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**Conifers as environmental biomonitors: a multi-residue method for the concomitant quantification of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls by LC-MS/MS and GC-MS/MS**

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## **Abstract**

The conifer is a natural vascular land plant composed of very particular leaves called needles that are able to accumulate, throughout years, a wide range of non-volatile, semi-volatile and volatile compounds of different structure and polarity such as pesticides and persistent organic pollutants. However, the extraction of these compounds from such matrix remains mainly uncommitted. In this paper, a QuEChERS based extraction procedure was developed for the concomitant extraction and analysis of 134 pesticides, 22 polychlorinated biphenyls and 16 polycyclic aromatic hydrocarbons residues from conifer needles. The method included a liquid-liquid extraction using acetonitrile (ACN) followed by a clean-up step using, Primary Secondary Amine (PSA), graphitized carbon black (GCB) and C<sub>18</sub> particles, in the presence of salts. The obtained extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for their contamination by non-volatile pesticides. However, volatile pesticides, polychlorinated biphenyls, organochlorine pesticides and polycyclic aromatic hydrocarbons were subjected to a concentration step using solid-phase microextraction (SPME) prior to their analysis by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The method was developed and validated, and the obtained results revealed a recovery rate ranged between 60% and 121% for all the targeted compounds. In addition, the method showed high sensitivity and precision with detection and quantification limits less than 20 ng g<sup>-1</sup> for most target compounds and low RSD for both inter and intra-day analysis. Once developed the method was applied on conifer samples collected from 15 different sites in northern Lebanon. The analysis of the collected samples showed a persistence of the different assessed pollutants depending of the characteristics of each site.

**Keywords:** Conifers, Environmental pollution, QuEChERS, Pesticides, Polycyclic aromatic hydrocarbons, Polychlorinated biphenyls.

## **1. Introduction**

Environmental pollution is a global common problem to both developed and developing countries, which attracts human attention to its severe long-term consequences [1]. Therefore, the monitoring of this pollution becomes a worldwide necessity. Among the different monitoring techniques used, figures the biomonitoring. In fact, biomonitoring requires the use of responses from natural species at multiple levels to identify or predict environmental changes and to observe their evolution as a function of time [2]. Consequently, biological monitoring can be defined as the measurement of the response of living organisms to changes in their environment [3]. In terrestrial environments, many species or groups of species can be used for monitoring purposes [4,5]. For instance, lichens, mosses, vascular plants and fungi have been widely used as passive biomonitors for air pollution [6,7].

As a follow, the use of vegetation for environmental monitoring can be considered as a simple, efficient and cost-effective monitoring technique for detecting and assessing environmental pollution [8,9]

Among the different vegetation species, conifer needles can play an important role as passive samplers. They have been widely used for the consecutive biomonitoring of several pollutants and are characterized by a high capacity to accumulate one or more pollutants in their tissues such as pesticides [9,10], PAHs [11,12] and PCBs [13,14]. Moreover, conifers are widespread and can be found in a large area that can be difficult to access [15]. Furthermore, conifers are characterized by a waxy layer allowing the adsorption and the accumulation of organic air

contaminants through the years and therefore, making them efficient for environmental passive sampling [16].

For all of these reasons, the development of extraction tools for the analysis of traces of organic pollutants in such matrix seems efficient in order to establish a specific environmental control.

In fact, a large variety of approaches have been used for determination of environmental pollutants in vegetables [17]. For instance, some modern techniques such as ultrasound-assisted extraction (UAE) [18,19], accelerated solvent extraction (ASE) [20-23], Soxhlet [11], ultrasonic solvent extraction [24], or pressurized liquid extraction [11] were used.

However, all these traditional methods present some major drawbacks and practical limitations which are mainly overcome by the multi residues extraction method QuEChERS. In fact, this method known as quick, easy, cheap, effective, rugged, and safe extraction procedure, has proved its efficiency for the extraction of organic pollutants, including non-polar and polar pesticides, in several matrix. [25,26]. This technique is divided in two steps, the first considered as a soft extraction method using ACN as extraction agent while the second as an optional clean-up procedure by dispersive solid-phase extraction (d-SPE) [27]. However, even that QuEChERS extraction procedure has proven its efficiency in many environmental studies, this method, was to the best of our knowledge never applied on conifer needles.

