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Risk assessment of polycyclic aromatic hydrocarbons and organochlorine pesticides in olive oil in Jordan

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Abstract

Many organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) pose risks to human health; so, their levels in foods should be constantly monitored. In the present work, the potential health risks of 21 OCPs residues and 16 carcinogenic PAHs in Jordanian olive oil were evaluated. A total of 27 olive oil samples were obtained from nine olive mills in Jordan. The levels of PAHs and OCPs were evaluated by gas chromatography coupled with mass spectrometry detector. Among the studied pesticides, only *4,4*-Dichlorodiphenyldichloroethylen (*4,4*-DDE) was found in the tested samples. The estimated average dietary intake (EADI) and hazard risk index (HRI) were then assessed for the *4,4*-DDE. The estimated HRI value of *4,4*-DDE was less than 1, thus indicating no health risk to consumers. Regarding PAHs, the average concentration of 16 PAHs in the tested olive oil was 36.5 µg/kg. Health risks due to PAH contamination were estimated by determining the dietary daily intake (DDI) and toxic equivalent quotient (TEQ). The values ranged from 0.139×10^{-2} to 7.70×10^{-2} and 0.01 to 0.57 µg/kg for DDI and TEQ, respectively. Light PAHs were predominant in the samples, while no heavy PAHs were detected. The incremental lifetime cancer risk (ILCR) was estimated, and the values ranged from 0.1×10^{-7} to 5.62×10^{-7}, and none of the olive oil samples exceeded the limit value of 10^{-6}, thus indicating insignificant potential risk.

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Introduction

Olive trees are one of the most important fruit trees in the Mediterranean region (Al-Hiary, 2015). In the current era, global awareness of the benefits of olive oil, and the importance of including it in the diet have grown. Consequently, the use of olive oil is no longer confined to the Mediterranean region and olive-producing countries, but rather olive oil is now exported to all parts of the world. This led to an increased demand for olive oil production, and consequently an increase in the consumption of chemicals that help in improving the product; and in resisting pests, the most important of which is pesticides. Pesticides are classified into groups based on their chemical composition. One of the most important of these groups which has been widely used is organochlorine pesticide (OCP). OCPs have been used to control vectors of diseases, fungi, insects, parasites, or weeds at various phases of cultivation (Bempah and Donkor, 2011; Tilman *et al*., 2011; Stanton *et al*., 2018). Using high doses of OCPs or not following their application guidelines can lead to food safety issues, endanger consumers' health, and harm the environment. The high lipophilicity and biological stability of OCPs led to their easy accumulation in oilseeds, and consequently their transfer to olive oil during the pressing process, then passing to the consumers. Bioaccumulation, high persistence, and toxicity of OCPs may result in many types of human cancers, endocrine disruption, mutagenicity, and diseases of the nervous and respiratory systems (Jayaraj *et al*., 2016). In 2002, the Stockholm Convention on Persistent Organic Pollutants (POPs) listed several OCPs as potential environmental risk, and prohibited their use (Perkins, 2002). Although Jordan signed the Stockholm Convention, and banned the use of most of the OCPs, the peculiarity of these compounds in terms of their environmental stability and danger to humans and animals, as well as the possibility that some farmers may use them illegally, indicate that we need to

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constantly monitor their levels in foods and the environment.

People are becoming more conscious of the probable environmental and health troubles accompanying the accumulation of poisonous chemicals, principally in food commodities. It is therefore mandatory to monitor the levels of OCPs in some food products to be aware of the potential risks, and take appropriate measures to avoid them.

Nowadays, food monitoring programs for OCPs are implemented globally. Cui *et al*. (2020) explored the levels of OCPs in olive oil in China, and they obtained mean value of 28.3 µg/kg. In Egypt, El-Shinawy *et al*. (2017) found the mean value of 14 OCPs to be around 1.9 µg/L. In Jordan, limited studies have reported OCP existence in various kinds of foods including human milk, dairy products, as well as fruits and vegetables (Hamid *et al*., 2017; Tarawneh *et al*., 2019). However, as far as we know, very little studies have been conducted on the levels of OCPs in olive oil in Jordan.

Polycyclic aromatic hydrocarbons (PAHs) are a group of large number of organic compounds containing two or more fused aromatic rings (Princewill-Ogbonna and Adikwu, 2015). Several organisations such as the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) have estimated the probable cancer risk and toxicity of many PAHs (IARC, 2010; EPA, 2013). Despite the low toxicity of some PAHs, the EPA incorporated 16 PAHs in its list of priority pollutants based on repeated exposure as found in environmental monitoring samples including: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbFlu), benzo[*k*]fluoranthene (BkFlu), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IP), dibenz[*a,h*]anthracene (DBahA), and benzo[*ghi*]perylene (BghiP) (EPA, 2013); these compounds are denoted as 16EPA PAHs. According to international organisations, PAHs have carcinogenic and genotoxic effects upon long-term exposure. Other health problems may include kidney and liver failures, and respiratory difficulties (EFSA, 2008; EPA, 2013; Bertoz *et al*., 2021).

BaP has been used by the EFSA (2008) as a marker of PAHs in foods, and the lower limits were set based on the concentration of this compound. In 2011, the European Union (EU) modified previous regulation by introducing acceptable levels of ΣPAH4 (BaA, Chr, BbFlu, and BaP) and ΣPAH8 (BaA, Chr, BbFlu, BkFlu, BaP IP, DBahA, and BghiP) as more appropriate markers (EC, 2011). The European Commission has imposed limits of 2.0 µg/kg for BaP, 5.0 µg/kg for PAH8, 10.0 µg/kg for PAH4, and 25 µg/kg for total PAHs in edible oils (EC, 2011). The main way that non-smokers are exposed to these compounds is through foods such as meats, vegetables, fruits, and oils.

