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## Method Article – Title Page

<b>Title</b>	<b>Simple extraction methods for pesticide compound-specific isotope analysis from environmental samples</b>
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<b>Keywords</b>	Pesticide extraction, stable isotopes, CSIA, degradation, transformation products
<b>Direct Submission or Co-Submission</b>	<i>Direct Submission</i>

### ABSTRACT

Compound-specific isotope analysis (CSIA) is a powerful approach to track the fate of organic pollutants in the environment. However, the application of CSIA to micropollutants, such as pesticides, remains limited because appropriate extraction methods are currently lacking. Such methods should address a wide range of pesticides and environmental matrices and allow recovering sufficient mass for reliable CSIA without inducing stable isotope fractionation. Here, we present simple extraction methods for carbon (<sup>13</sup>C/<sup>12</sup>C) and nitrogen (<sup>15</sup>N/<sup>14</sup>N) CSIA for different environmental matrices and six commonly used herbicides, i.e., atrazine, terbutryn, acetochlor, alachlor, butachlor, and S-metolachlor, and three fungicides, i.e., dimethomorph, tebuconazole, and metalaxyl. We examined the potential of several extraction methods for four types of soils or sediments, three types of environmental waters and aerial and root plant samples for multielement (ME)-CSIA.

- The pesticide extraction recovery varied depending on physical characteristics of pesticides or matrix properties for environmental water (77 to 87%), soil and sediment (35 to 82%), and plant (40 to 59%) extraction.
- The tested extraction methods did not significantly affect the carbon and nitrogen stable isotope signatures of pesticides ( $\Delta\delta^{13}\text{C} < 0.9\text{‰}$  for  $\Delta\delta^{15}\text{N} < 1.0\text{‰}$ ).

### SPECIFICATIONS TABLE

<b>Subject Area</b>	Environmental Science
<b>More specific subject area</b>	Compound-specific isotope analysis
<b>Method name</b>	Extraction methods for pesticide compound-specific isotope analysis from environmental samples
<b>Name and reference of original method</b>	Modified ultrasonic-assisted extraction (MUSE) Quick, Easy, Cheap, Effective, Rugged and Safe procedure (QuEChERS) Solid- phase extraction (SPE) [1–3]

Resource availability	Gas chromatography/isotope ratio mass spectrometer (GC/IRMS)
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## Method details

### Background

The constant growth of the population and increasing food demands have led to the widespread use of pesticides to sustain food supply. However, pesticide use has resulted in widespread pollution and a negative impact on ecosystems and human health [4]. Most of the world's agricultural ecosystems are at risk from one or more pesticides [5]. Pesticides can move away from the target organism and enter into different environmental compartments, including water, soil, plants, air. However, knowledge on how and whether pesticides degrade *in situ* so far remains limited [6]. Relying only on concentrations of parent compounds and their transformation products to follow up pesticide degradation in the environment is often poorly informative because the concentration decrease can be attributed to both degradative and non-degradative dissipation processes. Whenever known, the dissipation of formed transformation products can severely limit the identification of transformation pathways of pesticides. Novel monitoring approaches are thus required to understand pesticide behaviour in the environment.

Compound-specific isotope analysis (CSIA) may help to identify degradation pathways of pesticides and even to estimate the extent of pesticide degradation [7]. CSIA is based on the gradual change of stable isotope ratios of elements (e.g.,  $^{13}\text{C}/^{12}\text{C}$ ) in parent molecules during chemical or biological reactions leading to bond cleavage. However, the stable isotope fractionation during physical processes, such as sorption, generally remains within the total analytical uncertainty [8]. The application of pesticide CSIA, in contrast to CSIA of legacy pollutants, is still emerging due to analytical challenges posed by low (ng to  $\mu\text{g}/\text{L}$ ) environmental concentrations of nonpoint sources and complex environmental matrices [7]. A multielement (ME)-CSIA approach may help to identify reaction pathways in complex pollution scenarios, such as multiple contamination sources or concurrent degradation processes. However, applications of ME-CSIA for pesticides require validated and efficient extraction methods from environmental matrices. Extraction methods for pesticide residues from environment matrices for ME-CSIA should: (i) provide sufficient analyte mass for reliable isotope analysis, (ii) cause no stable isotope effect ( $\Delta\delta^{\text{H}}\text{E}$ ), (iii) be applicable to a wide range of pesticides and matrices, and (iv) limit matrix co-enrichment to avoid co-elution during chromatographic separation.

Previously, solid-phase extraction (SPE) for pesticide extraction from water for CSIA has been tested for atrazine, acetochlor, S-metolachlor, metalaxyl, butachlor, alachlor, terbutryn, chlordizon, and several of their transformation products [9–15]. Pesticide extraction methods from soil, sediment and plants for reliable pesticide CSIA have been used in both laboratory and field studies [16–19]. Ivdra et al. (2014) proposed a modified ultrasonic-assisted extraction (MUSE) method without carbon isotope effect ( $\Delta\delta^{13}\text{C} \leq 0.4\%$ ). Other studies used accelerated solvent extraction (ASE) for the extraction of hexachlorocyclohexanes (HCHs) from soil and plants for C, H, and Cl isotopes analysis [19–21]. The application of Quick, Easy, Cheap, Effective, Rugged and Safe procedure (QuEChERS) for the extraction of metolachlor from two agricultural soils led to a systematic but reproducible isotope fractionation for Cl ( $\Delta\delta^{37}\text{Cl}$  between +2.5 and +3.5%) [22].

