The Journal of Agricultural Sciences - Sri Lanka
Vol. 19, No 1, January 2024. Pp 142-156
https://doi.org/10.4038/jas.v19i1.9825

Assessment of Bee Honey in some Districts in South-Western Nigeria for Agricultural Pesticide Residues and Polycyclic Aromatic Hydrocarbons (PAHs)

K. A. Fasasi[1*], O. F. Awodiran[1], D. J. Ayeni[1], O. I. Awoniyi[2], and S. H. Awojide[3]

Received:21st June 2023 / Accepted: 21st November 2023

ABSTRACT

Purpose: *Honey is produced by honeybees which are expected to be free from contaminants or pollutants in the environment. However, different degrees of anthropogenic activities in regions where apiary domiciles have raised issues of primary health importance because of the increasing contents of pesticide residues, polycyclic aromatic hydrocarbons (PAHs), and other related hydrocarbon compounds in honey. Osun State is an agrarian state where agricultural herbicides and insecticides are used. The study focused on organophosphate and organochlorine compounds and PAHs.*

Research Method: *Honey samples sourced from selected zones in Osun State, were screened for organophosphate, organochlorine compounds, PAHs as well as other related hydrocarbon compounds using Gas Chromatography-Mass Spectrometry (GC-MS).*

Findings: *There were no traces of highlighted pesticide residues except for PAHs and other related compounds. Ninety-two PAHs and other related hydrocarbon compounds were identified. The number of PAHs and related compounds per sample ranged from 9 to 35. While Cyclohexane, Decodane, and Naphthalene were the most common with higher concentrations in all the samples. The Mn±S.D values of Cyclohexane, Naphthalene and Decodane obtained were 0.2976±0.1253µgkg^{-1}, 0.471±0.119µgkg^{-1}, and 12.516±3.120µgkg^{-1} respectively. Other PAHs and other related hydrocarbon compounds identified had insignificant concentrations.*

Originality/value: *The study recommends that bee farmers should locate their apiaries 3km to 5km away from regions where there are serious hazardous anthropogenic activities including industrial areas. Also, there is a need for a strong understanding between the bee farmers and crop farmers on tolerable uses of agricultural pesticides, and herbicides on field crops during the flower bloom period where there are no options. Lastly, harvested honey should undergo quality assurance and control for safety reasons. All packaged honey for retail should bear labels including the compositions after quality analysis.*

Keywords: *Cyclohexane, Decodane, GC-MS, Honey, Naphthalene and Pesticide residues*

INTRODUCTION

Honeybees gather pollens and nectars from different vegetation within their environment and the surplus after ripening is stored in the honeycombs for the dearth period to depend on (Fasasi and Malaka, 2006; Fasasi and Afolabi, 2019). The physicochemical composition of fresh and well-preserved honey includes <20% moisture content, color, moisture, pH, ash

[1*] *Applied Entomology and Pest Management Unit, Department of Zoology, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, Osogbo, Osun State, Nigeria.*

ayo.fasasi@uniosoun.edu.ng

[2] *Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, Osogbo, Osun State, Nigeria.*

[3] *Department of Pure and Applied Chemistry, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, Osogbo, Osun State, Nigeria.*

https://orcid.org/0000-0001-8681-1864

content, a high percentage of carbohydrate, free and lactone acidity, nitrogen content, diastase activity, hydroxymethylfurfural (HMF) and the undetermined (insoluble matters) including certain antibiotic properties (Fasasi and Malaka, 2006; Fasasi, 2012). Honey is of high importance in cosmetics, beverages, and pharmaceutical productions. From the literature, honey was reported to have both nutritional and medicinal values and is known to have therapeutic actions against infections, wounds, cough and sore throat, ulcers, earache, measles, certain eye diseases and cancers in local communities (Al-Waili and Boni, 2003). Despite the valuable importance of honey, the sources of any type of honey in any region are of concern to the end-users to ensure such honey is not a contaminant or pollutant in the wake of global civilization, industrialization and high rate of technology exploration. However, the specific compositions of any batch of honey and contaminants/pollutants present are dependent on either wild or cultivated vegetation from which nectar, pollens and honeydew were sourced by honeybees and the ecological locations of the apiaries of such contaminated batches of honey (Tahboub *et al.,* 2006). Honeybees travel long distances to gather suitable and acceptable nectars, pollens, and water irrespective of whether such sources are exposed to environmental contamination or pollutants within their ecological locations. Based on these facts, the nutritional quality of honey may be reduced if contaminated with toxic chemicals such as pesticide residues, polycyclic aromatic hydrocarbons and other environmental contaminants that may mitigate the safety of consumers of such contaminated honey produced by *Apis mellifera*. Honey and honeybees are considered environmental indicators due to their ability to contain harmful pollutants such as pesticide residues, Polycyclic aromatic hydrocarbons and other trace elements (Anderson and Woitas, 1986; Conti and Botre, 2001; Dobrinas *et al*., 2008). Honey is expected to be free of any form of biological and chemical contaminants for safe consumption. Several studies reported the presence of pesticide residues in honey samples (Kadar and Faucon, 2006; Pirard, *et al.,* 2007). The most used pesticides identified are Imidacloprid, Cymiazole, Flumethrin, Bromopropylate, Coumafos, Fluvalinate, Amitraz, and Fipronil (Korta *et al*., 2001; Rial-Otero *et al.,* 2007; Derebaşı *et al*., 2014). Most pesticide residues and PAHs in honey occur because of foraging activities of honeybees in search of food by visitations to citrus plants which are likely treated with various agro-chemicals to control honeybees' pests or diseases or other pests of crops on the field (Bogdanov, 2006) or exposed to PAHs as a result environmental anthropogenic activity. The polycyclic aromatic hydrocarbons were reported to belong to the group of persistent organic pollutants with enormous properties militating against man and his environment (Ramesh *et al*., 2004). Atmospheric air, water bodies, and foodstuffs were identified as the main reservoirs of PAHs generated in the industrial environment (Alexander *et al*., 2008). Trace concentration of PAHs in honey depends strongly on the botanical and geographical origin of such honey produced by species and subspecies of *Apis mellifera* whose beehives are located close to industrial zones or agricultural and agro-allied zones and less dependent on the climatic and seasonal criteria (Čelechovská and Vorlová, 2001; Bogdanov *et al*., 2008). The benefits and uses of honey cannot be over-emphasized. Honey adulteration and contamination affect the consumption rate and threaten food security. Most honey sourced within the states is not subjected to quality assurance and control. Honey is freely sold and consumed without adequate quality control. Hence, the need to constantly monitor the presence of pesticide residues and PAHs in honey in different ecological zones to assess any potential health risk and ensure its quality, whether as food, as a therapeutic substance or agent, as emphasized (Blasco *et al*., 2011; Kujawski and Namiesnik, 2011; Wiest *et al*., 2011; Bargańska *et al*., 2013; Wang *et al*., 2017). Assessment of pesticide residues and polycyclic aromatic hydrocarbon in honey sold in some communities' markets may help researchers to achieve a measurable objective that will help to address quality and safety issues. It will also provide information that will be useful as a basis for determining the risk of consumers of such contaminated honey in selected sites chosen for the study. This study is aimed at identifying and assessing pesticide residues (organophosphates and organochlorine compounds), PAHs and other related hydrocarbons in honey from selected communities.