Following their extraction, the analytical control of pesticides and POPs residues in environmental matrices requires their analysis by efficient analytical instruments such as tandem mass spectrometry (MS / MS) associated with gas chromatography (GC) or liquid chromatography (LC) [28,29]. The use of such analytical tools plays a key role and provides the most effective and efficient means to assess hundreds of analytes in a variety of matrices in one

run. In fact, it is important to note that while GC-MS / MS is specifically targeted at non-polar and semi-polar, volatile and semi-volatile compounds, LC-MS / MS is more suitable for polar and semi-polar, non-volatile and thermolabile compounds [30,31].

For all these reasons, this manuscript proposes the development of QuEChERS, a multi-residue analytical method for the extraction of 134 pesticides, 22 PCBs and 16 PAHs followed by their analysis using LC-MS /MS and GC- MS /MS.. The application of this method to the analysis of coniferous matrices is considered to be challenging as well. In fact, the introduction of the QuEChERS method provides a high-throughput multi-residual approach to the routine pollutant monitoring of conifers samples.. The extraction technique used was followed by a pre-concentration step for volatile compounds using SPME prior for their analysis by GC-MS/MS while non-volatile compounds were directly analyzed, after their extraction, by LC-MS/MS. In fact, the extraction techniques used and the wide number and pollutants assessed, were, to the best of our knowledge, never reported before. The developed method presents, therefore, a new contribution to this field. Moreover, the developed method was applied on real samples collected from several regions in northern Lebanon in order to biomonitor the environmental state in these regions.

## **2. Experimental**

### ***2.1. Materials and reagents***

For LC-MS/MS pesticides analysis, the 31 non-volatile pesticides (Sigma Aldrich, St, Quentin Fallavier, France) were:

Acetamiprid, Carbendazim, Carbetamide, Chlorfenvinphos, Chloridazone, Chlortoluron, Cyazofamid, Diflubenzuron, Diflufenican, Dimethenamid-P, Diuron, Epoxyconazole, Flufenoxuron, Formasulfuron, Isoproturon, Isoxadifen, Metalaxyl-M, Nicosulfuron, Penconazole, Pendimethalin, Propiconazole, Prothioconazole, Pymetrozine, Pyraclostrobine, Spinosade-A, Spinosade-D, Sulcotrione, Tebuconazole, Terbutryn, Thiachlopid and Triflurosulfuron-methyl.

A stock solution of each of these standards at 1 g L<sup>-1</sup> was prepared in ACN.

For GC-MS/MS pesticides analysis, the 82 volatile pesticides except OCPs (Sigma Aldrich, St. Quentin Fallavier, France) were:

2,4-MPCA, Acetochlor, Aclonifen, Alachlor, Azinphos-ethyl, Azoxystrobin, Benoxacor, Bifenox, Bifenthrin, Boscalid, Bromoxynil-octanoate, Bupirimate, Buprofezine, Captan, Carbaryl, Chlorothalonil, Chlorpropham, Chlorpyrifos, Chlorpyrifos-Methyl, Clofentezin, Clomazone, Cypermethrin, Cyproconazole, Cyprodinil, Deltamethrin, Dicamba, Diclobenil, Diclofop-methyl, Diflufenicanil, Dimethachlor, Dimethanamid-P, Dimetomorph, Dimoxystrobin, Diphenylamine, Epoxyconazole, Ethofumesate, Etridiazole, Fenarimol, Fenoxycarb, Fenpropidin, Fluazinam, Fludioxynil, Flumioxazin, Flurochloridon, Flusilazole, Folpet, Indoxacarb, Iprovolincarb, Isoxaflutole, Kerosym-methyl, Lambda-cyhalothrin, Lenacil, Lindane, Malathion, Mecroprop-P, Metamitron, Metazachlor, Metolachlor-S, Myclobutanil, Oxadiazon, Penconazol, Pendimethalin, Picloram Piperonil-butoxide, Prochloraz, Procymidon, Propiconazole, Propyzamid, Prosulfocarb, Pyraclostrobin, Pyrimethanil, Quinoxifen, Spiroxamine, Tebuconazole, Tebufenpyrad, Tebutam, Tetraconazole, Tolyfluanid, Triadimenol, Trifloxystrobin, Trifluralin and Zoxamide.



