et al. 2019).

Increasing environmental pollution due to industrial developments affects living life, such as soil, air, water, and plants. Honey bees (Apis mellifera L.) go out of the hives approximately ten times a day to collect nectar and pollen.
from flowers every day in an area of 7 km² (Rissato et al. 2007). During this process, various microorganisms, particulates, pesticides, and chemicals are kept in the bodies of honey bees and transported by the honey bees to pollen and honey (Rissato et al. 2007; Calatayud-Vernich et al. 2018). Honey is a natural food consisting of amino acids, sugars and enzymes, organic acids, vitamins, carotenoids, minerals, and aromatic substances (Da Silva et al. 2016). Honey, used as flavoring and sweetener in many foods and beverages, has been known for its nutritional and therapeutic values since ancient times (Meo et al. 2017). Pollen is frequently used as a functional food by humans as it contains high amounts of compounds such as amino acids, antioxidants, vitamins, and lipids (Ares et al. 2018). Pollen is frequently referred to as “the world’s best food products” (Kieliszek et al. 2018).

Consumption of honey has increased significantly in the last 25 years due to its healing properties and high nutritional values (Huang et al. 2020). Turkey is the world’s second-largest honey-producing country, with approximately 105,727 tons of annual honey production (Tornuk et al. 2013; Cengiz et al. 2018). Also, according to the Food and Agriculture Organization of the United Nations (FAO), Turkey’s daily consumption of honey is the sixth most consuming country in the world, with 3.3 g (FAO 2017). Also, there are many studies in the world and Turkey in which the concentration values of POPs on honey are determined (Al-Alam et al. 2019; Al-Waili et al. 2012; Bargańska et al. 2016; Chiesa et al. 2016; dos Santos et al. 2021; Erdoğru 2007; Kargar et al. 2017; Panseri et al. 2020; Roszko et al. 2016). However, although there are few studies in the literature for calculating the cancer risk for PAHs based on honey and pollen ingestion, there are no studies in which there is an estimation of cancer risk for PCBs based on honey and pollen ingestion.

The purposes of this study were (i) to determine PAH and PCB concentrations in pollen and honey samples, (ii) to evaluate the risk of cancer that may occur by ingestion, and (iii) to determine relationship between honey and pollen samples.

**Material and Methods**

**Sampling areas and sampling program**

This study was conducted from May to September 2017 in the Ovaakca (Urban) and Cumalikizik (Semi-urban) areas within the borders of Bursa (Turkey). The Ovaakca sampling area (40° 17’11.16 “N–29° 5’13.20 “E) represents an urban area since it is approximately 1.5 km from the nearest highway, 3 km from the industrial zone, and 500 m from the settlement. Similarly, the Cumalikizik sampling area (40° 10’8.30 “N–29° 10’26.82 “E) represents a semi-urban area since it is 2 km from the nearest highway and 3 km from the residential area.

Pollen samples were collected between May and September 2017, and honey samples were collected between July and September 2017 when the honey production was excessive. Both pollen and honey samples were taken from hives, raised by two volunteer beekeepers in monthly 2-week periods. Two weeks were chosen as the sampling period. Thus, a total of ten pollen and six honey samples were obtained for a sampling area. The samples were collected homogeneously from at least 5–6 different beehives to represent the whole area and made into a single sample. In order to prevent any pollution that may occur during the transportation of the samples and bringing them to the laboratory environment, they were wrapped in aluminum foils and placed in closed bags. Samples brought to the laboratory were stored in freezers at –20 °C until extractions.

