

# A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors

Josephine Al-Alam, Firas Baroudi, Asma Chbani, Ziad Fajloun, Maurice Millet

▶ **To cite this version:**

Josephine Al-Alam, Firas Baroudi, Asma Chbani, Ziad Fajloun, Maurice Millet. A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors. Journal of Chromatography A, 2020, 1621, pp.461006 -. 10.1016/j.chroma.2020.461006 . hal-03490203

**HAL Id: hal-03490203**
**https://hal.science/hal-03490203**

Submitted on 20 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S0021967320302120
Manuscript_983b4fb2e786b2e70cdc64152ce07fde

# A multiresidue method for the analysis of pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in snails used as environmental biomonitors

JOSEPHINE AL-ALAM[1,2,3], FIRAS BAROUDI[2], ASMA CHBANI[1,4], ZIAD FAJLOUN[1,5], MAURICE MILLET[2*]

[1]Azm Center for Research in Biotechnology and its Applications, Doctoral School of Science and Technology, Lebanese University, El Mittein Street, Tripoli, Lebanon

[2]Institute of Chemistry and Processes for Energy, Environment and Health ICPEES UMR 7515 Group of Physical Chemistry of the Atmosphere, University of Strasbourg, Strasbourg, France

[3]Lebanese American University, School of Engineering, Byblos, Lebanon

[4]Faculty of Public Health III, Lebanese University, Tripoli, Lebanon

[5]Department of Biology, Faculty of Sciences 3, Lebanese University, Michel Slayman Tripoli Campus, Ras Maska 1352, Lebanon

---

*Address correspondence to Maurice Millet, Institute of Chemistry and Processes for Energy, Environment and Health ICPEES UMR 7515 Group of Physical Chemistry of the Atmosphere, University of Strasbourg, Strasbourg, France Tel.: + 33 (0)3 68 85 04 22, Fax: + 33 (0)3 68 85 04 02, E-mail: mmillet@unistra.fr



© 2020 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

**Abstract**

This paper reports an optimized multiresidue extraction strategy based on the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction procedure and on solid-phase microextraction (SPME) for the simultaneous screening of 120 pesticides, 16 polycyclic aromatic hydrocarbons, and 22 polychlorinated biphenyls from the terrestrial snail *Helix aspersa*. The optimized extraction method was based on QuEChERS using acetonitrile, followed by dispersive-Solid-phase extraction clean-up using primary secondary amine and octadecyl (C18) sorbents. The obtained extracts were analyzed by liquid chromatography coupled with tandem mass spectrometry and gas chromatography coupled with tandem mass spectrometry. This latest technique was preceded by a pre-concentration step using SPME with appropriate fibers. Afterwards, the method was validated for its linearity, sensitivity, recovery, and precision. Results showed high sensitivity, accuracy, and precision, with limits of detection and quantification lower than 20 ng $g^{-1}$ for most considered pollutants. Both inter and intra-day analyses revealed low relative standard deviation (%), which was lower than 20% for most targeted compounds. Moreover, the obtained regression coefficient ($R^2$) was higher than 0.98 and the recoveries were higher than 60% for the majority of the assessed pollutants.

**Keywords:** Snails, *Helix aspersa*, QuEChERS, SPME, environmental biomonitoring.

## 1. Introduction

Recent excessive polluting factors due to increased industrial activities, excessive agricultural practices (such as the use of pesticides and fertilizers), and intensive population growth and tobacco smoking need to be monitored to protect the ecosystem from their negative effects [1-4]. Environmental biomonitoring or biological environmental monitoring is generally defined as "the systematic use of living organisms or their responses to determine the state or changes in the environment" [5]. These samplers, known as biomonitors, are living organisms naturally present in the environment and characterized by a high ability to accumulate pollutants in their tissues [6]. These species should be accumulative and characterized by different criteria such as specificity, accumulation ratio, occurrence, time-integrative behavior, and biodiversity [7-9].

Among these species, mollusks are well known as filtering organisms, which have been successfully used in persistent organic pollutant (POP) monitoring programs due to their high bioaccumulation capacity, fixed location, and high population density [10, 11]. The use of snails as sentinel indicators is efficient due to their wide distribution, easy sampling, and ability to accumulate various type of pollutants [10, 12, 13]. Furthermore, snails live at the soil–plant–air interface and then integrate different sources and paths of contamination, which make them prone to various types of pollutants that accumulate in their soft tissues and become incorporated in their shells [14-19].

Among different type of mollusks, the terrestrial gastropod *Helix aspersa* is known for its biomonitoring properties, easy adaptation and manipulation in the laboratory, and sensitivity to genotoxicity assays [20, 21]. *H. aspersa* can accumulate different classes of

chemicals and serve as appropriate species for environmental biomonitoring for pesticides [22], trace metals [23-25], polycyclic aromatic compounds [26], industrial contamination [27], and urban pollution agents [28].

Therefore, the development of analytical tools for the quantification of traces of organic pollutants in such organisms seems efficient to establish a specific environmental control. Prior to chromatographic analysis, sample preparation is required to remove all types of interferences that may disturb the detection of the pollutants of concern, decrease the separation efficiency, or shorten the chromatographic column life [10, 29]. Although snails are well known for their ability to accumulate pollutants, studies on the extraction of pesticides and POPs from these matrices remain limited.

Accelerated solvent extraction (ASE) is an example of an extraction technique that has been used for the assessment of organic pollutants [30]. However, ASE requires efficient clean-up to remove all interfering impurities that give rise to high labor costs. SPE and solid-phase microextraction (SPME) are two well-known extraction/purification/concentration methods used to obtain a pure extract that can be analyzed by chromatographic techniques [31, 32].

In 2003, a new method was developed by Anastassiades et al. to overcome all critical flaws and practical limitations of the extraction procedures. This method known as QuEChERS was first used for the analysis of multiresidue pesticides in food [33]. This technique consists of soft liquid–liquid extraction followed by an optional clean-up step and requires few minutes and 5–15 mL of extracting solvent [34]. Although the original method showed remarkable efficiency for hundreds of analytes, several adjustments were

made to improve the method's performance and make it even more rugged and efficient for other difficult analytes in different complex matrices [35-37].

After extraction, organic pollutants are analyzed using commonly known analytical techniques, namely, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) and gas chromatography coupled with tandem mass spectrometry (GC–MS/MS) [38]. The choice of the separation technique depends mostly on the characteristics of the pollutants of interest [39, 40].

For all these reasons, this study aimed to develop a simple, fast, sensitive, and reliable analytical method for the trace analysis of a large number of environmental contaminants in the terrestrial snail *H. aspersa.* For this purpose, 120 pesticides, 16 polycyclic aromatic hydrocarbons (PAHs), and 22 polychlorinated biphenyls (PCBs) were selected to cover a wide number of organic environmental contaminants that can be monitored in such organisms. Two extraction protocols were compared to choose the method with a high extraction potential, allowing the best extraction recovery. The first protocol was based on ASE–SPE, and the second was based on QuEChERS. Both techniques were followed by a pre-concentration step using SPME. Both liquid and gas chromatography, coupled with MS/MS, were used to analyze this wide range of pollutants. The chosen matrices have yet to be studied for contamination by all these pollutants. Furthermore, the two extraction protocols were never carried out on snails, enabling their use as potential biomonitor candidates.