In this context, the purpose of this study was to test and compare simple extraction methods for pesticide ME-CSIA from various environmental matrices, with emphasis on carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) stable isotopes. Efficient pesticide extraction from diverse matrices can improve the use of ME-CSIA to assess pesticide degradation and help develop monitoring and remediation strategies. To develop an overview for the application of pesticide CSIA extraction methods for four types of soils and sediments, three types of environmental waters, and aerial parts and roots of plants were tested. We selected six commonly used

herbicides, i.e., atrazine, terbutryn, acetochlor, alachlor, butachlor, and S-metolachlor, and three fungicides, i.e., dimethomorph, tebuconazole, metalaxyl, representing a wide range of physicochemical properties and belonging to four chemical families (Table S1). Here, we extend previous findings for SPE and ME-CSIA to new compounds and environmental matrices. We further tested a MUSE method for extracting selected pesticides from soils, sediments, and plant material, thus paving the way for wider application of ME-CSIA in the environment.

## Chemicals

Extraction solvents: dichloromethane (DCM), acetonitrile (ACN), ethyl acetate (EtOAc), pentane, methanol (MeOH) were HPLC grade purity (>99.9%) and purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical standards (purity >98%) atrazine, terbutryn, acetochlor, alachlor, butachlor, S-metolachlor, S-metolachlor d-11, dimethomorph, tebuconazole, and metalaxyl were PESTANAL grade purchased from Sigma–Aldrich (St. Louis, MO, USA).

## Sampling and characterization of water, soil, sediment, and plants

*Water.* Three types of water matrices were tested: i) buffered [gG1] ultrapure water (>18 MΩ cm), ii) storm water from a vineyard catchment (Rouffach, France) [23], iii) runoff and river water from two different crop catchments (Alteckendorf and Souffel, France) [3,17]. Field samples were successively filtered at 11 μm through grade one cellulose (Whatmann 1001-047) and 0.45 μm cellulose acetate membrane filters, and stored at 4 °C until further analysis. The hydrochemical composition (Table S2) was analyzed by ionic chromatography (IC) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) using standard analytical procedures (NF/ISO), as described elsewhere [24].

*Soil and sediment.* The top 0–10 cm of i) river sediments from the Souffel river, France, [15], ii) vineyard soils from the Rouffach catchment [17,23], iii) vineyard storm water sediments (Rouffach, France [25]), iv) forest soils (Strengbach, France [17]) were sampled with a shovel cleaned with ultrapure water and ethanol and wiped between collections. Soil and sediment samples were mixed separately and sieved through a 2-mm mesh and stored at 4 °C before spiking. The physicochemical characteristics of the sieved soil and sediment samples (Table 1, Table S3) were measured using standard analytical procedures (NF/ISO) [24].

*Plants.* Mature common reed (*Phragmites australis* Cav. Trin. ex Steud., 1840) individuals ( $n=20$ ) were sampled from the Rouffach storm water wetland, separated into roots and aerial parts, which were extracted separately. Plant material was washed by shaking the roots and rhizosphere for 10 min in ultrapure water. The water was replaced several times until all the sediment rhizosphere was separated from the roots [26]. A 0.9 % NaCl solution was used to rinse twice the plant material to remove rhizosphere and rhizoplane sediment. Plant material was homogenized with a hand blender (Bosch MSM66110) and frozen at –20°C until further use.

## Pesticide extraction from water

Pesticide stock solutions (1 g/L) of pesticides in DCM or ACN were added to buffered (13.6 g/L monopotassium phosphate, 4 g/L sodium hydroxide) ultrapure water or environmental water and stirred until complete solvent evaporation to reach concentrations ranging from 0.5 μg/L to 6 mg/L. The extraction method was adapted from USA EPA method 525.2 using an AutoTrace 280 SPE system (Dionex®, CA, USA) for the simultaneous extraction of 6 samples [3] (details in SI). Tested water volumes were: 0.01, 0.5, 1, 2, 4, and 8 L.

## Soil, sediment and plant extraction methods

*MUSE method.* Pesticides in sediment, soil or plant were extracted using a solid-liquid extraction protocol adapted from [1,2]. Five grams of soil or sediment (dry weight) were placed in an amber glass centrifuge tube. Due to the large (~85%) water

content of plants, 3 g of plant dry weight was used for extraction. Sediment samples were all extracted after adjusting water content to 100%. Triplicate or duplicate samples were spiked (0.5 to 50 µg/g) with aqueous solutions of pesticides, vortexed, shaken (orbital shaker 80 rpm) for at least 30 min, and incubated in the dark for three days at 4°C. 1 mL of EtOAc or DCM:pentane (3:1) per gram of sample was then added and vortexed for 5 s, followed by 5 min in an ultrasonic bath (Branson 5510, 40 kHz) for homogenization. The sample was vortexed for 1 min, followed by centrifugation (2400 rpm) for 20 min. The supernatant was transferred to an amber glass vial, and the extraction method was repeated two more times. The supernatants were pooled and concentrated at room temperature under a gentle nitrogen stream to the last drop, before resuspension into ACN to a volume of 1 mL by vortexing (5 s) and ultrasonication (5 min) to collect pesticide residues. Then 75 mg of anhydrous magnesium sulfate (MgSO<sub>4</sub>) was added to remove residual water and 13 mg of primary-secondary amine (PSA bonded silica, Supelco P/N 52738) as a clean-up agent [1]. The vial was vortexed for 30 s, centrifuged at 2400 rpm for 5 min, and the supernatant was transferred to a clean amber glass vial for further analysis.