MATERIALS AND METHODS

Sample Collection

The study was carried out at the Honeybees' Farm Research Centre, Department of Zoology, Osun State University, Nigeria from September 2018 to July 2019. Twelve samples of honey were sourced locally from six districts which include Ede, Ife, Ikirun, Ilesha, Iwo and Osogbo (representatives of six local government areas - LGAs), Osun State, Southwest Nigeria. Each honey sample was locally harvested randomly from selected colonies per apiary and processed and bottled accordingly. All the honey samples are multiflora. Two samples per area were collected and preserved in a fridge (-4^{o}C) pending the laboratory analysis in the Postgraduate Biochemistry Laboratory, Department of Biochemistry, Osun State University. Osun state is located on 7^{o}30'0.00" N 4^{o}30'0.00" E and is bounded in the north by Kwara State, in the east by Ekiti and Ondo States respectively and in the south by Ogun State and in the west by Oyo state.

Sample Preparation and Chromatography Determination of the Agricultural Pesticide Residues and Polycyclic Aromatic Hydrocarbons (PAHs)

Table 01 indicates the total weight of each of the twelve honey samples before extraction and analyses (Each honey sample had varied weights (gm) with similar volume).

The pesticide residue and PAHs analysis was carried out using Soxhlet extraction procedures and Gas Chromatography-Mass Spectrometry extracting analysis for the honey samples (García-Chao *et al*., 2010). All glass wares were washed with detergent using hot water, rinsed with tap water and organic-free reagent water. The glass wares were drained and dried in an oven at 130^{o}C for three hours or rinsed with acetone and drained. All dried glass wares were stored in a clean and dust-free environment.

Table 01: Total weight of honey samples (H_1 – H_{12}) before extraction.

S/NO	Sample codes	Weight taken (gm)	Final volume (mL)
1	H_1	11.57	2
2	H_2	10.77	2
3	H_3	12.25	2
4	H_4	13.26	2
5	H_5	9.54	2
6	H_6	11.91	2
7	H_7	11.89	2
8	H_8	12.97	2
9	H_9	16.99	2
10	H_{10}	12.32	2
11	H_{11}	11.41	2
12	H_{12}	11.63	2

Ten milliliters of each honey sample were weighed using a digital meter scale balance to 4 decimal points into the already-known weight of a paper thimble covered with glass wool. This was transferred into a glass thimble; 200mL of dichloromethane was measured using a graduated measuring cylinder into each 250mL quick-fit round bottom flask. Set up was done while tap water was allowed to pass through a condenser attached to a glass thimble for cooling and easy condensing and extraction of the sample for almost twelve hours. The extracted sample in Dichloromethane was transferred into a rotary evaporator flask whose temperature was set to Dichloromethane boiling point and the organic solvent for extraction (Dichloromethane) was collected into the receiving flask to reduce the extracted content to about 2mL. The 2mL collected was transferred by using a glass glass-dropping pipette into set up for clean-up.

Column chromatography was firstly filled with glass wool at the tip to prevent silica gel from escaping into the receiving beaker, Silica gel was filled up to 10 cm in length and was dried in an oven for almost 8 hours at 230^{o}C. Glass wool was also packed above silica gel, followed by sodium sulphate to remove any water that might have escaped with the extract, the extract is continuously deposited on the sodium sulphate through glass dropping pipette. Thirty millimeters of hexane (Pesticide grade) was poured through the funnel to clean up the extract and collected in the beaker by opening the tap. This was repeated with 15mL of hexane, this filtrate was also concentrated with nitrogen gas. Two millimeters of the sample were transferred into the vial and sent for gas chromatography-mass spectrometer for (Organochlorine and Organophosphates) analysis, at the Chemical Engineering Laboratory, University of Ilorin, Ilorin, Kwara State, Nigeria. The result of the GCMS (Gas Chromatography-Mass Spectrometer) (Agilent 5977B GC/MSD) (USA) was presented and extracts were made with some basic calculations as follows:

The concentration of each chemical compound, and residue was calculated as follows:

IR- Instrument Reading; This was presented in area percentage in the results. This was converted to concentration (μgkg^{-1} or ppm) as follows:

IR × 2ML (volume of vial set)/Weight of sample taken = concentration (ppm or μgkg^{-1})

Constant: 2 ml of each sample.

The Concentration of each chemical compound residue =

$$\frac{\text{concentration } (\mu gkg^{-1})}{\text{Frequency of identified compound}} \times 1$$

$$= \mu gkg^{-1}$$

Data Analyzes

Descriptive statistics were used. Means and standard deviations were calculated using Excel sheet 2016.

RESULTS AND DISCUSSION

Based on the GC-MS analysis, 92 PAHs and other related hydrocarbon compounds were identified. According to Table 02, no agricultural pesticide residues or elements were observed in all the honey samples analyzed except for the presence of polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds. Total concentrations of the polycyclic aromatic hydrocarbons (PAHs) and related hydrocarbon compounds in all the honey samples ranged from 10.807μgkg^{-1} to 43.364μgkg^{-1} (Table 2).

Table 02: List of Polycyclic aromatic hydrocarbons (PAHs) and related hydrocarbon compounds.