## 2. Materials and methods

### *2.1 Materials and reagents*

Pesticide analysis included 30 nonvolatile compounds analyzed by LC–MS/MS and 90 semi-volatile compounds including 21 organochlorine pesticides (OCPs) analyzed by GC–MS/MS. All pesticides, except OCPs, were purchased from Sigma–Aldrich (L'Isle d'Abeau, France) with purity higher than 97%.

For LC–MS/MS pesticide analysis, the 30 pesticides were as follows: pymetrozine, carbendazim, chloridazon, acetamiprid, nicosulfuron, thiacloprid, chlortoluron, carbetamide, terbutryn, spinosad A, isoproturon, diuron, metalaxyl-M, spinosad D, dimethenamid-p, penconazole, isoxadifen, tebuconazole, diflubenzuron, epoxiconazole, prothioconazole, propiconazole, chlorfenvinphos, triflusulfuron methyl, pendimethalin, cyazofamid, pyraclostrobine, diflufenican, flufenoxuron, and lufenuron. A stock solution of each of these standards at 1 g $L^{-1}$ was prepared in acetonitrile.

For GC–MS/MS pesticide analysis, the pesticides (except OCPs) were as follows: clofentezine, dichlobenil, etridiazole, diphenylamine, trifluralin, chlorpropham, tebutam, clomazone, propyzamide, lindane, pyrimethanil, dimethenamid-P, dimethachlor, acetochlor, alachlor, fenpropidin, carbaryl, ethofumesate, malathion, fenpropimorph, metolachlor-S, chlorpyrifos, flurochloridone, cyprodinil, pendimethalin, tolyfluanid, metazachlor, penconazole, procymidone, captan, folpet, oxadiazon, buprofezine, kresoxim-methyl, bupirimate, flusilazole, myclobutanil, aclonifen, trifloxystrobin, bromoxynil octanoate, propiconazole, quinoxyfen, lenacile, diclofop-methyl, chloridazon, diflufenicanil, fluazinam, tebuconazole, bifenthrin, dimoxystrobin, epoxiconazole, fenoxycarb, isoxaflutole, tebufenpyrad, bifenox, lambda cyhalothrin, fenarimol,

pyraclostrobin, prochloraz, cypermethrin, boscalid, indoxacarb, difenoconazole, deltamethrin, azoxystrobin, dimethomorph, spiroxamine, and metamitron. A stock solution of each of these standards at 1 g $L^{-1}$ was prepared in acetonitrile.

For OCP analysis, a mixture at 0.1 g $L^{-1}$ of 21 OCPs, including α-HCH, γ-HCH, β-HCH, δ-HCH, heptachlor epoxide A, methoxychlor, o,p'-DDD, o,p'-DDT, p,p'-DDD, p,p'-DDT, α-endosulfan, o,p'-DDE, p,p'-DDE, aldrin, heptachlor, dieldrin, hexachlorobenzene, heptachlor epoxide B, *trans*-chlordane, and *cis*-chlordane, was purchased from Cluzeau Info Labo (St. Foy la Grande, France).

For PAH analysis, a mixture at 0.1 g $L^{-1}$ of 16 PAHs (i.e., naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo(a)pyrene, indeno (1,2,3-cd) pyrene, benzo[g,h,i]perylene, and dibenzo[a,h]anthracene) was prepared from individual standards purchased from Sigma–Aldrich (L'Isle D'Abeau, France).

For PCB analysis, a mixture at 0.1 g $L^{-1}$ of 22 PCBs (i.e., PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 70, PCB 81, PCB 101, PCB 123, PCB 118, PCB 114, PCB 105, PCB 126, PCB 149, PCB 153, PCB 138, PCB 167, PCB 156, PCB 157, PCB 169, PCB 180, and PCB 189) was purchased from Cluzeau Info Labo (St. Foy la Grande, France). All prepared solutions were stored at −18 °C.

Four internal standards for LC–MS/MS were obtained from CDN isotopes (Quebec, Canada): carbendazim-$d^4$ (99.3%), diuron-$d^6$ (99.8%), pendimethalin-$d^5$ (99%), and nicosulfuron-$d^6$ (99%). A standard solution of each compound at 0.05 g $L^{-1}$ in

acetonitrile was prepared, and a mixture of each compound at 0.01 g $L^{-1}$ in acetonitrile was prepared and stored at −18 °C for alternative use as internal standard (IS) solution.

Internal standards for GC–MS/MS were trifluralin-$d^{14}$, 4-nitrophenol-$d^4$, and naphthalene-$d^8$ (99%), and they were purchased from Sigma–Aldrich (L'Isle d'Abeau, France) and Cambridge Isotope Laboratories (Cluzeau Info Labo, France). A mixture of trifluralin-$d^{14}$ and 4-nitrophenol-$d^4$ at 0.01 g $L^{-1}$ in acetonitrile was used as IS solution for pesticide analysis except OCPs. A solution of naphthalene-$d^8$ at 0.01 g $L^{-1}$ in acetonitrile was used as IS solution for OCP, PAH, and PCB analyses.

HPLC-grade acetonitrile, toluene (TOL) from Biosolve (Dieuze, France), methanol (MeOH), ethyl acetate (EA), "Fontainebleau" sand from Prolabo (France), and silica gel (Merck, Germany) were used. LC–MS-grade acetonitrile, LC–MS-grade water, formic acid, and HPLC-grade acetonitrile were purchased from Sigma–Aldrich (L'Isle D'Abeau, France). Ultrapure water used was purified by an Elga system (Antony, France).

Kits for QuEChERS sample preparations were purchased as ready to use from RESTEK, France. Buffered extraction kits (EN 1566 method) containing 4 g of $MgSO_4$, 1 g of NaCl, 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogencitrate sesquihydrate were used. For clean-up, sample clean-up kits (AOAC 2007 method) containing 1.2 g of $MgSO_4$, 400 mg of PSA, and 400 mg of $C_{18}$ were used.

SPE CHROMABOND® EASY cartridges consisting of a polar-modified polystyrene divinyl benzene copolymer were used as adsorbents (mean pore diameter 60 Å, surface area 623 $m^2/g$, and mean particle size 91 μm) and purchased from Macherey–Nagel, France.

SPME polyacrylate (PA) fiber (65 µm) was purchased from Supelco-Sigma Aldrich (Saint Martin d'Hère France) and used for the extraction of semi-volatile pesticides except OCPs.

SPME polydimethylsiloxane (PDMS) fiber (100 µm) was purchased from Supelco-Sigma Aldrich (Saint Martin d'Hère France) and used for the extraction of OCPs, PCBs, and PAHs.

### *2.2 Sample collection*

Certified *H. aspersa* blank snails were purchased from "Cap' Hélix Escargots, 2, Bréharadec, 29770 GOULIEN, France." The snails were transported frozen to the laboratory where they were kept at −18 °C until used.

For real sample analysis, three samples of *H. aspersa* were collected from three different lands in Akkar region situated in northern Lebanon (34°33′02″N 036°04′41″E). This region is known for its high population rate and agricultural production.

