



**HAL**  
open science

## **Ultrasound-assisted QuEChERS-based extraction using EDTA for determination of currently-used pesticides at trace levels in soil Authors**

Florent Lafay, Gaëlle Daniele, Maëva Fieu, Céline Pelosi, Clémentine Fritsch, Emmanuelle Vulliet

### **► To cite this version:**

Florent Lafay, Gaëlle Daniele, Maëva Fieu, Céline Pelosi, Clémentine Fritsch, et al.. Ultrasound-assisted QuEChERS-based extraction using EDTA for determination of currently-used pesticides at trace levels in soil Authors. *Environmental Science and Pollution Research*, 2022, <10.1007/s11356-022-19397-3>. <hal-03746414>

**HAL Id: hal-03746414**

**<https://hal.science/hal-03746414v1>**

Submitted on 5 Aug 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.



HAL Authorization

1 **Title**

2 Ultrasound-assisted QuEChERS-based extraction using EDTA for determination of currently-used pesticides at  
3 trace levels in soil

4

5 **Authors**

6 Florent LAFAY<sup>1</sup>, Gaëlle DANIELE<sup>1\*</sup>, Maëva FIEU<sup>1</sup>, Céline PELOSI<sup>2,4</sup>, Clémentine FRITSCH<sup>3</sup>, Emmanuelle  
7 VULLIET<sup>1</sup>

8

9 **Affiliations**

10 <sup>1</sup>Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR 5280, 5 rue de  
11 la Doua, F-69100 VILLEURBANNE, France

12 <sup>2</sup>INRAe, Avignon Université, UMR EMMAH, F-84000, Avignon, France

13 <sup>3</sup>UMR 6249 Chrono-Environnement – CNRS / Université Bourgogne Franche-Comté Usc INRA - 16 Route de  
14 Gray, 25030 BESANCON Cedex

15 <sup>4</sup>INRAe, UMR1402 ECOSYS, pôle Ecotoxicologie Ecologie fonctionnelle et écotoxicologie des  
16 agroécosystèmes, RD 10 Route de St Cyr, 78026 VERSAILLES Cedex

17

18 Corresponding author :

19 gaelle.daniele@isa-lyon.fr

20 Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100 VILLEURBANNE, France

21 +33434423630

22

23 **Abstract**

24 It is essential to monitor pesticides in soils as their presence at trace levels and their bioavailability can induce  
25 adverse effects on soil's ecosystems, animals, and human health. In this study, we developed an analytical  
26 method for the quantification of traces of multi-class pesticides in soil using liquid chromatography-tandem mass  
27 spectrometry. In this way, 31 pesticides were selected, including 12 herbicides, 9 insecticides, and 10 fungicides.  
28 Two extraction techniques were first evaluated namely: the pressurized liquid extraction and the QuEChERS  
29 procedure. The latest one was finally selected and optimized, allowing extraction recoveries of 55 to 118 %. The  
30 addition of the chelating agent EDTA, which binds preferentially to soil cations that complex some pesticides,  
31 facilitates their extraction. Coupled with liquid chromatography-tandem mass spectrometry, the procedure  
32 displayed very high sensitivity, with limits of quantification (LOQ) in the range 0.01-5.5 ng/g. A good linearity  
33 ( $R^2 > 0.992$ ) was observed over two orders of magnitude (LOQ-100×LOQ) with good accuracy (80% – 120 %)  
34 for all compounds except the two pyrethroids lambda-cyhalothrin and tau-fluvalinate (accuracy comprised  
35 between 50 and 175%) and the cyclohexanedione cycloxydim (accuracy < 35%). Good repeatability and  
36 reproducibility were also achieved. The method was finally successfully applied to 12 soil samples collected  
37 from 3 land-use types. Among the 31-targeted pesticides, 24 were detected at least once, with concentration  
38 levels varying from LOQ to 722 ng/g. Many values were below 0.5 ng/g, indicating that the developed method  
39 could provide new knowledge on the extremely low residual contents of some pesticides.

40

41 **Keywords**

42 Pesticide residues; soil; QuEChERS; ultrasounds; LC-MS/MS

43

## 44 **1.Introduction**

45 Pesticides are a threat to the environment and human societies due to their potential toxicity, persistence, and  
46 their extensive use worldwide. In order to evaluate the potential fate and impacts of pesticides on non-target  
47 areas and animals, it is first necessary to determine their concentration levels in soils that have a high capacity to  
48 retain and store chemical substances like pesticides. Soil is the main and first recipient of the applied pesticides,  
49 and their contamination is a major issue due to the crucial role they play in ecosystems and food chains (Chiaia-  
50 Hernandez et al. 2017).

51 Nevertheless, it represents a real challenge, due to the complexity of the matrix, the wide range of  
52 physicochemical properties of pesticides, and the low concentration levels at which pesticides can cause a  
53 chronic effect. Sample preparation, which includes both the specific extraction of the target pesticides and the  
54 elimination of organic or inorganic interfering compounds, represents a key step for the development of  
55 analytical methods for pesticide residues determination in such complex environmental matrices.

56 Different extraction methods are commonly used to measure pesticides in soils, including pressurized liquid  
57 extraction (PLE), ultrasonic- or microwave-assisted extractions, solid-phase micro-extraction, dispersive liquid-  
58 liquid micro-extraction, or supercritical fluid extraction (Tadeo et al. 2012; Di et al. 2015; Pastor-Belda et al.  
59 2015; Ukalska-Jaruga et al. 2020). The so-called QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe)  
60 extraction has also gained more and more attractiveness (Łozowicka et al. 2017; Zaidon et al. 2019). Indeed, it  
61 has largely been used due to its green and cheap approach, along with its versatility for a wide range of  
62 pesticides in many matrices, including soils or sediments. Nevertheless, most methods deal with one or a few  
63 pesticide chemical families (Kemmerich et al. 2015; Muhammad et al 2017; Qu et al. 2019), whereas multi-  
64 residue analysis of a large extends of pesticides in soils are still limited, although this is the most common  
65 situation in cultivated soils (Łozowicka et al. 2017; Avendaño et al. 2018; Silva et al. 2019).

66 Gas chromatography combined to mass spectrometry (GC-MS) or Electron Capture Detector (ECD), and liquid  
67 chromatography-tandem mass spectrometry (LC-MS/MS) are the most commonly used techniques for the  
68 determination of trace organic contaminants in soil and environmental matrices (Pose-Juan et al. 2014; Karasali  
69 et al.2016; Joseph et al. 2020). The latter allows detection of extremely low concentrations (ng/g or lower) in  
70 various complex solid matrices, especially when it is used with the specific multiple-reaction monitoring (MRM)  
71 mode.

72 Interfering substances that can be co-extracted with the analytes may affect the detection of the target  
73 compounds. Indeed, it can result in the enhancement or the reduction of the analyte signal (the so-called matrix-  
74 effect) during the analysis using electrospray ionisation. Most of the studies dealing with the determination of  
75 pesticide residues in soil use a quantification through matrix-matched calibration to compensate for these  
76 resulting errors (Domínguez et al. 2015). This method implies the use of a soil similar to those analysed in order  
77 to have close matrix effects (Zhao et al. 2018), and free of the selected analytes for quantification purposes,  
78 which is not always applicable. Calibration using isotopically labelled internal standards often used for sediment,  
79 sludge, or wastewater analysis (Wiest et al. 2018a, b) is less developed for soil analysis. However, using internal  
80 standards with the same physicochemical characteristics as the target compounds for calibration is one of the  
81 most accurate solutions to correct for matrix effects and to perform quantification regardless of the soil  
82 characteristics.

83 Regarding literature on quantification of pesticides in soils, many studies still deal with organochlorine  
84 pesticides, although they are prohibited for decades. Indeed, detrimental effects to humans and non-target  
85 organisms are still observed as several dozen persistent ng/g are still detected in soils (Yadav et al. 2016; Qu et  
86 al. 2019; Ukalska-Jaruga et al. 2020). Only a few analytical methods include currently used pesticides, that is to  
87 say, last generation pesticides intended to be less bioaccumulative, less persistent, and used at lower doses. Here,  
88 the pesticides selection was based on agrochemicals practices in the area where the soils were collected.

89 In this study, we developed a reliable and sensitive method, combining ultrasounds and salting-out solvent  
90 (derived-QuEChERS) extractions assisted by EDTA, for the simultaneous analysis of 31 pesticides currently  
91 used and at agronomical rates, covering a wide range of physicochemical properties applicable to a huge range  
92 of soils. It was then validated under environmental conditions to soil samples collected from different  
93 landscapes, using isotopically labelled standards for quantification.

94

## 95 **2. Materials and Methods**

### 96 2.1. Chemicals and reagents

97 UPLC-MS grade acetonitrile (ACN), ammonium acetate, and methanol (MeOH) were obtained from Biosolve  
98 Chimie (Dieuze, France). Acetic acid (purity  $\geq 99\%$ ), heptane (purity  $\geq 90\%$ ) and dimethylsulfoxide (DMSO,  
99 LC grade) were acquired from Sigma-Aldrich (Saint Quentin Fallavier, France). A MilliQ® gradient A10 water

100 purification device including an EDS-PAK cartridge and a 0.2 µm Millipak® 40 filter from Merck-Millipore (St.  
101 Quentin en Yvelines, France) generated ultra-pure water.

102 The QuEChERS extract tubes containing citrate buffer were supplied from Agilent Technologies (Massy,  
103 France). The citrate buffer packet included 1 g of NaOCitrate, 4 g of MgSO<sub>4</sub>, 1 g of NaCl, and 0.5 g of disodium  
104 citrate sesquihydrate (pH = 5-5.5). Dispersive solid phase PSA/C18 (primary and secondary amines/C18)  
105 composed of 900 mg of MgSO<sub>4</sub>, 150 mg of PSA, and 150 mg of C18 was obtained from Macherey Nagel  
106 (Düren, Germany). Ethylenediaminetetraacetic acid disodium salt (Na<sub>2</sub>EDTA) was purchased from Sigma-  
107 Aldrich.

108 Relevant targeted pesticides (Table S1) were chosen according to agricultural surveys carried out in the "Zone  
109 Atelier Plaine et Val de Sèvres" (ZAPVS), Villiers-en-Bois, France area. They were also selected for their  
110 variety of physico-chemical properties. Pesticide analytical standards were acquired from Sigma-Aldrich, Riedel  
111 de Haën (Seelze, Germany), or Cluzeau Info Labo (Sainte Foy la Grande, France) with purity ≥ 97.0% (except  
112 for acetochlor, cycloxydim, and cypermethrin with purity of 96.8 %, 96.4%, and 92.0%, respectively.

113 Isotopically labelled internal standards (ISs, Table S2) were acquired from Sigma–Aldrich, A2S (Saint Jean  
114 d'Illac, France), or CDN isotopes (Pointe-Claire, Quebec, Canada) with purity ≥ 97.5% except for  
115 propiconazole-(phenyl-3) (≥ 94 % purity). Phenacetin-ethoxy-1-<sup>13</sup>C (98 atom % <sup>13</sup>C) used as injection standard  
116 was purchased from Isotec (Ohio, USA).

117

## 118 2.2. Stock solutions and calibration standards

119 Pesticide stock solutions of the 31 individual standards (Table S1) and ISs of the 13 isotopically labelled internal  
120 standards (Table S2) were prepared individually in ACN at 1000 mg/L. They were stored 3 months in the dark at  
121 -20°C without any measured degradation.

122 A fortifying mixture containing the 13-labelled standards at concentrations of 5, 50, or 500 µg/L (depending on  
123 the molecules) was prepared weekly in ACN and used for internal calibration. The fortifying mixture was added  
124 to soil samples before extraction to compensate for losses occurring during the overall process, the matrix  
125 effects, and for quantification purposes. It was left to soak the soil for 15 h (one night) before performing the  
126 extraction procedure.

127

128 2.3. Soils

129 Soil sampling was performed in April 2017, in different areas located in the ZAPVS. Soils were stored at -20°C  
130 until analysis. Samples were air-dried at room temperature, coarse particles were removed, and the soils were  
131 sieved (250 µm-mesh) to obtain homogeneous samples.

132 Standard soil 2.4 from LUFA Speyer (Speyer, Germany) was used for method development and validation.  
133 Properties of this soil are the following: 32.0% of sand, 42.1% of silt, 25.9% of clay, 1.95 organic carbon (% C),  
134 0.22 total nitrogen (% N), 21.2 meq/100g of cation exchange capacity, pH of 7.4 (0.1 M CaCl<sub>2</sub>). According to  
135 the United States Department of Agriculture this soil is classified as loam soil.

136

137 2.4. Liquid chromatography tandem mass spectrometry

138 The chromatographic separation of the pesticides was performed using a liquid chromatographic system H-Class  
139 UPLC from Waters (St. Quentin en Yvelines, France). The chromatographic column was a Kinetex Phenyl-  
140 Hexyl (100 × 2.1 mm; 2.6 µm) from Phenomenex (Le Pecq, France). The mobile phase was composed of (A)  
141 0.01% acetic acid with 0.04 mmol/L ammonium acetate in ultrapure water and (B) MeOH. The flow rate was  
142 fixed at 0.4 mL/min. The following gradient was used: 0-7 min, 5-90% B; 7-7.2 min, 90-100% B; 8.2-10.2 min,  
143 100% B, 10.2-10.5 min, 100-5 %B; 10.5-13.5 min, 5% B. The oven temperature was maintained at 60°C and the  
144 injection volume was set at 2 µL.