**Extraction, clean-up, and fraction**

The extraction, clean-up, and fraction processes of the pollen and honey samples were reported previously (Sari et al. 2020). Briefly, 2 g of pollen samples was taken into bottles and crushed thoroughly. Fifty milliliters of dichloromethane (Merck, Darmstadt, Germany)/petroleum ether (Merck, Darmstadt, Germany) (DCM/PE) (1/1, v/v) mixture was added to the crushed samples. Approximately 1 g of the weighed honey sample was dissolved in 10 mL distilled water (Lambert et al. 2012; Sari et al. 2020). At the beginning of the extraction processes, 1 mL surrogate standard (acenaphthene-d10, chrysene-d12, naphtalene-d8, perylene-d12, and phenanthrene-d10 for PAHs, and PCB#14, PCB#65, and PCB#166 for PCBs) was added to this solvent in order to determine the recovery efficiency values of the samples. The pollen samples were shaken for about 2 h (Sari et al. 2020). The sample bottles were then taken into an ultrasonic bath (Elasonic S 80 H, Germany) and extracted for 15 min. The dissolved honey samples were transferred to the separatory funnel and 50 mL methanol (MeOH), 50 mL saturated salt solution, 50 mL distilled water, and 40 mL DCM were added, respectively. Then, the honey samples were shaken for about half an hour (Evci et al. 2016). The pollen and honey samples extracted were concentrated using a rotary evaporator (Laborota 4001 Model, Heidolph, Germany). The rotary evaporator operating at 30–35 rpm and 22–23 °C reduced their volumes to approximately 2 mL (Esen et al. 2008). Thus, the solvent in the sample was converted to hexane (HEX) (Merck, Darmstadt, Germany). Until the next stage, the sample bottles were stored in the freezer at –20 °C.

The samples were fractionated by using glass fiber wool, 3 g of silicic acid (3%pure water), 2 g of alumina (6% pure water), and 2 gr of sodium sulfate (Na₂SO₄) (Merck,
The column was first purged with 20 mL of DCM followed by 20 mL of PE. Subsequently, 2 mL volumes of samples were passed through the column. Twenty-five milliliters of PE was used for the elution of target PCBs (fraction 1), and 20 mL DCM was used for the elution of target PAHs (fraction 2) (Sari et al. 2020; Esen et al. 2017). The samples were fractionated, separated, and then their volume was reduced to 5 mL with the rotary evaporator operating at 30–35 rpm and 22–23 °C. Finally, after adding 15 mL of HEX to the samples, their volume was reduced to 2 mL (Esen et al. 2017).

**Instrumental analysis**

PAHs were determined using a gas chromatograph (GC) (Agilent 7890A) device with a mass spectrometer (MS) (Agilent 5975C), and PCBs were determined using a GC device with a micro-electron capture detector (µECD). Field blanks with a ratio of at least 10% of the samples were collected to determine possible contamination during the transportation, storage, and preparation of the samples. The Agilent 7890A-µECD instrument determined the mass values of pollen and honey samples and blank samples. Helium gas was used as the carrier gas at a flow rate of 1.9 mL/min, and high purity nitrogen gas was used as the detector make-up gas with helium gas. In this study, 16 different PAHs (naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fln), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-c,d)pyrene (IcdP), dibenz(a,h)anthracene (DahA) ve benzo(g,h,i)perylene (BghiP)) were analyzed. Similarly, 8 different dioxin-like PCBs (dl-PCBs) (PCB#81, 105, 114, 118, 126, 156, 167, and 169) and 6 different non-dioxin-like PCBs (Ndl-PCBs) (PCB#28, 52, 101, 138, 153, and 180) were analyzed. Pearson correlation analysis and all figures were created in the SigmaPlot® version 13.0, R Studio® version 2021.09.01, and Adobe Photoshop version CC 2018®.

**QA/QC**

All glass materials used during the experiment were washed with special detergent water and rinsed under tap water. The rinsed materials were washed with pure water, ACE, and PE, respectively, and kept in the oven at 105 °C for approximately 24 h. After the glass materials were dried, they were wrapped in aluminum foil and stored until use. In the GC–MS and GC-µECD analysis, calibration standards at six different concentration levels were used during the calibration process. All calibration standards were prepared in HEX. Concentration values of prepared calibration standards were 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 µg/mL for PAHs, and 1.0, 2.5, 5.0, 10.0, 25.0, and 40.0 ng/mL for PCBs. \( R^2 \) values for all calibration levels were calculated as higher than 0.99. The calibration of the GC–MS and GC-µECD was tested with a medium calibration standard for approximately every 100 samples. At least 10% of the blank samples were taken to determine the contamination that may occur during the taking of samples to the laboratory and analysis. Blank samples of the pollen and honey were obtained by considering 2 g of \( Na_2SO_4 \) (Sari et al. 2020).