*Two-step extraction method for hydrophilic soil or sediment.* We tested a two-step extraction from soil and sediment when the extraction yield with DCM:pentane (3:1) was low, due to limited solvent penetration in the soil or the sediment. Recovery with DCM:pentane (3:1) in such case was not improved by prolonged vortexing or shaking, possibly due to the hydrophilic nature of organic carbon in the sediment, containing high proportion of polar carbon species (e.g., C–OH, C = O [27]). In the first extraction step, 10 mL of 0.01M calcium chloride (CaCl<sub>2</sub>) was added to 5 g of soil or sediment (dry weight). Samples were vortexed for 5 s, ultrasonicated for 5 min, vortexed again for 1 min, and centrifuged for 10 min at 2000 rpm before collecting 10 mL of the water fraction. The extraction was repeated twice to reach a final fraction of 30 mL of the aqueous soil extract. The remaining soil and sediment fraction was extracted in a second step using the above-described MUSE method with MeOH as an extraction solvent. While the 30 mL water supernatant fraction was extracted following a liquid-liquid extraction method by adding 3 mL of DCM, followed by vortexing for 5 min, ultrasonication for 5 min and centrifugation for 5 min (1500 rpm). Next, the solvent (DCM) was collected from the bottom of the vial. This step was repeated three times, yielding a final solvent fraction of 9 mL. The solvent extract was evaporated to dryness and resuspended in ACN, vortexed for 30 s, ultrasonicated for 5 min, and transferred into a GC vial for concentration analysis and CSIA.

## **Pesticide quantification**

Pesticides were analyzed with a gas chromatograph (GC, Trace 1300, ThermoFisher Scientific) coupled to a mass spectrometer (MS, ISQ™, ThermoFisher Scientific). Chromatographic separation was performed with a TG –5MS column (30 m × 0.25 mm ID, 0.25 µ film thickness). S-metolachlor d-11 or atrazine d-5 were used as internal standards and injected with every injection to account for reproducibility of the autosampler. Additional parameters are described in SI (Table S4). All samples were diluted to be within the linear calibration range (0.1 to 1 mg/L).

## **Pesticide CSIA**

Carbon ( $\delta^{13}C$ ) and nitrogen ( $\delta^{15}N$ ) stable isotope signatures of pesticides were measured using a GC-C-IRMS system consisting of a TRACE™ Ultra Gas Chromatograph (ThermoFisher Scientific) coupled via a GC IsoLink/Conflow IV interface to an isotope ratio mass spectrometer (DeltaVplus, ThermoFisher Scientific). Chromatographic separation was performed on a TG –5MS column (60 m × 0.25 mm ID, 0.25 µ film thickness). Samples were injected in split/splitless modes with an injection volume of 2 µL

and injector temperature of 250 °C. Samples were injected in triplicates and  $\delta^{13}\text{C}$  values are reported as the arithmetic mean. For both elements, target compounds were combusted to  $\text{CO}_2$  or  $\text{N}_2$  in a single combined reactor (P/N 1255321, NiO tube and CuO-NiO-Pt wires, Thermo Fischer Scientific) at 1000°C. For N, liquid  $\text{N}_2$  was used for cryogenic trapping of  $\text{CO}_2$ . Laboratory BTEX (Benzene, Toluene, Ethylbenzene and o-Xylene, carbon) and caffeine (nitrogen) standards were injected at the beginning of each session to check the performance of the instrument. Isotope values of pesticide standards were calibrated relative to Vienna Pee Dee Belemnite (VPDB) and AIR scales with EA-IRMS (Flash EA IsoLink™ CN IRMS, Thermo Fisher Scientific) using a two-point calibration with international reference materials AIEA600, USGS40, and USGS41. Standard injection with known isotopic signatures of in-house standards was injected at least every six samples for carbon and every three samples for nitrogen to follow up the measurement accuracy. The reproducibility of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements ranged, respectively, from  $\pm 0.4$  to  $\pm 0.6$  ‰ and from  $\pm 0.4$  to  $\pm 0.5$  ‰, while the total uncertainty, including accuracy and reproducibility, ranged, respectively from  $\pm 0.6$  to  $\pm 0.8$  ‰ and from  $\pm 0.6$  to  $\pm 0.7$  ‰. All isotopic measurements of element E (C and N) are reported in delta notation (Elsner, 2010):

$$\delta^{\text{H}E} = \left[ \frac{\left( \frac{\text{H}E}{\text{L}E} \right)_{\text{sample}}}{\left( \frac{\text{H}E}{\text{L}E} \right)_{\text{standard}}} - 1 \right] \times 1000 \quad \text{Equation 1}$$

where  $\delta^{\text{H}E}$  is the isotopic value (‰),  $\left( \frac{\text{H}E}{\text{L}E} \right)_{\text{sample}}$  is the ratio of heavy to light isotopes in the sample, and  $\left( \frac{\text{H}E}{\text{L}E} \right)_{\text{standard}}$  is the ratio of heavy to light isotopes in the standard in an international isotopic scale (Vienna Pee Dee Belemnite for carbon and AIR for N).

