Environmental Science and Pollution Research (2021) 28:39723–39741
https://doi.org/10.1007/s11356-021-13348-0

RESEARCH ARTICLE

Polycyclic aromatic hydrocarbons, pesticides, and metals in olive: analysis and probabilistic risk assessment

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Received: 31 October 2020 / Accepted: 3 March 2021

Abstract

In the present study, levels of 22 pesticides, eight metals, and 16 polycyclic aromatic hydrocarbons (PAHs) in 1800 Iranian olive samples (20 cultivars from six different cultivation zones), were determined; then, health risk posed by oral consumption of the olive samples to Iranian consumers was assessed. Quantification of PAHs and pesticides was done by chromatography-mass spectrometry (GC-MS), and metal levels were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES). There were no significant differences among the cultivars and zones in terms of the levels of the tested compounds. Target hazard quotients (THQ) were <1.0 for all pesticides, and total hazard indices (HI) indicated *di minimis* risk. At the 25th or 95th centiles, Incremental Life Time Cancer Risks (ILCRs) for carcinogenic elements, arsenic, and lead and noncarcinogenic metals did not exhibit a significant hazard (HI <1.0 for both cases). At the 25th or 95th centiles, ILCR and margins of exposure (MoE) for PAHs indicated *di minimis* risk. Sensitivity analysis showed that concentrations of contaminants had the most significant effect on carcinogenic and noncarcinogenic risks.

Keywords Agricultural management · Cancer · Toxicity · Food · Safety · Uncertainty assessment

Introduction

In the classic risk assessment, risk assessors examine health risks posed by exposure to individual compounds. In the last few years, considerable attention has been paid to determine and quantify risks posed by simultaneous exposure to several contaminants to human health. Therefore, risk assessment frameworks have been updated accordingly to better assess health risk based on real-life exposure scenarios (Taghizadeh et al. 2019b).

Uncertainties may affect risk determination, and their frequency and degree depend on insufficient data, values for parameters, and models applied. Since such uncertainties can limit applicability of assessments, it is useful to report ranges of risks. During the 1980s, the United States Environmental Protection Agency (USEPA) developed approaches to

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Responsible Editor: Lotfi Aleya

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determine the uncertainties in health risk assessment for chemical mixtures (Dong et al. 2015).

Approximately 85% of world production of agricultural products is exposed to herbicides, insecticides, fungicides, or rodenticides. Exposure to pesticides causes various diseases including birth defects, fetal mortality, and reduced birth weight, as well as cancer, asthma, allergies, different hormone disruption, and hypersensitivity. Pesticide exposure was shown to be associated with Alzheimer's and Parkinson's disease as well (Kim et al. 2017). Pesticides are categorized in a wide range of chemical and functional classes, and they can interact with each other depending on dose and target organs. Hence, the identification of pesticides with synergistic interactions is an important concern for cumulative risks assessment (Hernández et al. 2017).

Metals to which humans are exposed have negative accumulative health effects (Suomi et al. 2017). Metal contamination of high-quality agricultural crops might be related to soil, irrigation water, aerial deposition, industrial emissions, and harvesting, storage and sale conditions and processes (Huang et al. 2014; Wang et al. 2015). Several metals and metalloids may induce carcinogenesis, oxidative stress (OS), DNA and chromosomal damage, gene expression alterations, and epigenetic modifications (Bahrami et al. 2020). The International Agency for Research on Cancer (IARC) has classified arsenic (As) and cadmium (Cd) as Group 1 human carcinogens, while considered lead (Pb) as a probable human carcinogen (Group 2 A) (Renieri et al. 2019; Wang et al. 2020). IARC has classified Nickel (Ni) as a Group 2 B carcinogen as it induces ROS overproduction and oxidative stress. Chromium (Cr) is a Group 3 carcinogen with chromium VI being classified as a Group 1 human carcinogen; also, its hexavalent ion has a greater toxic potency than the trivalent one. Cr can cause adverse health effects such as hemolysis, hemorrhage, and gastrointestinal problems; also, in combination with Zinc (Zn), Cr can negative impacts on the respiratory system (Atamaleki et al. 2019; IARC 2017). Copper (Cu) and mercury (Hg) can pollute the environment and affect health of humans. Metals persist in soil and can accumulate in tissues of plants (Gu et al. 2019). Iron (Fe) is one the most important micronutrients in biological systems, but at high concentrations, it can cause toxicity through induction of the generation of free radicals by converting ferrous (Fe^{2+}) to ferric (Fe^{3+}), which results in cell damages and ultimately, death (Eid et al. 2017). Absence or excessive intake of elements that are essential for plants and humans hemostasis, such as Cu and Fe, can cause chronic conditions including metabolic syndrome, anemia, and developmental retardation (Gu et al. 2019).

Polycyclic aromatic hydrocarbons (PAHs) are hazardous to the environment and human health due, in part, to the induction of DNA damage via formation of reactive oxygen species (ROS) and DNA adducts (Tarafdar et al. 2019). Results of several studies have shown relationships between exposure to PAHs and cancer of the bladder, brain, breast, blood (leukemia), kidney, larynx, lung, prostate, and skin (Armstrong et al. 2004; Bosetti et al. 2007; Boström et al. 2002; Ifegwu and Anyakora 2015; Kim et al. 2016; Rota et al. 2014). IARC reported associations of 16 PAHs with different cancers and identified these chemicals as main contaminants in food. Among them, Benzo(*a*)Pyrene (B*a*P) is classified as a proven, complete carcinogen in humans (IARC Group 1). Both antagonistic and synergic interactions have been observed among PAHs. Total carcinogenic potency increases following exposures to a mixture of PAHs of various potencies. It is well documented by USEPA that 16 PAHs including naphthalene (Nap), fluorene (Fl), acenaphthylene (Acy), phenanthrene (Phe), acenaphthene (Ace), anthracene (Ant), fluoranthene (Flu), benzo[a]Anthracene (B*a*A), pyrene (Pyr), chrysene (Chr), benzo[b]fluoranthene (B*b*F), benzo[g,h,i] perylene (B*ghi*P), benzo[k]fluoranthene (B*k*F), benzo(a)pyrene (B*a*P), indeno[1,2,3-cd]pyrene (I*cd*P), and dibenzo[a,h]anthracene (D*ah*A) are the major contaminants in foods (IARC 2017; Petit et al. 2019).

Olive (*Olea europaea* L.) is cultivated in many parts of the world. The European Food Safety Authority (EFSA) considers olive an "edible peel fruit" (Anastassiadou et al. 2020). Olives are a rich source of valuable nutrients and bioactive compounds, such as phenolic compounds (including phenolic acids, phenolic alcohols, and flavonoids), secoiridoids, lignans, terpenic acid derivatives, fatty acids, triacylglycerols, sterols, and various volatile compounds. Olive is traditionally used to treat inflammation-derived diseases, urinary tract infections, asthma, and rheumatism (Song et al. 2019). Due to the popularity of this fruit and its oil in Iran and as recommended by the Food and Agriculture Organization of the United Nations (FAO) and the Scientific Committee on Food (SCF), monitoring contaminants levels in food is necessary to guarantee humans health. To the best of our knowledge, this is the first study to assess risk of oral exposure to pesticides, metals and PAH mixtures, through consumption of Iranian olive (Pissinatti et al. 2015). The current study presents (1) concentration of 22 pesticides, 8 metals, and 16 PAHs in 20 olive cultivars from six cultivation zones in Iran, (2) carcinogenic and noncarcinogenic health risk assessment done based on IARC classifications, and (3) sensitivity analysis done to discover input parameters with the greatest impact on risk assessment results.

Materials and methods

Sample collection

A total of 1800 olive samples of 20 cultivars (three samples each) including Amphisis, Arbequin, Coratina, Koroneiki, Leccino, Roghani and Shenge as oily olive, Amigdalolia, Abu-satl, Derak, Gordal, Manzanilla, Shiraz, and Tokhmekabki as canned olive, Dezfuli fars, Konservolia, Mari, Mission, Picual, and Zard as both oily and canned olive, from five different olive orchards located in six cultivation zones (Gorgan (Golestan province), Rijab (Kermanshah province), Rudbar (Gilan province), Semnan (Semnan province), Shiraz (Fars province), and Tarom (Zanjan province)), were analyzed (Fig. 1).

In fruit production, besides the soil structure and the production techniques, environmental factors are of crucial importance. It is known that environmental factors of different regions in the world affect the quality of the produced fruits. Olive trees are well adapted to various climatic conditions, but an increase in environmental pollution may affect the quality of the fruit. However, besides food safety aspects, the geographical origin of olive fruits is an important concern for consumers. Moreover, in different zones, many factors may affect olive quality, including agricultural practices such as fertilization, irrigation management and agronomic practices adopted in the field. In all above-mentioned cultivation zones, the period between early September and early November corresponds to the harvesting date of olive fruits. The geographical location, and topographic, climatic, and soil characteristics of the cultivation zones where the samples were collected are shown in Table 1.

Preparation of samples

After collection of olive samples in 2018–2019, fruits were directly transferred to the laboratory, and rinsed with deionized water. Olives were oven-dried at 40 °C and fresh and dry masses were measured before and after drying, respectively. Dried fruits were grounded, passed through 2-mm mesh size sieves, and stored at −20 °C in polyethylene covers until analysis.