145 A Xevo TQ-S triple-quadrupole mass spectrometer (Waters) equipped with a StepWave ion guide was coupled  
146 to the chromatographic system. The multiple reaction monitoring (MRM) mode was used for the MS/MS  
147 detection with an electrospray source in the positive ionization mode (ESI+). The optimum ESI-MS/MS settings  
148 were obtained by direct infusion of each individual pesticide and internal standard into the electrospray source.  
149 The declustering potential (DP) and collision energy (CE) leading to the highest signal intensities for each  
150 pesticide are shown in Tables S2 and S3. Two transitions were optimized in the MRM mode for each analyte.  
151 The transition that presented the most intense signal (MRM1) was chosen for quantification and the second one  
152 (MRM2) for confirmation of each target compound. MRM ratios between both target transitions and retention  
153 times were also used as identification parameters. Only one transition was selected for each labelled internal  
154 standard. Ionization source parameters were optimized for maximum intensity of the precursor ions as follows:

155 capillary voltage 3200 V, desolvation temperature 450°C, source offset 50 V, source temperature 150°C,  
156 nitrogen desolvation and nebulizer gas flows of 900 L/h and 150 L/h, respectively.

157

#### 158 2.5.Extraction protocol

159 The final procedure corresponded to a combination of derived-QuEChERS and ultrasounds, assisted by EDTA.  
160 Once thawed, dried, and sieved, 2.5 g of soils were introduced into a 50 mL-polypropylene centrifugation tube.  
161 Then, 500 µL of the mixture of labelled internal standards (at 5, 50 or 500 µg/L depending on their detection  
162 intensity) were added so as to work from chromatograms with quite similar intensity signals. After 15 h of  
163 contact in the dark, and total evaporation of the solvent, the extraction was carried out.

164 Soil samples were mixed with 6 mL of water containing EDTA (0.1 M) and put in an ultrasonic bath during 15  
165 min at 30°C. The water was added first to the dry soil in order to weaken the interactions between the analytes  
166 and the matrix, and to subsequently facilitate solvent accessibility in the matrix pores. Then, a ceramic bar and 5  
167 mL of ACN were added to the tube that was vortexed 15s. Then, the citrate buffering salt was added and the tube  
168 was straightaway vigorously manually shaken for 10s, then vortexed for 1 min and finally centrifuged for 3 min  
169 at 6000 rpm, at room temperature. A 3-mL aliquot of the ACN extract layer containing the compounds of  
170 interest was transferred into the sample cleanup tube (15 mL) containing PSA/C18.

171 The extraction was repeated once again with 5 mL of ACN. At the end of the extraction, 5 mL of the supernatant  
172 was again transferred into the same dSPE tube. This cleanup tube was vortexed 1 min and centrifuged (3 min at  
173 6000 rpm, at room temperature). Subsequently, 6 mL of supernatant were transferred to a 10 mL glass tube with  
174 100 µL DMSO in order to prevent evaporation of the target analytes. Then, ACN was evaporated under a gentle  
175 stream of nitrogen at 40°C. Finally, 800 µL of water and 100 µL of <sup>13</sup>C-phenacetin (200 ng/mL in MeOH) were  
176 added to the residual DMSO prior to LC-MS/MS analysis. The addition of <sup>13</sup>C-phenacetin in the vials as  
177 injection internal standard was systematically carried out to control any variations due to the injection step.

178

#### 179 2.6.Method performance

180 Method validation followed the European Commission SANTE/12682/2019 guidelines. The standard soil was  
181 used for the evaluation of the method performance in terms of linearity, specificity, limits of detection (LOD)

182 and quantification (LOQ), accuracy, repeatability, and reproducibility. Additionally, the matrix effect (MEs)  
183 and the extraction efficiency were assessed.

184 The method performances were determined by fortifying the standard soil with analytical and isotopically  
185 labelled standards. The pesticides standards were spiked at seven concentrations ranging from LOQ to 100×LOQ  
186 (different for each pesticide) whereas labelled IS were introduced at a fixed concentration (corresponding to  
187 50×LOQ of each pesticide). The linearity was assessed by considering the determination coefficient ( $R^2$ ) of the  
188 plot built by the ratio of the analyte to the IS signal versus the analyte concentration.

189 The specificity was ensured by the accurate retention time of each pesticide, both MRM transitions and the  
190 accurate ratio between them (with a tolerance <20% between the ratio of an analyte detected in the sample and  
191 those of the reference standard fortified in the sample matrix). It was checked by an injection of each individual  
192 standard that there were no interfering signals between the different MRMs. It was also verified that there were  
193 no residual signals when injecting unspiked soil extracts.

194 Intra-day precision, expressed as repeatability (five replicate extractions in the same day, n=5) and intra-day  
195 precision, expressed as reproducibility (three replicate extractions in three different days, n=9) were assessed at  
196 three different levels corresponding to 2×LOQ, 20×LOQ, and 100×LOQ, which seemed to be representative of  
197 the range of residual concentration potentially expected in soils. Precision corresponded to the Relative Standard  
198 Deviation RSD(%).

199 Accuracy was calculated on three days as the closeness of agreement between the theoretical fortified  
200 concentrations (2×LOQ, 20×LOQ, and 100×LOQ) and the concentrations actually obtained at the end of the  
201 analysis, from the calibration curves obtained the same day.

202 The LOD was determined as the analyte concentration that generated a peak signal corresponding to three times  
203 the background noise from the chromatogram, regarding MRM2. The LOQ was determined as the analyte  
204 concentration that produced a peak signal of ten times the background noise, regarding MRM1, and at which the  
205 MRM1/MRM2 ion ratio was consistent with the MRM1/MRM2 ratio of a standard (Relative Standard Deviation  
206 (RSD) < 20%) analysed in the same conditions. It has to be noticed that sometimes LOD and LOQ could be  
207 similar. This is due to the calculation of their values, as the LOD and LOQ were determined regarding MRM2  
208 and MRM1 transitions, respectively. Thus, the signal-to-noise of 3 for MRM2 (LOD) can be the same as a  
209 signal-to-noise of 10 for MRM1 (LOQ).

210 To correct for extraction efficiency and matrix effects, internal standard calibration was performed using 13  
211 stable isotope labelled analogues of target pesticides (Table S2), which means that more than two thirds of the  
212 compounds had a specific IS. For the analytes that did not have their own isotopic labelled standard, the most  
213 appropriate IS was selected among the ones available by considering the closest chemical classes or retention  
214 times to best account for matrix effect and extraction behavior.

215 To calculate extraction efficiencies of the sample preparation, the signals corresponding to standard soil samples  
216 fortified at the beginning of the protocol (A fortified before extraction) were compared to those of samples  
217 fortified after extraction (A fortified after extraction) as followed :

218

$$219 \quad \text{Extraction efficiency (\%)} = \frac{A_{\text{fortified before extraction}}}{A_{\text{fortified after extraction}}} \times 100$$

220

221 Matrix effects are one of the main aspects that must be addressed when evaluating a multi-residue method for  
222 pesticide analysis. The intensity of the signal in a complex matrix can be influenced (suppressed or enhanced) by  
223 matrix component that are not removed during sample preparation step. Matrix effects were calculated by  
224 comparing extracted samples fortified after extraction and the signal obtained in the solvent, without the  
225 presence of matrix component, as followed:

226

$$227 \quad \text{Matrix effect (\%)} = \left( \frac{A_{\text{fortified after extraction}}}{A_{\text{solvent}}} - 1 \right) \times 100$$

228

### 229 **3.Results and Discussion**

230 In order to guarantee an accurate and sensitive analysis of the target compounds in a complex matrix such as  
231 soil, an efficient and robust sample preparation is necessary. The aim of the extraction is to recover as much  
232 analytes as possible from the soil while minimizing the co-extraction of matrix interferences. It is therefore  
233 essential to optimize the extraction parameters. We conducted the following strategy: preliminary experiments  
234 were carried out for two extraction techniques, considering only the responses obtained in LC-MS/MS after each  
235 extraction. This first comparison led to select the most promising technique for further development.

236

237 3.1.Preliminary comparison

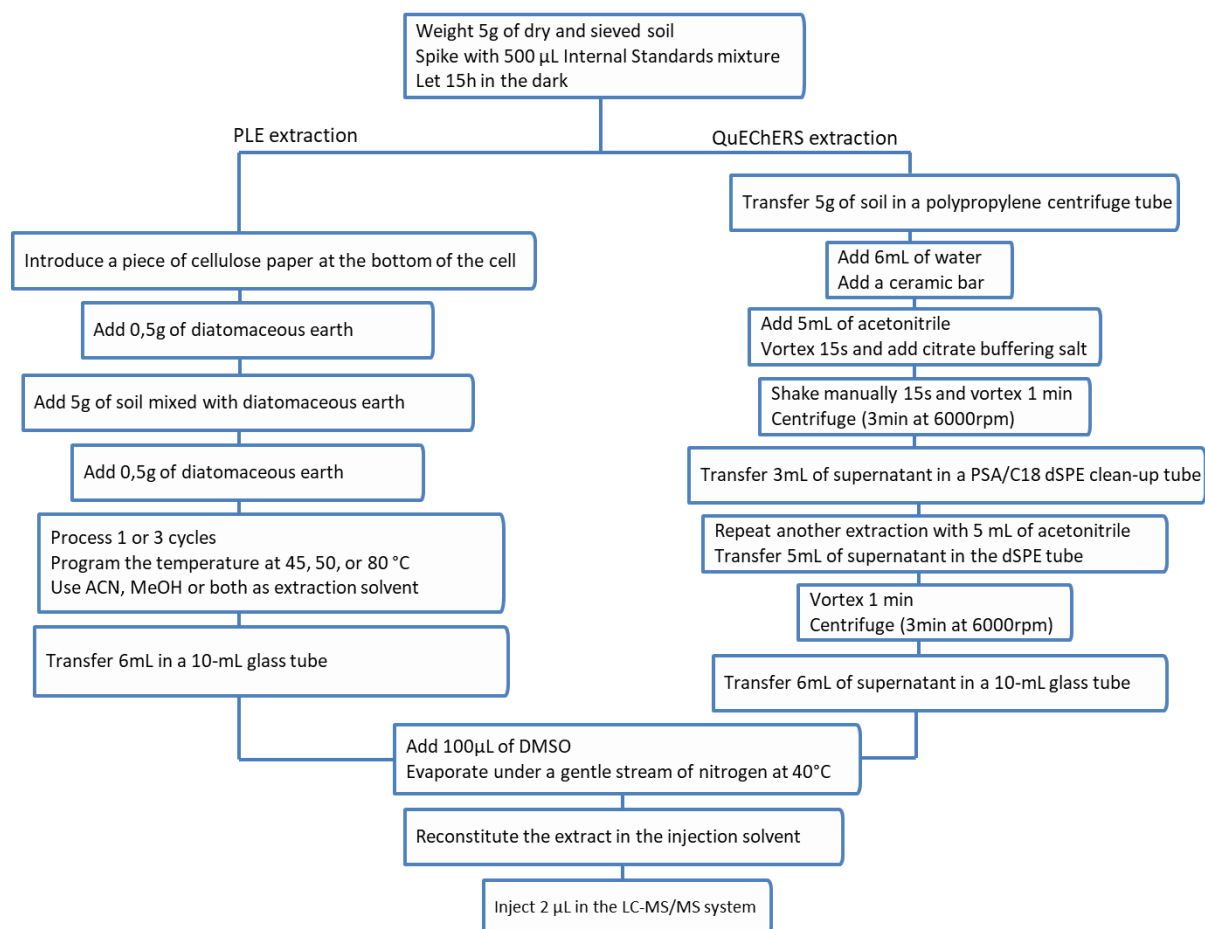
238 Firstly, two extraction methods commonly used in soil analyzes were compared : PLE which uses the  
239 combination of high temperature and high pressure to improve extraction efficiency, and QuEChERS which  
240 consists of a salt-assisted liquid-liquid extraction followed by a dispersive phase purification step.

241 Both extraction methods were carried out using 5 g of dry soil, milled and sieved at 250  $\mu\text{m}$ . At the end of  
242 extractions, the supernatant was removed. Then, 100  $\mu\text{L}$  of DMSO were added to the extract. After, it was  
243 evaporated, then reconstituted in the injection solvent for the LC-MS/MS analysis.

244 Both extraction protocols performed for the preliminary comparison are summarized in Figure 1.

245

246 **Fig.1:** PLE and QuEChers extraction protocols performed in the preliminary study in order to evaluate the best  
247 extraction for further developments



248

249

### 250 3.1.1. PLE

251 PLE experiments were performed on an Accelerated Solvent Extraction apparatus ASE Dionex 350 system  
252 (Thermo Scientific) with 10 mL stainless-steel vessels. Diatomaceous earth (0.5 g) was first introduced into the  
253 cell with a piece of cellulose paper in the bottom, then soil sample mixed with diatomaceous earth, and finally  
254 0.5 g of diatomaceous earth. Extractions were carried out at  $1.2 \times 10^7$  bars.

255 Several parameters of the PLE were assessed to improve the extraction of the targeted pesticides. It is established  
256 that the extraction solvent is one of the most important parameters to be optimized in PLE, and that the  
257 temperature has impacts on the equilibrium (solubility), mass transfer rate (diffusion coefficient), and the  
258 stability of pesticides (Masiá et al. 2015). Thus, different extraction solvents (MeOH, ACN, or a mix  
259 MeOH/ACN 50:50 (% v/v)), temperatures (45, 50, or 80°C) and number of cycles (1 or 3) were tested. In order  
260 to define the conditions for reaching the lowest detection limits, the LC-MS/MS response of each analyte was  
261 considered for this comparison as it includes both the contribution of the extraction efficiency and the matrix  
262 effect (Figure S1).

263 The temperature had a low impact on pesticide signal. Indeed, pesticides responses were quite similar when 1  
264 cycle with ACN/MeOH 50:50 (% v/v) was applied at 45, 50, or 80°C, except for pyrethrinoids, which exhibited  
265 higher signals at 80 °C. On the contrary, the number of cycles was important, as whole pesticide responses  
266 increased when 3 cycles were used instead of 1 cycle for ACN/MeOH extraction. Solvent composition was also  
267 an important parameter. MeOH led to the highest signals for 15 pesticides, especially for triazole,  
268 chloroacetamide and neonicotinoid families. The use of ACN and ACN/MeOH 50:50 (% v/v) conducted to  
269 highest responses of 7 and 8 compounds, respectively. The use of MeOH/ACN improved pyrethrinoids signals.