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	H_1 (μgkg^{-1})	H_2 (μgkg^{-1})	H_3 (μgkg^{-1})	H_4 (μgkg^{-})	H_5 (μgkg^{-1})	H_6 (μgkg^{-1})	H_7 (μgkg^{-1})	H_8 (μgkg^{-1})	H_9 (μgkg^{-1})	H_{10} (μgkg^{-1})	H_{11} (μgkg^{-1})	H_{12} (μgkg^{-1})
1	Dodecane	1.966	14.185	13.28	9.4593	18.2664	14.0509	11.8511	12.6918	9.1299	9.5429	14.378	10.2157
2	Naphthalene	0.415	0.2799	0.191	0.1438	1.238	0.3351	0.2719	0.2923	0.2653	0.392	0.373	0.5963
3	Azulene	0.214	0.1399	0.382	0.2921	-	-	0.1359	0.1566	0.1206	0.202	0.1865	-
4	Cyclohexasiloxane	0.115	0.1606	0.209	-	-	0.1295	0.5477	0.2388	0.3345	0.1336	-	0.205
5	Tri,Tetra,Penta,Hexa, Hepta, Octa, Nona-decane	10.86	10.689	-	0.0319	-	0.4037	0.2514	0.2107	0.0538	0.0769	0.0383	0.2509
6	Cyclohexane	0.142	0.4171	0.221	0.3197	0.1341	0.3191	0.1513	0.1762	0.2217	0.1666	0.5366	0.2734
7	Undecane	-	0.0425	0.032	-	-	0.1255	0.0347	-		0.0388	0.0638	0.0415
8	1-Nonadecene	0.339	0.5791	0.045	-	0.0883	-	-	-	0.1833	0.1638	0.1689	0.1567
9	5-Ethyldecane	0.032	-	-	-	-	-	-	-	-	0.0381	-	-
10	Sulfurous acid	0.046	-	-	-	0.1715		-	0.1376	-	0.0412	0.0604	0.0438
11	3,5,9-Eicosene	0.183	-	0.113	-	0.1677	-	0.1466	-	-	-	-	-
12	Decane	0.032	-	0.044	-	-	-	0.0882	0.3856	0.0705	0.0381	-	-
13	Formic acid	-	-	0.056	-	-	0.057		-	-	-	-	-
14	Pyrimidine	0.058	-	-		-			-		0.0668		-
15	Heptane	0.03	-	0.043	0.0319	-	-	-		-	0.0411	0.0383	-
16	Heptafluorobutyric acid	-	-	0.062	-	-	-	0.0411	-	-	-	-	-
17	Heptyl cyclohexane	0.029	-	-	-	0.0759	-	-	-	0.0269	-	-	-
18	Heptadecyltrifluoroacetate	-	-	-	-	0.0718	-	-	-	0.051	0.098	-	-
19	Pentafluoropropionic acid	-	-	0.062	-	-	-	0.066	-	-	-	-	-
20	Eicosane	0.639	-	0.038	0.3315	0.046	-	-	-	0.4164	0.0412	0.0492	-
21	2H-Pyran	-	-	-	0.0319	0.0615	0.0321	-	-	-	-	-	-
22	1,2,6,7-Tetradecene	0.05	-	-	0.1649	-	-	-	0.2427	-	0.0639	0.1298	-
23	1H-indene	-	-	-	-	0.2153	0.165	-	-	-	-	-	-
24	Cyclo-tetra,hexadecane	0.05	-	-	-	0.0702	0.0647	-	0.0559	-	0.1375	0.0649	0.0643
25	Heneicosane	0.061	-	0.113	-	-	0.0366	-	-	0.2082	-	-	-
26	Behenyl alcohol	-	-	-	-	0.0718	-	-	-	-	-	0.0702	-
27	3,5,9-Octadecene	-	-	-	-	0.1588	-	0.0974	-	0.0894	-	0.0723	-
28	1-Tridecene	-	-	-	-	-	-	0.0307	-	-	0.0717	-	-
29	1-Iodo-2-methyl-undecane	-	-	-	-	-	-	0.0339	0.0464	-	-	-	-
30	13-Tetradecen-1-0l acetate	-	-	-	-	-	-	0.0806	-	-	0.098	0.0441	-
31	1,9-nonadecene	0.34	-	-	-	-	0.1479	-	0.0464	-	0.2296	-	-
32	1-Hexadecanol	-	-	-	-	-	-	0.0487	-	0.1078	-	0.0723	-
33	3-methyl-4-undecane	-	-	-	-	-	-	0.0449	-	-	-	-	0.069
34	Tritriacontane	-	-	-	-	-	0.0366	-	0.0791	-	-	-	-
35	1-Docosene	0.157	-	-	-	0.0717	0.057	0.0411	-	-	-	-	0.107
36	Docosane	0.227	-	-	-	-	-	-	0.0791	0.0705	-	0.0383	
37	Cycloheptasiloxane	0.1523	0.0437	0.265	-	-	-	-	0.1661	-	-	-	0.1569
38	Cetene	0.108	-	-	-	-	-	-	-	-	0.0658	-	-
39	n-Nonadecanol-1	0.183	0.0614	-	-	-	-	-	-	-	-	-	-
40	13-Methylhentriacontane		-	-		0.046			-		-		-
41	1-Heneicosanol		-	-		-			-		-		0.107
42	1-Heneicosyl formate		-	-		-			0.0428		-		-
43	1-Pentadecene		-	-		-			0.0569		-		-

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	H_1 (μgkg^{-1})	H_2 (μgkg^{-1})	H_3 (μgkg^{-1})	H_4 (μgkg^{-})	H_5 (μgkg^{-1})	H_6 (μgkg^{-1})	H_7 (μgkg^{-1})	H_8 (μgkg^{-1})	H_9 (μgkg^{-1})	H_{10} (μgkg^{-1})	H_{11} (μgkg^{-1})	H_{12} (μgkg^{-1})
44	1-Propanol	-			-		-	-		0.0394		-	
45	2 & 3-Tetradecene		-	-		0.1404			-		0.0388		-
46	2&4-Dimethyldodecane		-	-		0.0418			-		-		-
47	2',6'-Dihydroxyacetophe-none	-			-		-	0.1006		-		-	
48	2-Butenedioic acid		-	-		-			-		-		0.0644
49	2-Methyl-2-docosene	-			-		0.057	-		-		-	
50	3,7-Hexadecene	0.05			-		0.1294	-		-		-	
51	3-Ethyl-3-methylhepta-decane	-			-		-	0.054		-		-	
52	4-Octanone		-	-		-			-		-		0.0416
53	5H-Naphthol(2,3-c) carbazole		-	-		-			0.0856		-		-
54	7-Hexadecene	0.05			-		-	-		0.0447		-	
55	9-methylheptadecane	-			-		-	0.0305		-		-	
56	Bacchotricuneatin c	0.031			-		-	-		-		-	
57	Bicyclo(2,2,1)heptan-2-one	0.042			-		-	-		-		-	
58	Bromoacetic acid		-	-		-			-		-		0.0809
59	Carbonic acid	-			-		-	0.0307		-		-	
60	Cyclobutene-3,4-dione	0.075			-		-	0.0306		-		-	
61	Cycloeicosane		-	-		0.0883			-		-		-
62	Cyclohexanecarbonyl chloride		-	-		-			0.3121		-		-
63	Cyclohexanone		-	-		0.0671			-		-		-
64	Cyclooctasiloxane	0.029			-		-	-		-		-	
65	Cylohexanecarboxylic acid	-			-		-	-		0.0394		-	
66	Dodecyclohexane	-			-		-	-		-		0.0638	
67	E-7-Octadecene		-	-		-			-		0.0657		-
68	Galaxolide 1 & 2		16.724	-		-			-		-		-
69	Heptacosyl acetate	0.157			-		-	-		-		-	
70	Heptacosyl acetate		-	-		-			-		0.0658		-
71	Hexane	-			-		0.0496	0.0379		-		-	
72	Hexatriacontan	-			-		-	-		0.0269		-	
73	Methyl6,8-dodecadienyl Esther	0.056			-		-	-		-		-	
74	n-Pentadecanol		-	-		-			-		0.0717		-
75	n-Tetracosanol-1	-			-		0.0739	-		-		0.0702	
76	n-Tridecylcyclohexane	-			-		-	-		-		0.0441	
77	Octacosanol	-			-		-	0.0411		-		-	
78	Octacosyl acetate		-	-		-			0.0428		-		-
79	Octadecane		0.0425	-		0.0459			-		-		-
80	Octadecyl	-			-		-	0.0339		-		-	
81	Octane		-	-		-			-		0.0388		0.0677
82	Oxalic acid	0.032			-		-	-		-		-	
83	Phenethylamine	0.029			-		-	-		-		-	
84	Phthalic acid	-			-		-	0.1806		-		-	
85	Pyrazole	0.029			-		-	-		-		-	
86	Tetrasiloxane		-	-		-			-0.1513		-		0.0785
87	Tetratetracontane	0.038			-		-	-		-		-	