A stock solution of each of these standards at 1 g L<sup>-1</sup> was prepared in ACN.

For OCPs analysis, a solution at 0.1 g L<sup>-1</sup> of 21 OCPs including: Aldrine, *cis*-chlordane, *trans*-chlordane, Dieldrine,  $\alpha$ -Endosulfan,  $\beta$ -Endosulfan, Heptachlore, Heptachlore-epoxyde A, Heptachlore-epoxyde B, Hexachlorobenzene, Metoxychlore, *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH and  $\delta$ -HCH was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

For PAHs analysis, a solution at 0.1 g L<sup>-1</sup> of 16 PAHs including: Acenaphthene, Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthrene, Benzo(e)pyrene, Benzo(g,h,i)perylene, Benzo(k)fluoranthrene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthrene, Fluorene, Indenol(1,2,3)pyrene, Naphtalene, Phenanthrene and Pyrene was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

For PCBs analysis, a solution at 0.1 g L<sup>-1</sup> of 22 PCBs including: PCB 18, PCB 28, PCB 31, PCB 44, PCB 52, PCB 70, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 123, PCB 126, PCB 138, PCB 149, PCB 153, PCB 156, PCB 157, PCB 167, PCB 169, PCB 180 and PCB 189 was purchased from Cluzeau Info Labo, St, Croix la Grande, France.

Internal standards for LC-MS/MS were obtained from Sigma Aldrich, St, Quentin Fallavier, France. These standards included: Carbendazim-d<sup>4</sup> (99.3%), Diuron-d<sup>6</sup> (99.8%), Pendimethalin-d<sup>5</sup> (99%), and Nicosulfuron-d<sup>6</sup> (99%). A standard solution of each compound at 1 g L<sup>-1</sup> in ACN was prepared. A mixture of these standards at 0.01 g L<sup>-1</sup> in ACN was also prepared for their alternative use as IS.

Internal standards for GC-MS/MS were obtained from Sigma Aldrich, St, Quentin Fallavier, France except Naphtalene-d<sup>8</sup> obtained from Cambridge Isotope Laboratories. A mixture of

Trifluralin-d<sup>14</sup>, 4-Nitrophenol-d<sup>4</sup>, 2,4-D-d<sup>3</sup>, Atrazine-d<sup>5</sup>, Pendimethalin-d<sup>5</sup> and *trans*-Cypermethrin-d<sup>5</sup> at 1 g L<sup>-1</sup> in ACN was used as IS for pesticides analysis except OCPs, while a mixture of Naphtalene-d<sup>8</sup>, Phenanthrene-d<sup>10</sup>, Chrysene-d<sup>12</sup> and Perylene-d<sup>12</sup> at 1 g L<sup>-1</sup> in ACN was used for OCPs, PAHs and PCBs analysis . A mixture of these two standard solutions at 0.01 g/L in ACN was prepared.

All prepared solutions were stored at -18°C.

Kits for QuEChERS (RESTEK France-EN 1566 method) were purchased as ready to use containing 4g MgSO<sub>4</sub>, 1g NaCl, 1g trisodium citrate dehydrate and 0.5g disodium hydrogen citrate sesquihydrate. For clean-up, the kits (AOAC 2007 method) containing 1.2g MgSO<sub>4</sub>, 400 mg PSA, 400 mg C<sub>18</sub> and 400 mg GCB were used.