Chemicals

All standards (of 99% purity) for pesticides (Acephate: CAS No. 30560-19-1; Azinphos-methyl: CAS No. 86-50-0; Chlorpyrifos: CAS No. 2921-88-2; Chlorpyrifos-methyl: CAS No. 5598-13-0; Diazinon: CAS No. 333-41-5; Dimethoate: CAS No. 60-51-5; Ethion: CAS No. 563-12-2; Fenitrothion: CAS No. 122-14-5; Fenthion: CAS No. 55-38-9; Malathion: CAS No. 121-75-5; Methamidophos: CAS No. 10265-92-6; Methidathion: CAS No. 950-37-8; Phenthoate: CAS No. 2597-03-7; Trichlorfon: CAS No. 52-68-6; Carbaryl: CAS No. 63-25-2; Carbofuran: CAS No. 1563-66-2; Methomyl: CAS No. 16752-77-5; Oxamyl: CAS No. 23135-22-0; Pirimicarb: CAS No. 23103-98-2; Propamocarb: CAS No. 24579-73-5; Deltamethrin: CAS No. 52918-63-5; Permethrin: CAS No. 52645-53-1), metals (As: CAS No. 7440-38-2; Cd: CAS No. 7440-43-9; Cr: CAS No. 7440-47-3; Cu: CAS No. 7440-50-8; Fe: CAS No. 7439-89-6; Hg: CAS No. 7439-97-6; Ni: CAS No. 7440-02-0; Pb: CAS No. 7439-92-1), and PAHs (Ace: CAS No. 83-32-9; Acy: CAS No. 208-96-8; Ant: CAS No. 120-12-7; B*a*A: CAS No. 56-55-3; B*a*P: CAS No. 50-32-8; B*b*F: CAS No. 205-99-2; B*ghi*P: CAS No. 191-24-2; B*k*F: CAS No. 207-08-9; Chr: CAS No. 218-01-9; D*ah*A: CAS No. 53-70-3; Fl: CAS No. 86-73-7; Flu: CAS No. 206-44-0; I*cd*P: CAS No. 193-39-5; Nap: CAS No. 91-20-3; Phe: CAS No. 85-01-8;

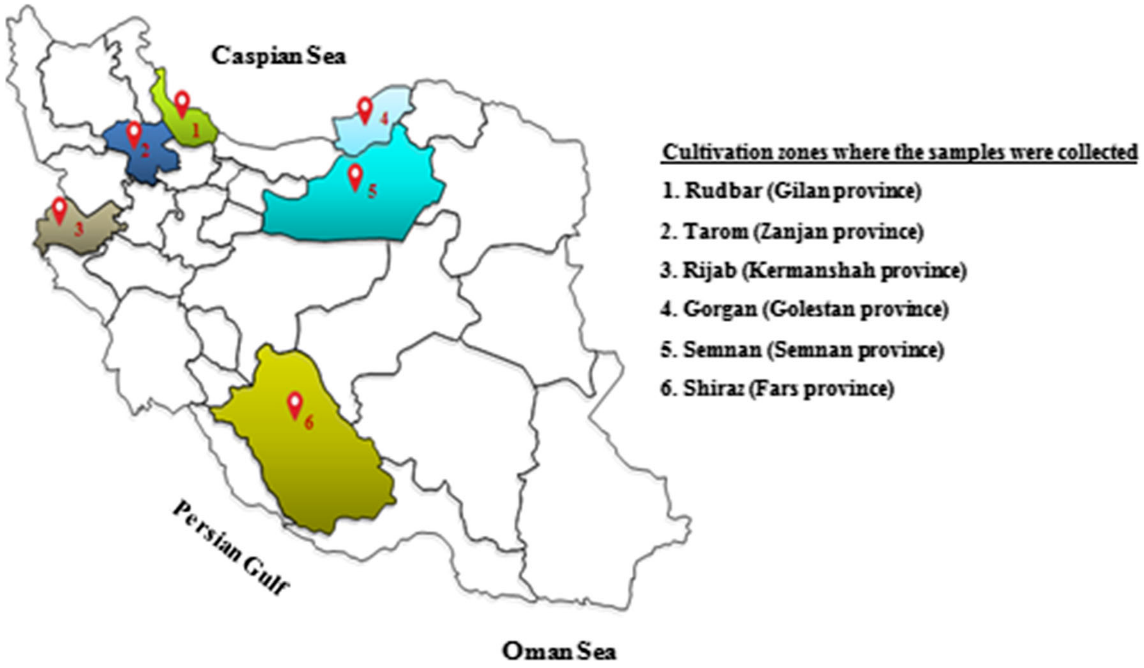


Fig. 1 Cultivation zones where the samples were collected

Table 1 Geographical location and topographic, climatic, and soil characteristics of cultivation zones where the samples were collected

Station	Latitude (N)	Longitude (E)	Altitude (m)	Average annual temperature (°C)	Mean annual precipitation (mm)	**Average of relative humidity (%)**	**Soil texture**	**pH**	**EC[a] (ds/m)**
Gorgan (Golestan province)	36°50′	54°26′	138.00	17.70	601.00	79.00	Silt-loam	7.10	1.60
Rijab (Kermanshah province)	34°18′	47°03′	1350.00	16.00	478.70	40.00	Silt-loam	7.13	1.81
Rudbar (Gilan province)	36°80′	49°41′	750.00	15.80	192.90	83.00	Silt-loam	6.84	1.00
Semnan (Semnan province)	35°57′	53°37′	1130.00	17.01	110.00	41.80	Loam	8.31	1.91
Shiraz (Fars province)	29°59′	52°58′	1585.00	22.00	346.00	38.00	Loam	7.75	1.56
Tarom (Zanjan province)	36°50′	48°39′	1638.00	12.00	314.00	51.00	Loam	8.12	1.90

[a] Electrolyte leakage

Pyr: CAS No. 129-00-0) were purchased from Sigma-Aldrich (Steinheim, Germany). Solvents (HPLC grade) including ethyl acetate (EtOAc), acetonitrile (MeCN), nitric acid (HNO_3), dichloromethane, tetrahydrofuran, and n-hexane were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany). Anhydrous sodium sulfate (99% purity; BDH, Poole, UK) and Sep-Pak silica plus long cartridges (690-mg sorbent per cartridge; 55–105-μm particle size; Waters, Wexford, Ireland) were also used.

Standard solutions

Pesticides

The stock solution of triphenylmethane (TPM, used as the internal standard at 1 mg/ml) was prepared in EtOAc and kept in glass vials in the dark at −20 °C. To determine recoveries, a standard mixture solution of pesticides (10 μg/ml) was prepared in EtOAc. Each of the pesticides and the pesticide mixture, which included all 22 pesticides, were injected individually into the Gas Chromatograph coupled to a Mass Spectrometer (GC-MS). Calibration curve for each pesticide was plotted by calculating the ratio of the peak area of standards to peak area of internal standard and compared to known concentrations.

Metals

Quantitation of metals was accomplished by multi-element analyses simultaneously for As, Cd, Cr, Hg, Ni, and Pb, based on a standard of 1000 mg/l prepared for calibration curves. The stock solution was diluted with 0.2% HNO_3 solution.

Polycyclic aromatic hydrocarbons

A stock solution of PAHs at 1000 mg/l was prepared in n-hexane and wrapped in aluminum foil. Triphenyl phosphate (TPP) stock solution was provided in acetone at 1000 mg/l. All the stocks were kept in a dark place at −20 °C.

Instrumentation

Gas chromatography-mass spectrometry (GC-MS)

For identification and quantification of pesticides and PAHs, an Agilent 7890A Turbo MSD 5975C (Agilent, Santa Clara, USA) was used; The GC-MS system was equipped with a PTV (programmed temperature vaporizer) inlet and a 7683B auto-injector. The carrier gas was helium (used at a flow rate of 1.0 ml/min). The system was equipped with an HP-5 MS 30 m × 0.25 mm × 0.25 μm column (Agilent, Santa Clara, USA.) for identifying and quantifying pesticides (Shakeri et al. 2019). The quadrupole analyzer measured the abundance of ions of m/z from 50 to 490, and detector voltage was 1294 V. Electron ionization (70 eV) with selected ion monitoring mode was used, and the most abundant ion from the molecular ion cluster was measured for each analyzed compound. Pesticides were identified by comparing the observed GC retention time with that of standard solutions of pesticides and use of characteristic ions (Farhadi et al. 2020; Taghizadeh et al. 2019d).

For quantification of PAHs, helium was used at a flow rate of 30.0 ml/min with 13.00 psi. Also, DB-5MS capillary column with 5% diphenyl- 95% dimethyl polysiloxane 30 m × 0.25 mm i.d., 0.25-μm film thickness, was used. The injector temperature was 300 °C, the flow rate of the carrier gas was 1.5 ml/min (constant flow), pulsed splitless, and injection volume was 1 μl. Quadrupole temperatures were 300, 280, and 180 °C. GC oven temperature was programmed as follows: 55 °C (for 1 min) and 55–290 °C (25 °C/min for 3 min). The range of m/z was measured from 45 to 450 and detector voltage was 1294 V. For each compound, the maximum abundant ion from the molecular ion cluster was measured (Badibostan et al. 2019).

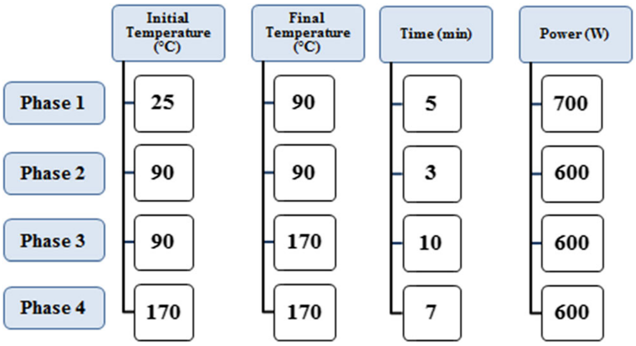
Microwave digestion and inductively coupled plasma-optical emission spectrometry (ICP-OES)

Samples used for quantification of metals were prepared by microwave digestion (Milestone Ethos Microsynth Oven, Germany) (Fig. 2). Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (SPECTRO ARCOS, Germany) was used for simultaneous, multi-element detection. The optical system chamber of SPECTRO ARCOS was filled with argon. Argon was circulated by a small membrane pump through a cleaning device which ensures excellent, long-term stability and eliminates disadvantages typical for systems. SPECTRO ARCOS records the spectrum with 32 linear CCD detectors aligned to cover the entire wavelength range relevant to ICP-OES from 130–770 nm (Taghizadeh et al. 2020b).