Several studies have been conducted to test the contamination of different types of oils with PAHs. The researchers in these studies tried to justify the presence of PAHs in the oils, and there were different points of view. Sakin *et al*. (2022) reported a level of 222 µg/kg of 16 PAHs in olive oil samples from Turkey. PAHs have been found in some edible oils in Nigeria which may have been formed during the production process of these oils (Princewill-Ogbonna and Adikwu, 2015). It is also possible that the cause of olive oil contamination with PAHs is the exposure of the oil mash to diesel fumes or through the deposition of dust and particulate matter contaminated with PAHs on olive fruits (Bertoz *et al*., 2021). Bogusz *et al*. 2004 suggested that the use of contaminating solvents during plant mash extraction, and high temperature during solvent evaporation caused PAH contamination. In another study, Romanić *et al*. (2011) showed that the olive tree blooms in May and the fruits develop on the tree within about five months, during which the waxy surface of the olives may come into contact with OCPs and PAHs from surrounding air. Therefore, these residues will frequently accumulate over time in olive fruits and edible oils. A few studies were published in Jordan related to the determination of PAHs in olive oil (Krajian and Odeh, 2018).

The potential risk of OCPs and 16EPA PAHs should be studied and evaluated to ensure consumer protection. Therefore, several studies in different countries have reported the human health risk assessments of OCPs and PAHs in different types of foods (Karthik and Vijayarekha, 2018; Lee *et al*., 2019). To the best of our knowledge, however, there are no studies evaluating OCP and 16EPA PAH levels in Jordanian olive oils, and no data on the potential health risks of these compounds in olive oils have been reported.

The present work was intended to provide information on the levels of PAHs and OCPs, and to

establish the distribution of PAHs and OCPs in olive oils from nine governorates in Jordan. In addition, the present work aims to estimate the effect of OCPs and 16EPA PAHs on human health even when exposed to low levels by consuming the olive oils. The risk assessment of dietary exposure to PAH and OCP residues in olive oils was accomplished by estimation of the risk hazard index (HRI) and evaluation of incremental lifetime cancer risk (ILCR) for OCPs and PAHs, respectively. The results will help give a clear picture of the potential health risks of the existence of OCP residues in olive oils in Jordan, thus helping the competent authorities in enacting legislation to maintain consumer health, improve agricultural resource management, and avoid economic losses.

Materials and methods

Chemicals

Certified reference materials were used through the whole analysis and validation process. PAH standard mixture (2,000 µg/L) and the OCP standard mixture (200 µg/L) were purchased from Supelco (Bellefonte, USA). PAH standard mixture contained: Nap, Acy, Ace, Fl, Phe, Ant, Flu, Pyr, BaA, Chr, BbFlu, BkFlu, BaP, IP, DBahA, and BghiP. OCP standard mixture contained: 2,4,5,6-Tetrachloro-m-xylene (TCMX), α-BHC, γ-BHC (lindane), β-BHC, δ-BHC, heptachlor (HC), aldrin (Ald), heptachlorepoxide (HE), γ-chlordane (γ-Chlo), endosulfan I alpha, α-chlordane (α-Chl), dieldrin, endrin, *4,4*-DDD, endosulfan II beta, *4,4*-DDT, *4,4*-DDE, endrin aldehyde, endosulfan sulphate, decachlorobiphenyl (DCB), and endrin ketone. 1-fluronaphthalene (99% purity) which was used as internal standard (I.S) in PAH determination, and isodrin (99% purity) which was used as internal standard (I.S) in OCP determination, were purchased from Supelco (Bellefonte, USA). All solvents (acetonitrile; ACN, acetone; AC, and *n*-hexane) used were of GC grade.

Sampling sites and samples

Olive oil samples were acquired from nine olive mills distributed over nine different governorates in Jordan namely Ajloon, Karak, Salt, Zarqa, Amman, Irbid, Jarash, Madaba, and Tafela (Figure 1). These mills usually receive olives that are harvested from farms owned by the owner of the mill, and from other nearby farms. Three samples from each mill with a total of 27 samples were collected during the months of October and November, 2018. Samples were collected in dark glass bottles with volume ranging between 100 - 110 mL. All samples were sealed and kept refrigerated at 4°C until further analyses.

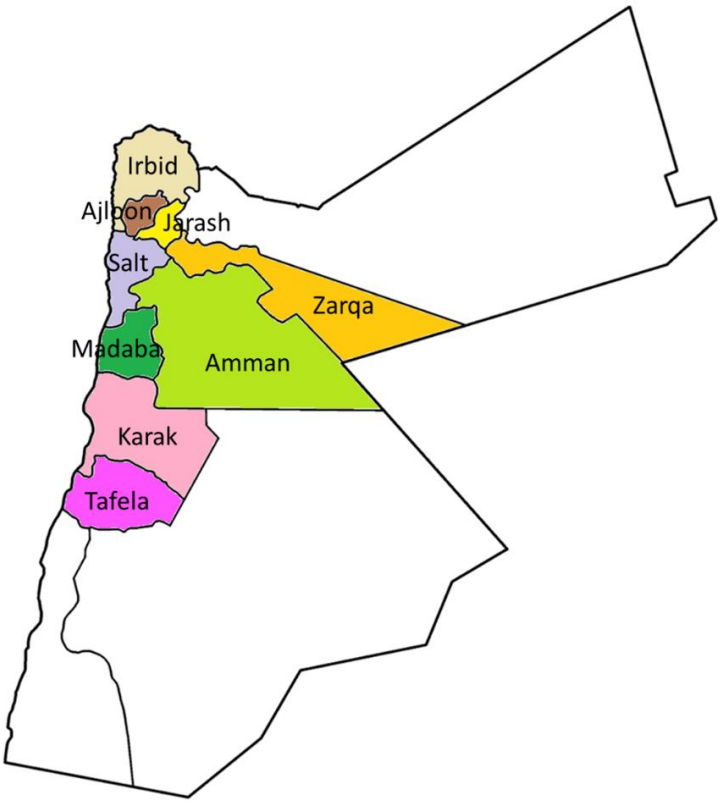

Figure 1. Sampling locations of olive oil in Jordan.