LC-MS grade ACN, LC-MS grade water and formic acid ≥ 99% were purchased from Avantor®, United States. The ACN and water solutions are prepared by mixing 500 mL of each solution with 0.1% (0.5 mL) of formic acid. The solvents used were all HPLC grade and the ultra-pure water was obtained through a Milli-Q system (18 MΩ.cm) from Merck, Germany.

## ***2.2. Sample collection***

Blank conifer matrix samples (*Pinus nigra*) were collected from the botanical garden of the university of Strasbourg, campus of Cronembourg, France and only the terminal parts of the branches, newly obtained and not exposed to environmental pollutants were collected. The samples were transported in polyethylene bags to the laboratory where they were finely cut and then washed with ACN for 15 min under the hood. This washing is repeated twice in order to remove all traces of contaminants on their surfaces. After washing, the conifers were well dried,

and were kept at -18 °C until their analysis. These needles were then analyzed by GC-MS / MS and LC-MS / MS in order to assess any previous contamination that may interfere with targeted pollutants. The results showed a complete absence of such interference and therefore these needles were used as for matrix matched calibration curves and for method development as well. Other conifers samples were collected from 15 different sites in northern of Lebanon (Bsharri, Abdine, Akkar, Koura and Tripoli) in August 2018. The samples were transported in polyethylene bags and stored frozen (- 18 °C) until analysis [23].

A map showing the location of the different sampling sites is shown in figure 1 in supplementary materials (S3).

### ***2.3. Extraction procedures***

Five grams of homogenized samples of washed conifer needles were weighed in 50 mL centrifuge tube then fortified with different concentrations of each mixture's solution (5, 10, 25, 50, 100, 500, 1000, 1500, 2000 and 3000 ng g<sup>-1</sup>). The samples were kept at 4°C overnight, and then extracted using the QuEChERS extraction procedure cited below. All extractions were done in triplicate.

The protocol chosen was as follows: to 5 grams of homogenized matrix, 15 mL of ACN were added and the tubes were shaken. After stirring, QuEChERS citrate buffered extraction salts were added, then the tubes were vortexed for 1 min and then centrifuged for 10 minutes at 5000 rpm. Afterwards, the supernatant was added to the 15 mL of PSA tube then vortexed and centrifuged for 10 minutes at approximately 5000 rpm. Finally, the obtained extract (around 3 mL), was sampled in a 10 mL glass tube, and then evaporated at approximately 100 µL.

### ***2.3.1. Extract reconstitution***

Once evaporated, the collected extracts were reconstituted with ACN to 1 mL, in order to prepare them to chromatographic analysis.

100 µL of this solution were transferred to LC vials and were directly injected into an LC-MS/MS system after the addition of 10 µL of the appropriate internal standards. The remained 900 µL were derivatized then pre concentrated by SPME prior to their analysis with GC-MS/MS.

#### ***2.3.1.1. Derivatization by silylation***

A silylation reaction from N-methyl-N-tert-butyltrimethylsilyltrifluoroacetamide (noted MTBSTFA) was used. The remained 900 µL were brought in contact with 50 µL of MTBSTFA at 80°C for 1 hour. After the derivation reaction, the obtained solution was diluted to 20 mL using of acidified water (pH 3) and 10 µL of both appropriated GC internal standards. The acidified water solution is prepared by mixing 30 grams of sodium chloride >99,8 % with 250 µL of nitric acid 68% and 2 L ultrapure water.

### ***2.3.2. Concentration and injection by solid-phase microextraction (SPME)***

Due to the large number of compounds with different chemical families, characteristics and polarities, two SPME fibers were used. The first was coated with polydimethylsiloxane (PDMS) of 100 µm and was used for the extraction of PAHs, PCBs, OCPs. The second was coated with

polyacrylate (PA) at 85  $\mu\text{m}$  and was used for the extraction of the remained semi-volatile pesticides. The immersion time for both fibers in the solution was 40 minutes.

