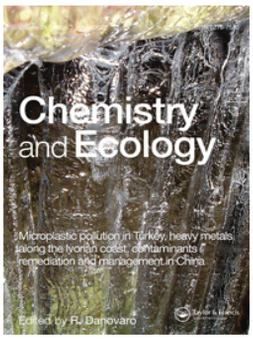

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Application of QuEChERS with GC/MS/MS for monitoring polycyclic aromatic hydrocarbon (PAHs) contaminants in Turkish flora honey produced in urban and rural areas

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ABSTRACT

Environmental pollution has been a problem waiting for solutions worldwide in recent years. Pollution with polycyclic aromatic hydrocarbon (PAHs), oil, and petroleum derivatives, may have long- and short-term environmental and health effects. Bee products are used as bioindicators to determine environmental pollution. In this work, 16 PAHs compounds were analyzed in honey samples from different Turkey regions by gas chromatography-tandem mass spectrometry (GC/MS/MS). In this work, the modern extraction method (QuEChERS) was selective and sensitive enough to extract 16 PAHs from honey samples. High amounts of naphthalene (22.5 µg kg^{-1}), acenaphthylene (16.2 µg kg^{-1}), acenaphthene (17.3 µg kg^{-1}), fluorene (13.3 µg kg^{-1}), phenanthrene (14.5 µg kg^{-1}), fluoranthene (11.9 µg kg^{-1}), benzo(b)fluoranthene (12.6 µg kg^{-1}), benzo(k)fluoranthene (12.7 µg kg^{-1}), and benzo(a)pyrene (11.7 µg kg^{-1}) were detected. Besides, PAHs levels in the Marmara region were very high. The differences in PAHs content between honey from rural and urban areas could be related to atmospheric conditions such as exhaust gases, dust, and airborne particles or from soils in which the plants grow.

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PAHs; Turkey; Bee products; Pollution; QuEChERS; GC/MS/MS

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more fused aromatic rings. They are produced by natural and anthropogenic processes [1–10]. Volcano eruption and forest and prairie fire are primary natural sources of PAHs in the atmosphere [11–13]. Anthropogenic sources for PAHs include straw and fuelwood combustion, waste incineration, asphalt production, coke and aluminum production, vehicle exhaust, combustion of coal, and fossil fuels [14,15]. Various PAHs exhibit toxic, mutagenic, and/or carcinogenic properties [15,16]. The highly lipid-soluble properties

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of PAHs ensure readily absorbed from the gastrointestinal tract of mammals. They are rapidly absorbed into humans' gastrointestinal tracts due to their high solution properties [15,17]. Honeybees contribute to plant fertilisation and crop growth while collecting pollen and nectar from flowering plants and fruit trees to meet their nutritional needs. In agricultural production, bees ensure the increase of product quality and the continuity of the plant population. Thus, they also help maintain the ecological balance. Turkey has a significant contribution to the development of beekeeping as it has rich vegetation, different climatic zones, and a suitable ecology. According to FAOSTAT [18], there is approximately 1.8 million honey production in the World. China was the major honey producer globally, followed by Turkey (114.113 tonnes). Also, Turkey contains the second largest number of honeybee colonies globally [19]. Honeybees and beekeeping are some of the traditional production tools of the rural population. Honeybees collect nectar from the flowers to a distance of up to 5 km. Thus, undesirable substances such as heavy metals, PAHs, pesticides, and antibiotics may also be found in honey [5,8,20–23]. Besides direct contamination, indirect contaminants such as contaminants from agricultural practices and, in general, from the environment could be present in honey samples [24].

Honey and honeybees could be used as a biological indicator to evaluate the level of PAHs atmospheric pollution. Perugini et al. [25] evaluated PAHs in honey from urban areas and wildlife reserves. The honey samples contained only three PAHs (phenanthrene, anthracene, and chrysene). There was no correlation found between specific PAHs and the botanical origin of the honey. Besides, there is very little information on the levels of PAH in honey. In these investigations, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method was used only in a few studies [26,27]. This method serves some advantages: extraction of a wide diversity of analytes and matrices, improvement of recoveries, and avoiding degradation and matrix effects [28].

The current study aimed to detect PAHs contaminates in different Turkish flora honey produced in the cities and the rural areas from different Turkish geographical regions using modern extraction method (QuEChERS), followed by gas chromatography-tandem mass spectrometry (GC/MS/MS).