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	H_1 (μgkg^{-1})	H_2 (μgkg^{-1})	H_3 (μgkg^{-1})	H_4 (μgkg^{-})	H_5 (μgkg^{-1})	H_6 (μgkg^{-1})	H_7 (μgkg^{-1})	H_8 (μgkg^{-1})	H_9 (μgkg^{-1})	H_{10} (μgkg^{-1})	H_{11} (μgkg^{-1})	H_{12} (μgkg^{-1})
88	Tri, Penta,hexa,hepta,Octa-decane		-	0.043		0.2263			-		0.0381		-
89	Trichloroacetic acid	-			-		-	-		0.0784		-	
90	Trifluoroacetic acid		-	-		-			-		-		0.107
91	Trifluoroacetoxy hexadecane		-	-		-			-		-		0.0758
92	z-5-Nonadecene		-	-		-			0.0464		-		-
	Total	16.89	43.364	15.2	10.807	21.5648	16.2706	14.5031	15.4406	11.579	12.0665	16.563	12.8034

The concentrations of Dodecane in the samples range from 1.9664 to 18.2664µgkg^{-1}, while the Naphthalene concentrations range from 0.1438 - 0.5963µgkg^{-1} and that of Cyclohexane ranged from 0.1341µgkg^{-1} to 0.5366µgkg^{-1}. Decodane was observed to exhibit the highest concentrations (1.9664µgkg^{-1} - 18.2664µgkg^{-1}) across all the honey samples while only one sample (OSU/DM/PR/H2) has Galaxolide 1 & 2 at 16.724 µgkg^{-1} which is relatively high (Table 02). Table 03 exhibits the statistical presentation (minimum, maximum, mean, and standard deviations) of identified polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds. Figure 01 shows the summary of the number of identified PAHs and other related hydrocarbon compounds per sample with the linear and logarithm trendlines. Sample H_1 has the highest number (36) of identified PAHs and other related hydrocarbon compounds while sample H_4 has the lowest number (9) of identified PAHs and other related hydrocarbon compounds. The linear and logarithm trendlines with $R^2 = 0.015$ predict that most of the samples safely and significantly contain an average of about 20 numbers of identified PAHs and other related hydrocarbon compounds. Hence, the honey samples are assumed to be safe on health grounds.

Table 03: Means and Standard deviations of Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds.

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	Minimum (μgkg^{-1})	Maximum (μgkg^{-1})	Mean (μgkg^{-1})	S.D (μgkg^{-1})
1	Dodecane	1.9664	18.2664	12.51584	3.119524
2	Naphthalene	0.2653	1.238	0.470488	0.118664
3	Azulene	0.1206	0.382	0.197267	0.045375
4	Cyclohexasiloxane	0.1295	0.5477	0.305257	0.104015
5	Tri,Tetra,Penta,Hexa,Hepta,Octa,Nona-decane	0.0383	10.8635	1.51865	1.11187
6	Cyclohexane	0.1341	0.5366	0.297688	0.1253
7	Undecane	0.0347	0.1255	0.071633	0.024313
8	1-Nonadecene	0.0883	0.5791	0.25036	0.0179
9	5-Ethyldecane	0.0381	0.0381	0.0381	0.01394
10	Sulfurous acid	0.0412	0.1715	0.0909	0.032842
11	3,5,9-Eicosene	0.1466	0.1829	0.16475	0.046434
12	Decane	0.0381	0.3856	0.1936	0.126893
13	Formic acid	0.0563	0.057	0.057	0.021244
14	Pyrimidine	0.0576	0.0668	0.0668	0.02488
15	Heptane	0.0295	0.0431	0.040833	0.014348
16	Heptafluorobutyric acid	0.0411	0.0623	0.0517	0.011438