## ***2.4. Extract analysis***

Extracted samples were analyzed, according to their properties, using LC-MS/MS for the 31 non-volatile pesticides and GC-MS/MS for the 103 volatile pesticides, the 22 PCBs and the 16 PAHs.

### *2.4.1. Liquid chromatography coupled to a tandem mass spectrometer*

A thermo Scientific TSQ Vantage Triple Quadrupole Mass Spectrometer coupled with a Surveyor pump and autosampler (Accela Autosampler) operating in electrospray ionization mode (ESI) was used. The sampler is equipped with a 20  $\mu\text{L}$  injection loop and the samples were kept at a temperature of 1  $^{\circ}\text{C}$ . The analysis was performed on a Nucleodur  $\text{C}_{18}$  Pyramid column (150 mm  $\times$  3 mm, 3  $\mu\text{m}$ ) thermostated at 25 $^{\circ}\text{C}$ . Samples were analyzed using a mobile phase ACN/water (0.1% formic acid) with a flow rate of 0.3  $\text{mL}\cdot\text{min}^{-1}$ . The gradient started with 30:70 (v/v) for 5 min, followed by 50:50 (v/v) for 6 min, 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 minutes was set in order to stabilize the column for any new injection.

The LC-MS/MS parameters for non-volatile pesticides analysis figure in supplementary materials S1-1.

### *2.4.2. Gas chromatography coupled to a tandem mass spectrometer*

A GC-MS / MS Trace GC Ultra / ITQ 700 coupling equipped with a Combi PAL equipped with SPME fiber was used for the analysis of semi volatile pesticides, PAHs, PCBs and OCPs. The analysis was carried out on an XLB (50% phenyl/ 50% methylsiloxane) capillary column of 30m x 0.25mm, 0.25 $\mu$ m film thickness. Injection was done in splitless mode at 250 °C for 15 minutes. The transfer line was maintained at 300 °C and the source of the MS at 210 °C. Helium was used as carrier gas at a flow rate of 1 mL.min<sup>-1</sup>.

#### *2.4.2.1. Semi volatile pesticides separation and analysis*

Injection of the sample was done by thermal desorption of the polyacrylate fiber. 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<sup>-1</sup>, followed by a ramp to 300°C at a rate 5.8°C min<sup>-1</sup>, where it was maintained for 10 minutes, leading to a total run time of 41 minutes.

The GC-MS/MS parameters for semi volatile pesticides analysis figure in supplementary materials S1-2.

#### *2.4.2.2. POPs separation and analysis*

Injection of the sample was done by thermal desorption of the polydimethylsiloxane fiber. 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<sup>-1</sup>, followed by a ramp to 330°C at a rate 20°C min<sup>-1</sup>, where it was maintained for 18 minutes, leading to a total run time of 45.25 minutes.

The GC-MS/MS parameters for POPs analysis figure in supplementary materials S1-3.

### ***2.5. Validation of the method***

Once developed, the method was validated for all analytical parameters. First, triplicate extraction of fortified samples with concentrations ranged from 5 to 3000 ng g<sup>-1</sup> (5-10-25-50-100-500-1000-1500-2000-3000) was performed to determine linearity. Then, five samples of spiked matrix with three level concentration (10, 100 and 1000 ng g<sup>-1</sup>) were extracted for three successive days in order to determine intermediate precision and repeatability. These two precision parameters were evaluated by their correspondent relative standard deviation (RSD %).

In fact, matrix-matched calibration curves were performed using the washed conifer needles. Matrix effect was assessed by the use of internal standards which gave the same intensity for both washed and real matrix and therefore we assumed that the wash had no effect on the matrix composition. For this, quantification was done using the matrix matched calibration curves developed on the washed conifer needles in order to ensure that the matrix was exactly the same as the samples. Concerning method validation limits, the limit of detection and quantification were calculated as the lowest concentration for which precision and accuracy has been demonstrated and which responds to the relationship respectively: signal / noise  $\geq 3$  and signal / noise  $\geq 10$ . In fact, the method limit of detection (LOD) was determined as the analyte concentration that produced a peak signal of three times the background noise from the chromatogram, and the method limit of quantification (LOQ) was determined as the analyte concentration that produced a peak signal of ten times the background noise from the

chromatogram. These limits were then determined graphically with:  $LOD = 3 \times [\text{min}] S/N$  and  $LOQ = 10 \times [\text{min}] S/N$ .

