J. Braz. Chem. Soc., Vol. 34, No. 11, 1717-1725, 2023 ©2023 Sociedade Brasileira de Química



# Influence of the Soil Composition on the Determination of 2,4-D and Fipronil in Environmental Samples by SLE-LC-MS/MS

Bianca V. Goulart,<sup>a</sup> Beatriz C. Vizioli,<sup>a</sup> Evaldo Luiz G. Espindola<sup>b</sup> and Cassiana Carolina Montagner<sup>®</sup> \*.<sup>a</sup>

<sup>a</sup>Instituto de Química, Universidade Estadual de Campinas (UNICAMP), 13083-970 Campinas-SP, Brazil

<sup>b</sup>Escola de Engenharia de São Carlos (EESC) e Hidráulica e Saneamento (SHS), Universidade de São Paulo (USP), 13560-970 São Carlos-SP, Brazil

The main objective of this work was to develop and validate an analytical method using solid-liquid extraction and liquid chromatography coupled with mass spectrometry in tandem (LC-MS/MS) for the simultaneous determination of the herbicide 2-(2,4-dichlorophenoxy)acetic acid (2,4-D) and the insecticide fipronil and its degradation products, fipronil sulfone and fipronil sulfide in different types of soil from Brazil (Oxisol and Entisol). Solid-liquid extraction was performed in two cycles using 20 mL of dichloromethane *per* cycle and sonication. The extraction efficiency of 2,4-D was more influenced by soil composition than the other compounds. The limits of quantification of the method were between 0.015 and 0.75 mg kg<sup>-1</sup> and recovery values from 61 to 118%, with a maximum relative standard deviation (RSD) value of 4%. The method was further applied to over 500 real soil and sediment samples. 2,4-D and fipronil concentrations varied from 0.03 to 1145  $\mu$ g kg<sup>-1</sup> and 1.1 to 282  $\mu$ g kg<sup>-1</sup>, respectively.

Keywords: pesticides, soil, solid-liquid extraction, method validation

## Introduction

The global use of pesticides has significantly increased in the last few decades, and this trend is expected to continue in the following years. Despite the extensive benefits to humanity, the indiscriminate use of pesticides introduces associated concerns due to their potential negative impacts on the environment and human health.<sup>1-3</sup>

Soil is usually the final depot of most pesticides, thus presenting a potential source of contamination. Pesticides mainly achieve the soil via direct application. Yet, offsite pesticide residue movements into soil from nearby applications, spills, leaks, and production sites may also be significant routes of entry. Also, pesticide residues may return to the soil during the culture cycle and after harvesting.<sup>4</sup> Therefore, monitoring the level of contamination of pesticide residues in the soil is important to maintain a safe environment while attaining a healthy production.

\*e-mail: ccmonta@unicamp.br

Dedicated to Prof Carol Collins for inspiring generations in Chromatographic Chemistry Typically, pesticide residues are strongly retained due to their interaction with soil particles and directly influence the extraction efficiency of the analytical method. Thus, the development and validation of multiresidue methods present a rather complex analytical challenge. Among the factors that influence the sorption of pesticides in the soil, the physicochemical properties of the pesticides, as well as the composition and characteristics of the soil (texture, soil organic matter (SOM), pH, and ion exchange capacity (IEC)) must be highlighted.<sup>5-7</sup>

Recently, several sample preparation techniques have been applied to soil analysis, including solid-liquid extraction (SLE), solid-phase extraction (SPE), solidphase microextraction (SPME), QuEChERS (quick, easy, cheap, effective, rugged, and safe) and methods that apply mechanical agitation in the presence of different solvents, sonication, and microwave irradiation.<sup>8-14</sup> Abraham and Gajendiran<sup>8</sup> and Baldaniya *et al.*<sup>9</sup> developed a method of extracting fipronil and its degradation products from soil by QuEChERS using acetonitrile and agitation in rotary shaker. Already, de Amarante *et al.*<sup>12</sup> and Prado *et al.*<sup>13</sup> employed SLE for extraction of 2-(2,4-dichlorophenoxy)

Editor handled this article: Carla B. G. Bottoli (Guest)

acetic acid (2,4-D) from soil samples using, respectively, 30 mL of dichloromethane with 1 h of sonication and methanol and water (4:1) acidified with acetic acid (85%) and agitation at 120 rpm during 4 h.

Although these methods are already published, sample preparation employed in the QuEChERS and solid-liquid extraction method involves several steps or high sonication time, which would be a limitation for its use in hundreds of real samples. In addition, the published studies evaluated the extraction efficiency of pesticides in only one type of soil. Thus, the optimization of simpler and faster extraction methods applied to the simultaneous determination of pesticides in soils of different chemical composition is important.