Samples were collected in propylene tubes and then transported frozen to the laboratory, where they were stored at −18 °C until analysis.

### *2.3 Extraction procedures*

About 5 g of homogenized blank snails samples was weighed in 50 mL centrifuge tubes and fortified with 200 µL of each mixture. Fortified samples were kept at 4 °C overnight, followed by the extraction procedure cited below. All extractions were conducted in triplicate.

*2.3.1 ASE–SPE-based extraction*

*2.3.1.1 ASE*

About 5 g of the fortified snails was extracted by ASE. For ASE, 33 mL cells were fitted at the bottom with filter paper, and a thin layer (1 cm height) of activated silica gel was added to provide a first purification step. A second filter was added on the top of the silica, and 5 g of snails mixed with "Fontainebleau" sand was added. Finally, a filter was added at the top of the cell, which was sealed well. Extraction was carried out with acetonitrile (100%), and the program was as follows: heating the cell for 7 min, 10 min of static cycle, temperature 100 °C, pressure 1500 psi, flushing 100%, and purging for 300 s. Around 30 min was required for each extracting cell.

ASE final extracts were collected in ASE bottles and prepared for SPE purification. Extracts were filtered and then diluted to 1000 mL with acidified (pH 3) ultrapure water.

*2.3.1.2 SPE*

The SPE procedure was as follows: conditioning of the cartridge with 5 mL of MeOH followed by 5 mL of ultrapure water, flushing 1000 mL of the sample into the cartridge at 10 mL $min^{-1}$, and drying by $N_2$ flushing for 30 min. Extracts were sequentially eluted with 2 mL of each of the following solvents: EA, TOL, and acetonitrile. The obtained

extract was sampled in a 10 mL glass tube and then evaporated to 100 µL. Around 1 h was needed for each sample to be extracted by SPE.

*2.3.2 QuEChERS-based extraction*

This extraction procedure was based on the work of Al Alam et al. in 2017 for the analysis of organic pollutant residues in honey [41].

First, 10 mL of acetonitrile was added to 5 g of fortified snails, and the tubes were shaken. When the mixture was homogeneous, 10 mL of QuEChERS citrate buffered extraction salts was added. The tubes were immediately shaken by hand, vortexed for 1 min, and centrifuged for 10 min at 5000 rpm. Second, the supernatant was added to the 15 mL PSA tube. This tube was immediately shaken by hand, vortexed for around 30 s, and centrifuged for 10 min at 5000 rpm. Finally, the obtained extract was sampled in a 10 mL glass tube and then evaporated to 100 µL. Around 20 min was needed for the whole procedure.

*2.3.3 Extract reconstitution*

Once evaporated, the collected extracts were reconstituted with acetonitrile to 1 mL to prepare them for chromatographic analysis.

About 100 µL of the final extract was transferred to an LC vial, where 10 µL of the appropriated IS solution was added. The extract was then analyzed by LC–MS/MS.

The remaining 900 µL was diluted to 20 mL with salted water (1.5% NaCl) to promote their adsorption on the SPME fiber. Subsequently, 10 µL of both appropriated GC IS solutions was added prior to this latest purification and extraction.

*2.3.4 SPME and concentration*

SPME fibers were soaked in 20 mL of the salted prepared solution and heated at 60 °C under agitation (500 rpm) for 40 min for the PA fiber and at 80 °C for 40 min for the PDMS fiber.

SPME was carried out by direct immersion in which the fiber was directly immersed into the liquid sample, and the analytes were divided between the fiber and the liquid sample. After extraction, the SPME fiber was transferred to the GC injection port where desorption of the analyte occurred and analysis was carried out.

***2.4 Sample analysis***

*2.4.1 LC–MS/MS*

The system used was an LC system (Thermo Scientific, Surveyor pump and autosampler) coupled with a tandem MS/MS system (TSQ Quantum Access Max equipped with a Hyper Quads Driven) operating in electrospray ionization (ESI) mode. Chromatographic separation was performed on a Macherey-Nägel Nucleodur $C_{18}$ Pyramid column (150 mm × 3 mm; 3 µm) thermostated at 25 °C. The chromatographic system was also equipped with an autosampler (Accela Autosampler) and a Surveyor LC Pump Plus

(Thermo Scientific). The flow rate was 300 µL $min^{-1}$. Samples were analyzed using a mobile phase of acetonitrile/water (0.05% formic acid). The gradient started with 30:70 (v/v) for 5 min, followed by 50:50 (v/v) for 6 min and then 80:20 (v/v) for 7 min to achieve 95:5 (v/v) for 10 min. Finally, a ratio of 30:70 (v/v) for 8 min was recommended to stabilize the column for a new injection. The injection volume was 20 µL.

Details on the analyzed pesticide figure are illustrated in supplementary materials S1.

*2.4.2 GC–MS/MS*

A Thermo Scientific Trace GC coupled with an MS/MS system (ITQ 700, Temperature source: 210 °C, transfer line temperature: 300 °C) operating in electron impact (EI) mode was used. The considered POPs (20 OCPs, 16 PAHs, and 22 PCBs) and the 70 remaining semi-volatile pesticides were analyzed on an XLB (50% phenyl/ 50% methylsiloxane) capillary column (30 m × 0.25 mm internal diameter and 0.25 µm as film thickness). Injections were made in splitless mode at 250 °C for 15 min. Helium was used as a carrier gas at a flow rate of 1 mL $min^{-1}$.

*2.4.2.1 POP separation and analysis*

Samples were injected by thermal desorption of the PDMS SPME fiber. The initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 255 °C at a rate of 10 °C $min^{-1}$ and another increase to 330 °C at a rate of 20 °C $min^{-1}$, where it was maintained for 18 min. The total run time was 45.25 min.

Details on the analyzed POP figure are in supplementary materials S2.

*2.4.2.2 Semi-volatile pesticide separation and analysis*

Samples were injected by thermal desorption of the PA SPME fiber. The initial oven temperature was set at 50 °C for 3 min, followed by a linear ramp to 160 °C at a rate of 36.6 °C $min^{-1}$ and another ramp to 300 °C at a rate of 5.8 °C $min^{-1}$, where it was maintained for 10 min. The total run time was 41 min.

Details of the semi-volatile pesticides analyzed by GC–MS/MS are shown in supplementary materials S3.

***2.5 Method validation***

*2.5.1 Validation parameters*

The method was validated for all quantification parameters. First, fortified samples with a concentration range of 5–3000 ng $g^{-1}$ were extracted in triplicate to determine linearity. Five samples of spiked matrix with three concentration levels (10, 100, and 1000 ng $g^{-1}$)

were extracted for three successive days to determine intermediate precision and repeatability. The intra- and inter-day precision and accuracy were estimated by analyzing five replicates at three different QC levels (10, 100, and 1000 ng $g^{-1}$). The intra-day precision of the assay was estimated by calculating the relative standard deviation (RSD) for the analysis of QC samples in five replicates, and inter-day precision was determined by the analysis of five replicates of QC samples on three consecutive days. This parameter was determined by varying processes conditions, which were the different days of extraction, different solvent bottles, and different extraction kits used. The intra-day and inter-day precision were evaluated by their corresponding RSD (%).