Extraction procedures

Pesticides

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique is usually used for pesticides analysis in fatty matrices (Association of Analytical Communities (AOAC.2007.01)). The method of Qin et al. (2016) with slight modifications was applied. Specifically, 2.5 g of each sample was placed in a 50-ml falcon tube, and then, 5 μl/ml of internal standard TPM in EtOAc was added. The solution was incubated for 1 h in cold and dark, then, 5 ml deionized distilled water was added and the solution was vigorously stirred for one min. Then, 10-ml MeCN and 5-ml n-hexane were added and shacked well. A mixture of 4-g $MgSO_4$ and 1-g NaCl was added to the tube and the tube was shaken for 3 min. The mixture was centrifuged at 4000 rpm for 10 min at −5 °C, and aliquots of the supernatant were transferred to a 15-ml falcon tube containing 1000-mg $MgSO_4$ and 200-mg primary–secondary amine (PSA) and 400-mg C_{18}. The tube was shaken for 2 min and then, centrifuged at 4000 rpm for 10 min at −5 °C. Afterward, 4 ml of the supernatant was transferred into a 5-ml flask. In order to get samples of 0.3–0.5 ml, all were concentrated under nitrogen atmosphere. Finally, 1 ml toluene was added to each sample and shacked for 3 min. Then, 2 μl of the final solution was prepared for GC-MS assay. MeCN and $MgSO_4$ were used for the extraction of the analytes. PSA and C_{18} were applied to remove nonpolar substances including lipids to prevent interference (Qin et al. 2016; Taghizadeh et al. 2020c)



	Initial Temperature (°C)	Final Temperature (°C)	Time (min)	Power (W)
Phase 1	25	90	5	700
Phase 2	90	90	3	600
Phase 3	90	170	10	600
Phase 4	170	170	7	600

Fig. 2 Operating program used for microwave digestion

Metals

Ten grams of each sample were digested using 60 ml of HNO_3 and 20 ml of concentrated H_2O_2 (30%) using a microwave digestion system (Milestone Ethos Microsynth Oven, Germany) for 25 min and then, diluted to 100 ml with 2% HNO_3. Blank preparation was done in the same way. Finally, clear liquid samples were analyzed by using ICP-OES (Taghizadeh et al. 2017)

Polycyclic aromatic hydrocarbons

Briefly, a 2-g aliquot of each sample was added to 10-ml acetonitrile/hexane (60:40 v/v) and shaken for 1 min. The mixture was ultra-sounded for 5 min at 60 °C. The homogenized solution was centrifuged at 4000 rpm for 5 min and then, the top layer was removed. The remaining oil was mixed with 10 ml acetonitrile/hexane. The solution with PAHs was passed through C_{18} cartridge, and evaporated under nitrogen at 35 °C. Consequently, in the second phase of purification, the solution was purified using a Florisil cartridge (500 mg) as follows: 1-ml sample from the previous step was added to the cartridge, and mixed with 4-ml hexane/dichloromethane (75:25 v/v). Reducing volume to 50 μl was completed under nitrogen at 35 °C. Using a microliter syringe loaded with tetrahydrofuran/methanol (50:50 v/v), the final volume was adjusted to 250 μl (Badibostan et al. 2019).

Analytical performance

Method validation was performed based on European Union (EU) guidelines SANTE 12682/2019, IMEP 114 and PAHs Factsheet for pesticides, metals and PAHs (EU 2019). Three scenarios including calibration curve and linearity, recovery and accuracy, limit of detection (LOD), and limit of quantification (LOQ) were considered for evaluation of the analytical performance (Figs. 3, 4, 5) (Taghizadeh et al. 2020c).

Data normalization

Relative potency factor (RPF) for pesticides

As suggested by the EFSA and the USEPA, one way to assess cumulative effects of mixtures of residues, is to apply a concentration addition method when relative potency factors (RPF) are used, to normalize concentrations of each compound present in food to equivalent residues of an index compound (IC) (Jardim et al. 2018). RPFs for various pesticide categories were taken from the USEPA, which were calculated by Bench Mark Doses (BMD10) related with a 10%

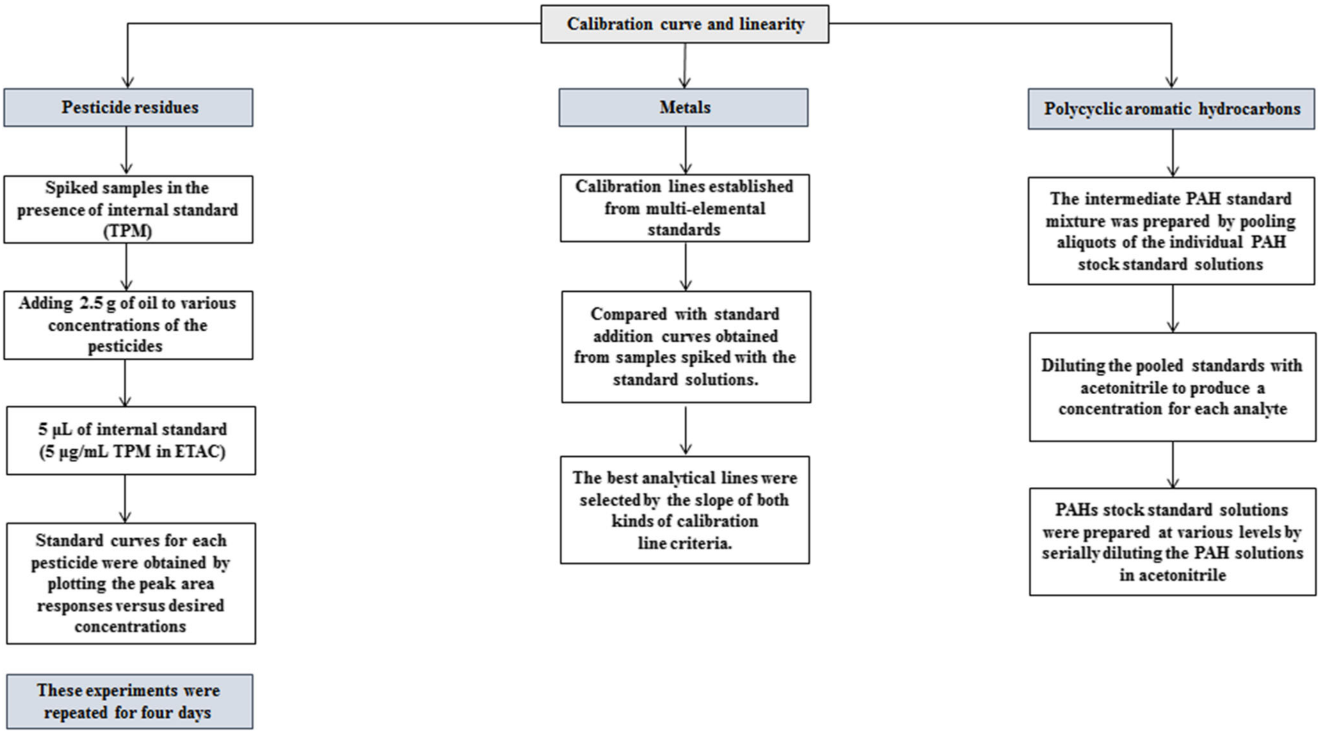


Fig. 3 Flowchart indicating the process of plotting calibration curve and determination of linearity of contaminants levels in olive samples

inhibition of AChE in the brain of rats. In this study, methamidophos or acephate was used for organophosphorus (OPs) as IC. Oxamyl and deltamethrin were considered ICs for carbamates (CBs) and pyrethroid (PYs), respectively (EPA U 2006, 2011). If BMD_{10} (for OPs and CBs) and/or BMD_{20} (PYs) were not available, No Observed Adverse Effect Level (NOAEL) from rats, dogs or humans published primarily by the EFSA, European Commission (EC), FAO/WHO Joint Meeting on Pesticide Levels (JMPR) or USEPA were used (Jardim et al. 2018). RPFs for the pesticides of different classes found in samples of olives are shown in Table 6.

Toxic equivalency factor (TEF) for PAHs

For cumulative assessment, TEF is a specific type of RPF that is extensively used. The reference compound such as B*a*P is used for binary mixture of PAHs for carcinogenic potency

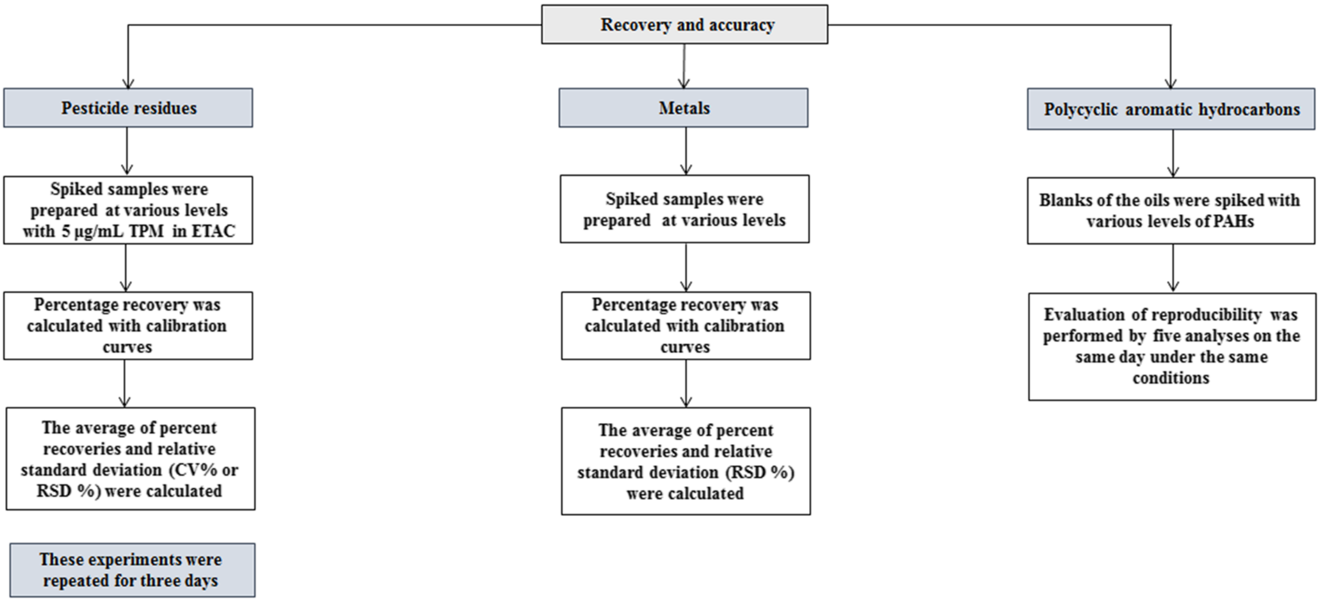


Fig. 4 Flowchart indicating the process of assessment of recovery and accuracy of contaminants determination in olive samples