Sample extraction

The olive oil samples were extracted according to Tarawneh *et al.* (2020). The oil in the three bottles collected from each mill was homogenised in the laboratory to ensure that the sample was representative. Next, 2.0 g of sample was placed in a 15 mL polypropylene, and treated with 10 mL of ACN:AC mixture (6:4). Then, the tube was shaken vigorously by hand for 10 min, followed by 0.5 h sonication (Aquasonic 250D). The tube was sonicated for 30 min, and centrifuged for 4 min at 4,000 rpm (Labocen Scanspeed 1236R). The supernatant was separated, and the extraction was repeated thrice. The extracts were combined and subjected to cleaning step by solid phase extraction (SPE). C18-SPE (Supelco, USA) cartridge was conditioned with ACN followed by 5.0 mL of (ACN:AC = 6:4). Next, 10 mL of the extract containing OCPs and PAHs was percolated through the SPE cartridge, and eluted with 12.0 mL of ACN at a flow rate of 3.5 mL/min. After elution, the solvents were evaporated in rotatory evaporator (Heidolph, LABORTA 4000). Finally, the residue was reconstituted with 1.0 mL of *n*-hexane containing 1 µg/mL of 1-fluorphthalate as I.S in PAH determination, or 25 ng/mL isodrin in OCP determination.

Chromatographic analysis

All target analytes were analysed according to Tarawneh *et al.* (2020) using gas chromatography with mass spectrometry detector (GC/MS) (Shimadzu QP2010 Ultra, Japan). HP-5Ms fused capillary column (30 m × 0.25 mm, 0.25 µm) was used for separation (Supelco, USA). The determination was accomplished by the selected ion monitoring (SIM) mode. The quadrupole ion analyser was operated on electron ionisation (EI) mode. For OCPs, the temperature program was as follows: 70°C for 2 min, 150°C at 25°C/min, then 200°C at 3.0°C/min, finally increased to 280°C at 9.0°C/min, then held for 10 min. For PAHs, the GC was programmed as follows: 70°C for 1.2 min, 280°C at 10°C/min, and held for 18 min. The injector was set on splitless injection mode at temperature 280°C. The temperature of the transfer line was 250°C, and fed into a 70-eV EI source at 200°C, for both OCPs and PAHs. Helium (purity 99.999) was employed as the carrier gas at flow rate of 1.0 mL/min.

Method validation

The method was validated with respect to linearity, precision, recovery, limit of detection (LOD), and limit of quantitation (LOQ). For linearity test, a series of PAH (1.0 - 100.0 µg/kg) and OCP (5.0 - 100.0 µg/kg) standard mixture solutions were injected into GC/MS in triplicate. Then, peak areas for each compound were measured relative to those of the internal standard, and plotted against concentrations to construct the calibration curves. The precision of the instrument was assessed by injecting standard solutions (25, 50, and 100 µg/L) of both PAHs and OCPs, each thrice. The recovery was evaluated by spiking a blank sample of olive oil with three different concentrations (25.0, 50.0, and 100.0 µg/L) of PAHs and OCPs, and analysed thrice. Limits of detection (LODs) and limits of quantitation (LOQs) were established by sequential dilution of standard solutions. LODs were designated as the concentration at which a signal-to-noise ratio of 3, and LOQs were designated as the concentration at which a signal-to-noise ratio of 10.

Estimation of dietary exposure, health risk, and risk assessment

The results from sample analysis were used for the assessment of consumers' risk upon exposure to olive oil contaminated with PAHs and OCPs.

The health risk of PAHs in olive oil was estimated by applying human intake models. The dietary daily intakes (DDI) of PAHs from ingestion of contaminated olive oil were evaluated. Cancer risks were also estimated by determining the toxic equivalent quotients (TEQs) and the increased lifetime cancer risk (ILCR).

The toxic equivalent quotients TEQs, expressed also as benzo[a]pyrene equivalent BaPeq, were calculated by multiplying the amount of PAH congener in each olive oil sample with its TEF as reported by Nisbet and La Goy (1992) using Eq. 1:

$$\text{TEQs} = \sum C_i \times \text{TEFi} \quad \text{(Eq. 1)}$$

where, C_i = concentration of PAHs congener i (µg/kg), while TEFi = toxicity equivalence factor for each PAHs congener i. TEF values are 1 for BaP and DBahA; 0.1 for BaA, BbFlu, IP, and BkFlu; 0.01 for Chr and BghiP; and 0.001 for Ace, Acy, Fl, Flu, Phe, Ant, and Pyr (Yousefi *et al*., 2018).

The daily dietary intake of (DDI) and the increased lifetime cancer risk (ILCR) for PAHs for each olive oil sample were estimated using Eqs. 2 and 3 (Xia *et al*., 2010):

$$DDI = \frac{\sum TEQs \times EF \times ED \times IR}{BW \times AT} \quad \text{(Eq. 2)}$$

$$ILCR = DDI \times SF \times CF \quad \text{(Eq. 3)}$$

In Eq. 2, DDI = daily dietary intake, ΣTEQs = total toxic equivalent quotients for all the congener, EF = exposure frequency (360 days/years), ED = exposure duration (70 years for adult), IR = amount of ingestion of olive oil per day (9.53 g/person/day), BW = average body weight for male adult (70 kg), and AT = average lifespan (25,550 days). While in Eq. 3, SF = oral cancer slope factor (7.3 per mg/kg/day), and CF = conversion factor (10^{-6} mg/ng).

The EPA (2013) designated that if ILCR value is less than 10^{-6}, cancer risk is trivial. However, if ILCR is more than 10^{-4}, risk of cancer is unacceptable, and when ILCR index is between 10^{-6} and 10^{-4}, carcinogenic risk assessment is acceptable for customers.