Concerning method validation limits, the limit of detection (LOD) and limit of quantification (LOQ) were calculated using the signal produced over the background noise obtained. These limits were determined using LOD =3 × [min] S/N and LOQ =10 × [min] S/N, where [min] = minimal concentration at which a signal was obtained, S = signal intensity obtained at this concentration, and N = noise intensity obtained at this concentration.

The recoveries were determined at the same levels as precision following the equation:

Recovery (%) = (A extracted spiked sample / A standard solution) ×100

where A= peak area obtained.

*2.5.2 Matrix-matched calibration curves*

To overcome the possible matrix effect and to obtain reliable data of analyzed samples, matrix-matched calibration curves were developed. Ten calibration points were prepared

using the validated extraction procedure. Homogenate matrices were spiked with standard solutions to cover a pollutant range between 5 and 3000 ng $g^{-1}$, extracted, and analyzed. The calibration points used included the following concentrations (in ng $g^{-1}$): 5, 10, 25, 50, 100, 500, 1000, 1500, 2000, and 3000.

Calibration curves were validated for their linearity by calculating the determination coefficient $R^2$.

Finally, the validation of each compound included several criteria such as fragmentations, retention time, and ion ratios for nonvolatile compounds. Furthermore, the extraction performance was determined following the signal areas of the IS solutions in each analyzed sample.

Calibrations and quantification analysis were conducted using XCalibur software.

## 3. Results and discussion

### *3.1 Method development*

To obtain a concentrated extract, a concentration step by evaporation and reconstitution was added prior to LC analysis. Moreover, SPME was added prior to GC analysis for arranging and discriminating volatile compounds. Among different SPME fibers, the PDMS fiber was used as it is well known for its usefulness for the extraction of analytes with high partition coefficients such as PAHs, PCBs, and OCPs, whereas PA was used for the extraction of pesticides due to its efficiency in extracting polar compounds. PA is

a moderated polar coating characterized by a stronger hydrogen bond than PDMS fiber, which makes it ideal for polar compounds with moderate hydrophobicity compared with other fibers [42].

These concentration steps were used following the two extraction procedures previously detailed. Both extractions were compared to choose the method enabling the highest recovery with the lowest RSD %.

Chromatograms of the assessed nonvolatile pesticides, volatile pesticides, PAHs, PCBs, and OCPs are shown in Figures 1s, 2s, and 3s in supplementary materials S4. These obtained chromatograms showed good separation of all sought compounds, which allowed the identification of the assessed pollutants on the basis of each parameter previously shown in Tables 1s, 2s, and 3s.

*3.1.1 Influence of the extraction procedure*

Fortified snails at a mid-level concentration of 1000 ng $g^{-1}$ of each pollutant mixture were extracted by ASE–SPE-based extraction and QuEChERS-based extraction. The two extractions were followed by a pre-concentration step using SPME prior to GC–MS/MS analysis.

All extractions were carried out in triplicate, and RSD % was calculated for each compound.

Figure 1 shows the recoveries obtained by the two methods at a concentration of 1000 ng $g^{-1}$. This figure shows the average recovery rate for each type of considered pollutant.

The obtained results showed that the recoveries obtained with ASE–SPE-based extraction were lower than those obtained with QuEChERS-based extraction. For semi-volatile pesticides, the recoveries obtained with ASE–SPE-based extraction were between 20% and 94% with a mean recovery of 61.24%, whereas those obtained with QuEChERS-based extraction were between 65% and 107% with a mean recovery of 85.72%. For OCP recoveries, results obtained with ASE–SPE-based extraction were between 17% and 82% with a mean recovery of 54.93%, whereas those obtained with QuEChERS-based extraction were between 62% and 104% with a mean recovery of 80.24%. For PAH recoveries, results obtained with ASE–SPE-based extraction were between 56% and 71% with a mean recovery of 63.39%, whereas those obtained with QuEChERS-based extraction were between 64% and 96% with a mean recovery of 84.31%. For PCB recoveries, results obtained with ASE–SPE-based extraction were between 17% and 110% with a mean recovery of 54.75%, whereas those obtained with QuEChERS-based extraction were between 50% and 117% with a mean recovery of 80.2%. For the remaining volatile pesticides, high recovery rates were obtained with QuEChERS-based extraction. Results obtained with ASE–SPE-based extraction were between 14% and 127% with a mean recovery of 51.06%, whereas those obtained with QuEChERS-based extraction were between 55% and 114% with a mean recovery of 78.06%.

These results clearly demonstrated the influence of the extraction procedure on pollutant analysis from the same matrix. These results were also proven by Blasco et al. in 2011, who proved that QuEChERS-based extraction allows higher recovery rates in comparison with PLE and SPE [43].

The RSD% of the two developed methods was also assessed. The RSD% of each pollutant type obtained using the two compared extraction methods is shown in Figure 2.

The results obtained from the analysis of the RSD% of both extraction protocols favored extraction based on QuEChERS to that based on ASE–SPE. The RSD% obtained with ASE–SPE was higher than 20% for all the analyzed pollutants, whereas this value was lower than 15% for all compounds extracted using QuEChERS. The mean RSDs % for ASE–SPE-based extraction were 33.12%, 43.66%, 43.71%, 54.71%, and 48.06% for the determination of nonvolatile pesticides, OCPs, PAHs, PCBs, and remaining volatile pesticides, respectively. These values were far lower for QuEChERS-based extraction; the RSDs % were 6.11%, 10.82%, 9.91%, 15.16%, and 15.55% for nonvolatile pesticides, OCPs, PAHs, PCBs, and remaining volatile pesticides, respectively.

The results provided by the calculation of the recoveries and the RSD % proved that QuEChERS-based extraction is a method of choice for the analysis of multiresidual pollutants from such matrices, providing high recovery rates with a low RSD. Compared with ASE-based methods, QuEChERS-based extraction is rapid and easy to use, providing better recoveries with fewer losses of volatile compounds [44].

For all these reasons, QuEChERS-based extraction was proved to be the method of choice in this work. Furthermore, several crucial points, such as the high cost of equipment, large volume used for cell rinsing and preparation before extraction, high temperature leading to low recoveries and decomposition of thermally instable analytes,

and several extraction steps increasing RSD% [45, 46], reduce ASE–SPE-based extraction's efficiency and give high credibility to QuEChERS-based extraction for the analysis of multi-residues of environmental pollutants.

*3.1.2 Influence of the nature of the extraction solvent*

As QuEChERS seems to be the most efficient extraction procedure, a selection of an appropriate extraction solvent for liquid–liquid extraction is crucial to improve recovery. The extraction solvent plays a main role in any extraction step as incomplete extraction and matrix effects can lead to an underestimation of the actual concentration in the sample [47]. The organic solvent chosen must be highly polar, miscible in water, and able to induce phase separation following the addition of the appropriate extraction salts. Moreover, the suitable salt must not be soluble in the extraction solvent [29]. Acetonitrile and EA were tested on fortified snails with the same precision level, and the extraction efficiency of both solvents was compared. To choose the best extraction solvent, recoveries were calculated. Figure 3 shows the recovery results obtained with the use of acetonitrile and EA as QuEChERS extraction solvent.