The instrumental detection limits (IDLs), which are the calibration standard concentration value at the lowest concentration level perceived by the device, are determined according to the signal:noise (S:N) ratio of the 3:1. The IDL values for 1 µL injection were 0.1 pg for PAHs and 0.15 pg for PCBs. The method of detection limits was calculated as the average mass values of the blank samples plus three times the standard deviation (Esen et al. 2017; Sari et al. 2020). The MDL values for sampling points ranged from 24.7 to 53.83 pg for PAHs and from 0.26 to 9.18 pg for PCBs. If any POP compounds had less than MDL values, then the concentration of that congener would have taken as half of the IDL values for statistical calculations. The surrogate standard was used to determine the recovery efficiencies. Average recovery efficiencies in all samples were higher than 60% except Nap and Acy. Nap and Acy were not included in the calculations of these compounds due to their low recovery efficiency (< 60%). The recovery efficiency and relative standard deviation (RSD) and linearity values of the pollen and honey samples have been added in Supplementary Material Table S1 and Table S2, respectively.

**Results and discussion**

**PAH concentration in pollen and honey**

Pollen and honey samples were taken from the urban and semi-urban areas to determine the total PAH concentrations in Bursa between May and September 2017. The concentrations of total 14 PAHs (\( \sum_{14} \)PAHs) compounds in pollen and honey samples were found to be 304.3 \( \pm \) 192.3 ng/g (ranged from 146.8 to 623.1 ng/g) and 650.2 \( \pm \) 539.3 ng/g (ranged from 413.1 to 192.3 ng/g) for the urban area, respectively. Similarly, for the semi-urban area, concentrations were found to be 329.6 \( \pm \) 118.1 ng/g (ranged from 194.7 to 598.6 ng/g) and 464.3 \( \pm \) 66.4 ng/g (ranged from 413.1 to 539.3 ng/g), respectively (Fig. 1).

The lowest PAH concentrations in pollen samples were measured in August for the urban area and July for the semi-urban area. The highest PAH concentrations were measured in May for both sampling areas. Concentration levels in urban and semi-urban areas were generally measured
low in the summer months. Plants are affected by both soil pollution and the atmosphere from PAH sources. Therefore, PAH concentrations in pollen samples are expected to vary depending on both present and old pollution of the soil (Lambert et al. 2012). The high PAH concentration in the pollen samples collected from the semi-urban area can be explained by the diversity of plant species grown in the region due to the presence of more species that are absorbed by PAHs. In a study conducted by Lambert et al. (2012), measured PAH4 concentration levels in pollen samples ranged from 0.33 to 129.41 ng/g in France. In our study, the PAH4 concentrations for the urban and semi-urban areas ranged from 8.23 to 72.38 ng/g and 16.27 to 135.40 ng/g, respectively. Since there are a limited number of studies in the literature in which PAHs were determined in pollen samples, further comparison with concentration values could not be made.

The highest PAH concentrations in honey samples for the urban area were observed in July, and the lowest PAH concentrations in August. In the semi-urban area, the highest PAH concentrations in honey samples were observed in September and the lowest PAH concentrations in July. A study conducted by Iwegbue et al. (2016) measured $\sum_{16}$PAH concentration levels ranging from 180 to 641 ng/g in industrial areas in Niger Delta, Nigeria. In another study conducted by Lambert et al. (2012), measured PAH4 concentration levels in honey samples ranged from 0.27 (PCB#28) to 0.92 ng/g (PCB#138) in South Italy. The concentration levels obtained in this study were similar to the various studies in the literature.

Ndl-PCB concentrations in pollen samples in the urban area were measured higher than dl-PCB concentrations in all other months except in September. In contrast, Ndl-PCB concentrations in honey samples in the urban area were measured lower than dl-PCB concentrations in all other months except August. In pollen samples in the semi-urban area, there was no significant difference between dl-PCB and Ndl-PCB concentrations ($t$-test, $p > 0.05$). In honey samples, Ndl-PCBs were dominant in all months in the semi-urban area (Fig. 2). Percentage distributions of dl-PCB and Ndl-PCB in pollen and honey samples are presented in both sampling areas for better explanation in Fig. 3.