To assess the health risk of OCP residues in olive oils in the present work, WHO guidelines were followed (WHO, 2017). The estimated average daily intake (EADI) was calculated to assess Jordanian consumers' exposure. Then, the value of EADI was compared with health safety limits such as the acceptable daily intake (ADI). ADI refers to the amount of specific substances such as OCPs in foods such as olive oil, that can be ingested (orally) daily over a lifetime without posing a significant health risk to the consumer. Then, the dietary daily intake of OCPs residues could be predicted. The EADI of OCP residues (expressed in mg/kg body weight/day) were calculated using Eq. 4:

$$EADI = Fi \times Ri \times Pi \quad \text{(Eq. 4)}$$

where, Fi = oil consumption rate in Jordan (kg/day) derived from monitoring data (9.53×10^{-3} kg/day); Ri = OCPs residue level (mg/kg) in the olive oil; Pi = processing factor for edible olive oil, the influence of the processing not determined in the present work (Pi = 1) (IRIS, 2017; WHO, 2017). The oil consumption rate was chosen by referring to the consumption data from the Department of Statistics on household expenditure in Jordan (Hundaileh and Fayad, 2019).

The hazard risk index (HRI) was calculated by dividing the EADI by ADI for each pollutant as shown in Eq. 5 (WHO, 2017):

$$HRI = EADI \div ADI \quad \text{(Eq. 5)}$$

Hazard Risk Index (HRI) > 1 indicates that the food in question poses a risk to consumers. If the HRI < 1, the food in question is considered safe for the health of the consumer (Cui *et al*., 2020).

Results and discussion

Method performance

In the present work, the levels of 21 OCPs and 16 PAHs were estimated using GC/MS method. Figures 2a and 3a show representative chromatograms of OCPs of PAHs, respectively, where the standard mixtures were spiked in blanks. The method was validated to ensure that its performance parameters were suitable to the circumstances in which it would be applied. Full validation results are presented in Tables 1 and 2 for both PAHs and OCPs, respectively. All the validation results were satisfactory and acceptable with respect to European Union guidelines SANTE 12682/2019 (Taghizadeh *et al*., 2021). Linearity of the method was reflected by the value of coefficient of determination which was higher than 0.99 for all the tested compounds. Precision was assured by calculating the coefficient of variation (CV%). The mean of CV% values were 4.30 and 2.78% for PAHs and OCPs, respectively. This indicated food precision since the accepted criteria demanded that CV% not exceeding 20%. Recovery of the spiked samples ranged from 70.1 - 96.0 for PAHs, and from 72.1 - 95.3 for OCPs. These values were within guideline requirements (70 - 120%). The mean values of LODs and LOQ were 0.278 and 1.16 µg/kg for PAHs and OCPs, respectively. While the mean LOQ values were 0.928 and 3.88, for PAHs and OCPs, respectively.

The validated method was used to determine the levels of 21 OCPs and 16 PAHs in olive oil samples obtained from nine olive mills in Jordan, and these levels were compared with international maximum residue levels (MRLs). These results were also used to determine the extent of the health risk arising from the consumption of this oil by estimation of HRI and ILCR values.

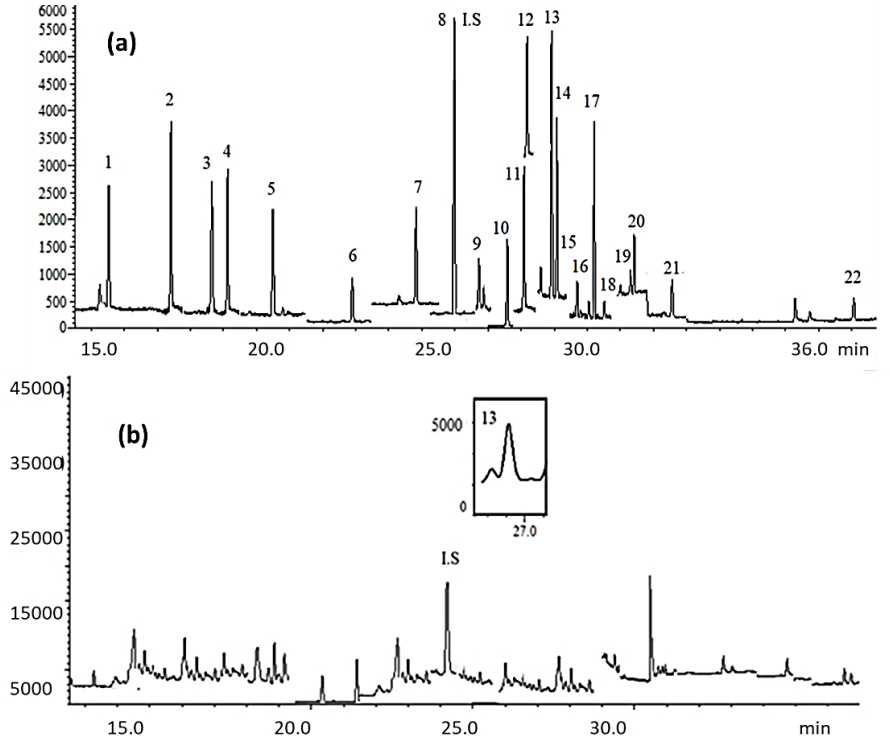

Figure 2. (a) Representative chromatogram of 21 OCPs standard mixture spiked in blank: 1 = TCMX; 2 = α-BHC; 3 = γ-BHC; 4 = β-BHC; 5 = δ-BHC; 6 = HC; 7 = Ald; 8 = I.S; 9 = HE; 10 = γ-Chlo; 11 = endosulfan I alpha; 12 = α-Chl; 13 = dieldrin; 14 = endrin; 15 = *4,4*-DDD; 16 = endosulfan II beta; 17 = *4,4*-DDT; 18 = *4,4*-DDE; 19 = endrin aldehyde; 20 = endosulfan sulfate; 21 = DCB; and 22 = endrin ketone. **(b)** Chromatogram of OCPs of olive oil sample obtained from Karak olive mill.