2. Materials and methods

2.1. Sample collection

Fifty honey samples from different botanical and geographical origins were collected from seven regions in Turkey to study their PAH levels. Twenty-seven of them came from locations away from roads and industrial sites, and twenty-three were collected from urban areas. Sample codes are given in Table 1.

2.1. Reagents and chemicals

Acenaphthene (Ace), acenaphthylene (Acy), anthracene (Ant), benz(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(g,h,i)perylene (BghiP), benzo (k)fluoranthene (BkF), chrysene (Chr), dibenz(a,h)anthracene (DBahA), fluoranthene (Fl), fluorene (F), indeno[1,2,3-cd]pyrene (IP), naphthalene (N), phenanthrene (Phe), and

Table 1. Sample code, geographical and botanical origin of honey samples.

Sample Code	Type	Geographical origin	Botanical origin
AH1	Urban	Aegeon	Multifloral
AH3	Urban	Aegeon	Fruit tree, pine
AH5	Urban	Aegeon	Fruit tree
MH7	Urban	Mediterranean	Fruit tree
MH9	Urban	Mediterranean	Fruit tree, multifloral
CH11	Urban	Central Anatolia	Multifloral
CH13	Urban	Central Anatolia	Multifloral
BH16	Urban	Black Sea	Multifloral, pine
BH18	Urban	Black Sea	Multifloral
BH21	Urban	Black Sea	Multifloral
BH23	Urban	Black Sea	Multifloral
BH25	Urban	Black Sea	Fruit tree
MAH27	Urban	Marmara	Multifloral
MAH29	Urban	Marmara	Multifloral
MAH31	Urban	Marmara	Fruit tree, multifloral
MAH33	Urban	Marmara	Fruit tree, multifloral
SEH35	Urban	South-Eastern Anatolia	Fruit tree
EH37	Urban	Eastern Anatolia	Multifloral
EH39	Urban	Eastern Anatolia	Fruit tree
EH42	Urban	Eastern Anatolia	Multifloral
EH44	Urban	Eastern Anatolia	Fruit tree, multifloral
EH46	Urban	Eastern Anatolia	Multifloral
EH48	Urban	Eastern Anatolia	Multifloral
AH2	Rural	Aegeon	Multifloral
AH4	Rural	Aegeon	Multifloral
AH6	Rural	Aegeon	Fruit tree
MH8	Rural	Mediterranean	Fruit tree
MH10	Rural	Mediterranean	Fruit tree, multifloral
CH12	Rural	Central Anatolia	Multifloral
CH14	Rural	Central Anatolia	Multifloral
CH15	Rural	Central Anatolia	Multifloral
BH17	Rural	Black Sea	Multifloral, pine
BH19	Rural	Black Sea	Multifloral
BH20	Rural	Black Sea	Multifloral
BH22	Rural	Black Sea	Multifloral
BH24	Rural	Black Sea	Multifloral
BH26	Rural	Black Sea	Fruit tree
MAH28	Rural	Marmara	Multifloral
MAH30	Rural	Marmara	Multifloral
MAH32	Rural	Marmara	Fruit tree, multifloral
MAH34	Rural	Marmara	Fruit tree, multifloral
SEH36	Rural	South-Eastern Anatolia	Fruit tree
EH38	Rural	Eastern Anatolia	Multifloral
EH40	Rural	Eastern Anatolia	Fruit tree
EH41	Rural	Eastern Anatolia	Multifloral
EH43	Rural	Eastern Anatolia	Fruit tree, multifloral
EH45	Rural	Eastern Anatolia	Multifloral
EH47	Rural	Eastern Anatolia	Multifloral
EH49	Rural	Eastern Anatolia	Multifloral
EH50	Rural	Eastern Anatolia	Multifloral

pyrene (Pyr) standards were obtained from Sigma-Aldrich (Steinheim, Germany). All the standards were of high purity grade (>95.0%). Individual stock solutions were prepared at 2000mg/L in acetonitrile and stored at −18°C. The calibration standards were prepared by dilution with acetonitrile on the analysis day. Ultrapure water was generated by a Millipore Milli-Q system (Milford, MA, USA). Methanol was HPLC grade from Merck, Germany. Individual stock solutions were prepared at 500 mg/L in methanol and stored at 4°C. The calibration standards were prepared by dilution with methanol on the analysis day. The

QuEChERS kits (part no.5982-5755) with 6 g magnesium sulfate, 1.5 g sodium acetate, and 15 mL centrifuge tubes with 1200 mg magnesium sulfate and 400 mg primary-secondary amine (PSA) for dispersive solid-phase extraction (dSPE, part no. 5982-5058) were purchased from Agilent Technologies (USA).