The method detection limit (MDL), i.e., the point with the lowest concentration within a linear interval of  $\pm 0.6$ ‰ of the mean value for the standard measured with GC-IRMS and with good reproducibility ( $1\sigma < 0.6$ ‰) from triplicate measurements.

## Method validation

### Extraction from water

**SPE recoveries.** The mean extraction recovery of pesticide for SPE ranged from  $77 \pm 8$  % (butachlor) up to  $115 \pm 12$  % (S-metolachlor) (Figure 1, Table S5) across all conditions tested. Extraction recoveries for atrazine, acetochlor, S-metolachlor, metalaxyl, and alachlor were similar than those previously obtained for different sorbents and amounts [9,10,13–15]. Overall, octanol-water partition constant of pesticides ( $\log K_{ow}$ ; Table S1) did not correlate with pesticide recoveries. Lower extraction efficiency (77%) of butachlor, in agreement with previous results [13], may be related to the length of its alkoxy chain and a 2-carbon alkyl chain bound to the aromatic ring. Indeed, the  $\log K_{ow}$  of butachlor is higher than that of the other compounds, indicating an apolar character. Hence, ACN and EtOAc may not be the optimal choice for butachlor (Table S1), and more apolar solvents, such as DCM, may be more appropriate for the extraction of apolar pesticides (Figure 4). Furthermore, physicochemical properties of parent compounds and their transformation products (TPs) may largely differ, implying that different sorbents or sorbent combinations are required to pre-concentrate both the parent compounds and the TPs [9].

Lower extraction recoveries for pesticides during SPE may be due to the breakthrough effect, i.e., the saturation of the sorbent capacity by the environmental matrix or washing away of sorbed compounds with water pumped through the cartridge [10]. No breakthrough effect was observed with increasing concentration or volume (Figure S1 and Figure S2) and for the different matrices (Figure S3). This indicates that environmental matrix, i.e., the co-enrichment of non-volatile matrix components from environmental sample, together with the target compounds during the extraction, did not lead to mass loading and reduction of the

extraction recovery. While SPE was conducted using one gram of sorbent (SolEx C18, Dionex®), increasing the mass of the sorbent up to 10 g may increase the extraction recovery and allow the extraction of larger volumes and/or heavily contaminated samples in point-source areas [9].

*Effect of SPE on stable isotope signatures.* Overall, our results show that SPE can be effectively used for the pre-concentration of tested pesticides prior to carbon and nitrogen CSIA. Both carbon and nitrogen isotopes signatures of pesticides were not affected by the matrix, volume or concentration of tested pesticides ( $\Delta\delta^{13}\text{C} < 0.9\text{‰}$  for and  $\Delta\delta^{15}\text{N} < 1.0\text{‰}$ ,  $p > 0.05$ , Kruskal-Wallis) (Figure 1, Figures S4 and S5, Table S5). These results confirm the applicability of SPE for both carbon and nitrogen CSIA of atrazine, acetochlor, S-metolachlor, metalaxyl, butachlor, and alachlor [3,9,10,13–15,17]. The findings also extend SPE methods for CSIA to fungicides dimethomorph and tebuconazole. Whenever larger water volumes were needed to reach the quantification limits for CSIA, water samples were divided into sub-samples  $\leq 4$  L for SPE and the extracts were recombined post-extraction. The maximum volume extracted may vary, depending on pesticide concentration, sorbent mass and matrix effect (discussed below).