270 The best responses were obtained with 3-extraction cycles using methanol at 50 °C. Therefore, it was retained  
271 for PLE technique, and compared to QuEChERS extraction technique.

### 272 3.1.2. QuEChERS

273 For preliminary QuEChERS extraction experiments, 6 mL of water were added to 5 g of soil in a 50-mL  
274 polypropylene tube. Then, two successive extractions were carried out with 5 mL of ACN, in the presence of  
275 citrate buffer (European method, EN 15662). The extract (8 mL) was then purified by dSPE with the PSA/C18  
276 phase. After vortex and centrifugation, the supernatant (6 mL) was evaporated. Finally, the evaporated extract  
277 was reconstituted in the injection solvent.

278 The LC-MS/MS responses obtained with both extraction methods were compared in Figure S2. The highest  
279 responses were obtained after the QuEChERS extraction, for whole targeted compounds. These preliminary  
280 steps therefore lead us to pursue further developments on a QuEChERS-based method.

281

### 282 3.2. QuEChERS optimization

283

284 Several parameters (e.g., addition of a non-volatile solvent before evaporation, additives in extraction solvents,  
285 mass of soils, etc.) were considered during improvement of the QuEChERS extraction. The extraction efficiency  
286 (recovery percentages of the pesticides) was determined in fortified blank soils by quadruplicate trials (n=4) for  
287 each test.

#### 288 3.2.1. Addition of DMSO

289 The first parameter considered was the interest of the addition of DMSO before the evaporation step. The DMSO  
290 is a solvent with low volatility and its addition could avoid the loss of volatile molecules during evaporation.  
291 Hence, extraction recoveries were evaluated with and without the addition of DMSO before evaporation (Figure  
292 S3). The results showed that the extraction yields of most pesticides are greater when DMSO was added before  
293 the final evaporation step, except for prochloraz, fenpropidin, and to lesser extent for two neonicotinoids. It is  
294 worth noting that some compounds were even completely lost during the evaporation step in the absence of  
295 DMSO, like the pyrethroids. So, the addition of DMSO before evaporation was necessary.

296 The extraction recoveries ranged from 15 to 85%, which was not satisfactory enough for some compounds. In  
297 particular, the pyrethroids and the herbicides pendimethalin and cloquintocet-mexyl were poorly extracted  
298 (recovery < 20%). These compounds have the highest log  $K_{ow}$  values (Table S1) meaning that they remain  
299 adsorbed onto the surface of organic matter present in soil (Wiest et al. 2018a).

#### 300 3.2.2. Addition of EDTA or formic acid

301 In order to improve the extraction yields, we evaluated the effect of the addition of EDTA (0.1 M) in water, and  
302 the addition of 200  $\mu$ L of formic acid (FA) in the extraction solvent.

303 The use of EDTA to improve the extraction of certain organic compounds from soil has already been proven.  
304 EDTA and its salts are chelating agents that can interact more strongly than analytes with soil metals, and thus  
305 exchange with analytes at active sites, or mask adsorption sites (Alcantara-Licudine et al. 1997). This property

306 has been used for the extraction of acidic pesticides (David et al. 2000; Campbell and Li 2001; Gaston and  
307 Locke 2001), but has rarely been used for multi-residue or multi-family analysis of pesticides.

308 On the other hand, the addition of a small amount of acid could promote the salting-out process and increase the  
309 pesticide stability in ACN (Zaidon et al. 2019). Moreover, at acidic pH, some anionic sites of the soil and some  
310 pesticides are protonated, leading to an electrostatic repulsion between them, increasing the extraction efficiency  
311 for these pesticides. Additionally, different authors indicated that, when using the EN method, the extraction  
312 efficiency of acetonitrile increased in acidic condition (EC 2015). The impacts of the different additives are  
313 illustrated in Figure S4.

314 The addition of formic acid led to the highest extraction recoveries for 15 pesticides. Moreover, it really  
315 improved the extraction of 12 pesticides, especially that of bifenthrin, pendimethalin, cloquintocet-mexyl,  
316 prochloraz, and diflufenican. It slightly improved that of the three neonicotinoids and the four chloroacetamides.  
317 However, when using formic acid the pyrethrinoids were no longer extracted.

318 The addition of EDTA increased extraction recoveries of 23 compounds compared to the QuEChERS extraction  
319 using water/ACN. It significantly enhanced the extraction of whole pyrethroids, as well as that of pyraclostrobin,  
320 pendimethalin, cloquintocet-mexyl, metrafenone, and diflufenican, corresponding to analytes the more difficult  
321 to extract with the highest  $K_{OC}$  values. Moreover, extraction recoveries were all higher than 60% when using  
322 EDTA, except for pyroxsulam (56%), aclonifen (59%) and fenpropidin (36%), and were the highest ones for 14  
323 pesticides.

324 Thus, for the final protocol, the addition of 0.1 M EDTA in water was retained unlike an addition of formic acid.

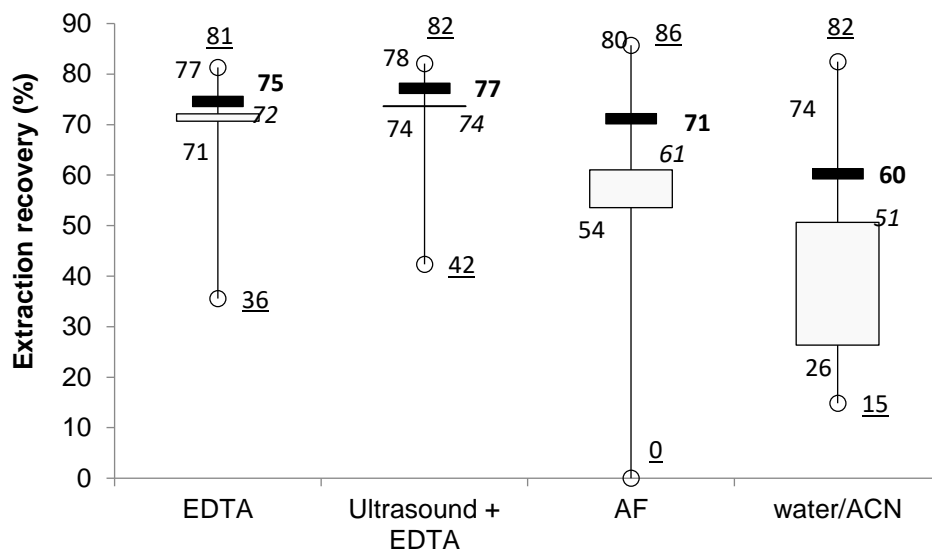
### 325 3.2.3. Addition of an ultrasonic step

326 Finally, as the use of high frequency can enhance the solvent extraction, the addition of an ultrasonic step (15  
327 min at 30 °C) after the addition of EDTA in water and before the addition of ACN was evaluated (Figure S5).  
328 Ultrasounds were performed on a Branson 5510 DTH Ultrasonic Cleaner, functioning at a frequency of 40 kHz.  
329 The results showed an overall slight positive effect of the ultrasonic step on the recoveries prior to derived-  
330 QuEChERS extraction. However, a significant enhancement was observed for aclonifen (from 59 to 82%) and  
331 fenpropidin in a lesser extent (from 36 to 42 %).

332 In conclusion, an extraction protocol combining ultrasounds with a derived QuEChERS extraction (in the  
333 presence of EDTA) was chosen, with DMSO addition before evaporation (Figure 2).

334 **Fig.2:** Extraction yields (%) of the targeted pesticides according to different QuEChERS extraction protocols  
 335 (median in bold; mean in italics; max and min values underlined; first and third quartiles in regular writing).  
 336 Extraction of 5 g of soil using 6 mL of water, 10 mL of ACN and citrate salt (corresponding to water/ACN plot),  
 337 addition of 0.1 M EDTA in water (EDTA plot), addition of 200 $\mu$ L of formic acid in ACN (FA plot), or addition  
 338 of an ultrasonic step (ultrasound+EDTA plot)

339



340

341

### 342 3.3. Ratio mass soil/extraction solvent optimisation

343 The extraction yields obtained after the derived-QuEChERS extraction of 2.5g soil/10 mL ACN and 5g soil/10  
 344 mL ACN were compared for the 31 pesticides (Figure S6). The best extraction yields were obtained with the  
 345 extraction of 2.5 g of soil. Decreasing the initial quantity of soil mainly enhanced the extraction of the  
 346 pyrethroids, and that of aclonifen, pendimethalin, or cloquintocet-mexyl of around 20%. The extraction  
 347 recoveries ranged from 55% (cycloxdim) to 118% (deltamethrin), and from 42 % (fenpropidin) to 82%  
 348 (aclonifen) for 2.5 and 5g, respectively.

349 Thus, the final extraction protocol was performed onto 2.5 g. With this protocol, all extraction recoveries were  
 350 equal to or higher than 65% except cycloxdim, pyroxsulam, and fenpropidin (55%, 56%, and 60%,  
 351 respectively).

352

### 353 3.4. Method performance

354 Validation parameters of the optimized analytical method, notified in the European Commission  
 355 SANTE/12682/2019 guidelines, are presented in Table 1.

356 Most of the LOQs are less than 0.5 ng/g and three-quarters of the LODs are less than 0.1 ng/g. This allows the  
357 detection of extremely low levels of pesticides, and should provide new and relevant environmental data. These  
358 LOQ are often lower than reported in previous multi-residue studies for the few pesticides in common with our  
359 work. For example, in their multi-residue method combining a QuEChERS extraction to a LC-MS/MS analysis,  
360 Masiá et al. (2015) obtained LOQ of 0.06, 3, and 10 ng/g for imidacloprid, prochloraz, and acetochlor,  
361 respectively, while Hvězdová et al. (2018) found LOQ from 3 to 10 ng/g, including 15 pesticides in common  
362 with our work. Similarly, Zaidon et al. (2019) obtained LOQ of 0.5 ng/g and of 0.8 ng/g for imidacloprid and  
363 propiconazole, respectively.

364 Linearity was evaluated using linear regressions and square correlation coefficients,  $R^2$ . Good linearity was  
365 obtained for whole compounds, with  $R^2$  greater than 0.992.

366 The precision of the method was assessed through the repeatability and reproducibility values. The calculated  
367 RSD are mainly less than 20%, and even less than 10% in most cases.

368 The accuracy is between 80 and 120% of the expected value, which means that the internal standards correctly  
369 compensate for partial extractions and matrix effects. However, for the two pyrethrinoids lambda-cyhalothrin  
370 and tau-fluvalinate the accuracy is between 59 and 175%, probably due to matrix effects only partially  
371 compensated by the internal standard. Cycloxydim, on the other hand, is difficult to quantify accurately, with a  
372 calculated value of only 15% of the expected value.

373 The majority of the compounds experience signal suppression due to the residual matrix. Some high signal  
374 enhancement effects are observed for lambda-cyhalothrin and tau-fluvalinate at the  $2\times$ LOQ spiking  
375 concentration (matrix effect of +263 and +177%, respectively). However, for most of the compounds and  
376 spiking levels, the matrix effects are below 40%.

377

### 378 3.5. Application of the method to actual soil samples

379 In order to evaluate the method under environmental screening conditions, a panel of 12 different soils collected  
380 in agricultural areas, hedgerows, or grasslands of the ZAPVS were analysed with the optimized conditions.

381 The selective identification of the pesticides was ensured by chromatographic and mass spectrometric  
382 characteristics. A method blank and a blank soil were systematically extracted and analyzed along with actual  
383 samples.

384 The method was successfully applied to soil samples, with 24 pesticides among the 31 selected compounds  
385 detected at least once (Table 2). Only seven pesticides, namely the herbicides cycloxydim and acetochlor, the  
386 fungicide fenpropidin and the pyrethroid insecticides cypermethrin, bifenthrin, lambda-cyhalothrin, and  
387 deltamethrin were not detected in the samples. All the soil samples (100%) contained at least one pesticide.

388 Samples from grasslands, hedgerows, and cropped fields contained 1 to 4, 3 to 13, and 7 to 18 pesticides,  
389 respectively (example of chromatograms from one soil is presented Figure 3). The highest concentrations were  
390 measured in soils from cropped fields, with values up to 601 and 722 ng/g for boscalid and diflufenican,  
391 respectively. The most frequently found pesticides were the herbicides diflufenican and napropamide, the  
392 fungicides epoxyconazole and boscalid, and the insecticide imidacloprid, with 7 to 10 detections in the analysed  
393 samples. The multi-family combination of herbicide, insecticide, and fungicide were observed in 9 samples. The  
394 simultaneous presence of diflufenican, epoxyconazole, and imidacloprid was the most often observed  
395 combination, in 7 samples, then that of napropamide, boscalid, and imidacloprid (6 samples), followed by  
396 diflufenican or napropamide with boscalid, and imidacloprid in 5 samples. These results are in accordance with  
397 data obtained by Riedo et al. (2021) who studied 46 pesticides in 100 soils. Among them 41 were found at least  
398 once, and 3 to 32 pesticides were detected per field. They detected napropamide, epoxyconazole, and  
399 imidacloprid in more than half of the studied soils, with concentrations up to 58, 150, and 24 ng/g, respectively.

400 In their work, Silva et al. (2019) evaluated 76 pesticide residues in 317 European agricultural topsoil samples.  
401 Their study revealed that the presence of mixtures of pesticide residues in soils were the rule rather than the  
402 exception, with 166 different pesticide combinations and up to 13 pesticides in a sample. They found that  
403 boscalid and epoxiconazole were among the most frequently found pesticides (in 87 and 75% of the samples,  
404 respectively) and those with the highest concentrations, up to 410 and 160 ng/g, respectively. Imidacloprid was  
405 also found in 23% of the samples.