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	Minimum ($\mu g kg^{-1}$)	Maximum ($\mu g kg^{-1}$)	Mean ($\mu g kg^{-1}$)	S.D ($\mu g kg^{-1}$)
17	methylcyclohexane	0.0269	0.0759	0.0514	0.002236
18	Heptadecyltrifluoroacetate	0.051	0.098	0.082333	0.028153
19	Pentafluoropropionic acid	0.0623	0.066	0.066	0.024056
20	Eicosane	0.0383	0.6394	0.28655	0.131547
21	2H-Pyran	0.0319	0.0615	0.0468	0.007121
22	1,2,6,7-Tetradecene	0.0501	0.2427	0.169775	0.06354
23	1H-indene	0.165	0.2153	0.19015	0.052444
24	Cyclo-tetra,hexadecane	0.0501	0.1375	0.087467	0.033445
25	Heneicosane	0.0366	0.2082	0.151	0.069077
26	Behenyl alcohol	0.0702	0.0718	0.071	0.02776
27	3,5,9-Octadecene	0.0723	0.1588	0.104475	0.024668
28	1-Tridecene	0.0307	0.0717	0.058033	0.021301
29	1-Iodo-2-methyl-undecane	0.0339	0.0464	0.042233	0.016464
30	13-Tetradecen-1-0l acetate	0.0441	0.098	0.080175	0.028737
31	1,9-nonadecene	0.0464	0.3395	0.19085	0.067017
32	1-Hexadecanol	0.0487	0.1078	0.08415	0.030567
33	3-methyl-4-undecane	0.0449	0.069	0.060967	0.023013
34	Tritriacontane	0.0366	0.0791	0.064933	0.023864
35	1-Docosene	0.0411	0.1565	0.0904	0.03017
36	Docosane	0.0383	0.2268	0.103675	0.013153
37	Cycloheptasiloxane	0.1569	0.2651	0.196033	0.04282
38	Cetene	0.0658	0.1079	0.08685	0.0179
39	n-Nonadecanol-1	0.0614	0.1829	0.020358	0
40	13-Methylhentriacontane	0.046	0.046	0.003833	0
41	1-Heneicosanol	0.107	0.107	0.107	0.0428
42	1-Heneicosyl formate	0.0428	0.0428	0.0428	0.01712
43	1-Pentadecene	0.0569	0.0569	0.0569	0.02276
44	1-Propanol	0.0394	0.0394	0.0394	0.01576
45	2 & 3-Tetradecene	0.0388	0.1404	0.0896	0.0048
46	2&4-Dimethyldodecane	0.0418	0.0418	0.003483	0
47	2’,6’-Dihydroxyacetophenone	0.1006	0.1006	0.1006	0.037725
48	2-Butenedioic acid	0.0644	0.0644	0.0644	0.02576
49	2-Methyl-2-docosene	0.057	0.057	0.057	0.021375
50	3,7-Hexadecene	0.0501	0.1294	0.1294	0.033656
51	3-Ethyl-3-methylheptadecane	0.054	0.054	0.054	0.02025
52	4-Octanone	0.0416	0.0416	0.0416	0.01664
53	5H-Naphthol(2,3-c) carbazole	0.0856	0.0856	0.0856	0.03424
54	7-Hexadecene	0.0447	0.0501	0.0474	0.0168
55	9-methylheptadecane	0.0305	0.0305	0.0305	0.011438
56	Bacchotricuneatin c	0.0306	0.0306	0.00255	0
57	Bicyclo(2,2,1)heptan-2-one	0.0418	0.0418	0.003483	0
58	Bromoacetic acid	0.0809	0.0809	0.0809	0.03236
59	Carbonic acid	0.0307	0.0307	0.0307	0.011513
60	Cyclobutene-3,4-dione	0.0306	0.0754	0.053	0.003075
61	Cycloeicosane	0.0883	0.0883	0.007358	0
62	Cyclohexanecarbonyl chloride	0.3121	0.3121	0.3121	0.12484

S/NO	Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds	Minimum (μgkg^{-1})	Maximum (μgkg^{-1})	Mean (μgkg^{-1})	S.D (μgkg^{-1})
63	Cyclohexanone	0.0671	0.0671	0.005592	0
64	Cyclooctasiloxane	0.0292	0.0292	0.002433	0
65	Cylohexanecarboxylic acid	0.0394	0.0394	0.0394	0.01576
66	Dodecyclohexane	0.0638	0.0638	0.0638	0.02552
67	E-7-Octadecene	0.0657	0.0657	0.0657	0.02628
68	Galaxolide 1 & 2	16.724	16.724	1.3477	0
69	Heptacosyl acetate	0.1565	0.1565	0.013042	0
70	Heptacosyl acetate	0.0658	0.0658	0.0658	0.02632
71	Hexane	0.0379	0.0496	0.0457	0.017254
72	Hexatriacontane	0.0269	0.0269	0.0269	0.01076
73	Methyl6,8-dodecadienyl Esther	0.0556	0.0556	0.004633	0
74	n-Pentadecanol	0.0717	0.0717	0.0717	0.02868
75	n-Tetracosanol-1	0.0702	0.0739	0.072667	0.029952
76	n-Tridecylcyclohexane	0.0441	0.0441	0.0441	0.01764
77	Octacosanol	0.0411	0.0411	0.0411	0.015413
78	Octacosyl acetate	0.0428	0.0428	0.0428	0.01712
79	Octadecane	0.0425	0.0459	0.007367	0
80	Octadecyl	0.0339	0.0399	0.0339	0.012713
81	Octane	0.0388	0.0677	0.058067	0.018087
82	Oxalic acid	0.0317	0.0317	0.002642	0
83	Phenethylamine	0.0291	0.0291	0.002425	0
84	Phthalic acid	0.1806	0.1806	0.1806	0.067725
85	Pyrazole	0.0292	0.0292	0.002433	0
86	Tetrasiloxane	0.0785	0.1513	0.127033	0.103059
87	Tetratetracontane	0.0382	0.0382	0.003183	0
88	Tri,Penta,hexa,hepta,Octa-decane	0.0381	0.2263	0.1322	0.017773
89	Trichloroacetic acid	0.0784	0.0784	0.0784	0.03136
90	Trifluoroacetic acid	0.107	0.107	0.107	0.0428
91	Trifluoroacetoxy hexadecane	0.0758	0.0758	0.0758	0.03032
92	z-5-Nonadecene	0.0464	0.0464	0.0464	0.01856
	Total	11.5786	43.3641	17.86156	3.187869

Mean: Mn; SD: Standard Deviation.

Figure 02 shows the cumulative concentrations of the common PAHs across the samples (Dodecane, Naphthalene and Cyclohexane), other identified PAHs and related hydrocarbon compounds in the samples. Dodecane, Naphthalene and Cyclohexane are common PAHs across all the samples. Dodecane has the highest cumulative concentrations (139.0134μgkg^{-1}) across the samples compared to all other PAHs and related hydrocarbon compounds.

From the GCMS analysis, there were no traces of pesticide contaminants in all the twelve honey samples from the selected study areas (Ede, Ife, Ikirun, Ilesha, Iwo and Osogbo). Polycyclic aromatic hydrocarbons (PAHs) and other related hydrocarbon compounds were identified. Polycyclic aromatic hydrocarbons were identified as pervasive pollutants of significant public health and environmental concerns. Cumulative concentrations of PAHs in the human body may exhibit acute and chronic toxicity, microbial recalcitrance, bioaccumulation potential and low efficiency of traditional detoxification processes of honey.