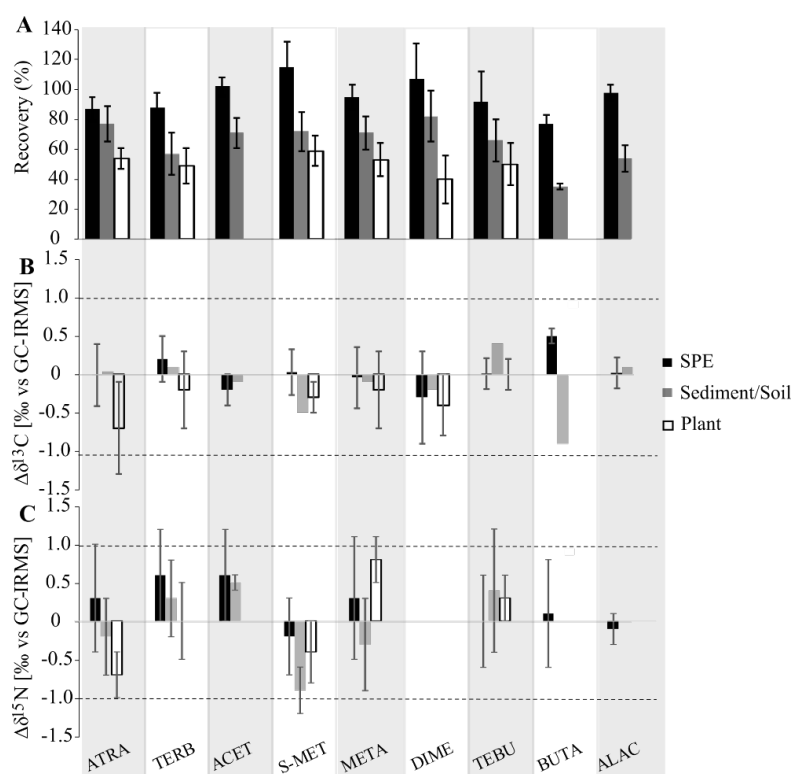


Figure 1. A - Extraction recovery, B – Effect on  $\delta^{13}\text{C}$  ( $\Delta\delta^{13}\text{C}$  [‰ vs GC-IRMS]), C – Effect on  $\delta^{15}\text{N}$  ( $\Delta\delta^{15}\text{N}$  [‰ vs GC-IRMS]) of SPE (water samples), and MUSE with DCM: Pentane (3:1) (sediment, soil, plant) for pesticides and all matrices combined. ATRA – atrazine, TERB – terbutryn, ACET – acetochlor, S-MET – S-metolachlor, META – metalaxyl, DIME – dimethomorph, TEBU – tebuconazole, BUTA – butachlor, ALAC – alachlor. Error bars denote standard deviation ( $1\sigma$ ,  $n \geq 8$ ). Dashed lines represent  $\pm 1\text{‰}$  (significance threshold) from reference isotope values of in-house standards measured with GC-IRMS.

Sample pre-concentration can co-enrich the target pesticide and organic compounds in environmental samples, leading to a matrix effect during GC-IRMS separation, especially for larger volumes ( $>4$  L). Hence, field variables, such as changes of the matrix composition, pesticide concentrations and the occurrence of vegetation, should be accounted for in a matrix screening prior to sampling. For instance, the co-elution of terbutryn with the wetland water matrix did not enable carbon CSIA when the water was collected in summer from the storm water wetland (data not shown). In contrast, no matrix interferences were observed for water

collected in winter. Typically, compounds eluting first, such as atrazine, metalaxyl, or terbutryn, were affected stronger by the co-elution peaks.

To address the issue of co-enrichment, several clean-up procedures can be applied in the future to maximize the analytical performance of pesticide CSIA extraction from environmental matrices without altering the isotope ratios of the target pesticide. These include: i) the addition of a sorbent, such as PSA, florisil, or graphitized carbon black, etc. [1,2,28,29], ii) chromatography HPLC separation [10], and iii) the use of molecularly imprinted polymers (MIP) [30]. MIP is likely the most effective cleanup method for CSIA. However, MIP is not commercially available for all classes of pesticides, and therefore must be specifically synthesized and validated prior to cleanup.

### Extraction from soil and sediment

*Solvent selection.* EtOAc and DCM:Pentane (3:1) were tested as extracting solvents in an adapted MUSE method for CSIA of alachlor, acetochlor, S-metolachlor, and metalaxyl from soil samples. The DCM:Pentane (3:1) mix gave significantly higher recoveries than EtOAc for all compounds, except metalaxyl where both solvents yielded similar extraction recoveries (Figure 2A). Due to its lower log  $K_{ow}$  (1.8, Table S1), metalaxyl can also be extracted using a more polar solvent (Figure 4), such as EtOAc. Since the method with DCM:Pentane (3:1) yielded generally higher pesticide recoveries, it was selected as extraction solvent for soil and sediments samples. Additionally, DCM:Pentane (3:1) forms separate layers with water, allowing efficient phase separation [2].

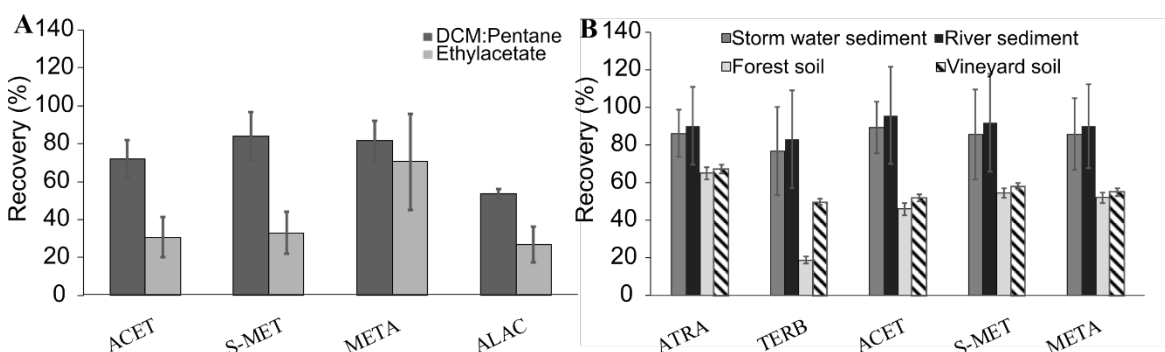


Figure 2. A- Recoveries of pesticide extraction from soil and sediment with DCM:Pentane and EtOAc; B- Recoveries of pesticide extraction with DCM:Pentane for different types of soil and sediment. ACET – acetochlor, S-MET – S-metolachlor, META – metalaxyl, ALAC – alachlor, ATRA– atrazine, TERB –terbutryn. Error bars denote standard deviation ( $1 \sigma$ ,  $n \geq 12$ ).