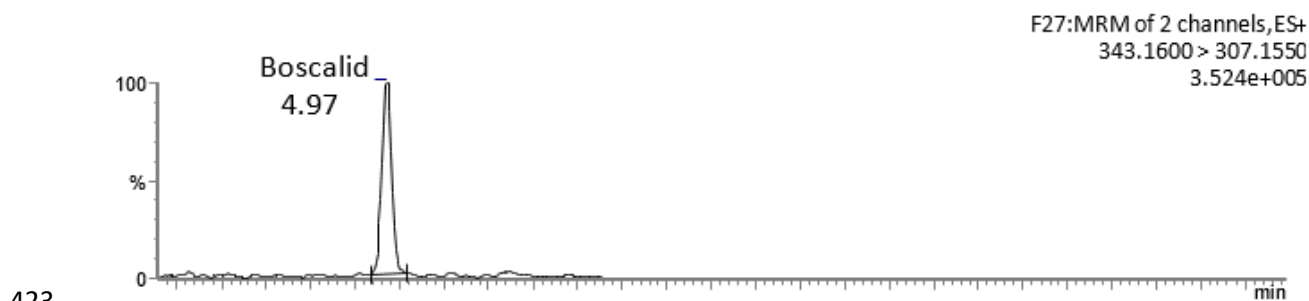
406 Many pesticide values were below 0.5 ng/g and lower than most of the LOQ previously reported. Indeed, the  
407 lower LOQ obtained in our study compared to that obtained in the study of Zaidon et al. (2019), allowed in our  
408 soil samples one more determination of imidacloprid. In the same manner, our lower LOQ compared to that  
409 obtained by Masia et al. (2015) allowed the determination of prochloraz in 4 more samples. Moreover, compared  
410 to the LOQ values obtained, for 15 compounds common with our study, by Hvězdová et al. (2018), our lower  
411 LOQs enabled the quantification of metazachlor, dimethachlor, thiacloprid, clomazone, cyproconazole,  
412 propiconazole, and S-metolachlor in the soil samples, whereas with their LOQ values these compounds would  
413 not have been detected. The detection frequencies of other pesticides like metconazole, diflufenican,

414 pendimethalin, boscalid, prochloraz, and epoxiconazole were also enhanced thanks to our lower LOQs, with 1 to  
415 6 more quantifications. In the same manner, in comparison with the study of Silva et al. (2019), the lower LOQs  
416 obtained here allow to quantify boscalid, epoxiconazole, imidacloprid, propiconazole, cyproconazole and  
417 prochloraz in respectively 6, 4, 3, 1, 4 and 4 more samples that would not have been detected with their LOQs.  
418 The very high sensitivity of our method therefore makes it possible to extend toxicity, risk assessment, sublethal  
419 effect studies, etc. to even lower concentration levels than those currently considered.

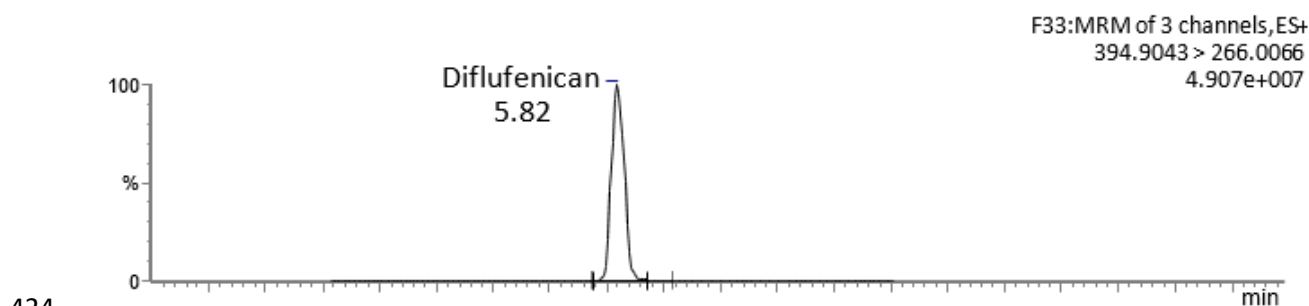
420

421 **Fig.3:** Example of chromatograms resulting from the analysis of the soil “Culture 4”

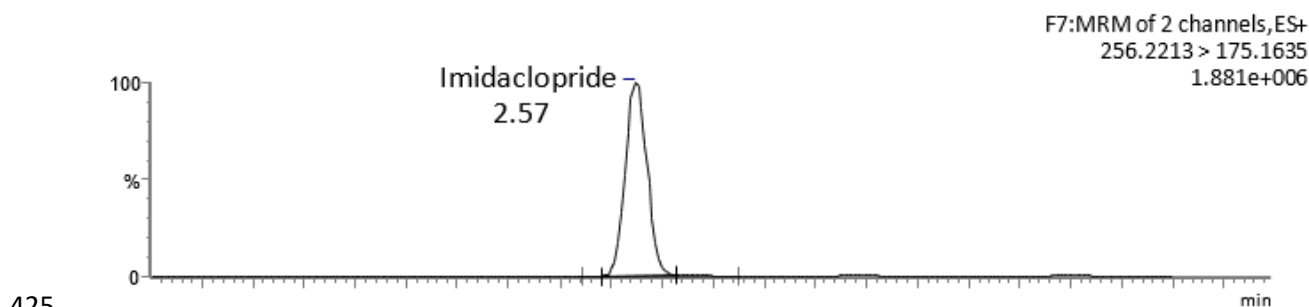
422

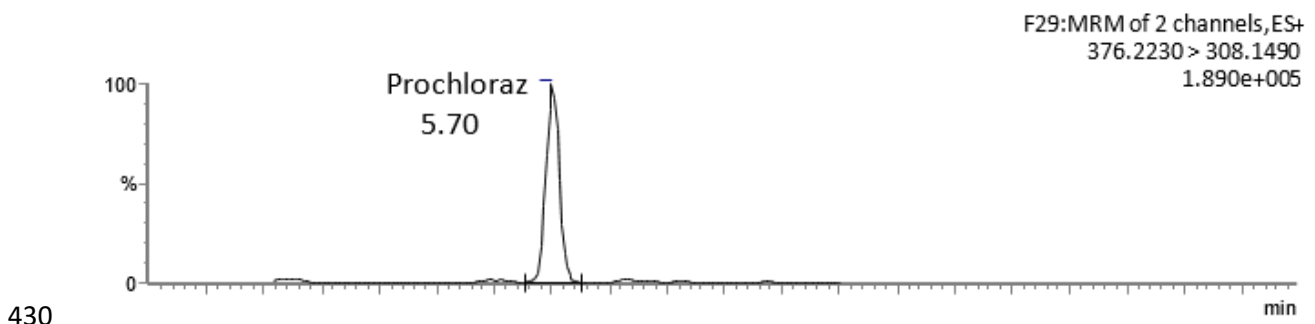
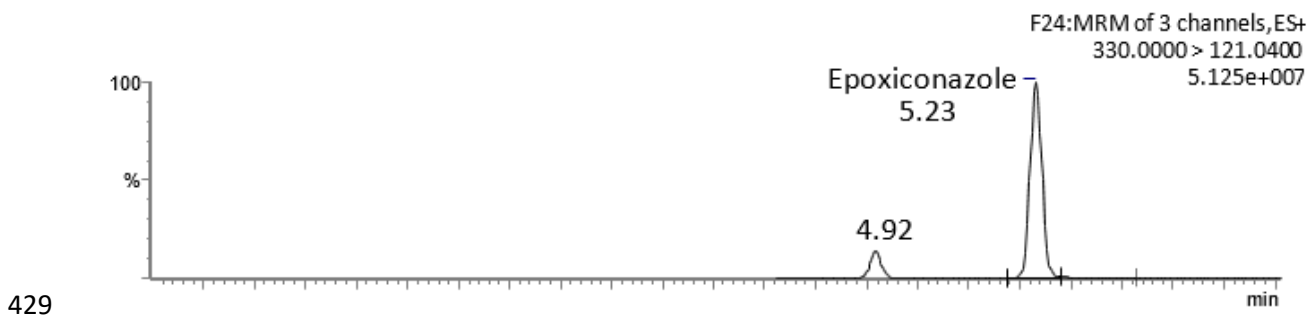
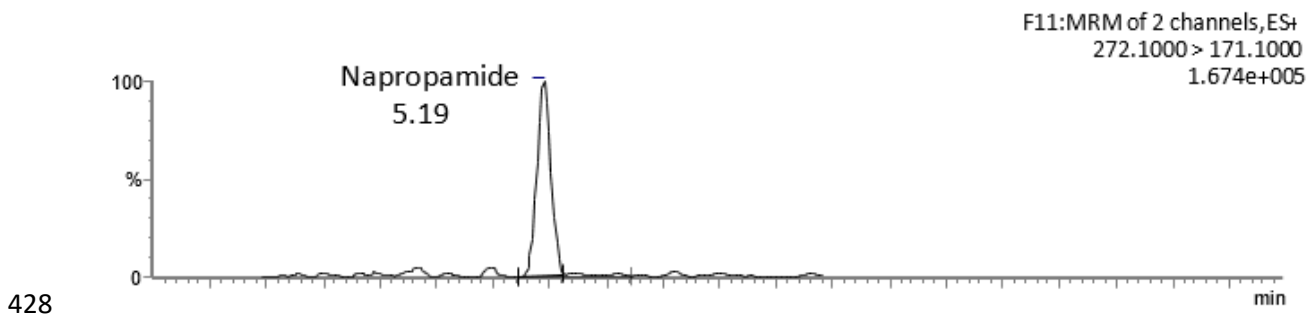
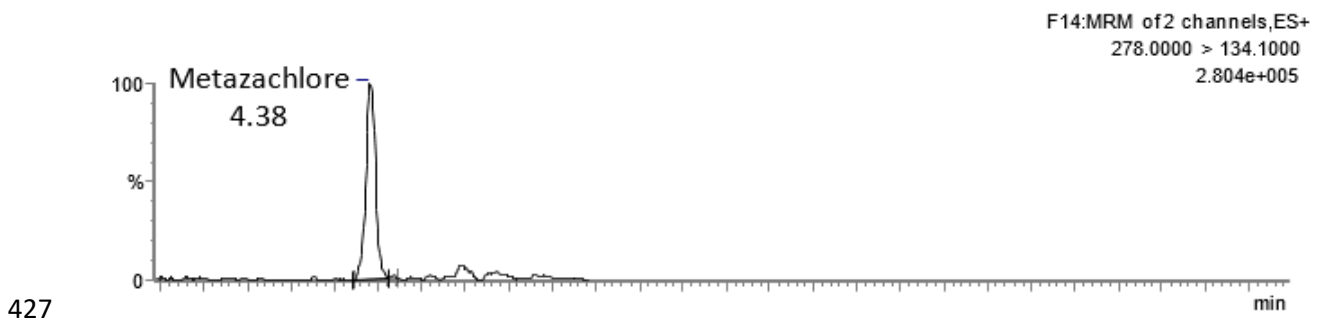
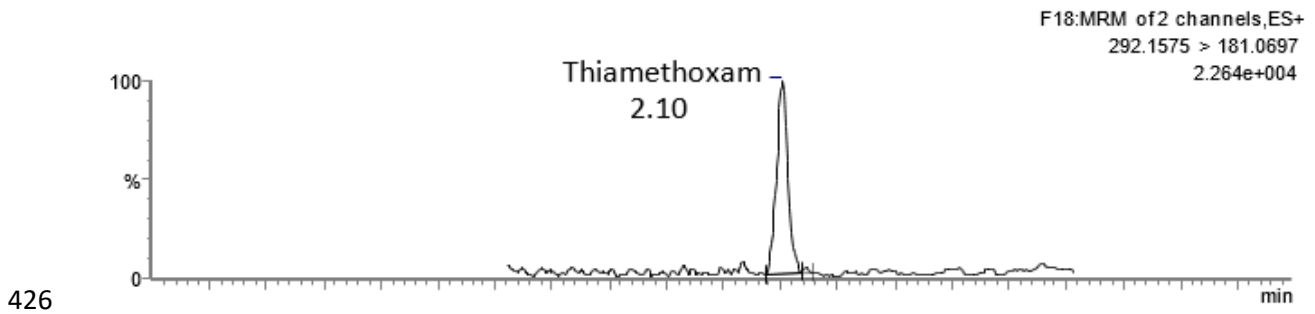