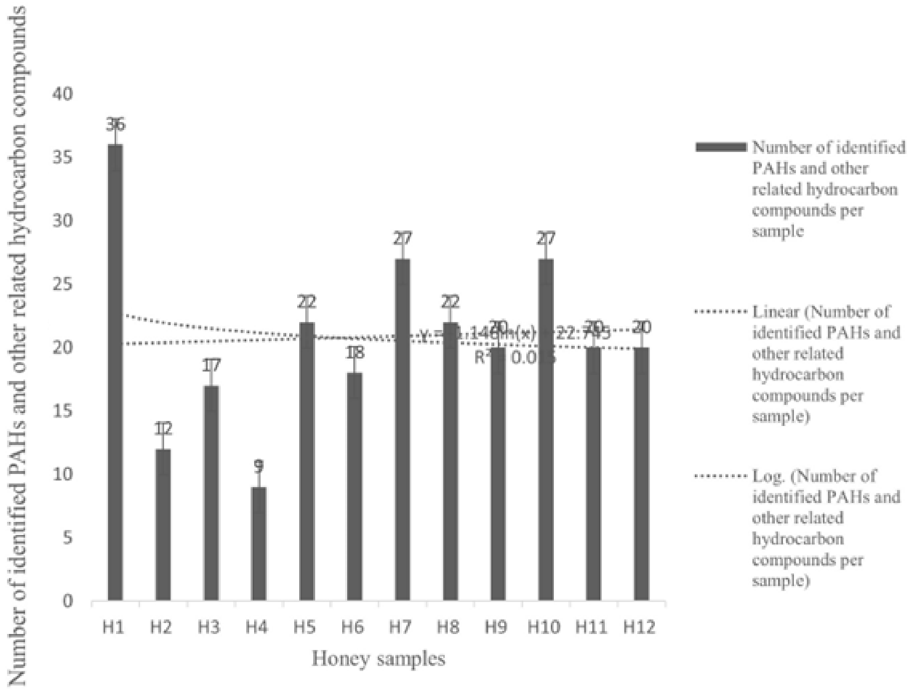

Figure 01: **Number of identified PAHs and other related hydrocarbon compounds per sample**

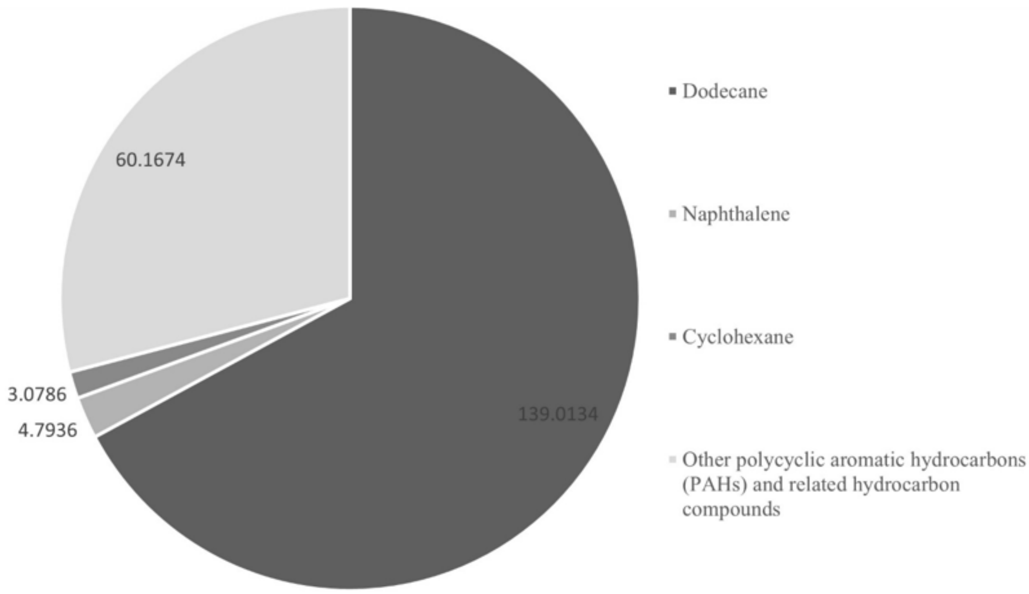

Figure 02: **Cumulative Conc. µgkg^{-1} of Dodecane, Naphthalene, cyclohexane and other polycyclic aromatic hydrocarbons and related hydrocarbon compounds in all the honey samples.**

All the honey samples show no traces of pesticide residues except the presence of polycyclic aromatic hydrocarbon compounds and other related hydrocarbons. This may probably be because agricultural herbicides and pesticides were not commonly used around the study zones but more anthropogenic activities such as clearing and burning of weeds and forest, mining, vehicular exhaust fumes, burning of unsorted wastes such as plastic and polythene materials, including processing and packaging of honey in plastic or polythene materials either fresh or re-used for storage and retail etc. These anthropogenic activities account for the presence of the PAHs. The observations of this study deviated from the reports of Dobrinas *et al*.,

(2006) and Yavuz *et al*., (2010) who reported the presence of organochlorine pesticides (Endrin, Dieldrin and Dichlorodiphenyltrichloroethane – DDT) without any record of PAHs. Corredera *et al*., (2011) observed and reported that the honey samples studied were not contaminated by PAHs at detectable levels, hence the studied samples posed no health risk and were saved for consumption as expected.

About 92 PAHs and other related hydrocarbon compounds were identified in 12 honey samples analyzed. Each of the samples has between 9 to 36 PAHs and other related hydrocarbons while the expected and safe average number of PAHs is 20 PAHs and other related hydrocarbons per sample. The list of identified PAHs and other related hydrocarbons in this study differs from those reported in some studies in different regions. Hence, detectable Polycyclic aromatic hydrocarbons and other related hydrocarbons vary from region to region based on different anthropogenic activities, levels of civilization, industrialization, and waste management capacity in such regions. In Nigeria, Okedere and Elehinafe (2022) reported that there are reported cases of different categories of polycyclic aromatic hydrocarbons (PAHs) present in the air space, soil and aquatic environment in Nigeria with varied concentrations hazardous to human health with the absence of regulatory standards for the PAHs in the environment. This may account for the identified polycyclic aromatic hydrocarbons in the honey samples but at tolerable and acceptable limits as observed in this study. Masclet, *et al*., (1995) and Vila-Escale *et al*., (2007) reported that most of the polycyclic aromatic hydrocarbons are persistent organic environmental contaminants and pollutants in different zones. But the health risk of food including honey with the presence of such PAHs depends on the level of their concentrations in such food whether is within the acceptable or tolerable limits set by the appropriate regulatory agencies in different countries.