[32,33].

Regarding the recoveries, they were also determined at the same three levels of concentrations as precision (10, 100 and 1000 ng. g<sup>-1</sup>). The recoveries were considered as the ratio of the area of the spiked samples to the area of the standard following the equation:

$\text{Recovery \%} = (\text{Sample concentration} / \text{Standard solution concentration}) * 100$ .

### **3. Results**

#### ***3.1. Method development***

A concentration step by evaporation and reconstitution was added before the liquid and gas chromatographic analysis to obtain a concentrated extract. For the volatile compounds, this process was followed by solid-phase microextraction extraction/concentration step prior to gas chromatographic analysis using the polyacrylate and polydimethylsiloxane fiber for the extraction of semi volatile pesticides and PAHs, PCBs, OCPs respectively [34,35]. . A validation procedure was performed, which showed good results for suitability, recovery and repeatability. The developed method was applied to the determination of real samples that some pollutants were detected.

#### ***3.2. Method validation***

Once identified and developed, the method was validated and verified in order to ensure its reliability and efficiency. Validation shows that the analytical method used to evaluate a component is reliable, accurate, reproducible and robust over the specific range and it is suitable



for its intended purpose [36]. Typical characteristics of validation to be considered were: accuracy, linearity, precision: repeatability and reproducibility, detection limit, quantitation limit and recovery.

All target compounds were validated with good linearity expressed by a regression coefficient higher than 0.98. LOD were lower than 15 ng g<sup>-1</sup> for all compounds except Indoxacarb and LOQ were lower than 15 ng g<sup>-1</sup> for all non-volatile pesticides and for the majority of volatile compounds analyzed by GC-MS/ MS. Moreover, results showed that all these pollutants were detected with high precision with RSD % lower than 20% for inter and intra-day analysis except nicosulfuron and diflubenzuron. Furthermore, the method showed good recoveries between 60.48 to 98.31%, 62.75 to 107.14%, 71.29 to 121.92%, 73.38 to 99.97% and 61.04 to 98.89% for non-volatile pesticides, volatile pesticides, OCPs, PCBs and PAHs respectively.

The validation parameters for non-volatile pesticides analyzed by LC-MS/MS, volatile pesticides and POPs analyzed by GC-MS/MS figure in supplementary materials S2-1, S2-2 and S2-3 respectively.

#### ***4. Application to real samples***

Once developed and validated, the method was applied on real samples. These samples were collected from several regions in Lebanon and were analyzed according to the previously developed method. Residue levels were calculated with Xcalibur using the previously plotted calibration curves.

Table 1 shows the majority of the pesticides, PAHs, OCPs and PCBs residues found in the samples analyzed.

**Table 1: Concentration of compounds (ng g<sup>-1</sup>) detected in real samples**

<b>Compounds</b>	<b>Average concentration</b>	<b>Lowest concentration</b>	<b>Highest concentration</b>
Chloridazon	2.66	0.3	6.72
Metalaxyl-M	38.66	3.64	51.24
Pendimethalin	3.93	1.59	11.91
Fluoranthrene	372.18	48.16	984.44
Boscalid	87.93	65.71	208.36
Naphtalene	11.68	4.51	39.6
Diflufenican	158.6	23.9	612.81
Acenaphthalene	7.21	13.39	82.19
Hexachlorobenzene	523.7	256.92	826.04
Terbutryn	5.33	0.81	56.22
Fenpropidin	238	75.27	918.68
Sulcotrione	78.23	4.72	287.18

**Table 1:**

## **5. Discussion**

To the best of our knowledge, this is the first article on the combination of QuEChERS-SPME extraction with LC-MS/MS and GC-MS/MS analysis for simultaneous quantification and confirmation analysis for 134 pesticides, 16 PAHs and 22 PCBs from coniferous matrix.