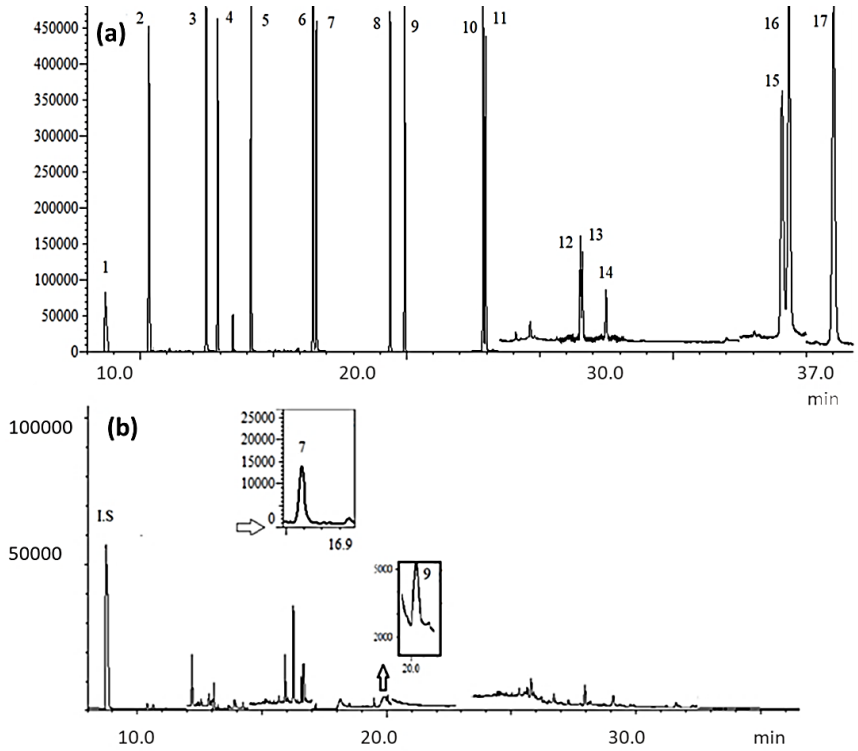

Figure 3. (a) Representative chromatogram for 16EPA PAHs standard mixture spiked in blank: 1 = I.S; 2 = Nap; 3 = Acy; 4 = Ace; 5 = Fl; 6 = Phe; 7 = Ant; 8 = Flu; 9 = Pyr; 10 = BaA; 11 = Chr; 12 = BbFlu; 13 = BkFlu; 14 = BaP; 15 = IP; 16 = DBahA; and 17 = BghiP. **(b)** Chromatogram of PAHs of olive oil sample obtained from Amman olive mill.

Table 1. Name, retention time, slop, intercept, R^2, CV%, recovery, LOD, and LOQ for 16 PAHs.

Peak No.	Compound name	Average retention time (min)	Slope	Intercept	R^2	CV%	Average recovery%	LOD (µg/kg)	LOQ (µg/kg)
1	1-Flu (I.S)	8.77	-	-	-	-	-	-	-
2	Nap	10.4	0.0109	0.0143	0.998	2.03	81.7	1.26	4.20
3	Acy	12.5	0.0089	0.0107	0.999	3.48	70.1	0.28	0.93
4	Ace	12.9	0.0052	0.0008	0.999	1.94	71.0	0.19	0.63
5	Fl	14.2	0.0059	0.0043	0.999	2.37	82.5	0.25	0.83
6	Phe	16.5	0.0075	0.013	0.999	2.53	94.3	0.23	0.77
7	Ant	16.6	0.0066	0.0042	0.999	3.01	92.7	0.21	0.70
8	Flu	19.5	0.0062	0.0014	0.999	4.80	79.5	0.26	0.87
9	Pyr	19.7	0.0072	0.0007	0.998	3.27	94.5	0.12	0.40
10	BaA	22.9	0.003	0.0007	0.999	4.97	93.0	0.17	0.57
11	Chr	23.1	0.0026	0.0008	0.999	6.13	89.2	0.19	0.63
12	BbFlu	26.4	0.0016	0.0006	0.999	5.38	96.0	0.23	0.77
13	BkFlu	26.5	0.0014	0.0003	0.999	7.01	86.9	0.07	0.23
14	BaP	27.7	0.0013	0.0002	0.999	5.41	99.0	0.26	0.87
15	IP	34.1	0.0008	0.00005	0.997	5.40	92.2	0.29	0.97
16	DBahA	34.4	0.0007	0.00008	0.999	5.70	91.0	0.17	0.57
17	BghiP	36.5	0.001	0.0001	0.999	5.20	93.4	0.27	0.90
		Mean			0.999	4.30	84.0	0.278	0.928

Table 2. Name, retention time, slop, intercept, R^2, CV%, recovery, LOD, and LOQ for 21 OCPs.