Naphthalene (0.2653µgkg^{-1} – 1.238 µgkg^{-1}; 0.471±0.119µgkg^{-1}) was one of the common PAHs (Dodecane, Naphthalene and Cyclohexane) detected across all the samples analyzed. The concentration of naphthalene is significantly less compared to dodecane (1.9664µgkg^{-1} - 18.2664 µgkg^{-1}; 12.516±3.120µgkg^{-1}) and cyclohexane has the lowest concentration (0.1341µgkg^{-1} – 0.5366µgk^{-1}g; 0.2976±0.1253µgkg^{-1}). Beyoğ-Lu and Omurtag (2007) detected naphthalene at a mean concentration of 1.13gkg^{-1} while Derebaşı *et al*., (2014) reported the mean concentration of naphthalene residues detected in the honey to be 4.04±0.48ppb which is far less to 0.471±0.119µgkg^{-1} (n = 12) of naphthalene concentration observed in this study although within acceptable limits.

The Mean±SD concentration of dodecane is 12.5158±3.1195 (Min.: 1.664; Max.: 18.2664) across all analyzed samples. The sources of this dodecane may probably be from the materials of burning firewood ignited by any of the petroleum products such as kerosene, premium spirit or dispensed engine oil or plastic materials used to harvest the honey from bee colonies. It was reported that dodecane is an isolate from the kerosene and gas oil fractions of crude oil by selective adsorption and subsequent desorption to yield mixtures of paraffin that were separated by fractional distillation (Bingham *et al.,* 2001). Hence, the suggested sources of the dodecane in the analyzed samples may be the actual suspects. Cyclohexane has a Mn±S.D concentration of 0.1341±0.1253 (Min.: 0.01253; Max.: 0.2976) in the samples. Like dodecane, cyclohexane is a petroleum-like product that may have its entry route into the honey samples via the same sources as the dodecane in the environment. Literature searches showed that there is scanty published information on the existence of detectable dodecane and cyclohexane in honey samples and some related agricultural foods and their toxicological effect. Other PAHs and other related hydrocarbon compounds identified had insignificant concentrations as presented in the study.

CONCLUSION

The honey samples were free of agricultural pesticide residues but commonly had traces of dodecane, naphthalene and cyclohexane. Hence, the honey from the studied districts poses no health risk and is safe for consumption. It is recommended that honey from colonies of *Apis mellifera* and other species should be harvested, processed, and analyzed hygienically during honey production. Detectable pesticide residues, PAHs, and other related hydrocarbon compounds analyses in honey are important for human health concerns. This will offer a simple and reliable approach to reducing or eliminating the risk of economic losses to honey-related food industries and threats to human health due to exposure to any forms and types of threatening pesticides, PAHs, and other related hydrocarbon compounds within the environment. Honeybee farmers need to seek the cooperation of crop farmers to avoid or reduce the use of pesticides on their farms and the burning of bushes with petroleum products or other related dangerous hydrocarbon materials during flower bloom and foraging period of bees to mitigate against contamination and pollution of hive products. Beehives may also be located, if possible, beyond almost 3 – 5 km away from agricultural crop plantations with regular pesticide treatment regimes or away from regions of heavy anthropogenic activities. This observation and assessment will enhance the patronage of honey in the area. However, there is a need for further research on the identification of more agricultural herbicides, pesticide residues and other PAHs in honey samples both from the apiaries and the wild. Such a study will help to uncover the critical area of quality control and assurance of honey in the region.

REFERENCES

Alexander, J., Benford, D., Cockburn, A., Cravedi, J., Dogliotti, E., Di Domenico, A., Fernández-Cruz, M.L., Fink Gremmels, J., Fürst, P., Galli, C., Grandjean, P., Azyl, J., Heinemeyer, G., Johansson, N., Mutti, A., Schlatter, J., Van Leeuwen, R., Van Peteghem, C. and Verger, P. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. *European Food Safety Authority Journal*. 724, 1–114. DOI: https://doi.org/10.2903/j.efsa.2008.724

Al-Waili, N.S. and Boni, N.S. (2003). Natural honey lowers plasma prostaglandin concentrations in normal individuals. *Journal of Medicinal Food*. 6(2), 129–133. DOI: https://doi.org/10.1089/109662003322233530.

Anderson, J.F. and Wojtas, A.A. (1986). Honeybees (Hymenoptera: Apidae) contaminated with pesticides and polychlorinated biphenyls. *Journal of Economic Entomology*. 79(5), 1200–1205. DOI: https://doi.org/10.1093/jee/79.5.1200

Bargańska, Ż., Ślebioda, M. and Namieśnik, J. (2013). Pesticide residue levels in honey from apiaries located in Northern Poland. *Food Control*. 31(1), 196–201. DOI: https://doi.org/10.1016/j.foodcont.2012.09.049

Beyoğ-Lu, D. and Omurtag, G.Z. (2007). Occurrence of naphthalene in honey consumed in Turkey as determined by high-pressure liquid chromatography. *Journal of Food Protection*. 70(7), 1735–1738. DOI: https://doi:org/10.4315/0362-028x-70.7.1735

Bingham, E. Cohrssen, B. and Powell, C.H. (2001). *Patty's Toxicology*. 5th ed. John Wiley & Sons. New York, N.Y. 6 Volumes. 66 pp. ISBN: 978-0-470-41081-3

Blasco, C., Vazquez-Roig, P., Onghena, M., Masia, A. and Picó, Y. (2011). Analysis of insecticides in honey by liquid chromatography-ion trap-mass spectrometry: comparison of different extraction procedures. *Journal of Chromatograph*. 1218 (30), 4892–4901. DOI: https://doi.org/10.1016/j.chroma.2011.02.045

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie*. 37(1), 1–18. DOI: https://doi.org/10.1051/apido:2005043

Bogdanov, S. Jurendic, T. Sieber, R. and Gallmann, P. (2008). Honey for Nutrition and Health: *Journal of American College of Nutrition*. 27(6), 677–894. DOI: https://doi.org/10.1080/07315724.2008.10719745

Čelechovská, O and Vorlová, L. (2001). Group of honey – Physicochemical properties and heavy metals. *Acta Veterinaria Brno*. 70, 91–95. DOI: https://doi:org/10.2754/avb200170010091

Conti, M.E. and Botre, F. (2001), Honeybees and their product as potential bioindicators of heavy metals contamination. *Environmental Monitoring and Assessment*. 69, 267-282. DOI: https://doi.org/10.1023/A:1010719107006

Corredera, L., Bayarri, S., Pérez-Arquillué, C., Lázaro, R., Molino, F. and Herrera, A. (2011). Multiresidue determination of carcinogenic polycyclic aromatic hydrocarbons in honey by solid-phase extraction and high-performance liquid chromatography. *Journal of Food Protection*. 74(10), 1692-1699. DOI: https://doi.org/10.4315/0362-028X.JFP-11-140

Derebaşı, E. Bulut, G., Melek, C., Güney. F., Yaşar, N. and Ertürk, Ö. (2014). Physicochemical and residue analysis of honey from the black sea region of Turkey. *Fresenius Environmental Bulle*. 23(1), 10–17.