As observed in Figure 3, the use of EA showed recoveries lower than those obtained with acetonitrile. For nonvolatile pesticides, the recovery obtained was 68.09% for the use of EA and 85.62% with the use of acetonitrile; for OCPs, the use of EA gave a mean recovery of 69.59%, whereas this value was 80.24% with the use of acetonitrile. For PAHs and PCBs, the mean recoveries were 77.91 and 72.49% with EA, respectively, and 84.31 and 80.2% with acetonitrile, respectively. Likewise, the recovery rate of semi-

volatile pesticides increased from 65.95% with the use of EA as QuEChERS extraction solvent to 78.06% with the use of acetonitrile.

The use of acetonitrile has proven its efficiency in several multiresidue analyses in biological samples in comparison with EA, exhibiting better recoveries and lower standard deviations between replicates [29, 48, 49].

For matrix effects, the analysis of biological matrices, such as snails, can lead to the co-extraction of a certain quantity of other compounds, such as lipids, sugars, and organic acids; their elimination prior to the final determination step is crucial [48].

Studies have shown that PSA allows the removal of all polar organic acids, polar pigments, sugars, and fatty acids from the extracts, whereas $C_{18}$ allows the elimination of nonpolar interfering substances such as lipids [34, 50].

The analyzed extracts showed good separation among all searched compounds, which allowed the identification of 120 pesticides, 16 PAHs, and 22 PCBs on the basis of the properties of each parameter previously shown in Tables 1s, 2s, and 3s.

### *3.2 Method validation*

Once chosen and developed, the method should be validated as efficient and useful. The validation proves that the used analytical methodology is accurate, specific, reproducible, and robust over the specified range that a compound will be analyzed [51]. Several parameters were tested: repeatability and reproducibility for method accuracy, LOD and LOQ for method limits, linearity for the ability of the method to elicit test results that are directly proportional to analyte concentration within a range between 5 and 3000 ng $g^{-1}$,

and recovery for extraction efficiency. To include the error due to the matrix effect in measurements, matrix-matched calibrations were conducted. Figure 4s in supplementary material S5 shows the calibration curves of some analyzed compounds.

Table 4s represents the results of the validation parameters for nonvolatile pesticides analyzed by LC–MS/MS.

For the nonvolatile pesticides analyzed by LC–MS/MS, all target compounds were validated with good linearity expressed by a regression coefficient higher than 0.99 for the 30 targeted pesticides. LOD and LOQ were lower than 15 ng $g^{-1}$ for all compounds, except lufenuron having an LOQ of about 20 ng $g^{-1}$. The 30 pesticides were detected with high precision with RSD % lower than 20% for inter- and intra-day analyses. Furthermore, the method showed good recoveries higher than 65% for all targeted compounds.

For semi-volatile compounds, Tables 5s and 6s represent the validation parameters for OCPs, PAHs, PCBs, and volatile pesticide analyzed by GC–MS/MS.

For the semi-volatile compounds analyzed with GC–MS/MS, compounds were validated for their good linearity expressed by regression coefficients higher than 0.99 for the majority of the compounds sought. Low LOD and LOQ were determined for the analyzed

compounds with an RSD lower than 20% for most of them. By contrast, the calculated RSD % was higher than the one obtained with the nonvolatile pesticides. The main explanation for these results is the introduction of SPME as a pre-concentration step; excluding this step increased the error and decreased the recovery rates [52]. Moreover, some fiber's saturation problems could be the main reasons for the high RSD % and low recovery observed with some compounds (PCB-169, PCB-157, methoxychlor, *o.p'*-DDT, and bupirimate).

### *3.3 Application to real samples*

The samples collected were analyzed in accordance with the previously developed method. Residue levels were calculated using Xcalibur software based on the previously plotted calibration curves.

Most of the assessed samples were contaminated with pesticides and PAHs, whereas no important PCB residues were found for all the samples analyzed.

Table 1 shows the most relevant pesticides and the PAH residues found in the samples analyzed.

## 4. Conclusion

The analytical method developed in this paper enabled the simultaneous analysis of 158 emerging environmental pollutants from the terrestrial gastropod *H. aspersa*. The

combination of a simple extraction method such as QuEChERS with an SPME process coupled with chromatographic analytical techniques allowed the extraction of this wide number of different types of pollutants. The comparison of the QuEChERS–SPME-based method with ASE–SPE–SPME proved the efficiency of the first method, especially in terms of being efficient and ecofriendly. The validation of the developed method yielded positive results, indicating good performance in terms of linearity, accuracy, and precision.

The presented extraction method followed by chromatographic analysis via LC–MS/MS and GC–MS/MS presents a reliable tool for the routine analysis of a large range of compounds at trace level in snails. The proposed method may be applied in further studies focusing on environmental biomonitoring.

**Acknowledgment**

We gratefully acknowledge AZM & SAADE association and the Lebanese University for funding the project, as well as Strasbourg University for the international mobility aid, without which the present study could not have been completed.

**References**

[1] S.I. Korfali, H. Karaki, Speciation of Metals in Soils and Water: Risk Assessment, Environ. Process., 5 (2018) 101-125. https://doi.org/10.1007/s40710-018-0328-1

[2] M. Wazne, S. Korfali, Spatial and temporal assessment of metal pollution in the sediments of the Qaraoun reservoir, Lebanon, Environ. Sci. Pollut. Res., 23 (2016) 7603-7614. https://doi.org/10.1007/s11356-015-6022-1

[3] S. Saha, R. Mistri, B.C. Ray, Determination of pyridine, 2-picoline, 4-picoline and quinoline from mainstream cigarette smoke by solid-phase extraction liquid chromatography/electrospray ionization tandem mass spectrometry, J. Chromatogr. A, 1217 (2010) 307-311. https://doi.org/10.1016/j.chroma.2009.11.050