The major advantage of the proposed method is the low volume of the organic solvent used allowing the extraction of a wide range of pollutants (172 pollutants) with a short time of sample preparation. Concerning the analytical procedure, it is more complicated to implement splitless

injection. The temperature of the oven, the solvent and the splitless time must be carefully selected. However, this method is suitable for trace analysis since the complete sample is introduced into the column. Once optimized, the methodology is easy to use, fairly robust and easy to automate [37].

In fact, the results provided by the developed method overcome the drawbacks presented by the traditional used extraction methods. For instance, this method proved to be fast, efficient, environmental friendliness and reliable mainly due to the reduction of the sample weight (5 g) and the amount of solvents used [25]. Moreover, recoveries obtained using QuEChERS appear to be even higher than those obtained by accelerated solvent extraction or solid-phase extraction especially with taking into consideration the high amount of solvent used in these latest techniques [38]. For instance, the recoveries obtained with our developed method were higher than those provided for the analysis of pesticides in conifer needles using S-PLE. In fact, the recoveries obtained for these two methods were for chlorpyrifos 73 and 50%, for trifluralin 86 and 70%, for Aldrin 96 and 65%, for heptachlor 87 and 70%, 86 and 70% for PCB 105 and for  $\beta$ -Endosulfan 96 and 65% respectively [39].

Furthermore, the developed method was evaluated and compared in terms of extraction time, accuracy, precision, sensitivity and versatility, with other procedures. The main differences between the developed procedures and other published methods for determination of pollutants residues in conifer samples pertain to the time of the stage of extraction and clean-up [40-41].

Based on the data presented in the literature it can be concluded that the developed methodology introduces a new trend in the process of determination of a wide range of pollutants (pesticides, PCBs, PAHs and OCPs) in conifer samples in comparison with other already published methods

such as ASE/S-PLE for pesticides and PCBs analysis [39], ASE mainly used for the analysis of PAHs [42], Soxhlet for the OCPs [43], polychlorinated dibenzo-p-dioxins/- furans [44] and ultrasonic extraction for PAHs [45].

Many researchers reported influence of different extraction solvents on the content of pollutants in matrix [46]. Efficiency of solvents and methods are strongly dependent on plant matrix used [47]. Solvents, such as methanol, ethanol, dichloromethane, ACN and ethyl acetate have been commonly used for the extraction of pollutants in conifer. Another main advantage between the QuEChERS-SPME procedure and other published methods for determination of pollutants residues in conifer samples is the number and the volume of solvents used for extraction. In the developed methodology, needles were extracted using ACN as solvent extraction while in other methods several solvents such dichloromethane, toluene, ethyl acetate and methanol were required for the extraction of pesticides and POPs from conifer needles [23,48,49].

In addition, the range of QuEChERS applications is very wide and allowed its comparison with reference methods in different applications such as liquid-liquid extraction, solid-liquid extraction, solid-phase extraction, accelerated solvent extraction, microwave assisted extraction and ultrasound assisted extraction that depend essentially by the nature of the analytes to extract and the complexity of the matrix. In fact, QuEChERS is a high-performance reasonable choice able to provide similar or better analytical performance without the drawbacks of the other methods, as well as the need for specific devices such as microwave or ultrasound [50]. For instance, Di et al. in 2015, analyzed organochlorine pesticides and confirmed that QuEChERS and microwave-assisted extraction methods generated higher results compared to the accelerated solvent extraction and ultrasound assisted extraction [51]. Moreover, the QuEChERS method showed better performance for the determination of pesticides in honey and honey bees [52] and

in roots and rhizomes of herbal medicines [53] than liquid-liquid extraction and solid-phase extraction.

A comparison of the %RSD for pesticides, PCBs and PAHs in the pine needle using conventional pressurized liquid extraction (PLE), selective pressurized liquid extraction (S-PLE) and QuEChERS method developed in our work showed that this latest provided higher precision than PLE and S-PLE with lowest % RSD for both  $\alpha$ -Endosulfan and  $\alpha$ -HCH [39].