2.2. Sample extraction and clean-up

Homogenised honey samples (15 g) were weighed into a 50 mL polypropylene tube, and 15 mL of hexane was added with the QuEChERS kit, then the tube was vortexed for 1 min immediately. The sample was centrifuged for 1 min at 5000 rpm, and during the clean-up step, 8 mL upper hexane layer was transferred into a dSPE polypropylene tube. The tube was vortexed for 1 min and centrifuged for 1 min at 5000 rpm. Finally, the supernatant was taken into an autosampler vial for GC/MS/MS analysis.

2.3. Gc/MS/MS analysis of PAH

An Agilent GC model 7890A coupled with an Agilent 7000B triple quadrupole mass spectrometer (Agilent Technologies USA) was used. The analytical column was a HP-5MS (15 m × 0.25 mm ID × 0.25 µm). The oven temperature was programmed from 70°C, held for 1 min, increased to 50°C/min to 150°C, ramped at 6°C/min to 200°C, then ramped at 16°C/min to 280°C held for 6 min. The mass spectrometer was operated at an ion energy of 70 eV, a filament current of 90 µA. In tandem mass spectrometry mode (MS/MS), multiple reactions monitoring (MRM) transitions of precursor ions fragmenting into product ions at specific collision energies were monitored. Helium quench gas and nitrogen (purity 99.9999) were used as the collision gas with the cell pressure of 1.7 mTorr.

3. Results

3.1. Method validation

Validation of the method was tested based on the European Union Commission Regulation No. 836/2011. The method was validated by assessing the linearity, recovery, precision, and specificity of peak areas. Limit of detection (LOD) and limit of quantification (LOQ) were defined, respectively, as the signal corresponding to 3 and 10 times the noise ratio. In order to reduce the matrix effect, calibration curves were created with matrix-matched (honey) covering the concentration range according to the external standardisation technique. Calibration curves were evaluated at the concentration range of 0.25–20 ng/mL (0.25, 0.5, 1, 2.5, 5, 10, and 20 ng/mL). Good linearity was achieved in all cases with correlation coefficients better than 0.990. The recovery and precision data were obtained for all the PAHs spiked at concentrations of 1, 5, and 10 µg kg^{-1}, and each concentration was conducted on six replicates. Table 2 shows the results for LOD, LOQ, and recoveries studies. The accuracy of the presented method was acceptable for all PAHs tested in the range of 50–120%, which fulfils the recommendation of EU 836/2011. The RSD values were less than 20% for all the concentration levels tested.

Table 2. The main analytical performances for the analysis of PAHs reproducibility relative standard deviation (RSDR of 1, 5, and 10 µg/kg standard value).

Compound	*r*	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)	RSD (%)
Ace	0.998	0.03	0.12	98	15, 6, 12
Acy	0.999	0.06	0.15	96	17, 5, 13
Ant	0.999	0.11	0.20	103	16, 9, 12
BaA	0.999	0.09	0.22	106	15,8,11
BaP	0.999	0.06	0.21	97	11, 5, 16
BbF	0.999	0.07	0.22	104	15, 5, 20
BghiP	0.998	0.04	0.12	98	11, 8, 18
BkF	0.999	0.08	0.27	102	9, 8, 14
Chr	0.999	0.04	0.14	92	12, 7, 16
DBahA	0.999	0.03	0.13	87	17, 10, 20
Fl	0.999	0.12	0.23	96	8, 9, 15
F	0.999	0.29	0.11	97	10, 6, 12
IP	0.999	0.09	0.16	102	13, 8, 18
N	0.998	0.15	0.23	93	18,9,17
Phe	0.996	0.13	0.24	92	12,6,13
Pyr	0.999	0.18	0.22	101	14,9,17

3.2. PAHs in the analyzed samples

PAHs concentrations in honey samples from rural and urban areas are given in Tables 3 and 4. PAHs analysis was made on 50 honey samples in the seven Turkish geographic regions (Figure 1). The most common PAHs detected were naphthalene, acenaphthylene, acenaphthene, fluorene, benzo(b)fluoranthene, and phenanthrene. PAHs concentration in honey samples is given in Tables 3 and 4. In addition, a chromatogram of PAH in honey samples is presented in Figure 2.