[4] S. Saha, R. Mistri, B.C. Ray, Rapid and sensitive method for simultaneous determination of six carcinogenic aromatic amines in mainstream cigarette smoke by liquid chromatography/electrospray ionization tandem mass spectrometry, J. Chromatogr. A, 1216 (2009) 3059-3063. https://doi.org/10.1016/j.chroma.2009.01.069
[5] Z. Yang, B. Chen, L. Li, B. Zheng, L. Liu, International Conference on Ecological Informatics and Ecosystem Conservation (ISEIS 2010) Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends, Procedia Environ. Sci., 2 (2010) 1510-1524. https://doi.org/10.1016/j.proenv.2010.10.164
[6] M. Marć, M. Tobiszewski, B. Zabiegała, M. de la Guardia, J. Namieśnik, Current air quality analytics and monitoring: A review, Anal. Chim. Acta, 853 (2015) 116-126. https://doi.org/10.1016/j.aca.2014.10.018
[7] B. Wolterbeek, Biomonitoring of trace element air pollution: principles, possibilities and perspectives, Environ. Pollut., 120 (2002) 11-21. https://doi.org/10.1016/S0269-7491(02)00124-0
[8] H.T. Wolterbeek, P. Bode, State of the Art of Trace Element Determinations in Plant Matrices Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution, Sci. Total Environ., 176 (1995) 33-43. https://doi.org/10.1016/0048-9697(95)04825-1
[9] S. Pérez, P. Sánchez-Marín, J. Bellas, L. Viñas, V. Besada, N. Fernández, Limpets (Patella spp. Mollusca, Gastropoda) as model organisms for biomonitoring environmental quality, Ecol. Indic., 101 (2019) 150-162. https://doi.org/10.1016/j.ecolind.2019.01.016
[10] A. Berlioz-Barbier, R. Baudot, L. Wiest, M. Gust, J. Garric, C. Cren-Olivé, A. Buleté, MicroQuEChERS–nanoliquid chromatography–nanospray–tandem mass spectrometry for the detection and quantification of trace pharmaceuticals in benthic invertebrates, Talanta, 132 (2015) 796-802. https://doi.org/10.1016/j.talanta.2014.10.030
[11] M. Gust, T. Buronfosse, O. Geffard, R. Mons, H. Queau, J. Mouthon, J. Garric, In situ biomonitoring of freshwater quality using the New Zealand mudsnail Potamopyrgus antipodarum (Gray) exposed to waste water treatment plant (WWTP) effluent discharges, Water Res., 44 (2010) 4517-4528. https://doi.org/10.1016/j.watres.2010.06.019
[12] A. Beeby, L. Richmond, Evaluating Helix aspersa as a sentinel for mapping metal pollution, Ecol. Indic., 1 (2002) 261-270. https://doi.org/10.1016/S1470-160X(02)00022-5
[13] R. Laskowski, S.P. Hopkin, Accumulation of Zn, Cu, Pb and Cd in the garden snail (Helix aspersa): implications for predators, Environ. Pollut., 91 (1996) 289-297. https://doi.org/10.1016/0269-7491(95)00070-4
[14] A. De Vaufleury, M. Coeurdassier, P. Pandard, R. Scheifler, C. Lovy, N. Crini, P.M. Badot, How terrestrial snails can be used in risk assessment of soils, Environ. Toxicol. Chem., 25 (2006) 797-806. https://doi.org/10.1897/04-560R.1
[15] B. Pauget, F. Gimbert, M. Coeurdassier, N. Crini, G. Pérès, O. Faure, F. Douay, A. Richard, C. Grand, A. de Vaufleury, Assessing the in situ bioavailability of trace

elements to snails using accumulation kinetics, Ecol. Indic., 34 (2013) 126-135. https://doi.org/10.1016/j.ecolind.2013.04.018
[16] A. Gomot-de Vaufleury, A. Bispo, Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails. 1. Sublethal effects on growth, Environ. Sci. Technol., 34 (2000) 1865-1870. https://doi.org/10.1021/es9907212
[17] K.S. El-Gendy, M.A. Radwan, A.F. Gad, A.E. Khamis, E.H. Eshra, Use of multiple endpoints to investigate the ecotoxicological effects of abamectin and thiamethoxam on Theba pisana snails, Ecotoxicol. Environ. Saf., 167 (2019) 242-249. https://doi.org/10.1016/j.ecoenv.2018.10.027
[18] D. Kowalczyk-Pecka, S. Pecka, E. Kowalczuk-Vasilev, Changes in fatty acid metabolism induced by varied micro-supplementation with zinc in snails Helix pomatia (Gastropoda Pulmonata), Ecotoxicol. Environ. Saf., 138 (2017) 223-230. https://doi.org/10.1016/j.ecoenv.2016.12.033
[19] J.L. Capinera, K. Dickens, Some effects of copper-based fungicides on plant-feeding terrestrial molluscs: A role for repellents in mollusc management, Crop Prot., 83 (2016) 76-82. https://doi.org/10.1016/j.cropro.2016.01.018
[20] F.R.d. Silva, B. Erdtmann, T. Dalpiaz, E. Nunes, A. Ferraz, T.L. Martins, J.F. Dias, D.P.d. Rosa, M. Porawskie, S. Bona, Genotoxicity of Nicotiana tabacum leaves on Helix aspersa, Genet. Mol. Biol., 36 (2013) 269-275. http://dx.doi.org/10.1590/S1415-47572013005000020
[21] M.R. de Souza, F.R. da Silva, C.T. de Souza, L. Niekraszewicz, J.F. Dias, S. Premoli, D.S. Corrêa, M.d.C. Soares, N.P. Marroni, M.I. Morgam-Martins, J. da Silva, Evaluation of the genotoxic potential of soil contaminated with mineral coal tailings on snail Helix aspersa, Chemosphere, 139 (2015) 512-517. https://doi.org/10.1016/j.chemosphere.2015.07.071
[22] C. Druart, M. Millet, R. Scheifler, O. Delhomme, C. Raeppel, A. de Vaufleury, Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard, Sci. Total Environ., 409 (2011) 4280-4288. https://doi.org/10.1016/j.scitotenv.2011.07.006
[23] A.G.d. Vaufleury, F. Pihan, Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: metal bioavailability and bioaccumulation, Environ. Toxicol. Chem., 21 (2002) 820-827. https://doi.org/10.1002/etc.5620210419
[24 A. De Vaufleury, M. Coeurdassier, P. Pandard, R. Scheifler, C. Lovy, N. Crini, P.M. Badot, How terrestrial snails can be used in risk assessment of soils, Environ. Toxicol. Chem., 25 (2006) 797-806. https://doi.org/10.1897/04-560R.1
[25] A.D. Campoy-Diaz, M.A. Arribére, S.R. Guevara, I.A. Vega, Bioindication of mercury, arsenic and uranium in the apple snail Pomacea canaliculata (Caenogastropoda, Ampullariidae): Bioconcentration and depuration in tissues and symbiotic corpuscles, Chemosphere, 196 (2018) 196-205. https://doi.org/10.1016/j.chemosphere.2017.12.145
[26] M. Ianistcki, J. Dallarosa, C. Sauer, C. Teixeira, J. Da Silva, Genotoxic effect of polycyclic aromatic hydrocarbons in the metropolitan area of Porto Alegre, Brazil,