In addition, the comparison of the developed method with the work of AL ALAM et al., 2017 in which ASE-SPE-SPME was used for the multi-residue's organic pollutants in conifer needles, showed that even if no big differences were found in term of analytical parameters, it is clear that the amount of solvent and time used in QuEChERS-SPME favor this latest and make it the method of choice for such studies [23,54].

On the other side, the analysis of organic pollutants residues in conifer by GC-MS/MS and LC-MS/MS proved the efficiency of these analytical tools in multi- residues analysis. In fact, results showed that the combination of the separating power of liquid and gas chromatography with the highly sensitive and selective mass analysis capability of MS/MS solve most of the problems associated with assessing pollutant residues such as the small amount of sample that can be detected, the relative analysis time, the precision, the broad range of samples, the continuous operation on a large scale and the simplicity of equipment [55,56]. In fact, chromatography is used in a wide range of applications due to its possibility to separate different components of a complex mixture based on polarity, molecular weight and ionic mobility. However, both techniques of chromatography are necessary in trace analysis for the determination of a wide

spectrum of environmental pollutants such as polynuclear aromatic hydrocarbons, dioxins, PCBs, chlorinated pesticides, and some other pesticides [57-59].

The results proved that the QuEChERS-SPME protocol in combination with LC and GC techniques can be used as a tool in environmental monitoring. The developed methods were successfully applied in monitoring real samples collected from 15 districts in Lebanon. Some pollutant concentrations in the conifer samples are summarized in table 1. The concentrations were expressed in ng g<sup>-1</sup>. Several studies have shown the currency of the use of conifer as an environmental biomonitor for pesticide and POPs pollution. Detectable pollutants have been found in most samples with varying residual levels by area and by different factors such as temperature, humidity, altitude and precipitation that can have a significant impact on the concentration of these pollutants [60]. In their study, Al-Alam et al., 2017 showed a direct effect of meteorological effects on pollutant concentrations found in conifer [23]. The levels of pesticides found in our study were comparable to those reported for honey samples from same sites in Northern Lebanon (Bsharri and Akkar) studied by Al Alam et al., 2017. However, the concentrations of diflufenican, fenpropidin, hexachlorobenzene and other pesticides present in this study were significantly identical as those above [61]. The results of the PAHs showed that the 15 sites tested were in accordance with the results provided by Al Alam et al., 2019 for determination of 16 PAHs and 22 PCBs in honey samples from different regions of Lebanon [62].

## **6. Conclusion**

This study aimed to develop an analytical method for the extraction and quantification of pesticides, PCBs and PAHs' residues from conifer needles. The protocol chosen consisted of the use of QuEChERS based extraction followed by SPME for extraction and concentration of multi- residues organic pollutants. Chromatographic analysis was done using salts with LC-MS/MS and GC-MS/MS. The developed method proves its efficiency especially being fast, simple and covering a large majority of the pollutants assessed. The results showed satisfactory quantification and detection limits, good recoveries rates with a reduction of the analysis time and the solvent consumption as well.

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## Tables

**Table 1: Concentration of compounds (ng g<sup>-1</sup>) detected in real samples**

<b>Compounds</b>	<b>Average concentration</b>	<b>Lowest concentration</b>	<b>Highest concentration</b>
Chloridazon	2.66	0.3	6.72
Metalaxyl-M	38.66	3.64	51.24
Pendimethalin	3.93	1.59	11.91
Fluoranthrene	372.18	48.16	984.44
Boscalid	87.93	65.71	208.36
Naphtalene	11.68	4.51	39.6
Diflufenican	158.6	23.9	612.81
Acenaphtalene	7.21	13.39	82.19
Hexachlorobenzene	523.7	256.92	826.04
Terbutryn	5.33	0.81	56.22
Fenpropidin	238	75.27	918.68
Sulcotrione	78.23	4.72	287.18