Dobrinas, S., Birghila, S. and Coatu, V. (2006). Determination of organochlorine and polycyclic aromatic hydrocarbon pesticides in honey from different regions in Romania. In: Simeonov and E. Chirila (eds.) Chemicals as Intentional and Accidental Global Environmental Threats. NATO Security through Science Series. Springer, Dordrecht. 413–415. DOI: https://doi.org/10.1007/978-1-4020-5098-5_37

Dobrinas, S. Birghila, S. and Coatu, V. (2008). Assessment of polycyclic aromatic hydrocarbons in honey and propolis are produced from various flowering trees and plants in Romania. *Journal of Food Composition and Analysis*. 21(1), 71-77. DOI: https://doi.org/10.1016/j.jfca.2007.07.003

Fasasi, K.A. and Malaka, S.L.O. (2006**).** Physicochemical attributes of local Honeys in Lagos metropolis, Nigeria. *Indian Bee Journal,* 68(1-4), 99-106.

Fasasi, K.A. (2012). Physicochemical Attributes of Nigerian Natural Honey from Honeybees (*Apis mellifera adansonii*) (Hymenoptera: Apidae) and their Shelf Life in Storage at Room Temperature. *Pakistan Journal Biological Sciences*. 15, 1027–1033. DOI: https://doi.org/10.3923/pjbs.2012.1027.10.33

Fasasi, K.A. and Afolabi, M.A. (2019). Comparative Study of Forage Plants by *Apis mellifera adansonii* (Hymenoptera: Apidae) in Osun State, Nigeria. *Journal Applied Sciences*. 19(2), 121–127. DOI: https://doi.org/10.3923/jas.2019.121.127

Ramesh, A., Walker, S.A., Hood, D.B., Guillen, M.D., Schneider, K., Weyand, E.H. (2004). Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology*. 23, 301–333. DOI: https://doi.org/10.1080/10915810490517063. PMID: 15513831.

García-Chao, M., Agruña, M.J., Flores Calvete, G., Sakkas, V., Llompart, M., and Dagnac, T. (2010). Validation of an offline solid phase extraction liquid chromatography-tandem mass spectrometry method for the determination of systemic insecticide residues in honey and pollen samples collected in apiaries from NW Spain. *Analytica Chimica Acta*. 672(1– 2), 107-113. DOI: https://doi.org/10.1016/j.aca.2010.03.011

Kadar, A. and Faucon, J.P. (2006). Determination of traces of fipronil and its metabolites in pollen by liquid chromatography with electrospray ionization–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*. 54(26), 9741–9746. DOI: https://doi.org/10.1021/jf062035+

Korta, E., Bakkali, A., Berrueta, L.A., Gallo, B. and Vicente, F. (2001). Study of Semi-automated solid-phase extraction for the determination of acaricide residues in honey by liquid chromatography. *Journal of Chromatography A*. 930, 21–29. DOI: https://doi:org/10.1016/S0021-9673(01)01184-0

Kujawski, M.W. and Namiesnik, J. (2011). Levels of 13 multi-class pesticide residues in Polish honey determined by LC-ESI-MS/MS. *Food Control*. 22(6), 914–919. DOI: https://doi.org/10.1016/j.foodcont.2010.11.024

Masclet, P., Cachier, H., Liousse, H. and Wortham, V. (1995). Emissions of polycyclic aromatic hydrocarbons by savanna fires. *Journal of Atmospheric Chemistry*. 22, 41–54. DOI: https://doi.org/10.1007/BF00708180

Okedere, B. O. and Elehinafe, F. B. (2022). Occurrence of polycyclic aromatic hydrocarbons in Nigeria's environment: A review. *Scientific African*. 16 e01144. DOI: https://doi.org/10.1016/j.sciaf.2022.e01144

Pirard, C., Widart, J., Nguyen, B.K., Deleuze, C., Heudt, L., Haubruge, E., De Pauw, E., Focant, J.F. (2007). Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid Chromatography– tandem mass spectrometry. *Journal of Chromatography A*. 1152(1-2), 116–123. DOI: https://doi.org/10.1016/j.chroma.2007.03.035

Ramesh, A., Walker, S.A., Hood, D.B., Guillen, M.D., Schneider, K., Weyand, E.H. (2004). Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology*. 23, 301–333. DOI: https://doi.org/10.1080/10915810490517063

Rial-Otero, R., Gaspar, E. M., Moura, I., and Capelo J.L. (2007). Chromatographic based methods for pesticide determination in honey: An overview. *Talanta*. 71(2), 503–514. DOI: https://doi.org/10.1016/j.talanta.2006.05.033

Tahboub, Y.A., Zaater, M.F., and Barri, T.A. (2006). Simultaneous identification and quantitation of selected organochlorine pesticide residues in honey by full-scan gas chromatography-mass spectrometry. *Analytica Chimica Acta*. 558(1-2), 62–68. DOI: https://doi.org/10.1016/j.aca.2005.11.004

Vila-Escale´, M., Vegas-Vilarrubia, T. and Prat, N. (2007). Release of polycyclic aromatic compounds into a Mediterranean creek (Catalonia, NE Spain) after a forest fire. *Water Research*. 41(10), 2171–2179. DOI: https://doi.org/10.1016/j.watres.2006.07.029

Wang, X. Shu, B. Li, S. Yang, Z. and Qiu, B. (2017). QuEChERS followed by dispersive liquid–liquid microextraction based on solidification of floating organic droplet method for organochlorine pesticides analysis in fish. *Talanta*. 162, 90–97. DOI: https://doi.org/10.1016/j.talanta.2016.09.069

Wiest, A.B., Giroud, B., Fratta, C., Amic, S., Lambert, O., Pouliquen, H., Arnaudguilhem, C. (2011). Multiresidue analysis of 80 environmental contaminants in honey, honeybees, and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. *Journal of Chromatography A*. 1218(34), 5743-5756. DOI: https://doi.org/10.1016/j.chroma.2011.06.079

Yavuz, H., Guler, G.O., Aktumsek, A., Cakmak, Y.S., Ozparlak, H. (2010). Determination of some organochlorine pesticide residues in honey from Konya, Turkey. *Environmental Monitoring and Assessment*. 168(1-4), 277-283. DOI: https://doi:10.1007/s10661-009-1111-6