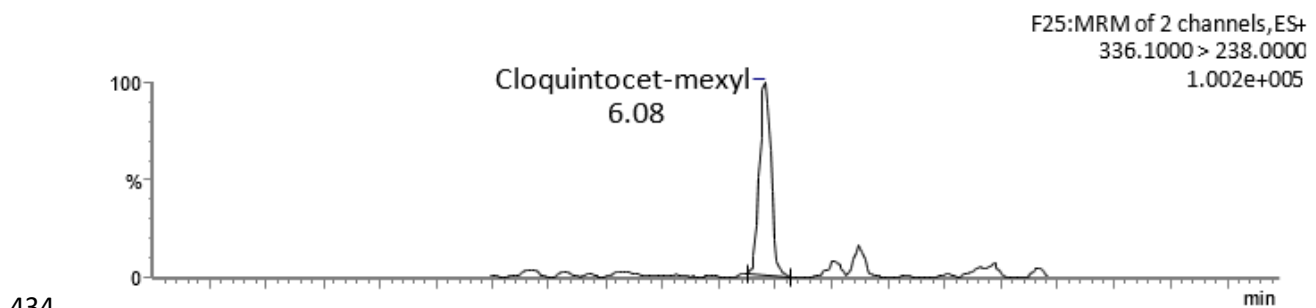
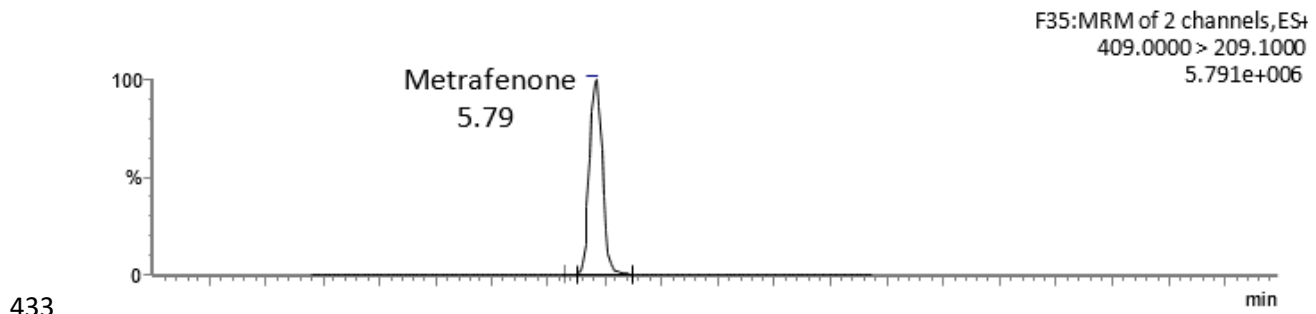
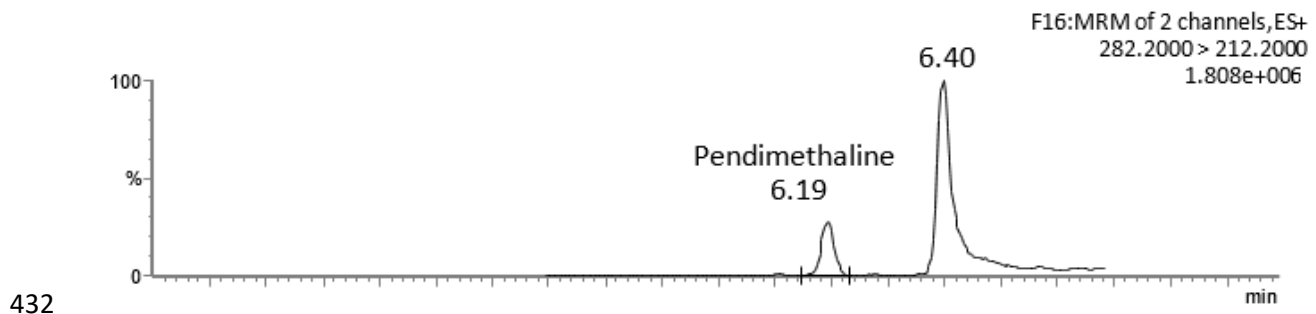
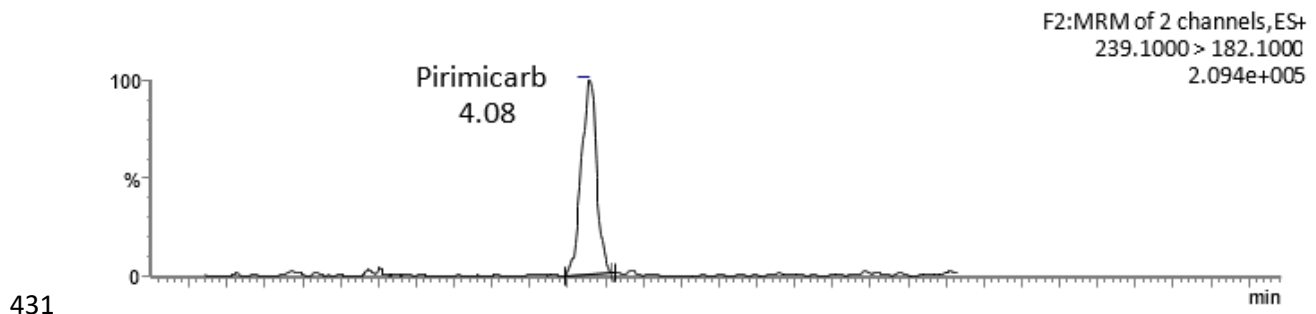
424



425







436 **5.Declarations**

437 *Ethics approval and consent to participate* : Not applicable.

438 *Consent for publication* : Not applicable.

439 *Availability of data and materials* : N/A, data supporting the results reported in the article can be found in the  
440 figures and tables included in this paper.

441 *Competing interests* : The authors declare that they have no competing interests.

442 *Funding* : This work has been financially supported by the French OFB (Office Français de la Biodiversité,  
443 formerly ONEMA) within the national call “Pesticides” (APR ECOPHYTO 2014).

444 *Authors' contributions* : FL, MF carried out the analytical work. GD and EV supervised the analytical  
445 development. GD wrote the original draft. CP and CF coordinated the research project, selected and organized  
446 the soil sampling. All authors read and approved the final manuscript.

447 *Acknowledgements* : The authors would like to thank the “Zone Atelier Plaine et Val de Sèvre” and especially  
448 Vincent Bretagnolle for the study site access and provision of site infrastructures. They also thank all the people  
449 who took part in the soil sampling in Chizé.

450

## 451 **6. References**

452 Alcantara-Licudine JP, Kawate MK, Li QX (1997) Method for the Analysis of Phloxine B, Uranine, and Related  
453 Xanthene Dyes in Soil Using Supercritical Fluid Extraction and High-Performance Liquid Chromatography. *J*  
454 *Agric Food Chem* 45:766-773.

455 Analytical quality control and method validation procedures for pesticide residues analysis in food and feed,  
456 SANTE/2017/11813. Implemented by 01/01/2020

457 Avendaño MC, Roqué P, Palomeque ME (2018) An Efficient Extraction Method of Persistent Organic  
458 Pesticides in Soil Samples for Their Chromatographic Determination. *Soil Sed Contam* 27(5):426-438.

459 Campbell S, Li QX (2001) Na<sub>4</sub>EDTA-assisted in situ derivatization pressurized fluid extraction of polar  
460 herbicides in soil. *Anal Chim Acta* 434:283-289.

461 Chiaia-Hernandez AC, Keller A, Wächter D, Steinlin C, Camenzuli L, Hollender J, Krauss M (2017) Long-term  
462 persistence of pesticides and TPs in archived agricultural soil samples and comparison with pesticide application.  
463 *Environ Sci Technol* 51(18):10642-10651.

464 David MD, Campbell S, Li QX (2000) Pressurized Fluid Extraction of Nonpolar Pesticides and Polar Herbicides  
465 Using In Situ Derivatization. *Anal Chem* 72:3665-3670.

466 Di S, Shi S, Xu P, Diao J, Zhou Z (2015) Comparison of different extraction methods for analysis of 10  
467 organochlorine pesticides: Application of MAE-SPE method in soil from Beijing. *Bull Environ Contam Toxicol*  
468 95(1):67-72.

469 Domínguez AM, Funes M, Fadic X, Placencia F, Cereceda F, Muñoz JP (2015) Evaluation of a buffered solid  
470 phase dispersion procedure adapted for pesticide analyses in the soil matrix. *Quim Nova* 38(7):884-890.

471 EC (2015). Analysis of Acidic Pesticides using QuEChERS 696 (EN15662) and acidified QuEChERS method.  
472 2015. EU Reference Laboratories for Residues of Pesticides, Fellbach, Germany, 2015

473 Joseph L, Paulose SV, Cyril N, Santhosh SK, Varghese A, Nelson AB, Kunjankutty SV, Kasu S (2020)  
474 Organochlorine pesticides in the soils of Cardamom Hill Reserve (CHR), Kerala, India: Geo spatial distribution,  
475 ecological and human health risk assessment. *Environ Chem Ecotoxicol* 2:1-11.

476 Gaston LA, Locke MA (2001) Comparison of methodologies for extraction of acifluorfen from soil. *Comm Soil*  
477 *Sci Plant Anal* 32:2953-2964.

478 Hvězdová M, Kosubová P, Košíková M, Scherr KE, Šimek Z, Brodský L, Šudoma M, Škulcová L, Sářka M,  
479 Svobodová M, Krkošková L, Vašíčková J, Neuwirthová N, Bielská L, Hofman J (2018) Currently and recently  
480 used pesticides in Central European arable soils. *Sci Tot Environ* 613-614:361-370.

481 Karasali H, Marousopoulou A, Machera K (2016) Pesticide residue concentration in soil following conventional  
482 and Low-Input Crop Management in a Mediterranean agro-ecosystem, in Central Greece. *Sci Tot Environ*  
483 541:130-142.

484 Kemmerich M, Bernardi G, Adaime MB, Zanella R (2015) A simple and efficient method for imidazolinone  
485 herbicides determination in soil by ultra-high performance liquid chromatography-tandem mass spectrometry. *J*  
486 *Chromatog. A* 1412:82-89.

487 Łozowicka B, Rutkowska E, Jankowska M (2017) Influence of QuEChERS modifications on recovery and  
488 matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD. *Environ Sci*  
489 *Poll Res* 24:7124-7138.

490 Masiá A, Vázquez K, Campo J, Picó Y (2015) Assessment of two extraction methods to determine pesticides in  
491 soils, sediments and sludges. Application to the Túria River Basin. *J Chromatogr A* 1378:19-31.

492 Muhammad M, Jan MR, Shah J, Ara B, Akhtar S, Rahman HU (2017) Evaluation and statistical analysis of the  
493 modified QuEChERS method for the extraction of pinoxaden from environmental and agricultural samples. *J*  
494 *Anal Sci Technol* 8:12.

495 Pastor-Belda M, Garrido I, Campillo N, Viñas P, Hellín P, Flores P, Fenoll J (2015) Dispersive liquid–liquid  
496 micro extraction for the determination of new generation pesticides in soils by liquid chromatography and  
497 tandem mass spectrometry. *J Chromatogr A* 1394:1-8.

498 Pose-Juan E, Herrero-Hernández E, Álvarez-Martín A, Sánchez-Martín MJ, Rodríguez-Cruz MS (2014)  
499 Development of a procedure for the multi-residue analysis of pesticides in vineyard soils and its application to  
500 real samples. *J Sep Sci* 37:2215-2224.

501 Qu C, Albanese S, Li J, Cicchella D, Zuzolo D, Hope D, Cerino P, Pizzolante A, Doherty AL, Lima A, De Vivo  
502 B (2019) Organochlorine pesticides in the soils from Benevento provincial territory, southern Italy: Spatial  
503 distribution, air-soil exchange, and implications for environmental health. *Sci Tot Environ* 674:159-170.

504 Riedo J, Wettstein FE, Rösch A, Herzog C, Banerjee S, Büchi L, Charles R, Wächter D, Martin-Laurent F,  
505 Bucheli TD, Walder F, van der Heijden MGA (2021) Widespread occurrence of pesticides in organically  
506 managed agricultural soils – the ghost of a conventional agricultural past? *Environ Sci Technol* 55:2919-2928.

507 Silva V, Mol HGJ, Zomer P, Tienstra M, Ritsema CJ, Geissen V (2019) Pesticide residues in European  
508 agricultural soils—A hidden reality unfolded. *Sci Tot Environ* 653:1532-1545.

509 Tadeo JL, Pérez RA, Albero B, García-Valcárcel AI, Sánchez-Brunette C (2012) Review of sample preparation  
510 techniques for the analysis of pesticide residues in soil. *J AOAC Int* 95(5):1258-1271.

511 Ukalska-Jaruga A, Smreczak B, Siebielec G (2020) Assessment of Pesticide Residue Content in Polish  
512 Agricultural Soils. *Molecules* 25(3):587.

513 Wiest L, Baudot R, Lafay F, Bonjour E, Becouze-Lareure C, Aubin JB, Jame P, Barraud S, Lipeme Kouyi G,  
514 Sébastien C, Vulliet E (2018a) Priority substances in accumulated sediments in a stormwater detention basin  
515 from an industrial area. *Environ Poll* 243:1669-1678.

516 Wiest L, Chonova D, Bergé A, Baudot R, Bessueille-Barbier F, Ayouni-Derouiche L, Vulliet E (2018b) Two-  
517 year survey of specific hospital wastewater treatment and its impact on pharmaceutical discharges. *Environ Sci*  
518 *Poll Res* 25:9207-9218.

519 Yadav IC, Devi NL, Li J, Zhang G, Shakya PR (2016) Occurrence, profile and spatial distribution of  
520 organochlorines pesticides in soil of Nepal: Implication for source apportionment and health risk assessment. *Sci*  
521 *Tot Environ* 573:1598-1606.

522 Zaidon SZ, Ho YB, Hamsan H, Hashim Z, Saari N, Praveena SM (2019) Improved QuEChERS and solid phase  
523 extraction for multi-residue analysis of pesticides in paddy soil and water using ultra-high performance liquid  
524 chromatography tandem mass spectrometry. *Microchem J* 145:614-621.

525 Zhao P, Zhao J, Lei S, Guo X, Zhao L (2018) Simultaneous enantiomeric analysis of eight pesticides in soils and  
526 river sediments by chiral liquid chromatography-tandem mass spectrometry. *Chemosphere* 204:210-219.