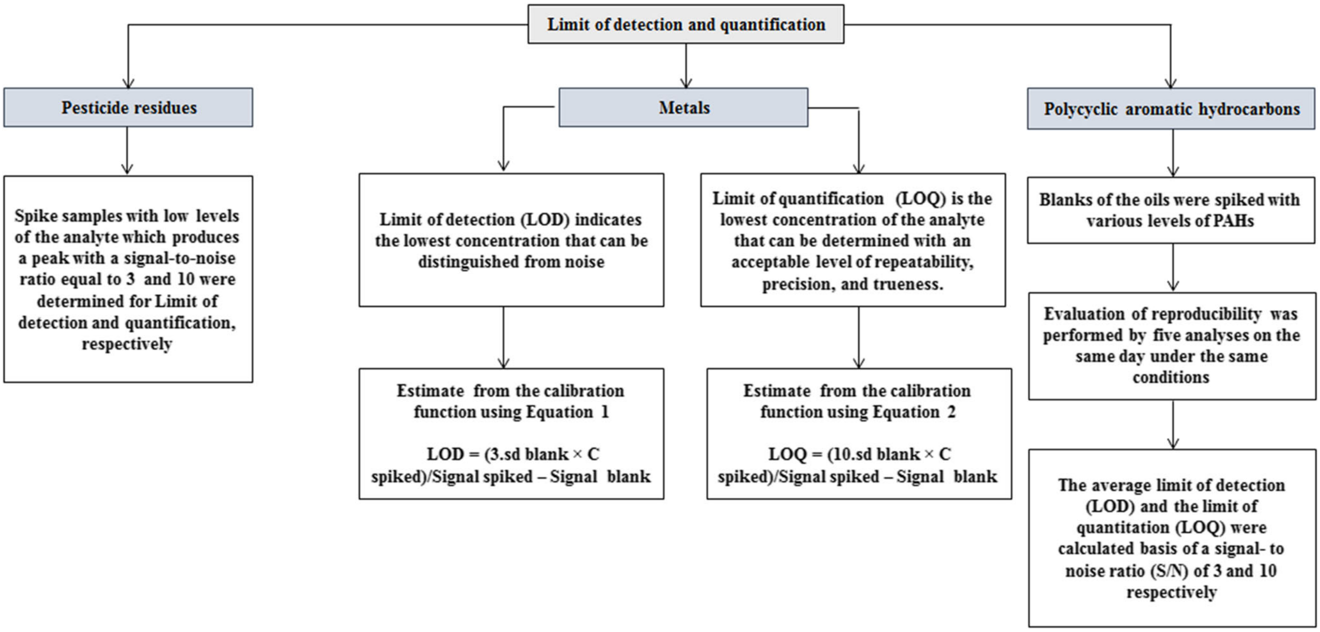


Fig. 5 Flowchart indicating the process of determination of LOD and LOQ of contaminant analysis in olive samples

calculation (Heys et al. 2016; Jiang et al. 2016). TEFs for the PAHs are presented in Table 8.

Assessment of risks to health

Noncarcinogenic scenario

Parameters of noncarcinogenic scenario were calculated using Eqs. 1–3 (Table 2). The accepted value for Hazard Index (HI) in estimation of the total risk of noncarcinogenic effects posed by a chemical mixture is equal to or less than 1.0 (i.e., *di minimis* risk to health) while an HI >1.0 indicates a chance for occurrence of noncancer effects and HI >10 indicates greater risk (Epa U 2009) (Table 2).

Carcinogenic scenario

Incremental life time cancer risk (ILCR)

Risks of additional cancers were evaluated based on ILCR assessment for intake of carcinogenic elements present in samples (Eq. 4). It was conducted using CDI, cancer slope factor of B*a*P (CSF) (geometric mean of 7.3 mg/kg, body mass (BM)/day) and CF is the conversion factor (10^{-6} mg/ng) (EPA A 2004; Ni and Guo 2013). For ILCR, values between 10^{-6} and 10^{-4} imply risk, whereas values greater than 10^{-4} indicate greater risks (JECFA 2006; Singh and Agarwal 2018).

Margins of exposure (MoE)

Recently, the EFSA applied another concept for assessment of genotoxic carcinogens. MoE compares the actual mean or maximum exposure in humans with the point of departure (POD) for example BMDL_{10} (Bench Mark Dose with 10% effect) derived from *in vivo* experiments where cancer has been considered an endpoint in animals. BMDL_{10} and CDI should have the dimension of a dose (mg/kg BM), producing a dimensionless MoE (Eq. 5). The EFSA suggests that an MoE >10,000 indicates '*di minimis* public health concern' while MoEs ≤10,000 imply 'of concern' risks (Lee et al. 2018). The EFSA has mostly used substitution methods for results reported to be less than the LOD, the value is imputed as the LOD (upper bound), zero (lower bound) or LOD/2 (medium or middle bound) (EFSA 2010). In this study, the middle bound was used to estimate exposures.

Monte Carlo method and sensitivity analyses

Monte Carlo (MC) methods provide distributions of uncertainties and present a range of responses with probabilities of occurrence. Monte Carlo simulations usually require a large number of model runs (Uusitalo et al. 2015). In the current study, MC with 10,000 iterations was used for evaluation of carcinogenic and noncarcinogenic risks due to exposure to pesticides, metals, and PAHs through oral consumption of olive. Another option is to apply sensitivity analysis (SA), which is a common way to explore which parameters in predictive models have the greatest contributions to variance. The goal of SA is to characterize how model outputs respond to changes in input, with an emphasis on finding the input parameters to which outputs are the most sensitive. The basic idea of the SA is to alter model input values (Taghizadeh et al. 2021). In the current calculations, distribution mode was "log-

Table 2 Equations used to assess risks

Equation	Noncarcinogenic scenario	Reference
1	CDI (chronic daily intake) $= \frac{CF \times IR \times EF \times ED}{BM \times AT}$	Taghizadeh et al. (2020b)
2	THQ (hazard quotient) $= \frac{CDI}{ADI}$	Taghizadeh et al. (2019b)
3	HI (hazard index) $= \sum_{n=0}^{i} THQn$	Taghizadeh et al. (2019a)
Carcinogenic scenario		
4	ILCR (incremental life time cancer risk)$=CDI \times CSF \times CF$	Badibostan et al. (2019)
5	MoE (margins of exposure)$=(BMDL10/CDI)$	Taghizadeh et al. (2019c)

CF contaminant concentration in olives (mg/kg, dry mass (dm))

IR ingestion rate is the daily olive consumption (kg)

The value of consumption used in the current study is 2.19 g/person/day for olive for Iranian general population. These values correspond to the average consumption based on tonnages of production and country population (FAO 2020)

EF exposure frequency (365 meals/year)

ED exposure duration (70 years) (Taghizadeh et al. 2019c)

BM average body mass for Iranian adult population is considered 70 kg (Taghizadeh et al. 2018)

AT average time [(365 days/ year × ED)= 25550] (Taghizadeh et al. 2020a)

ADI acceptable daily intake (ADI values set by various jurisdictions and agencies for the 22 pesticides and 8 metals are presented in Tables 6 and 7)

CSF cancer slope factor

CF conversion factor

BMDL10 bench mark dose with 10% effect (BMDL10 is equal to 0.49 mg/kg, bm/day for PAH8) (Lerda 2011)

normal” in SAS software JMP 8 (Campus Drive, Cary, NC 27513).

Statistical analyses

SPSS Statistics 16.0 was used for data analysis. Comparisons of mean residue levels were made using Mann-Whitney or Kruskal-Wallis nonparametric tests. Also, Monte Carlo simulation was performed for regenerating 10,000 samples for each contaminant using IBM SPSS Statistics simulation routines. A level of 0.05 was considered statistically significant.

Results

Analytical performance

Pesticides

Accuracy and precision of analytical methods were assessed (Table 3). Three matrices were spiked at three levels (10, 20, and 50 μg/kg) with 22 standard pesticides. Mean recoveries were in the range of 93.2–99.5%. Relative Standard Deviations (RSDs %) were ≤4.5%, which were consistent with European Union guidelines SANTE 12682/2019 and indicated successful validation and good method performance (EU 2019). Coefficients of determination (R^2), calculated for each pesticide from matrix calibration plots, ranged from 99.1–99.999% (Table 3).

LODs and LOQs were calculated based on signal-to-noise ratios of S/N=3 and S/N=10, respectively. LODs were 0.1 to 3.2 μg/kg and LOQs were in the range of 0.3–9.3 μg/kg (Table 3). Based on the QuECHERS method, the present method showed an acceptable LOD for determination of pesticides.

Metals

Recoveries from olive matrices were determined at three concentrations. Recoveries were in the range of 88.5–99.9%, with an associated RSD of ≤ 2.3%. The obtained recoveries confirmed the suitability of extraction with no significant losses of metals. Coefficients of determination (R^2) exhibited significant linear relationships (99.2–99.9%) in all the calibration curves (Table 4). LODs and LOQs were similar for all metals (1.0 and 3.0 μg/kg, dm, respectively) with the exception of Ni (7.0 and 21.0 μg/kg, dm, respectively).

Polycyclic aromatic hydrocarbons

Satisfactory recoveries were observed for PAHs (Table 5). Mean recoveries ranged from 88.1 to 100%, with an associated RSD of ≤5.0%. Analytical curves for PAHs presented appropriate R^2 values ranging from 99.1 to 100%. LODs were found to be 0.15–1.0 μg/kg and LOQs were in the range of 0.45 to 3.0 μg/kg (Table 5).