According to the percentage distribution of DI-PCBs, PCB#169 (ranged from 27.0% to 35.9%) is the dominant PCB congener for all sampling areas and sampling materials. Similarly, percentage distribution of Ndl-PCBs in pollen and honey samples, PCB#180 (ranged from 23.7 to 42.4%), and PCB#52 (ranged from 18.8 to 37.5%) are dominant.
PCB congeners in both sampling areas. In honey samples, PCB#180 (40.4%) and PCB#138 (20.0%) are the dominant PCB congeners in the urban sampling area, while PCB#52 (37.5%) and PCB#180 (31.2%) are the dominant PCB congeners in the semi-urban area. In the study conducted by Erdoğru (2007), the dominant PCB congeners in honey samples are PCB#52 and PCB#28, whereas, in the study conducted by Herrera et al. (2005), the dominant PCB congeners in honey samples are PCB#28 and PCB#138. In addition, in the study conducted by Herrera et al. (2005) and Chiesa et al. (2016), industrial activities were near the sampling areas. In the study conducted by Erdoğru (2007), the sampling area is relatively far from industrial activities. It has been thought that the differences between the sampling areas may cause differences between the PCBs. In a study conducted by Roszko et al. (2016), it was stated that the differences between PCB congeners are due to long-range air transport, accumulation on plant surfaces, and the distribution/transport of pollutants in the environment. Also, high chlorinated PCB congeners such as PCB#180 have lower evaporation properties compared to low chlorinated PCB congeners such as PCB#28. Therefore, the predominance
of low-chlorinated PCBs is due to atmospheric deposition, whereas the predominance of high-chlorinated PCBs is due to the effectiveness of local resources (de Souza et al. 2018). Since high chlorinated PCBs from both dl-PCB and Ndl-PCB congeners are predominant, local resources are thought to be more effective in pollen and honey samples from both sampling areas.

Health risk assessment of PAHs and PCBs

Food consumption is defined as the main pathway for humans to be exposed to pollutants (Khillare et al. 2012). Rapidly increasing industrialization causes adverse effects on the ecosystem and the environment, which leads to food pollution (Altunay et al. 2019). Foods contaminated with POPs present a high probability of posing a potential health risk for the consumers (Rusin et al. 2019). Exposure to PAH and PCBs in various ways causes carcinogenic effects, neurological, and immunological problems, and other adverse health effects (Fu et al. 2018). Cancer risk calculation refers to the incremental lifetime cancer risk (ILCR) calculated using the food ingestion dose with the following Eq. (1) (Wang et al. 2019):

\[
\text{ILRC} = \frac{C_i \times DC \times CSF \times EF \times ED}{BW \times AT} \times 10^{-6}
\]  

(1)

where \( C_i \) is the average \( i \)th PAH and PCB congener concentration for pollen and honey samples in the urban and semi-urban area (ng/g); \( DC \) is the daily consumption of pollen (1.4 g/day) and honey (3.3 g/day) (FAO 2017); \( CSF \) is the cancer slope factor (7.3 days × kg/mg for PAHs and 2 days × kg/mg for PCBs) (Fu et al. 2018; Ali 2019); \( 10^{-6} \) is a conversion factor (ng to mg); \( EF \) and \( ED \) are the exposure frequency (180 days/year), and exposure duration (24 years); \( BW \) is the body weight (61.5 kg), and \( AT \) is the average days (25,550 days) (Ghanavat et al. 2019). According to USEPA, if the ILCR values are higher than \( 1 \times 10^{-4} \), there is a high cancer risk, if the ILCR values are between \( 1 \times 10^{-6} \) and \( 1 \times 10^{-4} \), there is an acceptance of cancer risk, and if the ILCR values are lower than \( 1 \times 10^{-6} \), there is a non-cancer risk (Wang et al. 2019). The carcinogenic risk results obtained in this study are given in Table 1.