evaluated by Helix aspersa (Müller, 1774), Environ. Pollut., 157 (2009) 2037-2042. https://doi.org/10.1016/j.envpol.2009.02.025
[27] F. Pihan, A. De Vaufleury, The snail as a target organism for the evaluation of industrial waste dump contamination and the efficiency of its remediation, Ecotoxicol Environ Saf, 46 (2000) 137-147. https://doi.org/10.1006/eesa.1999.1891
[28] F. Regoli, S. Gorbi, D. Fattorini, S. Tedesco, A. Notti, N. Machella, R. Bocchetti, M. Benedetti, F. Piva, Use of the land snail Helix aspersa as sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach, Environ. Health Perspect., (2006) 63-69. https://doi.org/10.1289/ehp.8397
[29] A. Berlioz-Barbier, A. Buleté, J. Faburé, J. Garric, C. Cren-Olivé, E. Vulliet, Multi-residue analysis of emerging pollutants in benthic invertebrates by modified micro-quick-easy-cheap-efficient-rugged-safe extraction and nanoliquid chromatography–nanospray–tandem mass spectrometry analysis, J. Chromatogr. A, 1367 (2014) 16-32. https://doi.org/10.1016/j.chroma.2014.09.044
[30] J.L. Gómez-Ariza, M. Bujalance, I. Giráldez, A. Velasco, E. Morales, Determination of polychlorinated biphenyls in biota samples using simultaneous pressurized liquid extraction and purification, J. Chromatogr. A, 946 (2002) 209-219. https://doi.org/10.1016/S0021-9673(01)01534-5
[31] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review (Part I), Trends Anal. Chem., 80 (2016) 641-654. https://doi.org/10.1016/j.trac.2015.08.015
[32] J. Li, Y.-B. Wang, K.-Y. Li, Y.-Q. Cao, S. Wu, L. Wu, Advances in different configurations of solid-phase microextraction and their applications in food and environmental analysis, Trends Anal. Chem., 72 (2015) 141-152. https://doi.org/10.1016/j.trac.2015.04.023
[33] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J AOAC Int, 86 (2003) 412-431.
[34] A. Albinet, S. Tomaz, F. Lestremau, A really quick easy cheap effective rugged and safe (QuEChERS) extraction procedure for the analysis of particle-bound PAHs in ambient air and emission samples, Sci. Total Environ., 450 (2013) 31-38. https://doi.org/10.1016/j.scitotenv.2013.01.068
[35] F.P. Costa, S.S. Caldas, E.G. Primel, Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in canned and fresh peach, Food Chem., 165 (2014) 587-593. https://doi.org/10.1016/j.foodchem.2014.05.099
[36] P.-L. Cloutier, F. Fortin, P.E. Groleau, P. Brousseau, M. Fournier, M. Desrosiers, QuEChERS extraction for multi-residue analysis of PCBs, PAHs, PBDEs and PCDD/Fs in biological samples, Talanta, 165 (2017) 332-338. https://doi.org/10.1016/j.talanta.2016.12.080

[37] M. Mattarozzi, M. Milioli, F. Bianchi, A. Cavazza, S. Pigozzi, A. Milandri, M. Careri, Optimization of a rapid QuEChERS sample treatment method for HILIC-MS2 analysis of paralytic shellfish poisoning (PSP) toxins in mussels, Food Control, 60 (2016) 138-145. https://doi.org/10.1016/j.foodcont.2015.07.027
[38] D. Megson, E.J. Reiner, K.J. Jobst, F.L. Dorman, M. Robson, J.-F. Focant, A review of the determination of persistent organic pollutants for environmental forensics investigations, Anal. Chim. Acta, (2016). https://doi.org/10.1016/j.aca.2016.08.027
[39] M.W. Kujawski, Ż. Bargańska, K. Marciniak, E. Miedzianowska, J.K. Kujawski, M. Ślebioda, J. Namieśnik, Determining pesticide contamination in honey by LC-ESI-MS/MS–Comparison of pesticide recoveries of two liquid–liquid extraction based approaches, LWT-Food Sci Technol., 56 (2014) 517-523. https://doi.org/10.1016/j.lwt.2013.11.024
[40] P.A. Souza Tette, L. Rocha Guidi, M.B. de Abreu Glória, C. Fernandes, Pesticides in honey: A review on chromatographic analytical methods, Talanta, 149 (2016) 124-141. https://doi.org/10.1016/j.talanta.2015.11.045
[41] J. Al-Alam, Z. Fajloun, A. Chbani, M. Millet, A multiresidue method for the analysis of 90 pesticides, 16 PAHs, and 22 PCBs in honey using QuEChERS–SPME, Anal. Bioanal. Chem., 409 (2017) 5157-5169. https://doi.org/10.1007/s00216-017-0463-y
[42] Solid Phase Microextraction: Recent Developments and Applications, 2016.
[43] C. Blasco, P. Vazquez-Roig, M. Onghena, A. Masia, Y. Picó, Analysis of insecticides in honey by liquid chromatography–ion trap-mass spectrometry: Comparison of different extraction procedures, J. Chromatogr. A, 1218 (2011) 4892-4901. https://doi.org/10.1016/j.chroma.2011.02.045
[44] F. Rouvière, A. Buleté, C. Cren-Olivé, C. Arnaudguilhem, Multiresidue analysis of aromatic organochlorines in soil by gas chromatography-mass spectrometry and QuEChERS extraction based on water/dichloromethane partitioning. Comparison with accelerated solvent extraction, Talanta, 93 (2012) 336-344. https://doi.org/10.1016/j.talanta.2012.02.048
[45] H. Sun, X. Ge, Y. Lv, A. Wang, Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed, J. Chromatogr. A, 1237 (2012) 1-23. https://doi.org/10.1016/j.chroma.2012.03.003
[46] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: A critical review. part ii, Trends Anal. Chem., 80 (2016) 655-667. https://doi.org/10.1016/j.trac.2015.08.014
[47] K. Sharmili, S. Jinap, R. Sukor, Development, optimization and validation of QuEChERS based liquid chromatography tandem mass spectrometry method for determination of multimycotoxin in vegetable oil, Food Control, 70 (2016) 152-160. https://doi.org/10.1016/j.foodcont.2016.04.035

[48] M. Saraiva, J. Cavalheiro, L. Lanceleur, M. Monperrus, Synthetic musk in seafood products from south Europe using a quick, easy, cheap, effective, rugged and safe extraction method, Food Chem., 200 (2016) 330-335. https://doi.org/10.1016/j.foodchem.2016.01.017
[49] S. Ramos, V. Homem, L. Santos, Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge, Sci. Total Environ., 651 (2019) 2606-2614. https://doi.org/10.1016/j.scitotenv.2018.10.143
[50] A. Wilkowska, M. Biziuk, Determination of pesticide residues in food matrices using the QuEChERS methodology, Food Chem., 125 (2011) 803-812. https://doi.org/10.1016/j.foodchem.2010.09.094
[51] G.A. Shabir, Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization, J. Chromatogr. A, 987 (2003) 57-66. https://doi.org/10.1016/S0021-9673(02)01536-4
[52] K.M. Dimpe, P.N. Nomngongo, Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices, Trends Anal. Chem., 82 (2016) 199-207. https://doi.org/10.1016/j.trac.2016.05.023