527

528

Table 1: Validation parameters for the multi-residue LC-MS/MS method for the determination of 31 pesticides in soils, using internal calibration (H=herbicide, I=insecticide, F=fungicide)

Family	RT min	Validation level	Sensitivity		Linearity	Accuracy (%)	Repeatability (%RSD)	Reproducibility (%RSD)	Matrix effect ME (%RSD)					
			LOD (ng/g)	LOQ (ng/g)	R <sup>2</sup>									
Boscalid	5.0	20×LOQ	2×LOQ	0.01	0.06	> 0.9959	109.1	21.0	17.7	-19				
			100×LOQ								102.3	3.8	5.1	-19
Diflufenican	5.8	20×LOQ	2×LOQ	0.02	0.06	> 0.9996	80.0	15.8	17.6	-29				
			100×LOQ								111.7	0.5	3.2	-21
Fluoxastrobin	5.5	20×LOQ	2×LOQ	0.02	0.06	> 0.9938	99.5	13.8	17.9	-13				
			100×LOQ								111.3	1.6	4.4	-2
Pyraclostrobin	5.8	20×LOQ	2×LOQ	0.01	0.03	> 0.9953	86.1	12.3	13.1	-20				
			100×LOQ								100.7	2.2	3.3	-26
Imidacloprid	2.6	20×LOQ	2×LOQ	0.02	0.41	> 0.9994	88.8	5.1	7.9	-31				
			100×LOQ								98.0	1.3	2.7	-11
Thiacloprid	3.2	2×LOQ	0.01	0.01	> 0.9986	86.0	29.2	18.8	-28					

			20×LOQ				91.3	4.9	8.5	-40
			100×LOQ				115.5	1.7	6.7	3
			2×LOQ				103.7	6.3	9.4	-28
Thiamethoxam	Neonicotinoid	2.1	20×LOQ	0.01	0.02	> 0.9990	99.8	1.4	3.3	-7
			100×LOQ				115.1	3.4	3.9	-5
			2×LOQ				104.4	6.8	16.3	-16
Acetochlor	Chloroacetamide	5.1	20×LOQ	0.53	0.53	> 0.9977	101.8	4.1	6.5	-24
			100×LOQ				94.6	2.5	16.7	-28
			2×LOQ				89.2	4.9	8.0	-32
Dimethachlor	Chloroacetamide	4.4	20×LOQ	0.02	0.08	> 0.9989	93.0	2.1	4.5	-24
			100×LOQ				94.1	2.9	3.2	-25
			2×LOQ				91.3	1.7	15.5	-19
Metazachlor	Chloroacetamide	4.4	20×LOQ	0.01	0.07	> 0.9994	118.0	1.9	7.9	-5
			100×LOQ				110.2	2.0	5.7	-10
			2×LOQ				100.1	4.4	16.3	-15
S-Metolachlor	Chloroacetamide	5.1	20×LOQ	0.02	0.12	> 0.9951	108.1	2.8	6.6	-24
			100×LOQ				99.0	2.4	9.3	-24
			2×LOQ				80.0	2.4	15.9	27
Napropamide	Amide	5.2	20×LOQ	0.02	0.05	> 0.9977	105.0	3.4	4.5	-21
			100×LOQ				107.7	1.2	4.6	-15
Cyproconazole	Triazole	4.8	2×LOQ	0.05	0.05	> 0.9985	83.3	12.9	17.6	-35

			20×LOQ				92.6	4.1	5.8	-19
			100×LOQ				102.5	0.9	3.5	-24
			2×LOQ				82.1	2.9	10.8	21
Epoxiconazole	Triazole	5.2	20×LOQ	0.05	0.14	> 0.9967	112.2	5.5	8.3	23
			100×LOQ				106.9	6.5	8.7	22
			2×LOQ				99.2	8.3	8.7	-21
Metconazole	Triazole	5.4	20×LOQ	0.06	0.12	> 0.9977	113.6	9.9	9.3	-33
			100×LOQ				109.3	4.9	6.1	-19
			2×LOQ				83.0	21.2	31.7	13
Propiconazole	Triazole	5.5	20×LOQ	0.01	0.01	> 0.9947	114.4	8.6	18.4	-8
			100×LOQ				119.8	5.5	6.9	-3
			2×LOQ				101.8	11.5	12.4	-1
Prochloraz	Imidazole	5.7	20×LOQ	0.01	0.03	> 0.9925	112.4	11.9	13.6	-51
			100×LOQ				111.7	2.9	19.8	-13
			2×LOQ				118.3	16.7	14.1	15
Pyroxsulam	Triazolopyrimidine	3.9	20×LOQ	0.01	0.01	> 0.9986	100.7	3.4	8.8	3
			100×LOQ				101.8	2.1	6.3	11
			2×LOQ				107.2	12.8	12.6	-13
Bifenthrin	Pyrethroid	6.8	20×LOQ	0.86	0.86	> 0.9951	102.7	7.8	22.4	-69
			100×LOQ				82.6	11.8	22.2	-31
Cypermethrin	Pyrethroid	6.6	2×LOQ	0.66	0.66	> 0.9966	97.4	14.7	13.6	41

			20×LOQ				98.3	7.1	11.9	-55
			100×LOQ				107.9	3.5	7.8	-26
Deltamethrin	Pyrethroid	6.7	2×LOQ	0.42	2.7	> 0.9973	110.9	13.6	14.5	50
			20×LOQ				103.0	6.2	6.9	-49
			100×LOQ				120.1	3.6	5.2	-17
λ-cyhalothrin	Pyrethroid	6.5	2×LOQ	3.0	3.0	> 0.9945	175.2	7.7	12.4	263
			20×LOQ				110.3	6.8	11.8	-42
			100×LOQ				131.5	1.4	10.0	-6
τ-fluvalinate	Pyrethroid	6.8	2×LOQ	0.03	0.43	> 0.9956	136.0	3.6	5.9	177
			20×LOQ				66.5	11.6	19.2	-81
			100×LOQ				58.8	10.3	22.5	-59
Pirimicarb	Carbamate	4.1	2×LOQ	0.01	0.01	> 0.9984	100.0	19.4	23.3	-30
			20×LOQ				82.0	7.4	19.0	-57
			100×LOQ				88.8	4.8	8.1	-38
Clomazone	Isoxazolidinone	4.5	2×LOQ	0.02	0.02	> 0.9997	105.0	11.5	13.7	-18
			20×LOQ				80.0	5.1	9.0	-33
			100×LOQ				90.4	5.3	8.2	-27
Aclonifen	Diphenyl-ether	5.4	2×LOQ	0.77	2.49	> 0.9885	102.8	14.8	17.5	-0.5
			20×LOQ				89.8	22.3	27.5	-35
			100×LOQ				97.9	14.2	20.5	-29
Pendimethalin	Dinitroaniline	6.2	2×LOQ	0.86	5.5	> 0.9918	114.5	0.5	13.4	-24

			20×LOQ				113.6	9.4	14.4	-36
			100×LOQ				111.4	4.2	9.9	-21
			2×LOQ				124.6	7.4	7.5	74
Fenpropidin	Piperidine	6.5	20×LOQ	0.08	0.36	> 0.9906	117.3	6.2	15.8	5
			100×LOQ				120.3	2.1	19.8	38
			2×LOQ				35.0	15.6	38.3	-33
Cycloxydim	Cyclohexanedione	5.8	20×LOQ	0.12	0.15	> 0.9971	19.2	11.1	29.9	-20
			100×LOQ				30.9	11.4	17.7	-14
			2×LOQ				100.4	4.8	9.3	-26
Metrafenone	Benzophenone	5.8	20×LOQ	0.02	0.12	> 0.9997	98.2	2.6	6.1	-46
			100×LOQ				107.9	3.5	4.3	-26
			2×LOQ				91.1	8.1	9.6	-24
Cloquintocet- mexyl	Quinoleine	6.1	20×LOQ	0.02	0.03	> 0.9993	86.4	4.0	10.4	-53
			100×LOQ				116.3	1.4	10.2	-9

Table 2: Concentrations (ng/g) of the 31-targeted pesticides measured in a panel of 12 soils with different surrounding landscape

	Grassland1	Grassland2	Grassland3	Grassland4	Hedgerows1	Hedgerows2	Hedgerows3	Hedgerows4	Culture1	Culture2	Culture3	Culture4
Boscalid	< LOQ	0.61	nd	nd	8.6	9.1	1.4	2.2	284	nd	600	4.8
Diflufenican	nd	0.70	nd	0.34	1.6	0.67	0.20	nd	66.7	3.5	12.7	722
Fluoxastrobin	nd	nd	nd	nd	nd	0.63	nd	nd	nd	nd	nd	nd
Pyraclostrobin	nd	nd	nd	nd	1.5	nd	0.19	nd	0.36	0.18	nd	nd
Imidacloprid	nd	nd	nd	0.43	0.70	6.3	nd	< LOQ	54.3	57.2	56.1	15.2
Thiacloprid	nd	nd	nd	nd	nd	0.10	nd	nd	0.07	nd	nd	nd
Thiamethoxam	nd	0.10	0.20	nd	nd	nd	nd	nd	nd	nd	nd	0.19
Acetochlor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dimethachlor	nd	nd	nd	nd	1.5	nd	nd	nd	0.46	nd	0.38	nd
Metazachlor	nd	nd	nd	nd	nd	nd	nd	nd	2.4	nd	0.87	0.42
S-Metolachlor	nd	nd	nd	nd	nd	nd	0.15	nd	<LOQ	nd	0.40	nd
Napropamide	nd	0.32	nd	nd	4.6	1.7	nd	0.30	1.8	nd	1.8	0.48
Cyproconazole	2.3	nd	nd	nd	2.1	nd	nd	nd	0.59	nd	0.73	nd
Epoxiconazole	nd	nd	nd	7.4	8.7	1.3	1.5	nd	151	119	58.1	117
Metconazole	nd	nd	nd	nd	0.97	nd	nd	nd	3.6	75.2	0.84	nd
Propiconazole	2.0	nd	nd	nd	<LOQ	nd	nd	nd	nd	nd	nd	nd
Prochloraz	nd	nd	nd	nd	0.39	nd	nd	nd	19.1	0.23	1.7	0.50
Pyroxsulam	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.19	nd	nd
Bifenthrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cypermethrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Deltamethrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
λ-cyhalothrin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
τ-fluvalinate	nd	nd	nd	nd	nd	nd	nd	nd	1.6	nd	nd	nd
Pirimicarb	nd	nd	nd	nd	nd	nd	nd	nd	0.15	nd	0.09	0.27
Clomazone	nd	nd	nd	nd	0.21	0.11	nd	nd	0.25	nd	0.17	nd
Aclonifen	nd	nd	nd	nd	<LOQ	nd	nd	nd	nd	nd	17.1	nd
Pendimethalin	nd	nd	nd	nd	nd	nd	nd	nd	11.5	nd	127	9.4
Fenpropridin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cycloxydim	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Metrafenone	nd	nd	nd	nd	nd	nd	nd	nd	35.1	nd	nd	18.9
Cloquintocet-mexyl	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.13

nd : not detected

## SUPPLEMENTARY INFORMATION

### **Ultrasound-assisted QuEChERS extraction using EDTA for determination of currently-used pesticides at trace levels in soil**

*Environmental Science and Pollution Research*

Florent LAFAY<sup>1</sup>, Gaëlle DANIELE<sup>1\*</sup>, Maëva FIEU<sup>1</sup>, Céline PELOSI<sup>2</sup>, Clémentine FRITSCH<sup>3</sup>, Emmanuelle VULLIET<sup>1</sup>

#### **Affiliations**

<sup>1</sup>Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100 VILLEURBANNE, France

<sup>2</sup>INRAE, Avignon Université, UMR EMMAH, F-84000, Avignon, France

<sup>3</sup>UMR 6249 Chrono-Environnement – CNRS / Université Bourgogne Franche-Comté Usc INRA - 16 Route de Gray, 25030 BESANCON Cedex

<sup>4</sup>INRA, UMR1402 ECOSYS, pôle Ecotoxicologie Ecologie fonctionnelle et écotoxicologie des agroécosystèmes, RD 10 Route de St Cyr, 78026 VERSAILLES Cedex

**\*Corresponding author:** [gaelle.daniele@isa-lyon.fr](mailto:gaelle.daniele@isa-lyon.fr)

Table S1: List and physico-chemical properties of the target pesticides (Herb. = herbicide, Insect. = insecticide, Fung. = fungicide)

<b>Pesticide</b>	<b>Class</b>	<b>Family</b>	<b>CAS</b>	<b>Mw (g.mol<sup>-1</sup>)</b>	<b>log K<sub>ow</sub></b>	<b>K<sub>oc</sub> (L/kg)</b>
<b>Acetochlor</b>	Herb.	Chloroacetamide	34256-82-1	269.77	4.14	211
<b>Aclonifen</b>	Herb.	Diphenyl-ether	74070-46-5	264.66	4.37	7126
<b>Bifenthrin</b>	Insect.	Pyrethroid	82657-04-3	422.87	>6.00	35712
<b>Boscalid</b>	Fung.	Carboxamid	188425-85-6	343.21	2.96	772
<b>Clomazone</b>	Herb.	Isoxazolidinone	81777-89-1	239.70	2.54	293
<b>Cloquintocet-mexyl</b>	Herb.	Quinoleine	99607-70-2	335.82	5.12	13257
<b>Cycloxydim</b>	Herb.	Cyclohexanedione	101205-02-1	325.47	3.23	59
<b>Cypermethrin</b>	Insect.	Pyrethroid	52315-07-8	416.30	6.30	85572
<b>Cyproconazole</b>	Fung.	Triazole	94361-06-5	291.78	2.91	364
<b>Deltamethrin</b>	Insect.	Pyrethroid	52918-63-5	505.20	5.43	1024000
<b>Diflufenican</b>	Herb.	Carboxamid	83164-33-4	394.29	2.50	1996
<b>Dimethachlor</b>	Herb.	Chloroacetamide	50563-36-5	255.74	2.33	69
<b>Epoxiconazole</b>	Fung.	Triazole	133855-98-8	329.76	3.33	1073
<b>Fenpropidin</b>	Fung.	Pipéridine	67306-00-7	273.46	2.90	3808
<b>Fluxastrobin</b>	Fung.	Strobilurine	361377-29-9	458.83	2.86	1003
<b>Imidacloprid</b>	Insect.	Neonicotinoid	138261-41-3	255.66	0.57	225
<b>λ-Cyhalothrin</b>	Insect.	Pyrethroid	91465-08-6	449.85	7.00	157000
<b>Metazachlor</b>	Herb.	Chloroacetamide	67129-08-2	277.75	2.49	66
<b>Metconazole</b>	Fung.	Triazole	125116-23-6	319.83	3.85	1166
<b>Metrafenone</b>	Fung.	Benzophenone	220899-03-6	409.27	4.30	3105
<b>Napropamide</b>	Herb.	Amide	15299-99-7	271.35	3.36	300
<b>Pendimethalin</b>	Herb.	Dinitroaniline	40487-42-1	281.31	5.18	16637
<b>Pirimicarb</b>	Insect.	Carbamate	23103-98-2	238.29	1.70	800
<b>Prochloraz</b>	Fung.	Imidazole	67747-09-5	376.66	4.10	849