*Pesticide recoveries.* The mean extraction recoveries for MUSE with DCM:Pentane (3:1) ranged from  $35 \pm 2$  % (butachlor) up to  $82 \pm 17$  % (dimethomorph) across the soil and sediment samples (Figure 1, Table S5). The extraction recoveries were above 70 % for atrazine, acetochlor, S-metolachlor, metalaxyl, and dimethomorph. The results partly mirror the main parameters governing pesticide sorption capacities in soil/sediment matrices, including the physicochemical characteristics of the pesticide, such as the hydrophobicity and the acid dissociation constant (pKa, Table S1), and soil characteristics, such as soil pH, organic matter content and its surface functional group (Table 1) [31]. The lowest extraction recovery was observed for butachlor, correlating with the highest log  $K_{ow}$  (Table S1). The other pesticides, i.e. terbutryn, alachlor, tebuconazole, with log  $K_{ow}$  above 3 also had an extraction recovery below 70%.



Table 1. Physicochemical properties of tested soils and sediments (see also Table S3), mean value  $\pm$  standard deviation (1  $\sigma$ ) for all measurements.

	Clay [%]	Silt [%]	Sand [%]	Organic carbon [%]	pH	Water content [%]	Cation exchange capacity (CEC) [cmol <sup>+</sup> kg <sup>-1</sup> ]
Forest soil, Strengbach	7 $\pm$ 0.3	32 $\pm$ 1	62 $\pm$ 2	4.5 $\pm$ 0.2	3.4 $\pm$ 0.1	47 $\pm$ 12	14
Vineyard soil, Rouffach	23 $\pm$ 2	68 $\pm$ 9	9 $\pm$ 7	1.1 $\pm$ 0.2	7.9 $\pm$ 0.1	13 $\pm$ 7	18
River sediment, Alteckendorf	14 $\pm$ 0.4	73 $\pm$ 2	13 $\pm$ 0.4	2.0 $\pm$ 0.2	7.8 $\pm$ 0.1	39 $\pm$ 3	15
Storm water sediment, Rouffach	24 $\pm$ 8	45 $\pm$ 19	32 $\pm$ 22	2.5 $\pm$ 0.4	7.6 $\pm$ 0.1	41 $\pm$ 6	14

Here, we tested different pesticides with amines or amides functional groups, which do not ionize at pH 7 (Table S1). The sorption mechanism governing the action of nonionic pesticides includes physical interaction, e.g. the hydrophobic effect, when a nonpolar compound interacts with a nonpolar soil organic component, or chemical interactions, e.g. hydrogen bonding, interactions with humic substances and clay in the soil [31]. Parameters of hydrophobicity (i.e.,  $K_{ow}$ ) and soil sorption properties (i.e.,  $K_{oc}$ , adsorption coefficient of soil) are thus prevailing factors influencing the extraction recovery.

In addition, the physicochemical characteristics of the soil or the sediment affect the extraction efficiency. Figure 2B shows the variation of extraction efficiencies among environmental samples. For all compounds, extraction recoveries were above 70% in sediment samples (Figure 2B). In contrast, the forest soil with the highest organic carbon (OC) content (Table 1), displayed the lowest extraction recovery. Interestingly, the forest soil was characterized by a pH=3.4. The low soil pH may increase the amount of protonated functional groups in soil, such as carboxylic or thiol, thereby enhancing sorption of nonionic pesticides [32,33]. The low soil pH may also enhance ionic interactions between soil components and pesticides, such as terbutryn, with a pKa value of 4.3, which may reduce extraction recovery (Figure 2B, Table S1).

The vineyard soil (Rouffach, France) featured low initial water content (13  $\pm$  7%) and lower extraction yields (Figure 2B), although the OC contents and pH of the vineyard soil, and both the river and wetland sediments (Rouffach and Alteckendorf) were similar (Table 1). The fraction of non-extractable residues (NER) may increase with lower water content due to the more hydrophobic nature of soil organic matter controlling pesticide extraction [34–37]. Therefore, together with changes in soil texture and structure, pH, soil OC content and the initial soil water content appears as fundamental parameters controlling pesticide aging in soil [38] and thus extraction from soil and sediment samples.