According to the calculated ILCR values for pollen and honey samples, PCBs did not pose any risk for both sampling areas. However, the calculated cancer risk among PAHs was found to be within an acceptable cancer risk. This situation is thought to be due to the use of smoke in the collection of samples. In general, lower ILCR values were obtained in pollen samples compared to honey samples for both PAHs and PCBs. Cancer risk has been calculated for PCB concentrations in honey and pollen samples, but no study has been found to compare with the literature. In a study conducted by Iwegbue et al. (2016) in Nigeria, it was stated that there was no risk of cancer as a result of ingestion of honey for PAHs. The reason for the acceptable cancer risk in this study was considering 14 PAH compounds in cancer risk calculations. In addition, due to the limited number of studies in the literature, pesticide residues were compared with the results we obtained. In a study by Blasco et al. (2003), it was stated that pesticides did not pose any risk in honey samples, but in a study by Sanchez-Bayo and Goka (2014), especially lindane (γ-HCH) carries a high risk in honey samples. It is stated that the different results obtained by using the same sampling materials, especially the concentrations of the OCPs and the pollutant profiles, vary according to the areas (Thompson et al. 2017).

The relationship between honey and pollen samples

In this study, the Pearson correlation coefficient (PCC) method determined the relationship between honey and pollen samples. The PCC values obtained in the study are shown in Fig. 4.

According to the PCC results, positive correlation coefficients were obtained for PAHs and PCBs. Positive and significant results were observed among honey samples from urban and semi-urban areas for PAHs and PCBs. Honey samples are in the hive and, unlike pollen samples, they are in less contact with the ambient air. It is thought that the high correlation coefficient results between the two sampling points are due to this situation. Also, honey samples were thought to have a positive relationship because they were immobile and therefore less affected by environmental factors (Sari et al. 2021). Positive and significant correlation coefficients were observed at both sampling sites for PAHs and PCBs in pollen. This means that pollen samples are affected by similar sources at both sampling points, because PAHs and PCBs have low water solubility. For

|          | Urban area | Semi-urban area |
|----------|------------|-----------------|
|          | Pollen     | Honey           | Pollen | Honey |
| PAHs     |            |                 |        |       |
| Minimum  | 0.12       | 0.27            | 0.13   | 0.74  |
| Maximum  | 1.06       | 3.45            | 0.97   | 2.31  |
| Mean     | 0.54       | 1.97            | 0.61   | 1.78  |
| Ndl-PCBs |            |                 |        |       |
| Minimum  | 0.01       | 0.07            | 0.01   | 0.05  |
| Maximum  | 0.11       | 0.85            | 0.13   | 0.12  |
| Mean     | 0.05       | 0.18            | 0.05   | 0.09  |

Table 1 Carcinogenic risk for PAHs and PCBs via digestion of honey and pollen (×10^-6)
this reason, it is not possible for plants and plant organs (pollen, leaf, etc.) grown in soils contaminated with both PAH and PCBs to absorb these compounds from the soil (Sanchez-Bayo and Goka 2016; Sari et al. 2021).

**Conclusion**

In this study, the concentration levels of PAHs and PCBs and cancer risks that may occur as a result of the consumption of pollen and honey were evaluated in the urban and semi-urban areas in Bursa. PAH and PCB concentrations were measured close to each other in pollen and honey samples in both sampling areas. Besides, the concentration values obtained were similar to those previously conducted in the literature. High correlation coefficients were obtained in pollen and honey samples from both sampling points. This means that pollen and honey at both sampling points are affected by similar pollutant sources. According to the cancer risk calculations developed by the US-EPA, the cancer risk for PCBs due to ingestion of honey and pollen was not determined in either sampling area, while the cancer risk values for PAHs were within the acceptable range. On the other hand, this situation causes the smoke used during the collection of samples to slightly increase the PAH concentrations and, accordingly, the risk of cancer to be slightly higher. Since this study is one of the first to evaluate the health risk of PAHs and PCBs in pollen and honey samples, it is thought to guide future studies. Also, we recommend that researchers who want to identify POP sources in pollen and honey samples take samples from dominant plant species and soils in the sampling areas.

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Fatma Esen: methodology, data curation, writing — original draft, writing — review and editing, visualization, supervision

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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