**Figures**

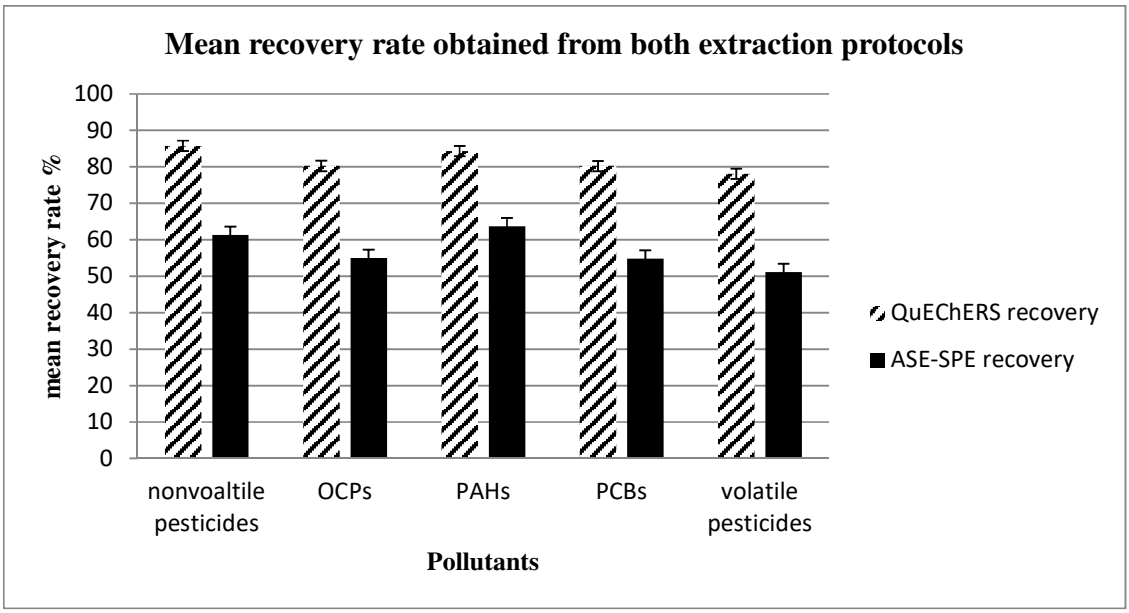



**Figure 1.**

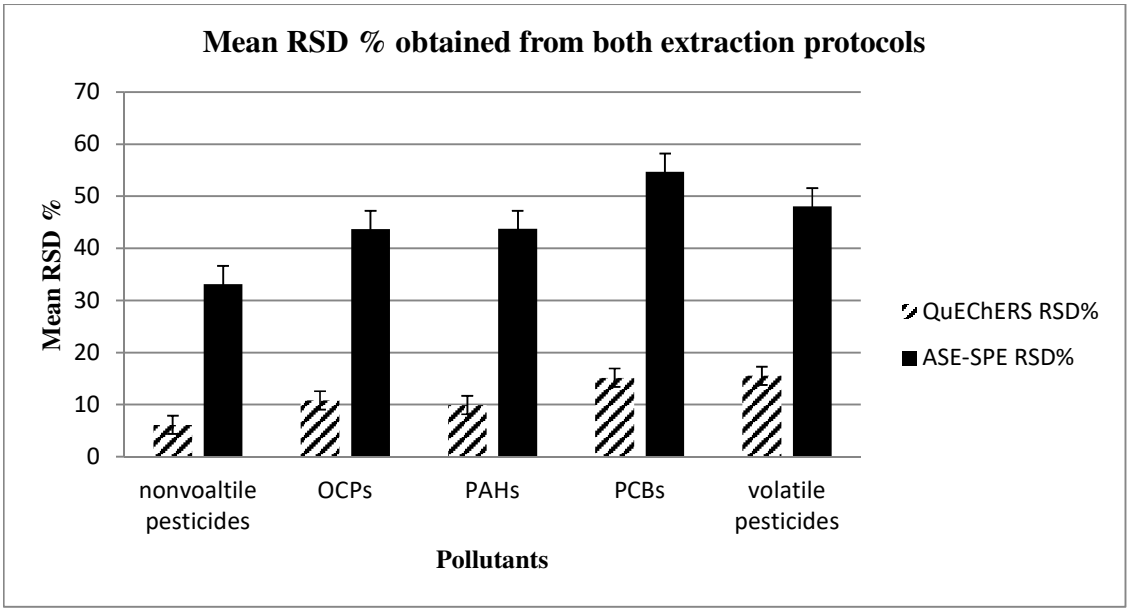



**Figure 2.**

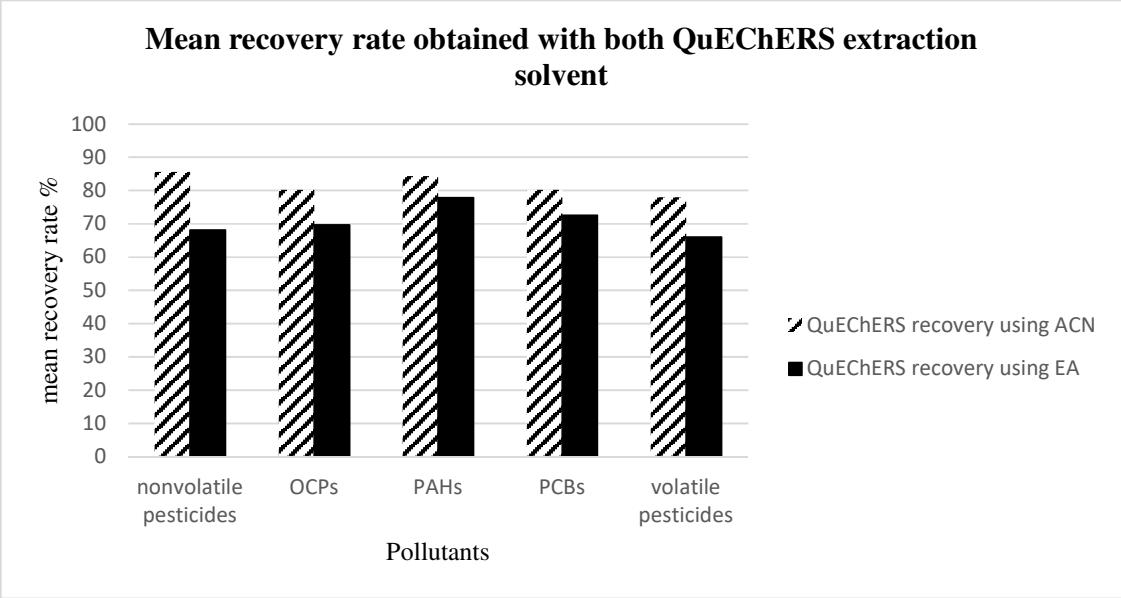

Mean recovery rate obtained with both QuEChERS extraction solvent
mean recovery rate %
100
90
80
70
60
50
40
30
20
10
0
nonvolatile pesticides
OCPs
PAHs
PCBs
volatile pesticides
Pollutants
QuEChERS recovery using ACN
QuEChERS recovery using EA

**Figure 3.**

## Tables:

**Table 1: Compounds detected in real samples: percentage of samples contaminated and the average quantified concentration**

| | Average concentration (ng $g^{-1}$) | Percentage of samples contaminated |
|---|---|---|
| carbendazim | 38.5 | 100 |
| chloridazon | 0.56 | 66.6 |
| pymetrozine | 30.6 | 100 |
| acetamiprid | 0.43 | 66.6 |
| terbutryn | 2.17 | 66.6 |
| metalaxyl-M | 3.4 | 66.6 |
| sulcotrione | 85.6 | 100 |
| trifluralin | 26.6 | 100 |
| chlorpropham | 33.5 | 66.6 |
| tebutam | 8.63 | 33.3 |
| clomazone | 53.71 | 100 |
| propyzamide | 59.1 | 100 |
| naphthalene | 8.54 | 100 |

| | | |
|---|---|---|
| Acenaphthene | 6.82 | 100 |
| fluorene | 9.16 | 66.6 |
| anthracene | 42.9 | 100 |
| phenanthrene | 18.73 | 100 |
| fluoranthene | 314.33 | 100 |