In addition, seasonal variations of soil and sediment properties may affect the extraction recovery. For instance, the extraction recovery of dimethomorph from storm water sediment was lower in summer than in winter (Rouffach, France), which may be due to higher OC content of sediment in summer (Figure S6). In addition, only partial mixing of the solvent (DCM:Pentane) and storm water sediment sampled in summer was observed. This may explain the decrease by three-fold of the dimethomorph recovery from the storm water sediment (Figure S6). In this case, a more polar extraction solvent, such as MeOH, can be used in combination with a CaCl<sub>2</sub> extraction step to replace organic compounds sorbed to negatively charged organic matter with cations

[39]. A two-step extraction method for storm water sediment collected in summer and the MUSE extraction method used for the storm water sediment collected in winter both yielded recoveries of about 80% (Figure S7). In this method, tailoring extraction solvent to the soil type (i.e., use of  $\text{CaCl}_2$  and  $\text{MeOH}$ ), help to increase extraction recovery (Figure 4). Extraction recoveries were similar to those with ASE extraction of dimethomorph from two types of sediment from California, using DCM as extraction solvent [40].

*Effect on stable isotope signatures.* Carbon and nitrogen stable isotope composition of pesticides were not significantly affected ( $<0.5\text{‰}$  for  $\Delta\delta^{13}\text{C}$  and  $<0.8\text{‰}$  for  $\Delta\delta^{15}\text{N}$ ) by the soil type or the pesticide concentrations (Figure 1, Figure 3). This is in line with the literature for soil extraction with MUSE method for both carbon and nitrogen CSIA of atrazine, acetochlor and S-metolachlor, metalaxyl, butachlor, and alachlor (Alvarez-Zaldivar et al., 2018; Drouin, 2021; Droz, 2021; Droz et al., 2021; Masbou et al., 2018b; Pérez-Rodríguez et al., 2021). This study is the first report of soil extraction of dimethomorph and tebuconazole for carbon and nitrogen CSIA.

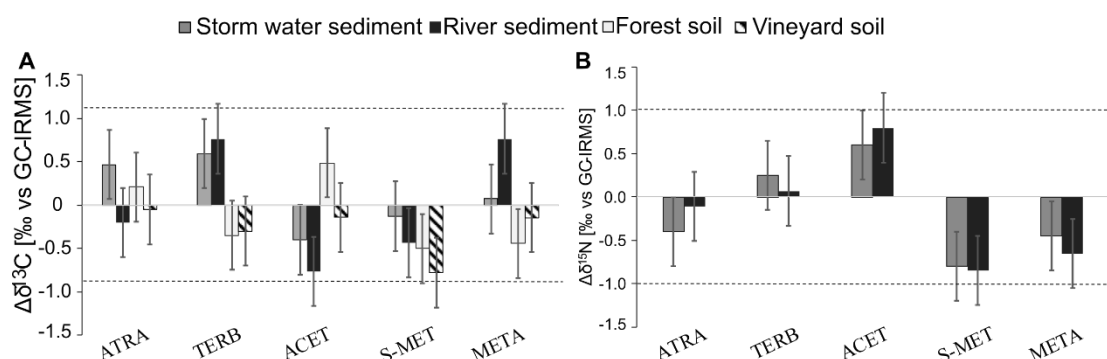


Figure 3. Effect of the MUSE extraction method on A – Carbon isotope values ( $\Delta\delta^{13}\text{C}$  [‰ vs GC-IRMS]) for the different types of soils and sediments and B – Nitrogen isotope values ( $\Delta\delta^{15}\text{N}$  [‰ vs GC-IRMS]) for the different types of soil and sediment. Dashed lines represent  $\pm 1\text{‰}$  (significance threshold) from the measured isotope values of in-house standards. ATRA– atrazine, TERB –terbutryn, ACET - acetochlor, S-MET - S-metolachlor, META – metalaxyl. Error bars denote standard deviation for all concentrations.

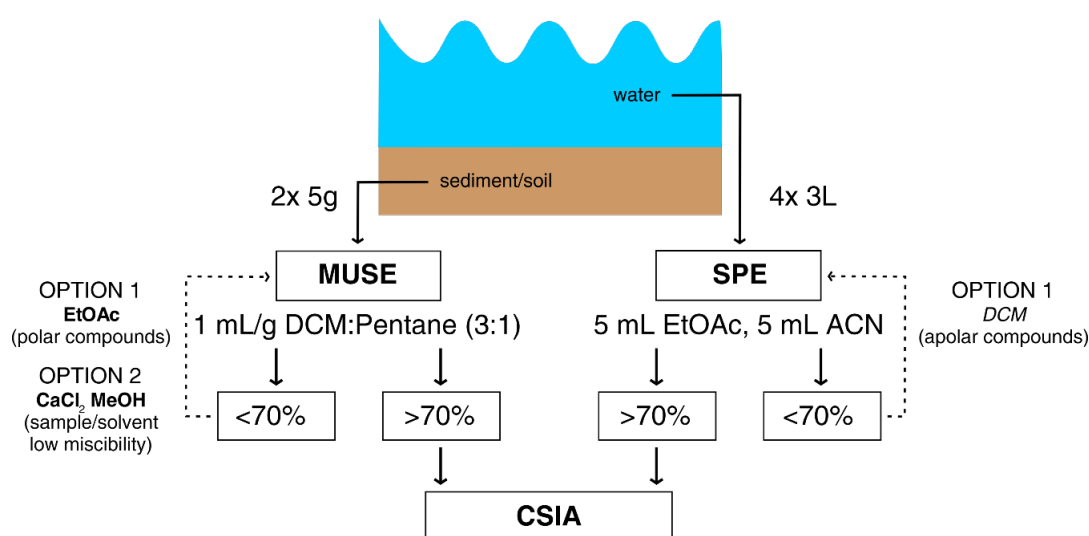


Figure 4. Pesticide extraction from sediment/soil and water samples for CSIA. Possible solvent alternatives (suggested – italicized, tested in this study– bolded) are mentioned when extraction recovery is  $<70\%$ . MUSE - modified ultrasonic-assisted extraction, SPE - solid-phase extraction, CSIA - compound-specific isotope analysis