Table 3 Mean recoveries (%), relative standard deviation (RSD, %) of 22 pesticides at three spiked concentrations (μg/kg, dm), correlation coefficients (R^2), and LOD and LOQ (μg/kg, dm) of pesticides

Pesticide	Spiked concentration			R^2	LOD[d]	LOQ[e]
	10 μg/kg	20 μg/kg	50 μg/kg			
OP[a] pesticides						
Acephate	94.2 (1.3)	96.1 (3.3)	99.3 (3.2)	1.000	2.1	6.0
Azinphos-methyl	93.5 (1.2)	96.5 (2.5)	99.0 (3.3)	0.991	3.3	9.5
Chlorpyrifos	94.0 (2.1)	97.5 (3.5)	99.2 (4.1)	0.994	0.1	0.3
Chlorpyrifos-methyl	93.4 (1.5)	97.1 (4.1)	99.1 (4.3)	1.000	0.1	0.3
Diazinon	95.1 (2.1)	97.5 (3.2)	99.5 (3.3)	1.000	1.0	3.0
Dimethoate	95.0 (2.0)	96.1 (3.4)	98.5 (4.0)	0.991	3.1	10.0
Ethion	93.2 (1.4)	97.3 (3.0)	99.4 (3.5)	1.000	0.5	1.5
Fenitrothion	94.1 (2.1)	96.5 (4.3)	99.3 (4.5)	0.993	0.1	0.3
Fenthion	94.5 (2.2)	97.1 (3.7)	99.0 (4.3)	0.998	0.1	0.3
Malathion	93.2 (2.0)	97.4 (2.2)	99.2 (4.1)	0.999	3.3	9.5
Methamidophos	95.2 (1.1)	97.5 (4.1)	99.4 (3.5)	1.000	3.3	9.5
Methidathion	94.2 (2.3)	96.5 (3.5)	98.5 (3.5)	0.993	3.3	9.5
Phenthoate	95.5 (1.2)	96.2 (3.1)	99.1 (4.2)	1.000	3.2	9.5
Trichlorfon	94.3 (1.5)	96.4 (4.5)	98.2 (3.2)	0.994	3.1	9.5
CB[b] pesticides						
Carbaryl	95.5 (2.2)	97.2 (2.3)	98.5 (4.1)	0.992	3.3	9.5
Carbofuran	95.1 (2.1)	96.5 (4.1)	98.1 (3.5)	0.992	0.1	0.50
Methomyl	96.1 (1.5)	98.5 (3.5)	99.5 (4.5)	1.000	0.2	0.65
Oxamyl	95.4 (2.4)	97.5 (4.1)	99.1 (4.0)	0.999	3.0	10.0
Pirimicarb	96.3 (3.1)	98.1 (4.5)	99.5 (3.2)	1.000	3.3	9.5
Propamocarb	95.2 (4.5)	98.5 (4.3)	99.3 (3.5)	1.000	3.2	10.0
PY[c] pesticides						
Deltamethrin	93.4 (2.5)	97.5 (3.5)	98.5 (4.2)	0.994	1.0	3.0
Permethrin	94.5 (3.4)	98.1 (2.5)	99.1 (4.5)	0.998	3.2	9.5

[a] Organophosphorus

[b] Carbamate

[c] Pyrethroid

[d] Limit of detection

[e] Limit of quantitation

Concentrations of contaminants

Pesticide concentrations

The mean of pesticide concentrations of olive cultivars and sample collection zones is shown in Fig. 6. OP, CB, or PY levels did not show statistically significant differences among olive cultivars (Fig. 6a). Moreover, the mean level of pesticides did not significantly vary among the cultivated zones (P =0.7–0.99) (Fig. 6b).

Metals

Concentrations of metals of olive cultivars and sample collection zones are shown in Fig. 7. Mean concentrations of metals did not vary among cultivars (P =0.990–0.999) (Fig. 7a).

Table 4 Mean recoveries (%), relative standard deviation (RSD, %), and coefficients of determination (R^2) for eight metals at three spiked concentrations (μg/kg, dm)

Metal	Spiked concentration			R^2
	50 μg/kg	100 μg/kg	150 μg/kg	
As	95.5 (1.2)	99.5 (1.0)	99.9 (1.5)	0.999
Cd	93.5 (1.5)	96.2 (1.3)	99.3 (1.3)	0.999
Cr	92.3 (1.2)	95.0 (1.4)	98.4 (1.4)	0.999
Cu	89.4 (1.0)	93.1 (1.0)	97.0 (1.0)	0.995
Fe	n.d.[a]	88.5 (1.2)	93.5 (1.5)	0.995
Hg	90.5 (2.3)	93.2 (2.1)	98.0 (2.2)	0.992
Ni	94.3 (1.1)	97.3 (1.5)	99.4 (2.0)	0.999
Pb	93.1 (2.1)	95.4 (2.4)	99.0 (1.5)	0.999

[a] Not detected: this selected isotope could not be determined at 50 μg/kg due to lesser sensitivity

Table 5 Mean recoveries (%), relative standard deviation (RSD, %) of 16 polycyclic aromatic hydrocarbons (µg/kg, dm), correlation coefficients (R^2), and LOD and LOQ (µg/kg, dm) for PAHs at three spiked concentrations (µg/kg, dm)

Polycyclic aromatic hydrocarbons (PAHs)	Spiked concentration					
	5 µg/kg	10 µg/kg	20 µg/kg	R^2	LOD[a]	LOQ[b]
Acenaphthene (Ace)	88.3 (2.5)	96.1 (4.1)	99.4 (2.5)	0.996	0.25	0.70
Acenaphthylene (Acy)	91.2 (3.1)	95.0 (4.1)	99.5 (2.3)	0.997	0.25	0.70
Anthracene (Ant)	94.1 (5.0)	96.5 (3.0)	99.5 (4.5)	0.996	0.30	0.85
Benzo[*a*]Anthracene (B*a*A)	95.5 (4.2)	97.4 (2.4)	100 (3.3)	1.000	0.25	0.80
Benzo(*a*)Pyrene (B*a*P)	92.3 (2.0)	95.4 (4.1)	100 (2.5)	0.999	1.00	3.00
Benzo[*b*]Fluoranthene (B*b*F)	90.1 (2.2)	94.4 (2.0)	98.4 (2.0)	0.999	0.25	0.85
Benzo[*g,h,i*] Perylene (B*ghi*P)	90.4 (3.1)	94.4 (2.4)	97.2 (4.4)	0.999	0.25	0.60
Benzo[*k*]Fluoranthene (B*k*F)	93.2 (4.1)	96.1 (3.1)	100 (3.5)	0.997	0.25	0.70
Chrysene (Chr)	92.1 (3.1)	97.4 (2.4)	100 (2.4)	0.999	0.15	0.50
Dibenzo[*a,h*]anthracene (D*ah*A)	90.5 (4.3)	96.2 (4.2)	99.4 (3.1)	0.993	0.25	0.60
Fluorene (Fl)	94.1 (2.1)	99.0 (2.4)	97.3 (2.4)	0.991	0.15	0.50

[a] Limit of detection

[b] Limit of quantitation

However, statistically signficant differences in concentrations were observed among cultivation zones (P =0.990 to 0.999) (Fig. 7b).

Polycyclic aromatic hydrocarbons

Mean concentrations of PAHs in olive cultivars from various cultivation zones are given in Fig. 8. Concentrations of PAHs in olives were not significantly different among cultivars (P >0.05) (Fig. 8a) or zones of cultivation (Fig. 8b).

Assessments of risks

Pesticides

For THQs and HIs (in noncarcinogenic risk scenario), the 25th and 95th centiles are presented in Table 6. Based on MCS, hazard indices (25 and 95%) for OPs in olive were less than 1.0 (7.09×10^{-5} and 3.13×10^{-4}, respectively). At the 25th centile for CBs, HI (25%) was 2.32×10^{-4} and at 95th centile of concentrations was 9.43×10^{-4}. The 25th centile of HIs for PYs was 3.55×10^{-6}, whereas, the HI at the 95th centile of concentrations was 4.21×10^{-5}. Similarly, total HI values were less than 1.0 at both centiles (Table 6).

Metals

Estimated exposure centiles for carcinogenic and noncarcinogenic effects of metals are given in Table 7. At the 25th and 95th centiles, estimated ILCRs were as follows: As (9.50×10^{-9} and 6.86×10^{-8}, respectively) and Pb (2.20×10^{-11} and 4.78×10^{-10}, respectively). For the carcinogenic metals, estimated ILCRs at the 25th and 95th centiles were 9.52×10^{-9} and 6.90×10^{-8}, respectively. The THQs at the 25th centile ranged from 1.21×10^{-6} to 4.30×10^{-5}. Similarly, at the 95th centile, THQs ranged from 5.11×10^{-6} to 5.00×10^{-4}. Total HI (sum of 25, 50 and 95 centiles) was less than 1.0 (Table 7).

Polycyclic aromatic hydrocarbons

Centiles of estimated ILCRs and MoEs for 16 PAH in three matrices are reported in Table 8. Total ILCR values were 4.90×10^{-14} and 3.30×10^{-13} at the 25th and 95th centiles, respectively. Based on the model, the 25th and 95th centile values for MoE were 1.13×10^{12} and 1.97×10^{12}, respectively (Table 8).

Sensitivity analysis

The MCS sensitivity analysis of THQ, ILCR and MoE data is presented in Table 9. Concentration of residues explained 0.96% of the total variability and was the single most significantly influential variable in determining risk of exposure to pesticides in olive samples, while body mass contributed less (−14.00%) (Table 9). Also, concentration was the key input parameter for exposure to metals. Based on an evaluation of carcinogenic effects of metals, concentrations of metals explained 13.71% of the total variance. For THQ, the influence of concentration was 17.10%. For the ILCR, concentration was the most significant parameter (0.89%). For MOE, body mass had the greatest effect on risk of exposure to PAHs (0.56%) (Table 9).