4. Discussion

The nectars, considered a biological indicator of environmental pollution, are collected by bees within a radius of 3 km; if beekeepers place their hives 3 km away from agriculture,

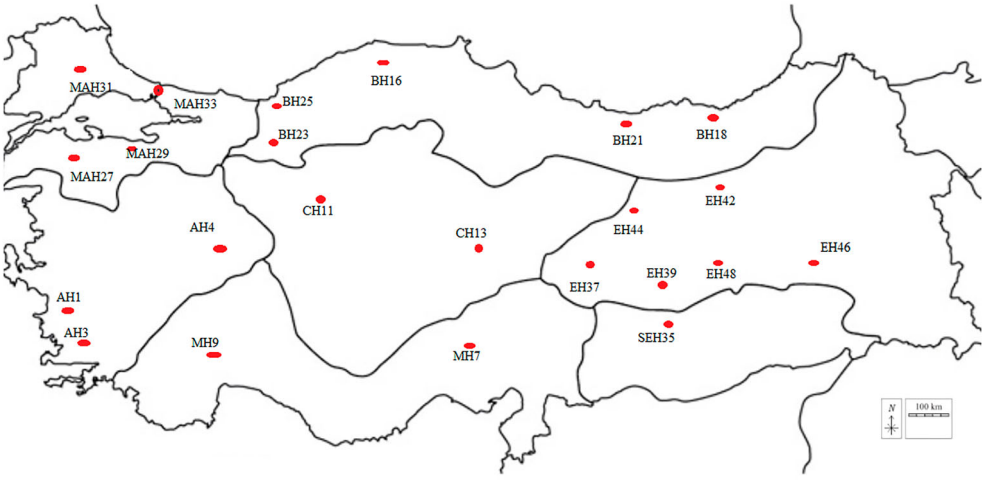

Figure 1. Locations where honey samples collected.

Table 3. PAHs detected in GC/MS/MS-analyzed urban honey.

Sample code	Ace	Acy	Ant	BaA	BaP	BbF	BghiP	BkF	Chr	DBahA	Fl	F	IP	N	Phe	Pyr
AH1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
AH3	5.50	8.96	nd	nd	nd	nd	nd	nd	nd	nd	8.33	1.37	nd	7.57	3.83	nd
AH5	nd	nd	nd	nd	nd	12.46	nd	10.89	nd	nd	nd	nd	nd	nd	nd	nd
MH7	15.89	12.64	nd	nd	nd	12.05	nd	12.69	nd	nd	nd	nd	nd	11.37	nd	nd
MH9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.31	nd	nd
CH11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.20	nd	nd
CH13	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.60	nd	nd
BH16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH18	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.10	nd	nd
BH25	8.59	11.05	nd	11.70	nd	nd	nd	nd	nd	nd	nd	6.09	nd	12.09	nd	11.02
MAH27	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.43	7.11	nd
MAH29	11.58	16.20	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.34	nd	12.11	1.44	nd
MAH31	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.47	nd	nd
MAH33	17.37	13.18	nd	nd	nd	11.80	nd	11.78	nd	nd	11.78	12.19	nd	15.84	nd	nd
SEH35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.37	nd	nd
EH37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.66	nd	nd
EH39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.86	nd	nd
EH42	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
EH44	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.71	nd	nd
EH46	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.63	nd	nd
EH48	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4. PAHs detected in GC/MS/MS-analyzed rural honey.

Sample code	Ace	Acy	Ant	BaA	BaP	BbF	BghiP	BkF	Chr	DBahA	Fl	F	IP	N	Phe	Pyr
AH2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
AH4	11.91	11.91	nd	nd	nd	nd	nd	nd	nd	nd	11.90	11.81	nd	22.54	14.59	nd
AH6	nd	nd	nd	nd	nd	3.78	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MH8	3.74	6.29	nd	nd	nd	0.85	nd	1.94	nd	nd	nd	nd	nd	5.72	nd	nd
MH10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.93	nd	nd
CH12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.42	nd	nd
CH14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.78	nd	nd
CH15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.81	nd	nd
BH17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH22	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BH24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.46	nd	nd
BH26	6.57	12.60	nd	nd	0.52	nd	nd	nd	nd	nd	nd	5.03	nd	8.90	nd	nd
MAH28	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.92	0.73	nd
MAH30	5.87	1.60	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.04	nd	4.22	2.30	nd
MAH32	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.56	nd	nd
MAH34	nd	0.85	nd	nd	nd	0.83	nd	1.47	nd	nd	1.47	2.85	nd	8.34	nd	nd
SEH36	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.25	nd	nd
EH38	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.54	nd	nd
EH40	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.37	nd	nd
EH41	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.19	nd	nd
EH43	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
EH45	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.56	nd	nd
EH47	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.34	nd	nd
EH49	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
EH50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