Overall, these results highlight that pesticide extraction from soil samples for CSIA requires considering several practical aspects. First, pre-concentration of soil extracts may increase the chromatogram baseline during GC-IRMS measurements, especially for  $\delta^{13}\text{C}$  analysis (Figure S10). This may severely compromise the analytical performance (higher MDL and lower accuracy), even when co-elution is limited. The application of MIP with high specificity in the pre-concentration step may significantly reduce the matrix effect of soil compounds and thus reduce the MDL. Finally, injection of high carbon loads, especially during  $\delta^{15}\text{N}$  measurements, may significantly increase the maintenance of IRMS system. This may imply frequent oven and column replacement and capillaries blockage.

### Extraction from plants

*Extraction recovery and effect on stable isotope signatures.* The mean extraction recovery for plant material ranged from  $40 \pm 16\%$  (Dimethomorph) up to  $59 \pm 10\%$  (*S*-metolachlor) (Figure 1, Table S5). Extraction recoveries were slightly higher for the roots than for the aerial parts (data not shown). Overall, extraction recoveries from plants were lower than those for soil and sediment, suggesting that pesticide sorption in the plant matrix was higher. However, extraction efficiencies were similar to those reported for ACE extraction for HCHs from wheat and grass [19–21]. Despite low extraction efficiencies,  $\delta^{13}\text{C}$  of  $\delta^{15}\text{N}$  of pesticides were not affected by the extraction from plant material (Figure 1, Table S5).

In the future, other extraction methods of pesticides from plants, such as solid-phase microextraction, QuEChERS, superfluid extraction, and Soxhlet extraction, routinely tested for concentration analysis, could also be evaluated for CSIA in order to increase the recovery [41–43].

### Implication for ME-CSIA from environmental matrices

Overall, reliable ME-CSIA primarily requires both a decent extraction recovery ( $\geq 70\%$ ) and the absence of isotope effects associated with the extraction method. For all tested extraction methods, the stable isotope composition of pesticides was not significantly altered ( $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N} < 1\text{‰}$ ) during pesticide extraction from water, soil, sediment, and plant matrixes (Figure 1, Table S5).

The applicability of extraction methods for ME-CSIA to environmental samples is an essential step. The tested SPE methods allowed carbon CSIA from water samples for all studied pesticides at concentrations above 100 to 200 ng/L (Table S6). In contrast, nitrogen CSIA required several  $\mu\text{g/L}$  of pesticides in water. In a previous study, concentrations of *S*-metolachlor and acetochlor in water ranged from  $<100$  to  $66100$  ng/L at the plot or at the outlet of the agricultural catchment [44]. In urban catchments, biocides used in paints and renders, such as terbutryn, may leach from the facade materials and result in heavily polluted leaching and runoff (up to several mg/L biocides) [45]. Hence, carbon CSIA of pesticides and biocides appears feasible from agricultural samples across the agricultural season, and from most leachates from construction material in urban settings. For some compounds, the SPE method for carbon CSIA can also be used for nonpoint sources of pollution [46], although wider application would require reaching levels of pg/L to several ng/L. In contrast, nitrogen CSIA may be restricted to areas close to the source, following application, and/or during runoff events (Table S6).

The tested extraction method enabled carbon CSIA from soil/sediment and plant samples with pesticide concentrations ranging from 300 to 1000 ng/g and 300 to 500 ng/g, respectively (Table S6). Although *S*-metolachlor concentrations in soil can

reach 8 µg/g following application [18], pesticide concentrations typically range from pg/g to several ng/g in agricultural soils [47]. This mainly restricts pesticide CSIA to source areas, following application. Furthermore, nitrogen CSIA in soils and plants would typically require more than 10 µg/g, currently restricting its application to laboratory studies.

Developing simple and free of isotope-effect clean-up methods for environmental matrices and pesticides might reduce current concentration ranges of ME-CSIA. In spite of promising results [30], MIP is still not widely used due to its high selectivity and the requirements of self-preparation of the polymer. Nevertheless, recent MIP phase offers excellent selectivity for a broad range of triazines (SupelMIP Triazines, Merck, model 53208-U) has been introduced. Altogether, this study illustrates the diversity of candidate compounds and environmental matrices for ME-CSIA and the current potential and limitations of pesticide extraction methods, paving the way for wider use of ME-CSIA to assess pesticide degradation in environmental compartments.

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**Supplementary material:** Supporting information contains physicochemical properties of tested waters, soils and sediments, method detection limits and detailed method validation graphs.

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