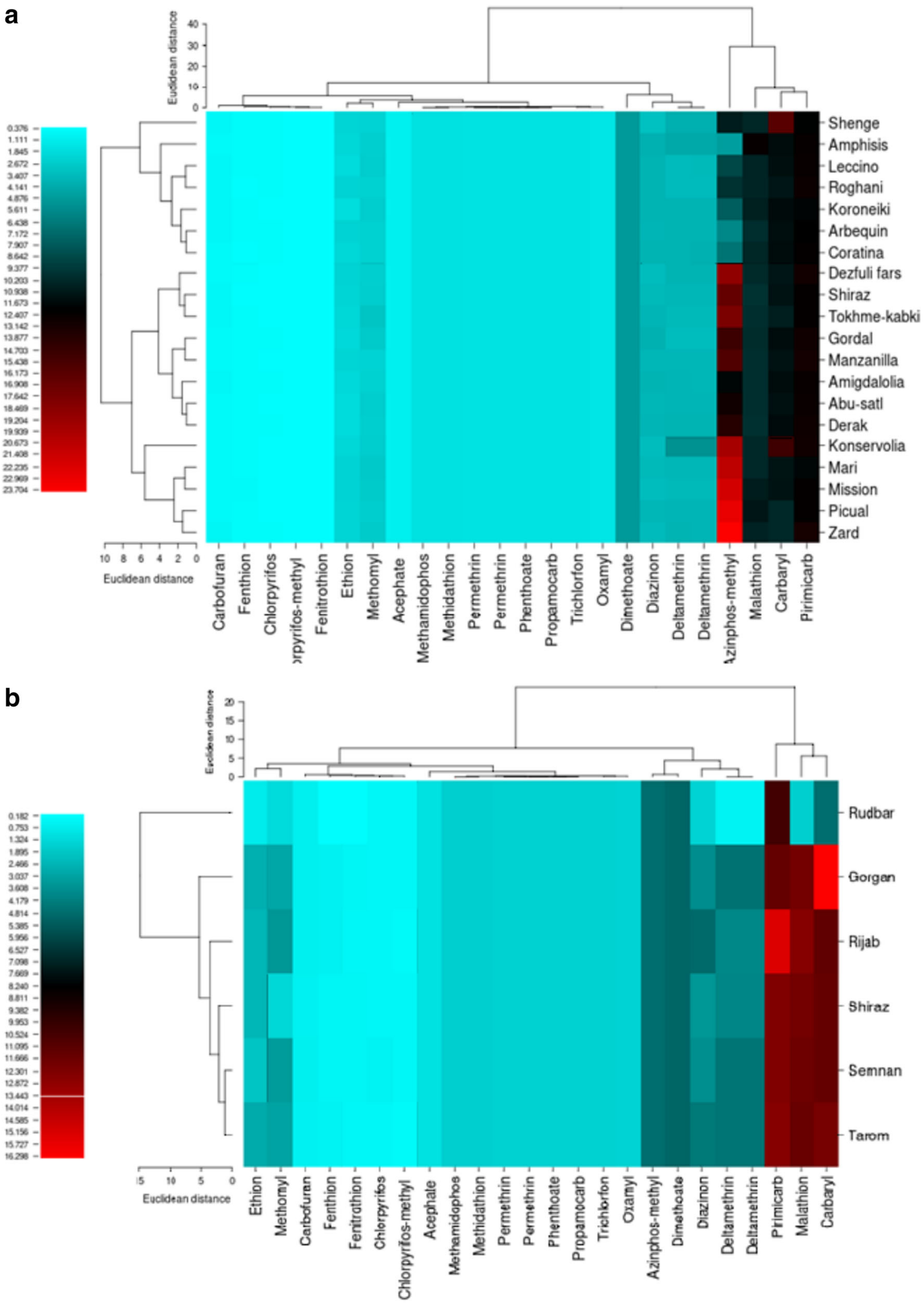


Fig. 6 Heatmap pictorial presentation of pesticide concentrations in olive samples plotted for each cultivar (**a**) and each cultivation zone (**b**)

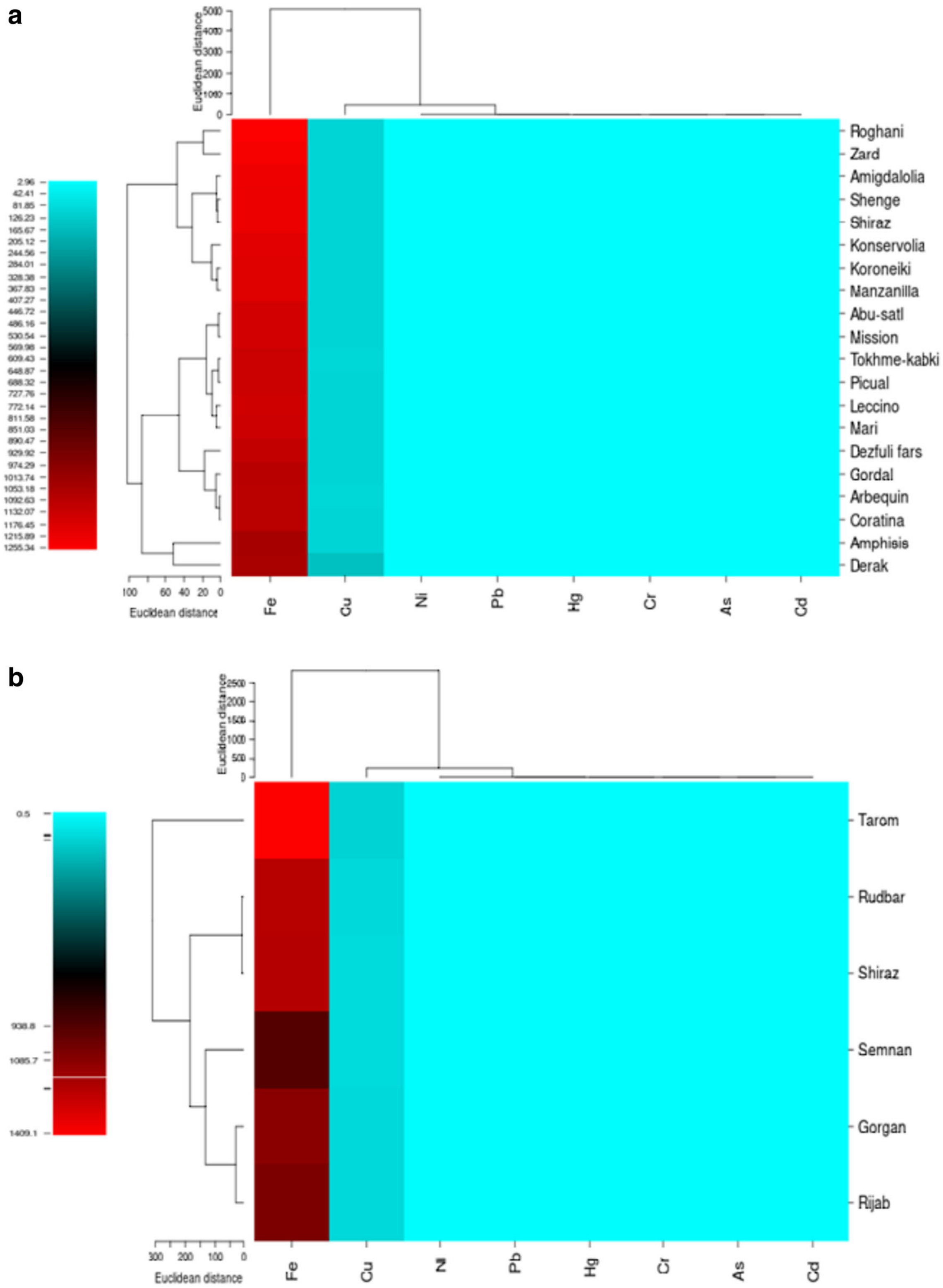


Fig. 7 Heatmap representation of concentrations of metals in various cultivars of olive (**a**) from different cultivation zones (**b**)

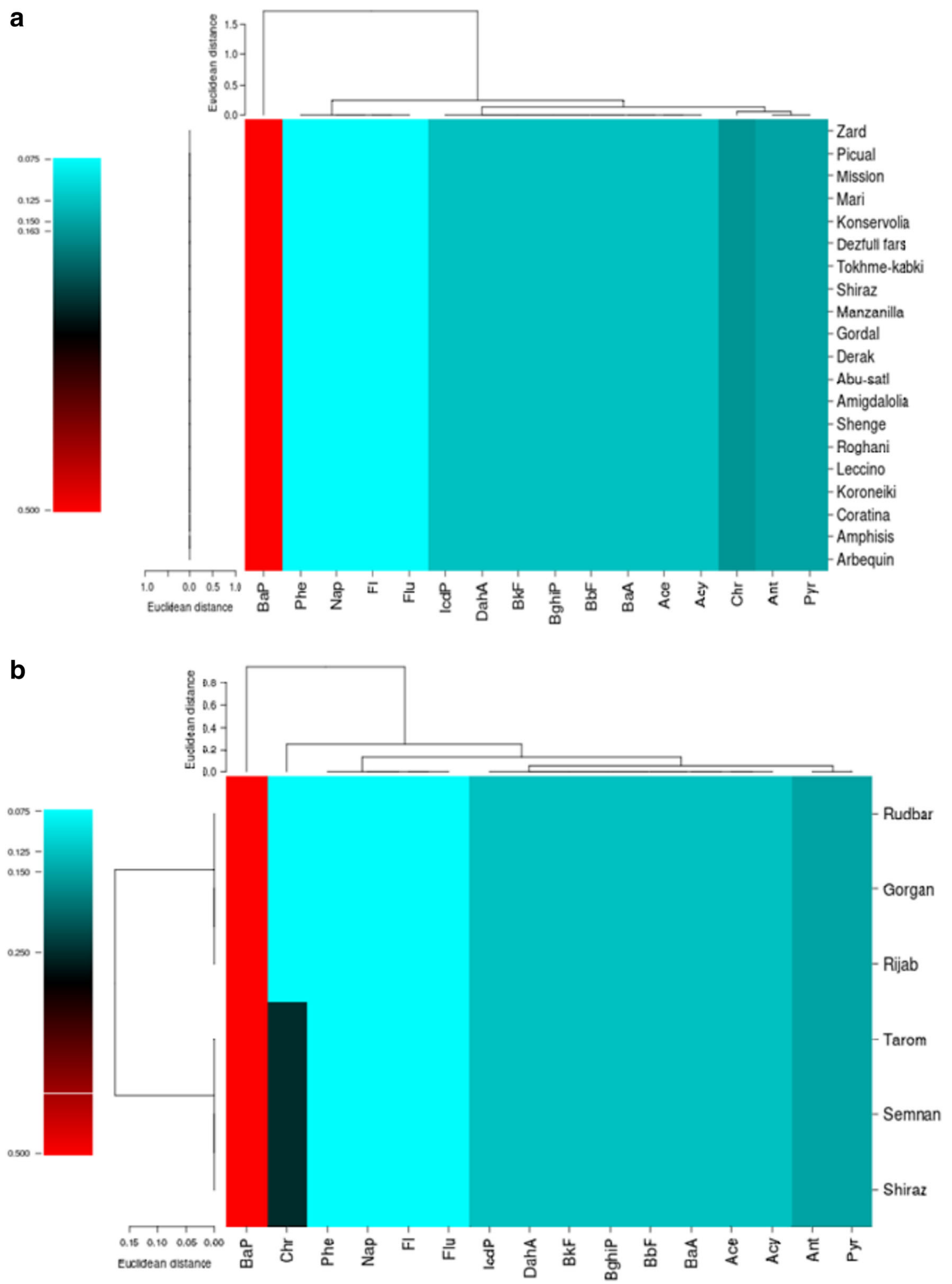


Fig. 8 Heatmap representation of concentrations of polycyclic aromatic hydrocarbons (PAHs) in olive samples plotted for each cultivar (a) and zone of cultivation (b)