<b>Propiconazole</b>	Fung.	Triazole	60207-90-1	342.22	3.72	1564
<b>Pyraclostrobin</b>	Fung.	Strobilurine	175013-18-0	387.82	3.99	11000
<b>Pyroxsulam</b>	Herb.	Triazolopyrimidine	422556-08-9	434.35	1.08	6000
<b>S-metolachlor</b>	Herb.	Chloroacetamide	87392-12-9	283.79	2.90	226
<b>□-fluvalinate</b>	Insect.	Pyrethroid	102851-06-9	502.91	4.26	504123
<b>Thiacloprid</b>	Insect.	Neonicotinoid	111988-49-9	252.72	1.26	615
<b>Thiamethoxam</b>	Insect.	Neonicotinoid	153719-23-4	291.72	-1.16	68

Table S2: List of internal standards used for quantification and MS parameters

<b>Internal standard (IS)</b>	<b>RT (min)</b>	<b>Cone voltage (V)</b>	<b>MRM 1</b>	<b>Collision Energy (eV)</b>	<b>Corresponding analysed pesticide</b>
<b>Thiamethoxam-d3</b>	2.1	14	294.9 → 183.9	23	Thiamethoxam
<b>Imidacloprid-d4</b>	2.6	11	260.3 → 213.2	15	Thiacloprid; Imidacloprid
<b>Pirimicarb-d6</b>	4.1	2	245.2 → 78.0	22	Pirimicarb; Pyroxsulam ; Fenpropidin
<b>Metazachlor-d6</b>	9.8	4	279.1 → 105.0	12	Clomazone; Diméthachlore; Metazachlor
<b>Cyproconazol-(methyl-d3)</b>	4.8	40	294.9 → 69.9	17	Cyproconazole
<b>Napropamide-d10</b>	5.2	10	282.2 → 171.1	20	Acetochlor; Napropamide; S-Metolachlor; Boscalid
<b>Aclonifen-d5</b>	5.4	32	272.2 → 186.9	24	Aclonifen
<b>Propiconazole-(phenyl-d3)</b>	5.5	16	347.2 → 69.1	18	Metconazole; Epoxiconazole; Propiconazole
<b>Prochloraz-(ethylene-d4)</b>	9.8	94	380.6 → 312.9	11	Prochloraz
<b>Metrafenone-(2,3,4-trimethoxy-d9)</b>	5.8	32	418.2 → 218.1	12	Cycloxydim; Metrafenone ; Pendimethalin
<b>Pyraclostrobin-(N-methoxy-d3)</b>	5.8	34	391.2 → 163.0	24	Pyraclostrobin; Fluoxastrobin
<b>Diflufenican-d3</b>	5.8	2	397.9 → 185.1	50	Cloquintocet-mexyl; Diflufenican

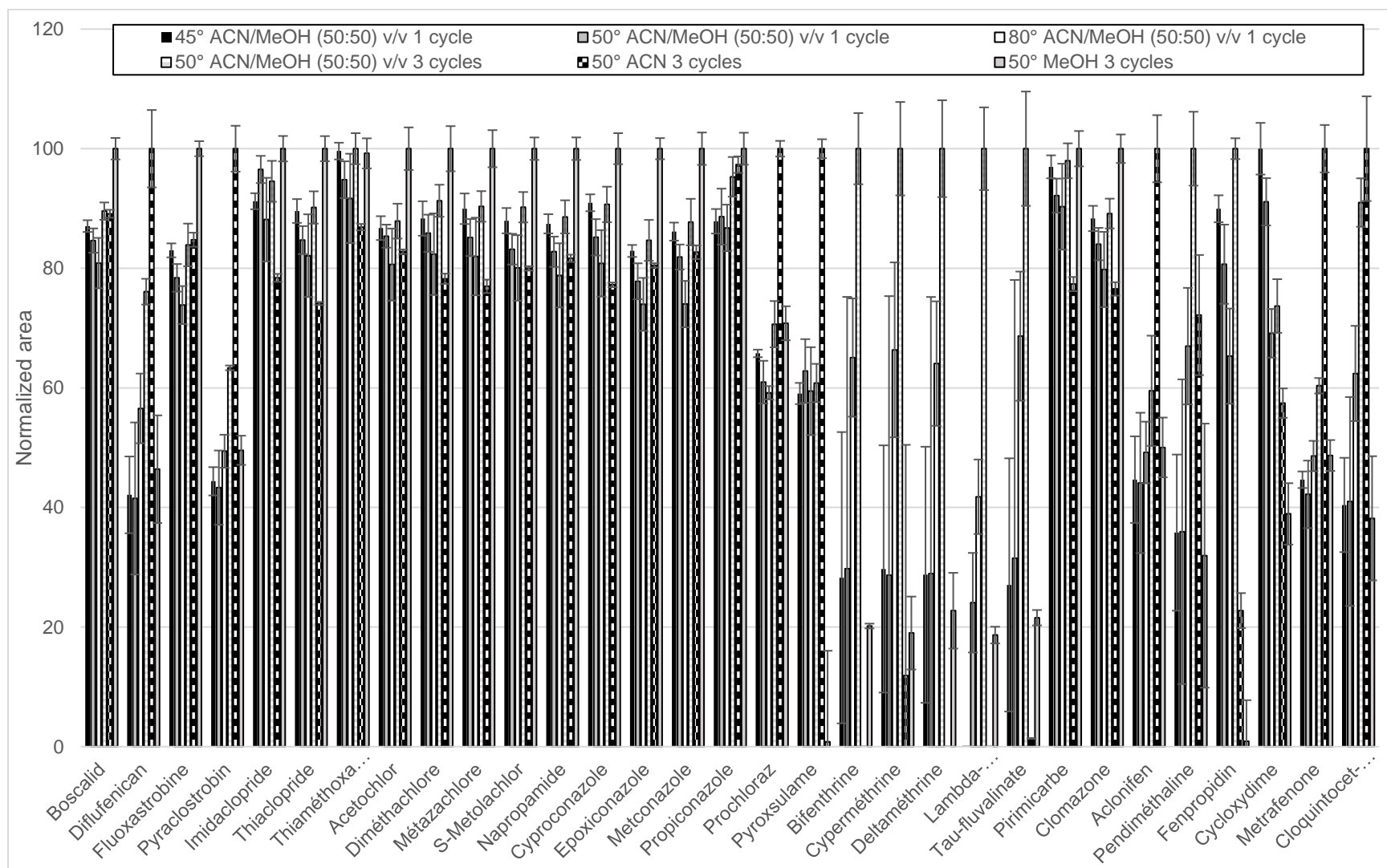
<b>Deltamethrin-phenoxy-d5</b>	9.8	40	269.9 → 104.9	15	Cypermethrin; Bifenthrin; □-cyhalothrin; □-fluvalinate; Deltamethrin
--------------------------------	-----	----	---------------	----	---

Table S3: MS parameters for the target pesticides

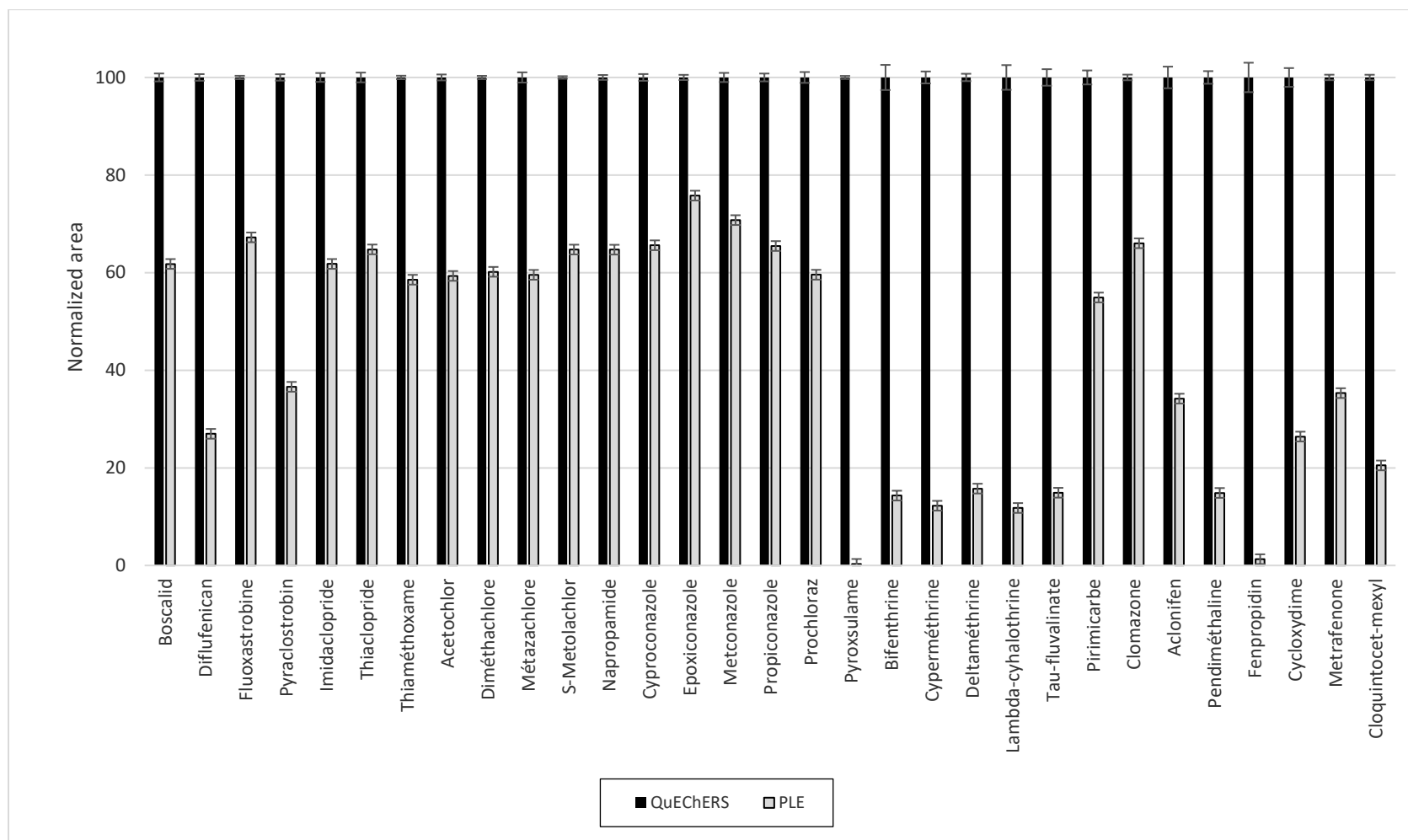
<b>Pesticide</b>	<b>DP (V)</b>	<b>MRM 1</b>	<b>CE (V)</b>	<b>MRM 2</b>	<b>CE (V)</b>	<b>MRM1/MRM 2</b>
<b>Acetochlor</b>	22	269.9 > 224.0	10	269.9 > 148.1	18	2.3
<b>Aclonifen</b>	20	264.8 > 182.1	28	264.8 > 79.9	32	2.4
<b>Bifenthrin</b>	32	440.3 > 181.2	14	440.3 > 166.0	44	2.5
<b>Boscalid</b>	30	343.2 > 307.2	20	343.2 > 140.1	20	2.8
<b>Clomazone</b>	23	240.0 > 125.0	18	240.0 > 89.0	46	18.9
<b>Cloquintocet-mexyl</b>	30	336.2 > 238.0	18	336.1 > 192.0	35	2.4
<b>Cycloxydim</b>	23	326.0 > 180.0	16	326.0 > 280.0	22	1.1
<b>Cypermethrin</b>	20	433.3 > 191.1	12	433.3 > 416.2	8	1.9
<b>Cyproconazole</b>	27	292.2 > 70.2	18	292.2 > 125.1	24	1.9
<b>Deltamethrin</b>	10	523.2 > 281.0	14	523.2 > 506.1	8	1.6
<b>Diflufenican</b>	30	394.9 > 266.0	28	394.9 > 246.0	32	3.8
<b>Dimethachlor</b>	20	256.2 > 224.2	15	256.2 > 148.2	25	2.4
<b>Epoxiconazole</b>	30	330.0 > 121.0	23	330.0 > 123.0	20	3.4
<b>Fenpropidin</b>	47	274.2 > 147.1	28	274.2 > 86.1	28	2.0
<b>Fluoxastrobin</b>	27	459.0 > 427.0	18	459.0 > 188.0	36	2.9
<b>Imidacloprid</b>	11	256.2 > 175.2	15	256.2 > 209.2	17	1.1
<b>□-Cyhalothrin</b>	6	467.3 > 225.1	12	467.3 > 141.1	46	3.3
<b>Metazachlor</b>	15	278.0 > 134.1	22	278.0 > 210.0	10	1.4
<b>Metconazole</b>	29	320.1 > 70.0	22	320.1 > 125.0	36	6.0
<b>Metrafenone</b>	19	409.0 > 209.1	14	409.0 > 226.9	16	2.0
<b>Napropamide</b>	21	272.1 > 129.1	16	272.1 > 171.1	18	1.2
<b>Pendimethalin</b>	12	282.2 > 212.2	10	274.2 > 86.1	17	2.6
<b>Pirimicarb</b>	30	239.1 > 72.0	18	239.1 > 182.1	18	1.8
<b>Prochloraz</b>	27	376.2 > 308.1	11	376.2 > 70.0	23	2.4