Peak no.	Name	Average retention time (min)	Slope	Intercept	R^2	CV%	Average recovery%	LOD (µg/kg)	LOQ (µg/kg)
1	TCMX	13.6	0.0721	0.0047	0.999	2.19	95.3	1.38	4.60
2	α-BHC	15.4	0.077	0.0018	0.999	2.40	95.6	1.42	4.73
3	γ-BHC	16.7	0.0545	0.0092	0.999	2.81	91.5	1.23	4.10
4	β-BHC	17.2	0.0445	0.0245	0.999	2.69	92.6	1.37	4.57
5	δ-BHC	18.6	0.0432	0.005	0.999	3.08	94.3	1.03	3.43
6	HC	20.8	0.0237	0.0005	0.999	4.26	74.6	1.07	3.57
7	Ald	22.9	0.0516	0.0067	0.998	1.99	72.1	1.32	4.40
8	Iso (I.S)	24.1	-	-	-	-	-	-	-
9	HE	24.7	0.0204	0.009	0.991	1.80	86.1	0.86	2.87
10	γ-Chlo	25.6	0.0101	0.0135	0.998	2.06	87.5	1.26	4.20
11	Endosulfan I alpha	26.10	0.0161	0.0133	0.999	1.88	80.7	1.31	4.37
12	α-Chlo	26.2	0.012	0.007	0.993	2.29	75.5	0.86	2.87
13	Diel	27.1	0.0655	0.0066	0.999	2.36	79.7	1.19	3.97
14	Endrin	27.8	0.0128	0.0066	0.997	2.47	83.1	1.28	4.27
15	pDDD	28.2	0.139	0.0125	0.999	3.29	90.9	1.36	4.53
16	Endosulfan II beta	28.4	0.0101	0.0094	0.997	1.68	92.8	0.69	2.30
17	pDDT	28.6	0.0205	0.0168	0.998	3.62	81.1	1.34	4.47
18	pDDE	28.8	0.1134	0.0167	0.999	3.13	93	1.17	3.90
19	Endrin aldehyde	29.3	0.0081	-0.0099	0.999	3.53	89.9	0.98	3.27
20	Endosulfan sulfate	29.5	0.0165	0.0113	0.999	3.11	80.6	0.63	2.10
21	DCB	30.1	0.0086	0.0062	0.999	3.61	91.3	1.29	4.3
22	Endrin ketone	36.2	0.0.171	0.0152	0.999	4.09	93.4	1.39	4.63
		Mean			0.998	2.78	86.7	1.168	3.88

Level of OCPs and risk assessment

Among the 21 OCPs examined, only one with *4,4*-DDE was detected. It was present in three of the analysed olive oil samples. Figure 2b shows a GC/MS chromatogram of *4,4*-DDE in olive oil sample from Karak. Table 3 shows the *4,4*-DDE concentrations in different olive oil samples. The highest concentration was in Karak mill (21.4 µg/kg).

By comparing the concentrations of *4,4*-DDE that we obtained in the present work with the levels recorded in previous studies in other countries, we found that there was a clear difference, and that the levels of *4,4*-DDE that were recorded in Jordan were significantly higher than those recorded in Croatia (0.44 µg/kg; Romanić *et al*., 2011), Spain (1.8 µg/kg; Yagüe *et al*., 2005), and Italy (0.59 µg/kg; Guerranti *et al*., 2008); and not detected in Egypt (El-Shinawy *et al*., 2017). On the other hand, other compounds of the OCPs were detected in the samples of those countries that were not found in the Jordanian olive oil samples. This discrepancy might have been due to the different practices of farmers, their awareness of the type of pesticides that must be applied, their commitment to the permissible quantities, and the appropriate application times.

For *4,4*-DDE, DDT can be degraded by solar radiation or metabolised in living organisms, giving *4,4*-DDE and DDD as major metabolites (Bempah and Donkor, 2011). *4,4*-DDE is the major metabolite of DDT in oxic environment (with oxygen), whereas the major metabolite in anoxic environment is DDD (Tolosa *et al*., 1995). Hitch and Day (1992) reported that once the ratio of DDD/DDE is lower than 1, this indicates that aerobic conditions are prevalent for the biodegradation of DDT, whereas, if the ratio is greater than 1, the biodegradation more probably occurs under anaerobic conditions. Therefore, in the present work, the potential source of *4,4*-DDE contamination in olive oil samples was the biodegradation of DDT under aerobic conditions.

Codex maximum residue limits (MRLs) for pesticides were used to judge the estimated residue levels in several previous studies, and we followed them in the present work as well. The MRL value for *4,4*-DDE is 0.05 mg/kg. In the present work, none of the examined olive oil samples exceeded this limit, thus indicating the absence of potential risks (WHO, 2017). Inappropriately, the rate of violation may vary depending on the maximum residue limits for consumer safety assessment. Therefore, the estimated average daily intake (EADI) was calculated to evaluate the health risks of OCP residues in olive oil samples in the present work. The calculated values for EADI intake per body weight and health risk index (HRI) are summarised in Table 3.

The mean of EADI in all the tested samples was 0.124 µg/kg/day. The HRI was calculated for each sample, and the results are shown in Table 3. We observed that the EADI of *4,4*-DDE was below the ADI limit, and the HRI was always less than 1. Therefore, it was assumed that *4,4*-DDE with such levels not exceeding reference values could not have the potential for systemic toxicity to humans, which meant that it would not pose an immediate danger to human health. Cui *et al*. (2020) reported EDI value of 0.000656 µg/kg/day for 11 OCPs in olive oil samples purchased from local markets in China with HRI value less than 1, thus also indicating low human health risk. In Egypt, researchers reported EDI value of 0.000006 µg/kg/day for 14 OCPs in olive oil samples from El Minia governorate (El-Shinawy *et al*., 2017).

Table 3. Levels of OCPs (4.4-DDE) in Jordanian olive oil samples and their health risk assessment.

Olive mill	4.4-DDE concentration range (µg/kg)	Mean 4.4-DDE concentration (µg/kg)	EADI (µg/kg/day)	Intake per body weight × 10^{-2} (µg/kg(bw)/day)	(HRI) × 10^{-2}	HR
Karak	12.13 - 21.4	15.7 ± 0.33	0.149	0.248	0.025	No
Madaba	8.33 - 15.1	10.0 ± 0.28	0.095	0.158	0.016	No
Tafela	11.02 - 17.8	13.6 ± 0.14	0.129	0.215	0.022	No
Mean		13.1	0.124			

ADI for 4.4-DDE is 10 µg/kg(bw)/day

Level of PAHs in olive oil, dietary exposure, and health risk estimation

Among the 16EPA PAHs that we studied, eight congeners were detected in the analysed samples namely Nap, Fl, Phe, Ant, Flu, Pyr, BaA, and Chr. Figure 3b shows a GC/MS chromatogram of the detected PAHs in olive oil sample from Amman olive mill. Figure 4 shows the distribution of these eight compounds in the different samples. The values in Figure 4 are the mean levels of the individual EPAs PAHs in olive oils based on the regions from which the samples were collected. Table 4 shows the values for the total 16EPA PAHs (ΣPAH), genotoxic PAHs (ΣPAH8), and 4 PAHs (ΣPAH4) (mean ± standard deviation for each compound). Note that out of the eight PAH8 compounds, only four compounds were identified in our samples, and they are the same four compounds designated as PAH4, and therefore ΣPAH4 were the same as ΣPAH8. The compounds Acy, Ace, BbFlu, BkFlu, BaP, IP, DBahA, and BghiP were not detected in any of the analysed olive oil samples.