Table 6 Estimated exposure centiles for organophosphorus pesticides (OPs), carbamates (CBs), and pyrethroids (PYs)

Pesticide	European union		Reference	RPF[a]	THQ[b]		
	ADI[c]	MRL[d]			25%	50%	95%
OP pesticide				IC[e]= Methamidophos			
Acephate	0.03	0.01	Reg. (EU) No 899/2012	0.08	5.05×10^{-8}	8.00×10^{-8}	1.00×10^{-7}
Azinphos-methyl	0.005	0.05	Reg. (EU) No 839/2008	0.1	1.54×10^{-6}	2.71×10^{-6}	5.10×10^{-6}
Chlorpyrifos	0.001	0.01	Reg. (EU) No 686/2018	0.06	4.83×10^{-7}	7.30×10^{-7}	1.03×10^{-6}
Chlorpyrifos-methyl	0.01	0.01	Reg. (EU) No 686/2018	0.005	3.72×10^{-9}	5.45×10^{-9}	1.09×10^{-8}
Diazinon	0.0002	0.01	Reg. (EU) No 834/2013	0.01	3.61×10^{-6}	5.15×10^{-6}	9.16×10^{-6}
Dimethoate	0.002	3.0	Reg. (EU) No 1135/2017	0.32	1.04×10^{-5}	2.28×10^{-5}	9.00×10^{-5}
Ethion	0.002	0.01	Reg. (EU) No 310/2011	1	1.42×10^{-5}	3.07×10^{-5}	8.80×10^{-5}
Fenitrothion	0.005	0.01	Reg. (EU) No 8990/2012	0.083	8.00×10^{-8}	1.79×10^{-7}	4.79×10^{-7}
Fenthion	0.007	0.01	Reg. (EU) No 310/2011	0.33	2.95×10^{-7}	6.15×10^{-7}	1.06×10^{-6}
Malathion	0.03	0.02	Reg. (EU) No 399/2015	0.00	0	0	0
Methamidophos	0.001	0.01	Reg. (EU) No 899/2012	1	3.11×10^{-5}	4.71×10^{-5}	7.10×10^{-5}
Methidathion	0.001	0.02	Reg. (EU) No 310/2011	0.32	0.82×10^{-5}	1.50×10^{-5}	4.30×10^{-5}
Phenthoate	0.003	-	Reg. (EU) No 839/2008	0.1	9.00×10^{-7}	1.57×10^{-6}	3.70×10^{-6}
Trichlorfon	0.002	0.01	Reg. (EU) No 899/2012	0.003	4.00×10^{-8}	6.64×10^{-8}	8.61×10^{-8}
Hazard Index (HI)					7.09×10^{-5}	1.26×10^{-4}	3.13×10^{-4}
CB pesticide				IC= Oxamyl			
Carbaryl	0.008	0.01	Reg. (EU) No 1096/2014	0.15	4.03×10^{-6}	6.88×10^{-6}	1.09×10^{-5}
Carbofuran	0.00015	0.003	Reg. (EU) No 399/2015	2.4	1.95×10^{-4}	4.10×10^{-4}	8.00×10^{-4}
Methomyl	0.0025	0.01	Reg. (EU) No 1822/2016	0.67	1.02×10^{-5}	2.45×10^{-5}	6.00×10^{-5}
Oxamyl	0.001	0.01	Reg. (EU) No 552/2019	1	2.23×10^{-5}	4.28×10^{-5}	7.20×10^{-5}
Pirimicarb	0.02	0.01	Reg. (EU) No 71/2016	0.02	9.30×10^{-8}	2.02×10^{-7}	5.02×10^{-7}
Propamocarb	0.4	0.01	Reg. (EU) No 832/2018	0.00	0	0	0
Hazard Index (HI)					2.32×10^{-4}	4.84×10^{-4}	9.43×10^{-4}
PY pesticide				IC= Deltamethrin			
Deltamethrin	0.01	1.0	Reg. (EU) No 832/2018	1	3.50×10^{-6}	1.05×10^{-5}	4.20×10^{-5}
Permethrin	0.05	0.05	Reg. (EU) No 623/2017	0.09	5.41×10^{-8}	8.22×10^{-8}	1.10×10^{-7}
Hazard Index (HI)					3.55×10^{-6}	1.06×10^{-5}	4.21×10^{-5}
HI[e] (SUM)					3.06×10^{-4}	6.20×10^{-4}	1.30×10^{-3}

[a] Relative potency factors

[b] Target hazard quotient

[c] Acceptable daily intake (mg/kg bm/day)

[d] Maximum concentration limits (mg/kg bm/day)

[e] Index compound

Discussion

Awareness about adverse effects of daily-life exposure to chemical mixtures has been increased recently (Rotter et al. 2018).

Exposure of humans to certain chemicals is a challenging subject and extensive monitoring data for an exposure is rarely available. Since consumers are exposed to complex chemical mixtures, assessment of exposure to a single compound is not expressing realistic exposure scenarios and could lead to under estimation of safety issues. Studies reporting interactions between pesticides from different classes, or within the same class, have shown that toxicokinetic interactions may occur and can lead to potentiation and synergism that may produce unpredicted toxicological responses. However, these interactions unlikely occur at low doses around ADI (Tsatsakis et al. 2017).

Olives exert a huge variety of local and regional types and olive cultivation is a common economic activity in the Mediterranean countries. Olive oil and table olives accounts for about 98% of global production of olives (Rodrigues et al. 2015). Results of the present study indicated an HI <1.0

Table 7 Estimated exposure centiles for carcinogenic and noncarcinogenic metals

Metal	EU[a]		ILCR[b]			THQ[c]		
	ADI[d]	CSF[e]	25%	50%	95%	25%	50%	95%
As	0.0001	1.5	9.50×10^{-9}	2.14×10^{-8}	6.86×10^{-8}	4.30×10^{-5}	1.42×10^{-4}	5.00×10^{-4}
Cd	0.001	-			-	4.40×10^{-6}	1.42×10^{-5}	3.64×10^{-5}
Cr	0.005	-			-	1.21×10^{-6}	2.85×10^{-6}	5.11×10^{-6}
Cu	0.5	-			-	3.54×10^{-6}	5.92×10^{-6}	1.00×10^{-5}
Fe	0.8	-			-	1.90×10^{-5}	4.11×10^{-5}	9.83×10^{-5}
Hg	0.0005	-			-	1.32×10^{-5}	2.85×10^{-5}	5.11×10^{-6}
Ni	0.005	-			-	9.40×10^{-6}	2.00×10^{-5}	4.38×10^{-5}
Pb	0.003	0.0083	2.20×10^{-11}	1.18×10^{-10}	4.78×10^{-10}	2.15×10^{-6}	4.76×10^{-6}	1.61×10^{-5}
Hazard Index (HI)	-	-			-	9.59×10^{-5}	2.60×10^{-4}	7.15×10^{-4}
ILCR (SUM)	-	-	9.52×10^{-9}	2.15×10^{-8}	6.90×10^{-8}		-	-

[a] European union

[b] Incremental life time cancer risk

[c] Target hazard quotient

[d] Acceptable daily intake (mg/kg BM/day)

[e] Cancer slope factor (mg/kg BM/day)

calculated for simultaneous oral exposure to 22 pesticides, which is indicative of minimal hazard and the concentration of each pesticide was less than respective Maximum Residue Limits (MRLs). In the current study, the levels of pesticides residue in 1800 samples analyzed were comparing to EU standards. The present extensive risk assessment indicated that

Table 8 Estimated exposure centiles for polycyclic aromatic hydrocarbons (PAHs)