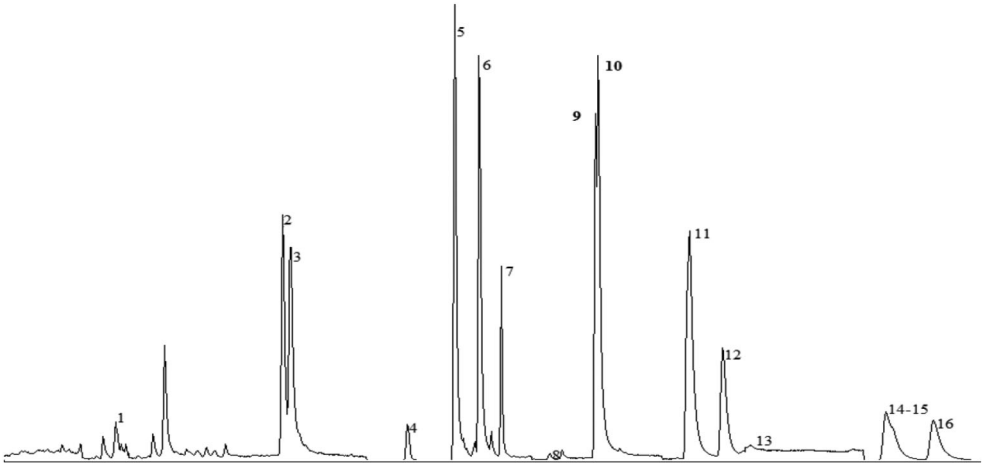

Figure 2. Chromatogram of (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo(a)anthracene, (10) chrysene, (11)benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo(a)pyrene, (14) dibenz[a,h]anthracene, (15) ındeno[1,2,3-cd]pyrene, and (16) benzo[g,h,i]perylene.

they can prevent residues [29,30]. PAHs in plants originate mainly from the atmosphere and soil, accumulating in pollen and to a lesser extent in nectar. Nectar is characterised by a low potential for bioaccumulation of PAHs due to its low lipid content. Since the lipid content in pollen is different from nectar, it causes PAHs accumulation. PAHs contamination is highest, especially in bees, given their direct exposure to air pollution and contact with plants. Compared to pollen and nectar, honey has the lowest levels of PAHs; the leading cause of contamination of honey is of botanical origin. The origin of the flower, on the other hand, determines how much the nectar has been exposed to contamination [30].

Contamination of honey with PAH occurs either directly through the use of naphthalene, which is a PAH group, or indirectly from forest fires, stubble burning, industrial establishments close to beehives, and lack of knowledge of beekeepers. Honeycomb contaminated with naphthalene and other pesticides are very risky since it can be used for several seasons. Because pesticides and naphthalene slowly mix with honey, old combs become a potential source of pesticides and naphthalene in the hive. Napthalene was detected in 34 samples from 50 samples and ranged from 0.37 to 22.5 µg/kg. Naphthalene, used against wax moth (*Galleria mellonella*) in beekeeping that is not properly stored after the honey, is filtered in autum. When the combs are reintroduced to the colony, the naphthalene they contain turns back into honey. Naphthalene has been detected in all regions. The legal limit for napthalene in honey as listed in Turkısh Food Codex is 10 µg/kg.

Dobrinas et al. [31] detected naphthalene in most samples at high concentrations (up to 665.0 ng/g). Our results are lower than those previously reported by Dobrinas et al. [31]. The results shown here are similar to those previously published by Dobrinas et al. [31] and Ozoani et al. [32], who reported that a higher naphthalene content was found in the urban honey than in rural honey.