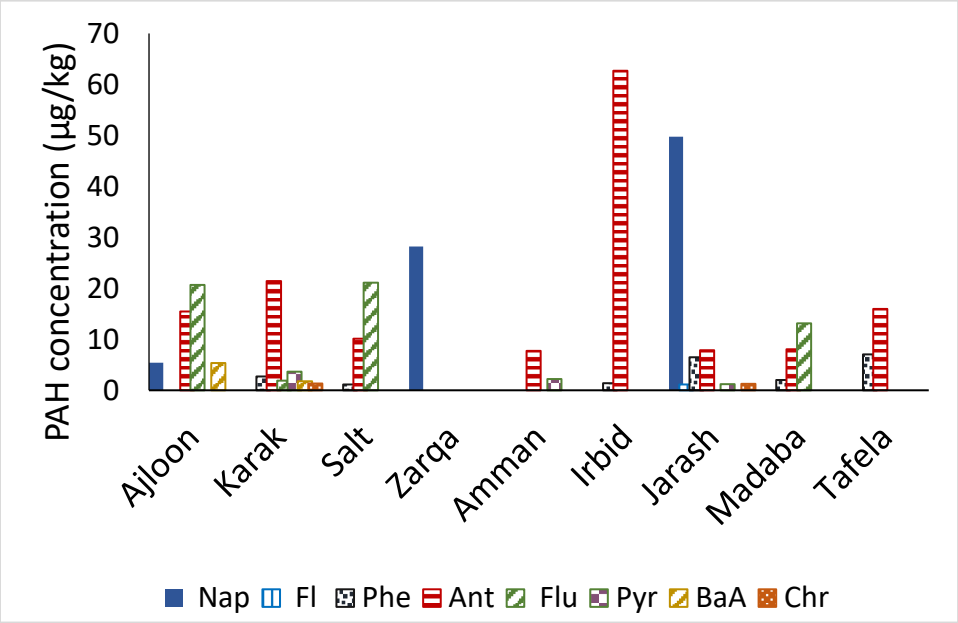

Figure 4. Distribution of PAHs in olive oil samples from different olive mills in Jordan.

Table 4. ∑4PAHs, ∑8PAHs, ∑16PAHs, TEQs, DDI, and ILCR for PAHs in olive oil samples.

Olive mill	Σ8PAHs (= 4PAHs) (µg/kg ± s)	Σ16PAHs (µg/kg ± s)	TEQs or B[a]Peq (µg/kg)	DDI*10^{-2} ng/kg/day	ILRC*10^{-7}
Ajloon	5.32 ± 0.32	46.82 ± 1.19	0.574	7.70	5.62
Karak	3.07 ± 0.43	32.8 ± 1.50	0.220	2.95	2.15
Salt	N.D.	32.4 ± 1.18	0.0324	0.435	0.318
Zarqa	N.D.	28.2 ± 0.23	0.0282	0.378	0.276
Amman	N.D.	9.95 ± 0.84	0.00995	0.134	0.0975
Irbid	N.D.	64.0 ± 0.57	0.0641	0.860	0.628
Jarash	1.30 ± 0.76	67.7 ± 1.04	0.0793	1.07	0.777
Madaba	N.D.	23.2 ± 0.83	0.0232	0.311	0.227
Tafela	N.D.	23.0 ± 1.17	0.0230	0.309	0.225
Mean	3.23	36.5			

N.D. = not detected.

The mean concentration of Σ16PAHs in the examined olive oil samples was 36.5 µg/kg from nine different locations. In general, the quantities of PAHs found in the olive oil samples were quite low. This can be attributed to the fact that preparing olives for pressing is not accompanied by processes that require raising the temperature, such as roasting or drying, in addition to the fact that the pressing process itself does not require heating to high temperatures. Another factor affecting the levels of these compounds is that most of the examined oil samples were taken from mills far from industrial cities.

Significant variation in PAH concentrations in olive oil samples can be observed across the different mills where the concentrations ranged from 1.12 µg/kg (Phe) to 62.70 µg/kg (Ant). Figure 4 shows that regardless of the Σ16PAHs concentration level, Phe and Ant were the most abundant in all investigated olive oil samples with concentrations ranging from 1.12 - 7.06 and 7.75 - 62.7 µg/kg, respectively. This can be attributed to the lipophilicity of these two compounds (EPA, 2013), and their high solubility in the oily environment (Karcher, 2013).

In the present work, PAHs ratios were used to determine the sources of PAHs. Yunker *et al*. (2011) stated that ratio (Ant/Phe + Ant) < 0.1 is an indicator of the petroleum source, while a ratio > 0.1 suggests combustion as the major source of PAHs. In addition, a ratio of fluoranthene to fluoranthene plus pyrene (Flu/Flu + Py) of less than 0.40 indicates that the petroleum source is predominant. Based on our results the ratio (Ant/Phe + Ant) was 0.88, and thus the combustion of liquid fossil fuels (automobiles and crude oil) was the predominant source of anthracene and phenanthrene. While the ratio (Flu/Flu + Py) was 0.89, thus indicating that combustion was the major source of fluoranthene and pyrene as well.