The main objective of this work was to develop and validate an analytical method using solid-liquid extraction (SLE) and liquid chromatography coupled with mass spectrometry in tandem (LC-MS/MS) for the simultaneous determination of the herbicide 2,4-D and the insecticide fipronil and its degradation products, fipronil sulfone and fipronil sulfide in different types of soil from Brazil (Oxisol and Entisol).

Fipronil and 2,4-D are two of the main pesticides used in Brazil, especially in sugarcane crops. In 2020, 2,4-D was the second most applied herbicide in the country, whereas fipronil was the twelfth insecticide.<sup>15,16</sup> Table 1 presents the physical-chemical properties of 2,4-D and fipronil and their degradations products.<sup>17,18</sup>

### Experimental

#### Reagents and materials

High purity standards of fipronil (97.9%), fipronilsulfide (99%), fipronil-sulfone (99%), 2,4-D (99.9%) and internal standard fipronil-(pyrazole-<sup>13</sup>C<sub>3</sub>, cyano-<sup>13</sup>C) (99%) were acquired from Merck (Darmstadt, Germany). Internal standard 2,4-D (ring <sup>13</sup>C<sub>6</sub>) 100 µg mL<sup>-1</sup> in acetonitrile was purchased from Cambridge Isotope Laboratories Inc. (Massachusetts, USA). Dichloromethane high performance liquid chromatography (HPLC) grade was obtained from Honeywell (Charlotte, USA), sulfuric acid ACS grade was purchased from Synth (São Paulo, Brazil), and ammonium hydroxide was obtained from Fluka Analytical (Buchs,

Table 1. Physical-chemical properties of 2,4-D, fipronil, fipronil sulfide and fipronil sulfone

Property	2,4-D	Fipronil	Fipronil sulfide	Fipronil sulfone
Chemical formula	$C_8H_6Cl_2O_3$	$C_{12}H_4Cl_2F_6N_4OS$	$C_{12}H_4C_{12}F_6N_4S\\$	$C_{12}H_4Cl_2F_6N_4O_2S$
Chemical structure	CI CI CI	$\begin{array}{c} F_3C-S \\ H_2N \\ CI \\ CF_3 \end{array} \begin{array}{c} CI \\ CF_3 \end{array}$	$F_3C^{S}$ $CN$ $H_2N$ $N^{N}$ $CI$ $CI$ $CF_3$	$ \begin{array}{c} O \\ F_3C-S \\ O \\ H_2N \\ C \\ $
CAS	94-75-7	120068-36-2	120067-83-6	120068-36-2
IUPAC name	2-(2,4-dichlorophenoxy) acetic acid	5-amino-1-[2,6-dichloro- 4-(trifluoromethyl)phenyl]- 4-(trifluoromethylsulfinyl) pyrazole-3-carbonitrile	5-amino-1-[2,6-dichloro- 4-(trifluoromethyl)phenyl]- 4-trifluoromethylsulfanyl- 1 <i>H</i> -pyrazole-3-carbonitrile, 5-amino-3-cyano- 1-[2,6-dichloro- 4-(trifluoromethyl)phenyl]- 4-(trifluoromethyl)phenyl]- 4-(trifluoromethylthio) pyrazole	5-amino-1-[2,6-dichloro- 4-(trifluoromethyl)phenyl]- 4-[(trifluoromethyl)sulfonyl]- 1 <i>H</i> -pyrazole-3-carbonitrile
Pesticide class	herbicide	insecticide	-	-
Molar weight / (g mol <sup>-1</sup> )	221.03	437.10	421.1	453.1
Water solubility / (mg L <sup>-1</sup> at 20 °C)	24 300	3.78	0.54	-
pK <sub>a</sub>	2.64	not applicable	not applicable	not applicable
Log K <sub>ow</sub>	-0.82	3.75	_	4.7
Vapor pressure / mPa	0.0009	0.0002	_	_
$K_{oc} / (mg g^{-1})$	31 to 275	427 to 1248	_	4209

IUPAC: International Union of Pure and Applied Chemistry; Kow: octanol-water partition coefficient, Koe: soil organic carbon-water partitioning coefficient.

Switzerland). Methanol and acetonitrile HPLC grade were from J. T. Baker (Phillipsburg, USA). Ultrapure water was obtained from Millipore's Synergy Water Purification System (Burlington, USA). Hydrophobic polytetrafluoroethylene (PTFE) (0.45 µm pore size) syringe filters were purchased from Analítica (São Paulo, Brazil). Individual stock solutions (400 µg mL<sup>-1</sup>) were prepared in methanol for fipronil, fipronil-sulfide, fipronil-sulfone, 2,4-D, and fipronil-(pyrazole-<sup>13</sup>C<sub>3</sub>, cyano-<sup>13</sup>C). The stock solution (10 µg mL<sup>-1</sup>) for 2,4-D (ring <sup>13</sup>C<sub>6</sub>) was prepared in acetonitrile. All stock solutions were stored in amber glass bottles at -4 °C.<sup>19</sup>