The other widespread PAHs in honey samples were acenaphthylene and acenaphthene. Twenty percent of the samples were contaminated with acenaphthylene at levels from 0.85 to 16.2 µg/kg, and 18% of the samples with acenaphthene at levels from 3.74 to 17.37 µg/kg. Ozoani et al. [32] reported that acenapthylene was detected in only urban honey samples, wherein the highest value for acenapthylene was 2.72 mg/kg. Dobrinas et al. [31] reported honey samples to have higher values of acenapthylene (0.9–32 ng/g). Our results showed similarity with the results reported by Dobrinas et al. [31] and Ozoani et al. [32]. In our study, the higher values for acenapthylene found in urban samples compared with rural samples are compatible with the results of Dobrinas et al. [31] and Ozoani et al. [32]. Like acenapthylene, acenaphthene was found in urban honey at a higher concentration than rural honey. Similar results were reported by Dobrinas et al. [31] and Ozoani et al. [32], who demonstrated that higher acenaphthene content was detected in urban honey samples than in rural honey.

Benzo(b)fluoranthene was detected in 6 honey samples. The higher content was determined among contaminated samples in the urban samples with the range of 11.8–12.4 µg/kg, while rural samples contained lower values ranging from 0.83 to 3.78 µg/kg. Like benzo(b)fluoranthene, fluorene (F) was found at a higher amount in urban honey (1.37–13.3 µg/kg), while the lowest values were in rural honey (2.85–11.8 µg/kg). Similar results have been previously observed in the study of Dobrinas et al. [31] and Ozoani et al. [32].

Accordingly, the results of benzo(a)pyren in the study, Dobrinas et al. [31] reported that benzo(a)pyren results in regions close to the centre are much lower than the results in rural honey, almost the same. Moret et al. [33] reported that benzo[a]pyrene (BaP) concentrations ranging from 0.3 to 20.3 µg/kg in propolis. The concentration was higher in places near the road and industrial sites. A similar result in the study of Batelkova et al. [34] determined the BaP value as 0.81 µg/kg in honey samples taken from beekeepers.

Benz(a)anthracene (BaA) and pyrene (Pyr) were detected only in one sample from urban areas with a concentration of 11.7 and 11.0 µg/kg, respectively. Similar results were observed in the study of Dobrinas et al. [31] and Ozoani et al. [32].

Fluoranthene (Fl) and phenanthrene (Phe) were determined in 3 and 4 samples of each urban and rural samples, respectively. Contrary to other PAHs, these PAHs were found at higher content in rural samples. The highest fluoranthene (Fl) and phenanthrene (Phe) values were 11.9 and 14.5 µg/kg, respectively. Dobrinas et al. [31] and Perugini et al. [25] detected phenanthrene as a major contaminant in honey samples from urban and rural samples.

Various studies have been conducted on PAHs contamination in honey. Ciemniak et al. [20] did not report a significant difference in PAHs content in collected honey samples. Gnonsoro et al. [35] detected 6 samples containing benzo(k)fluoranthene (BkF) varied between 12.55 and 27.28 µg kg^{-1}, and only 1 sample was found to contain Benzo (a)pyrene (BaP) at a rate of 5.29 µg kg^{-1}. Petrovic et al. [30] reported that the content of PAHs were measured using a gas chromatography-mass spectrometric method and chrysen 140.6 µg/kg, benzo[ghi]perylene 136.3 µg/kg, benzo-[a]pyrene 120.1 µg/kg, benz[a]anthracene 87.2 µg/kg and 79.6 benzo[k]fluorantene µg/kg. Russo et al. [36] reported PAH residues in honey using ultrasound-vortex-assisted liquid–liquid microextraction followed by GC-FID/IT-MS.

5. Conclusions

Turkey has a rich flora in nectar plants. Therefore, it is a very suitable country for beekeeping. In the study, Locations close to and far from the city centre were taken into account in the collection of honey. PAHs residue analyzes were made in these honey samples, and their amounts were determined. This study determined that honey collected from rural locations was less contaminated than honey collected from locations close to the centre. In addition, there are risks associated with the PAHs content in honey. Soil, air and water pollution, and chemical applications lead to residues. The PAHs component naphthalene, used against wax moth (*Galleria mellonella*) in beekeeping, was detected in all regions. The main problem with PAHs is the uncertainty of the upper limit found in foods. While there is a legal limit for benzo(a)pyrene and naphthalene, there is no legal limit for other PAHs components. Therefore, it is thought that it would be beneficial to introduce these limits by the authorised institutions. In addition, beekeeping should be carried out professionally, preferably nomadic, in rich flowery, away from industry and residential areas (cities) where heavy traffic should be supported and encouraged.

Authors contributions

İ. TOPTANCI, A. BAYRAK, and M. KIRALAN have performed the analysis and written the article. M. F. Ramadan reviewed and edited the article.

Disclosure statement

No potential conflict of interest was reported by the authors.

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