The fluorene was detected only in one sample taken from the Jarash olive mill (1.13 µg/kg). These results were slightly lower than those reported by authors in similar study in China who determined values in the range of 3.36 - 3.70 µg/kg (Qin *et al*., 2011).

BaP was not detected in any of the examined samples in the present work; thus, Σ4PAH and Σ8PAH systems were used as good indicators of PAHs in olive oil samples. The Σ4PAH values in all samples were below the suggested tolerance limit of 10.0 µg/kg in all olive oil samples (Table 4). Meanwhile, the Σ8PAH values ranged between 1.29 and 5.32 µg/kg. Only Ajloon samples slightly exceeded the suggested tolerance limit of 5.0 µg/kg in olive oil samples. By comparing the levels of PAHs in olive oil samples obtained in the present work with those of previous studies, we noted inconsistencies in the results depending on the country in which the study was conducted, and even in studies conducted in the same country, some differences were observed. For example, in Turkey, Sakin *et al*. (2022) reported a level of 222 µg/kg for Σ16 PAHs, while Ergönül and Sánchez (2013) reported a value of 30.7 µg/kg. In Spain, Σ16 PAHs was 5.40 µg/kg (Rascón *et al*., 2018), and in Tunisia 63.7 µg/kg (Krajian and Odeh, 2018). This discrepancy can be attributed to the different temperatures at which the olives were pressed. In addition, the location of the mills or olive mills from which the samples were collected, the extent of their proximity was to the main streets, and the possibility of PAHs contamination from vehicle exhaust could also stand as possible reason.

The results showed that ∑16PAHs in six areas exceeded the maximum allowable limit of 25 µg/kg. The value of ∑16PAHs in Madaba and Tafela areas was within the permissible limits with values of 23.16 and 23.04 µg/kg, respectively. While in the capital Amman, the ∑PAHs were significantly less than the suggested limit, with a value that did not exceed 9.95 µg/kg. The increase in the concentrations of PAHs in the six olive mills was due to the proximity of these mills to the public street, and thus the olive fruits in them were more susceptible to car exhaust and incomplete combustion products.

The results showed that light PAHs (2 - 4 rings) were predominant (100% of the total amount of PAHs) in all tested olive oil samples. Meanwhile, none of the heavy PAHs (5 - 6 rings), which are considered the most carcinogenic, were detected; these results agreed with previously published results (Wu and Yu, 2012).

Among the 16 studied PAHs, anthracene was detected in at least 66.7% of samples (n = 18), followed by phenanthrene which was detected in 37.04% of samples (n = 10), naphthalene in 22.2% of samples (n = 10), fluorene in 18.5% of samples (n = 5), and pyrene, chrysene, and benz[a]anthracene which were detected in 14.8% of samples (n = 4).

We used the values of ∑16PAHs in Table 4 to estimate the health risks resulting from the ingesting of olive oil contaminated with PAHs by calculating TEQs, DDI, and ILCR using Eqs. 1, 2, and 3, respectively, and we included the results in Table 4. Results showed that Ajloon samples had the highest

TEQs (0.574 µg/L), while Amman samples had the lowest TEQs (0.00995 µg/L).

Based on the international guidelines, an ILCR of 10^{-6} or less indicates that the risk level is considered low, while an ILCR of 10^{-4} indicates that the potential risk will be increased (Xia *et al*., 2010). The mean value of ILCR in the present work was estimated to be 1.15×10^{-7} as shown in Table 4, and therefore none of the tested olive oil exceeded the specified ILCR limit. Hence, the possibility of cancer occurring by ingesting olive oil contaminated with PAHs in the samples examined was considered to be very low. Therefore, the consumption of olive oil from the olive mills assessed in the present work did not pose a significant risk of cancer. The ILCR was reported at 2.18×10^{-6} by Sakin *et al*. (2022) who studied the cancer risk of PAHs in olive oil in Turkey, and indicated that the values were also within acceptable limits for safe consumption.

Conclusion

In the present work, we determined the concentrations of 21 OCPs and 16EPA PAHs in Jordanian olive oil samples. We also evaluated the health risks arising from the consumption of this oil as a result of the presence of these compounds. For pesticides, only *4,4*-DDE was found in the tested samples, which is a major metabolite of DDT, and has been banned internationally. This indicated that some farmers were still using this pesticide to control pests. Fortunately, the levels of *4,4*-DDE in samples did not pose a serious health risk as revealed by risk assessment results. Regarding 16EPA PAHs, their levels varied in the different samples; exceeding the permissible limits in some samples, while not exceeding the permissible limits in others. Fortunately, the level of risk towards health was considered low. The ILCR and DDI of all olive oil samples were studied following international programs. Depending on the sampling locations, the reported levels of PAHs fluctuated, and the calculated ILCR values fluctuated accordingly. ILCR values decreased in the following order in olive oil: Ajloon > Jarash > Karak > Irbid > Zarqa > Salt> Madaba ≈ Tafela > Amman. The risks for all samples were lower than the 10^{-6} limit value. Therefore, a Jordanian olive oil consumer is not at risk of exposure to carcinogenic OCPs and 16EPAs PAHs from olive oil.

The present work can be considered as a preliminary study to assess the risk and presence of OCPs and PAHs residues in Jordanian olive oil, which is the main edible fat source used in the Mediterranean area. Monitoring of these levels must continue to protect consumer health. These results can be used for effective environmental management, and to draw the attention of legislators to the need to take precautionary measures so that pollutants do not exceed the internationally permissible limits. On the other hand, we must also consider the limit of the present work, as it included only nine of the olive mills in Jordan out of nearly 140 olive mills. Therefore, we must be wary of generalising, and making sure that there are broader studies that include a larger number of samples from different sources, and the frequency of sampling must be increased. It is also desirable that the studies included different regions of the world. The method of evaluating the risk of exposure to trace levels of several contaminants simultaneously should also be considered. We also need more understanding of the metabolic mechanism of various pollutants in the human body. The risks arising from exposure to pollutants with different pathways should also be investigated.

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