<b>Propiconazole</b>	37	342.0 > 159.0	34	342.0 > 69.0	22	3.9
<b>Pyraclostrobin</b>	20	388.1 > 193.9	12	388.1 > 163.0	25	1.1
<b>Pyroxsulam</b>	16	434.9 > 195.0	26	434.9 > 123.9	46	7.5
<b>S-metolachlor</b>	2	283.9 > 252.0	14	283.9 > 176.1	26	3.8
<b>□-fluvalinate</b>	15	503.0 > 181.1	30	503.0 > 208.1	12	1.2
<b>Thiacloprid</b>	20	253.0 > 126.0	20	253.2 > 186.1	12	15.9
<b>Thiamethoxam</b>	7	292.2 > 211.1	11	292.2 > 181.1	19	1.8

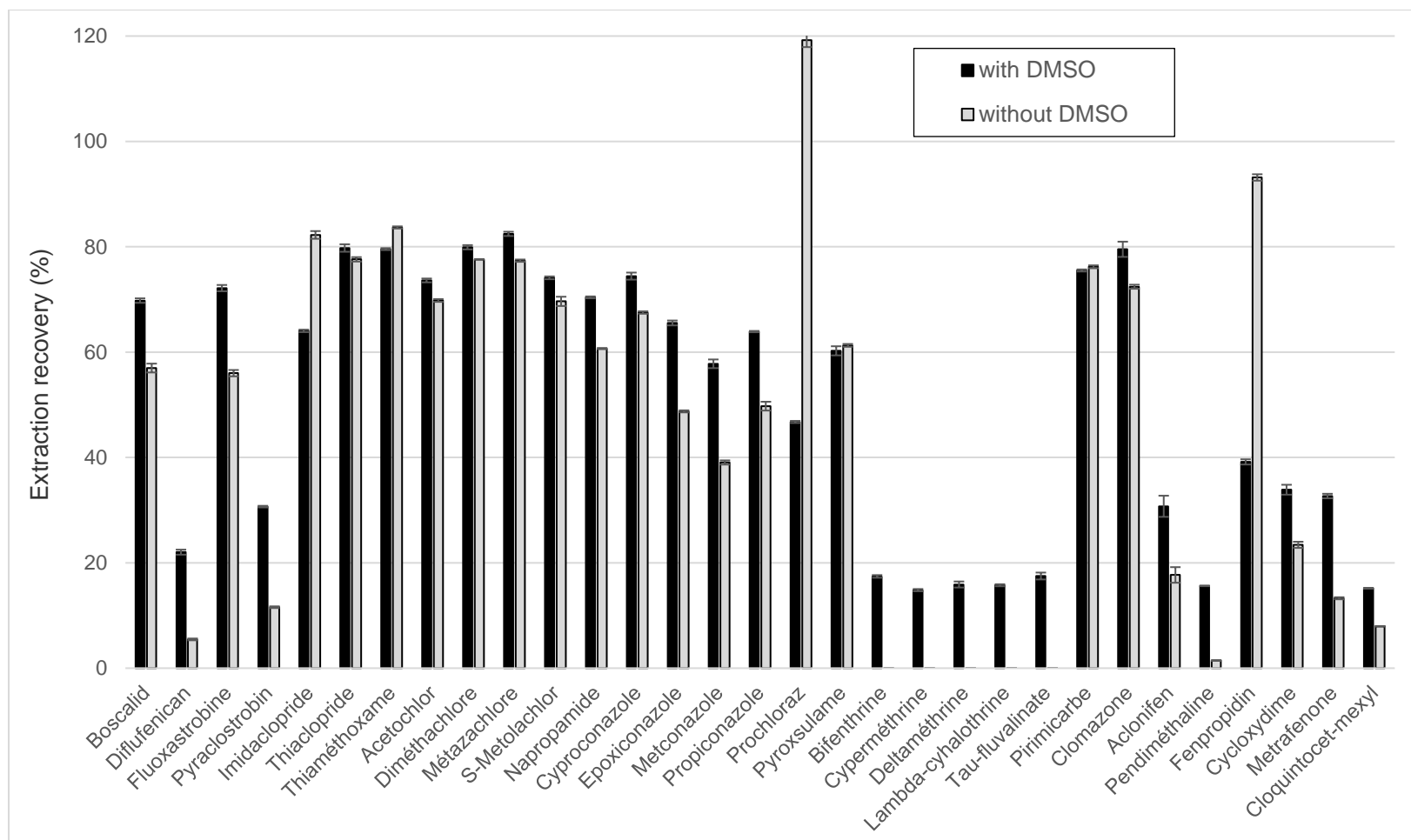
**Fig.S1:** Normalized responses of the targeted pesticides obtained after different PLE extraction conditions onto 5g of soil



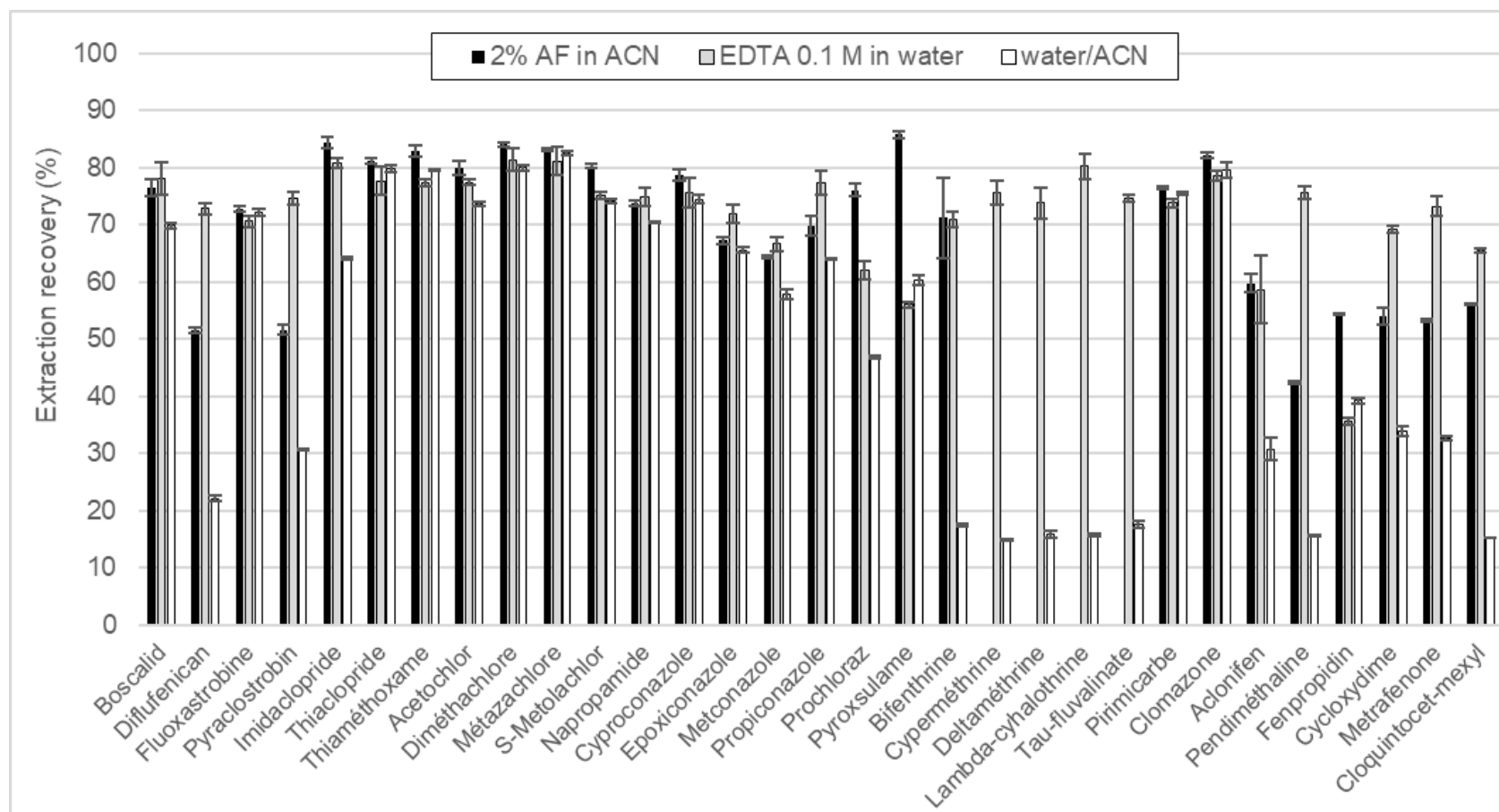
**Fig.S2:** Normalized responses obtained for the extraction of the 31-targeted pesticides in 5g of soil using PLE (using 3 extraction cycles with methanol at 50°C) or QuEChERS (using 6 mL of water, 10 mL of acetonitrile and citrate salt) extraction methods (n=4)



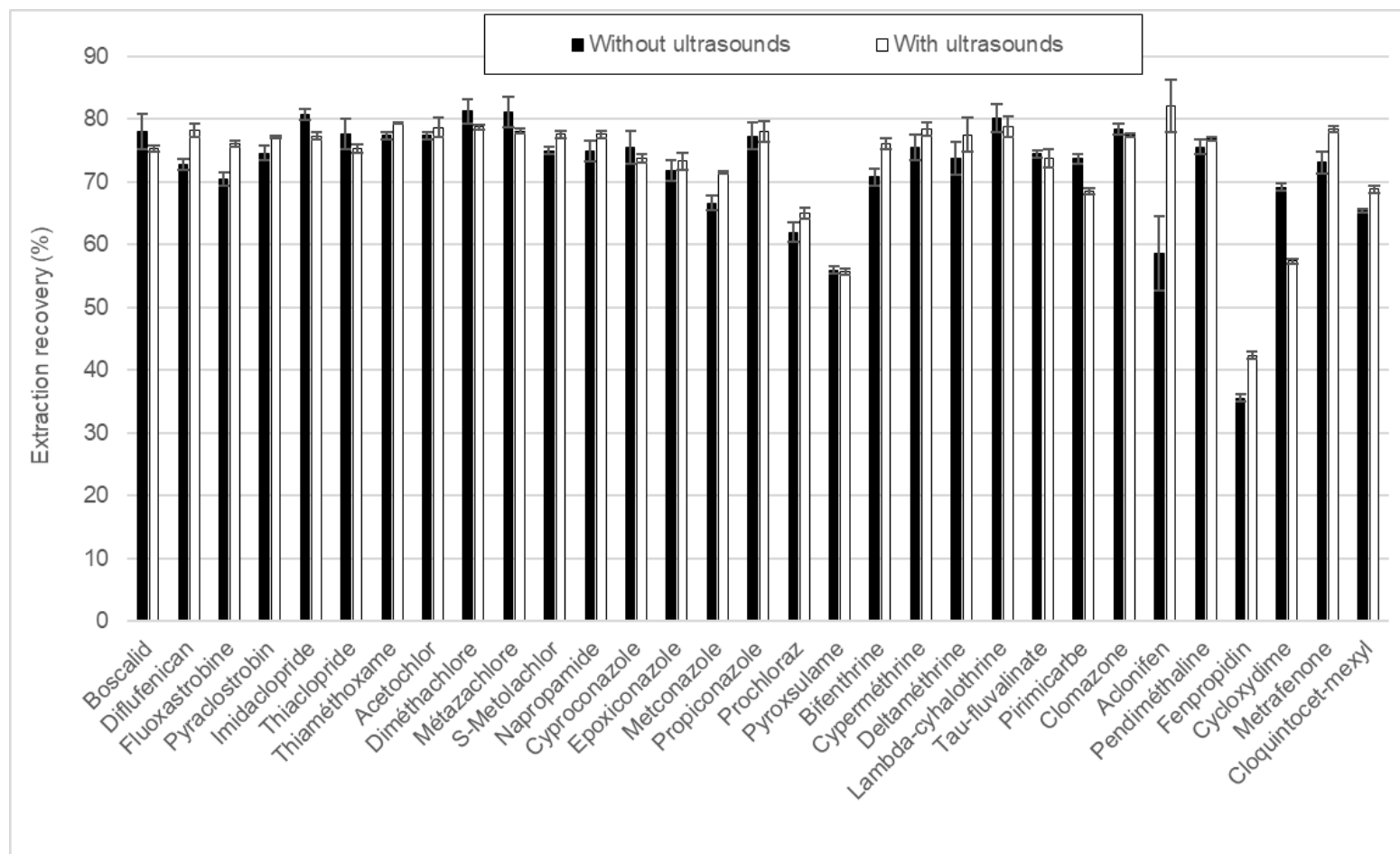
**Fig.S3:** Extraction recoveries of the selected pesticides after QuEChERS extractions of 5 g of soil using 6 mL of water, 10 mL of acetonitrile and citrate salt, with and without addition of DMSO before the final evaporation step



**Fig.S4:** Extraction recoveries (%) of the 31-targeted pesticides using QuEChERS extractions of 5g of soil using water (6 mL), acetonitrile (10 mL), citrate salt, and 100  $\mu$ L of DMSO before the final evaporation step, compared with those obtained after addition of 0.1 M EDTA in water, or of 200  $\mu$ L of acid formic in acetonitrile



**Fig.S5:** Extraction recoveries (%) of the 31 targeted pesticides after QuEChERS extraction of 5g of soil, using 6 mL of water containing 0.1M EDTA, 10 mL of acetonitrile, citrate salt, and 100  $\mu$ L of DMSO before the final evaporation step, compared with or without additional ultrasound step (15 min at 30  $^{\circ}$ C) before acetonitrile addition



**Fig.S6:** Extraction yields of the 31-targeted pesticides after derived-QuEChERS extraction of 2.5g and 5g of soil using 6 mL of water containing 0.1M EDTA, ultrasounds (15 min), 10 mL of acetonitrile, citrate salt, and 100  $\mu$ L of DMSO before the final evaporation step