PAH	TEF[a]	ILCR[b]			MoE[c]		
		25%	50%	95%	25%	50%	95%
Ace	0.001	1.70×10^{-17}	2.61×10^{-17}	5.66×10^{-17}	0.96×10^{11}	1.37×10^{11}	2.13×10^{11}
Acy	0.001	1.70×10^{-17}	2.60×10^{-17}	5.61×10^{-17}	0.96×10^{11}	1.37×10^{11}	2.13×10^{11}
Ant	0.01	1.83×10^{-16}	3.12×10^{-16}	7.13×10^{-16}	0.66×10^{10}	1.14×10^{10}	2.25×10^{10}
B*a*A	0.1	1.70×10^{-15}	2.60×10^{-15}	6.66×10^{-15}	0.90×10^{9}	1.30×10^{9}	1.95×10^{9}
B*a*P[h]	1.00	0.43×10^{-13}	1.04×10^{-13}	3.04×10^{-13}	2.73×10^{7}	3.40×10^{7}	4.20×10^{7}
B*b*Fj	0.1	1.23×10^{-15}	2.60×10^{-15}	5.66×10^{-15}	0.94×10^{9}	1.37×10^{9}	1.40×10^{9}
B*ghi*P	0.01	1.23×10^{-16}	2.60×10^{-16}	5.40×10^{-16}	0.94×10^{9}	1.37×10^{9}	1.40×10^{9}
B*k*F	0.1	1.23×10^{-15}	2.60×10^{-15}	5.60×10^{-15}	0.94×10^{9}	1.37×10^{9}	1.40×10^{9}
Chr	0.01	2.00×10^{-16}	3.38×10^{-16}	6.33×10^{-16}	0.73×10^{10}	1.00×10^{10}	1.60×10^{10}
D*ah*A	0.001	1.24×10^{-17}	2.60×10^{-17}	2.54×10^{-16}	0.95×10^{11}	1.37×10^{11}	2.13×10^{11}
Fl	0.001	1.04×10^{-17}	1.56×10^{-17}	3.10×10^{-17}	1.82×10^{11}	2.28×10^{11}	2.88×10^{11}
Flu	0.001	1.04×10^{-17}	1.56×10^{-17}	3.51×10^{-17}	1.82×10^{11}	2.28×10^{11}	2.88×10^{11}
IcdP	0.1	1.22×10^{-15}	2.60×10^{-15}	5.60×10^{-15}	1.00×10^{9}	1.37×10^{9}	1.40×10^{9}
Nap	0.001	1.04×10^{-17}	1.56×10^{-17}	5.56×10^{-17}	1.82×10^{11}	2.28×10^{11}	2.88×10^{11}
Phe	0.001	1.04×10^{-17}	1.56×10^{-17}	5.57×10^{-17}	1.82×10^{11}	2.28×10^{11}	2.88×10^{11}
Pyr	0.001	1.82×10^{-17}	3.12×10^{-17}	6.11×10^{-17}	0.65×10^{11}	1.14×10^{11}	1.38×10^{11}
ILCR (SUM)		4.90×10^{-14}	1.16×10^{-13}	3.30×10^{-13}	-	-	-
MoE (SUM)		-	-	-	1.13×10^{12}	1.48×10^{12}	1.97×10^{12}

[a] Toxic equivalency factor

[b] Incremental life time cancer risk

[c] Margins of exposure

Table 9 Parameters (%) influencing THQ and ILCR for pesticides, metals, and polycyclic aromatic hydrocarbons

Influential parameters (%)	Pesticides	Metals		Polycyclic aromatic hydrocarbons	
	THQ[a]	THQ	ILCR[b]	ILCR	MoE[c]
Ingestion rate	0.72	5.83	9.85	0.20	−0.83
Body mass	−14.00	−5.60	−10.03	−1.40	0.56
Concentration	0.96	17.10	13.71	0.89	−0.03

[a] Target hazard quotient

[b] Incremental life time cancer risk

[c] Margins of exposure

exposure to none of these 22 pesticides due to consumption of olives grown in Iran is likely to pose risks to consumers health.

Based on our results, estimated centiles for exposure to carcinogenic and noncarcinogenic metals pose *di minimis* risks to human health and concentrations of metals were less than their respective ADIs. Based on ILCRs and MoEs calculated in the present study, concentrations of PAHs posed *di minimis* risks. Various indicators of risk can provide extremely different risk probabilities for a given concentration. In order to increase the reliability of risks assessments, uncertainties must be considered. Differences in predicted risks that exist between and within groups can derive from several factors that influence exposure, including raw materials, type of processing and temperature (Petit et al. 2019). Humans are continuously exposed to mixtures of chemicals via water, food, and commercial products consumption. Currently, several international regulatory authorities consider new potential associations between exposure to chemical mixtures and adverse health outcomes (Tsatsakis et al. 2017).

Spraying mixture of synthetic pesticides by farmers can result in residues persisting in agricultural products, as well as soil. Humans can be exposed to those residues through dietary intake (Sakthiselvi et al. 2020). In a study performed in Italy, in 65 olive oil samples tested, only two samples contained dimethoate at concentrations greater than FAO/WHO Codex Alimentarius MRLs (Rastrelli et al. 2002). Based on an assessment of OPs residues in olive oils from Greece, a total of 30.5% of samples contained detectable residues, although only one sample contained dimethoate residues at concentrations greater than the MRLs and HI and TEF indices indicated no acute nor chronic risk for the Greek population through consumption of olive oil (Tsoutsi et al. 2008). Similar results were found in olive and olive oil samples from Portugal, where the QuEChERS method was applied to quantify 16 pesticides (Cunha et al. 2007). Another report showed residues of five pesticide including chlorpyrifos, cypermethrin, diazinon, profenofos and L-cyhalothrin in 50 samples of black and green olives (Ryad and Mahmoud 2016). Results of the present study are consistent with another study that analyzed 37 commercially available olive oils in Iran which demonstrated that consumers were not at risk of noncarcinogenic effects via ingestion of olive oil (Razzaghi et al. 2018). In a previous study, residues of fenthion and dimethoate pesticides were determined in organic and conventional olive oils collected from Crete; results showed that residue levels were lower than the maximum residue levels according to the FAO/WHO Codex Alimentarius (Tsatsakis et al. 2003). Also, Tsakiris et al. showed that a significant degree of sample contamination may occur during industrial production in factories (Tsakiris et al. 2010b). In another study, 16 pesticides were detected in olive samples, and pyrethrins were found to be the most commonly used insecticide (Tsakiris et al. 2010a).

Due to restrictions of fresh water for agricultural irrigation, wastewater has been used as alternative water resources in arid and semiarid regions. However, long-term wastewater irrigation can cause contamination of soils with metals and metalloids that can contaminate crops, posing a threat to humans health (Zaanouni et al. 2018). According to the findings of a study that analyzed 23 olive samples from Turkey, industrial pollution was suggested as a potential source of metals contamination. Similar to results reported in the present work, Fe was the most abundant metal (Yücel and Kılıçoğlu 2020). Another report showed that concentrations of metals in black olives in polluted areas of Turkey exceeded limits suggested to be safe for humans health (Şahan et al. 2007). Another report from Turkey showed olive contamination with Pb and Cd (Sahan et al. 2007). In a previous study that determined levels of various metals (e.g., Pb, Ni, Cd, Co, Cr, Cu, Fe, Zn, Mn, Mg, Ca, P, and K) olives in Turkey , concentrations of Cu and Pb were less than limits established by the Codex Alimentarius and Turkish local table olive standards (Tuna and Gecgel 2011). While metals in olive fruits can cause toxic effects and this can pose a risk to health of humans, considering the rates of ingestion of olives, dietary intake of olives was not found a significant exposure route to metals (Yücel and Kılıçoğlu 2020).

TEF is one of the beneficial tools for cumulative risk calculation posed by multi-component PAH (Liao and Chiang

2006). Analyses of various olive oils in Kuwait showed that the mean concentration of BaP was 0.92-μg/kg wet mass (wm) (Alomirah et al. 2011). Agricultural products can be contaminated by PAHs present in the air, soil, or water. In a previous study that analyzed various olive oils from Spain for nine PAHs, contamination of olive depended directly on environmental pollution, and was inversely associated with the size of fruits. Concentrations of PAHs were increased by contamination during several preparation procedures. Relatively great concentrations of PAHs were found as a consequence of accidental exposure to contamination, such as direct contact of olives with diesel exhaust and oil extraction in a polluted environment (Rodríguez-Acuña et al. 2008). Concentration was found the most significantly important factor in risk assessment of the three categories of chemicals that were studied in the present work as increasing contaminant concentrations were directly associated with higher risks (Rotter et al. 2018). In probabilistic exposure assessment, exposure to chemical mixtures in food depends on the concentrations of mixture components in different food items, as well as the consumption rate of these foods in a specific population (Taghizadeh et al. 2021).

It should be emphasized that general knowledge of Iranian farmers need to be upgraded to be more cautious about utilization of pesticides and fertilizers in agricultural practices (Amini et al. 2014; Azizi and Fuji 2005). Agricultural policies can be applied to manage risks by using cultivars with lower contaminant-accumulation probability, avoidance of farming near roads, and urban, industrial, and waste mining zones, and soil remediation, to make a more appropriate balance between environmental protection, food safety improvement, and health risks (Kheirabadi et al. 2020; Taghizadeh et al. 2021).

Conclusions

In the present work, risk of exposure of the Iranian population to 22 pesticides, eight metals, and 16 PAHs through consumption of olives, was assessed. In a total of 1800 olive samples, for none of the assessed chemicals, levels exceeded the MRLs of EU. Chronic cumulative risk examined in term of HI showed no concern for pesticide concentrations (i.e., HI<1). Metals did not show potential risks as shown by ILCRs and HI values. ILCR and MOE values did not imply potential risk to health for exposure to PAHs. Based on sensitivity analysis, concentration of the contaminants was the main factor influencing the calculated risk. Agricultural and environmental policies such as using eco-friendly components, biofertilizers, and alternative ways should be practiced to reduce chemical residue and further diminish the already minimal risks.

Acknowledgements The authors are thankful to Research Unit, Imam Raza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, for their help and support.

Availability of data and materials All data generated or analyzed during this study are included in this article.

Author contribution Author Taghizadeh, a Postdoctoral Fellow at Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran, conducted laboratory analyses, wrote the article, calculated the risk, simulation, and did statistical analyses. Author Azizi, a full-time professor of Department of Horticultural Science, Ferdowsi University of Mashhad, Mashhad, Iran, designed and supervised the work and participated in calculations. Author Rezaee, an assistant professor at Mashhad University of Medical Sciences, critically reviewed contaminants analyses and risk calculations, and revised the manuscript. Author Giesy, a full-time professor of Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, funded the project, and the corresponding author Karimi, a full-time professor of Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran, designed and funded the project. All authors read and approved the final version of the manuscript. Direct inquiries should be sent to author Karimi at Karimig@mums.ac.ir.

Funding Prof. Giesy was supported by the Canada Research Chairs Program of the Natural Sciences and Engineering Research Council of Canada (NSERC) and a visiting distinguished professor position in Environmental Science at Baylor University, Waco, Texas, USA.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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