#### Sampling site and sample collection

Samples were obtained from two types of soil with different textural classes (Oxisol and Entisol, according with Soil Taxonomy system).<sup>20</sup> Oxisol samples were used in aquatic mesocosm systems located in the Center of Water Resources and Environmental Studies (CRHEA, Itirapina-SP, Brazil), in which a semi-field study was conducted after controlled application of 2,4-D and fipronil (experimental design described in Pinto et al.<sup>21</sup>). Entisol samples were used in aquatic mesocosm systems located in an experimental farm in São Paulo Agribusiness Technology Agency (APTA, Brotas-SP, Brazil), in which a study was conducted during the conventional management of sugarcane and pastures (experimental design described in Goulart et al.<sup>19</sup> and Girotto et al.<sup>22</sup>). Oxisol samples were collected from November 2018 to April 2019, whereas Entisol samples were collected from November 2018 to October 2020.

Sampling was conducted as follows: Oxisol samples were collected three times using a soil core sampler (5 cm diameter) as the mesocosm system sediment, whereas Entisol samples were collected five times around the mesocosm system using a soil auger sampler (10 cm depth) and as the mesocosm system sediment. Final composite samples consisting of a mixture of the individual grab samples were stored in 0.5 L plastic containers and kept at 4° C. Physicochemical properties of Oxisol and Entisol are presented in Table 2.

### Sample preparation

Analytes were extracted from soil and sediment matrices by SLE following de Amarante Jr. *et al.*<sup>12</sup> Firstly, soil and sediment samples were dried under ambient conditions and granulometrically separated with a 1.0 mm particle size sieve. Next, a portion of 10 g of each sample was added to a 50 mL Falcon tube and acidified with 1 mL of sulfuric acid 0.1 mol L<sup>-1</sup>. SLE was performed in

**Table 2.** Physicochemical properties of soil organic matter (SOM), pH,

 ion exchange capacity (IEC), metal content, and soil texture for soils

Parameter	Oxisol	Entisol	
SOM / (g dm <sup>-3</sup> )	114.0	26.7	
pН	5.2	4.5	
IEC / (mmol c dm <sup>-3</sup> )	34.7	39.9	
	Metal content / (g dm-3)		
Copper (Cu)	< 0.1	0.6	
Iron (Fe)	26	79	
Manganese (Mn)	5.7	1.9	
Zinc (Zn)	< 0.1	1.0	
	Soil texture / %		
Clay	31.9	8.2	
Sand	46.4	89.8	
Silt	21.7	2.0	
Clay	31.9	8.2	

two cycles using 20 mL of dichloromethane per cycle. For each cycle, samples were homogenized in a vortex mixer (Morse, São Paulo, Brazil) for 1 min, sonicated in an ultrasonic (Untronique, Indaiatuba, Brazil) bath for 10 min, and separated in a centrifuge (MPM, Warsaw, Poland) at 4000 rpm for 10 min. The organic phase was collected at the end of each cycle. After both cycles, the aliquots were combined, reduced to dryness under a gentle stream of nitrogen gas 5.0, then brought to a final volume of 1500 µL using water: methanol 70:30 (v/v) with fipronil- $(pyrazole^{-13}C_3, cyano^{-13}C)$  and 2,4-D  $(ring {}^{13}C_6) (50 \ \mu g \ L^{-1})$ each). The final extracts were filtered using a syringe filter of hydrophobic PTFE (13 mm diameter, 0.45 µm) (Analítica, São Paulo, Brazil), quantitatively transferred to a vial, and stored below 4 °C until instrumental analysis was performed. On condition that the samples could present concentration levels in different orders of magnitude, all samples were subjected to all sample preparation steps for better comparative purposes. When necessary, extracts were diluted (70:30 extract:solvent v/v) with a mixture of 70:30 (v/v) water: methanol with fipronil-(pyrazole- $^{13}C_3$ , cyano-<sup>13</sup>C) and 2,4-D (ring  ${}^{13}C_6$ ) (50 µg L<sup>-1</sup> each).

### Instrumental analysis and method validation

An Agilent 1200 liquid chromatograph coupled with an Agilent 6410B triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA) with electrospray ionization (ESI) and a Zorbax SB-C18 column with 2.1 × 30 mm and particle size of 3.5  $\mu$ m (Agilent Technologies) were used for analysis as fully described in Goulart *et al.*<sup>19</sup> The analytes were quantified by internal standardization with curve in the solvent using fipronil-(pyrazole-<sup>13</sup>C<sub>3</sub>, cyano-<sup>13</sup>C) and 2,4-D (ring <sup>13</sup>C<sub>6</sub>) (50  $\mu$ g L<sup>-1</sup> each) as internal standards. Method validation was carried out according to the National Institute of Metrology Standardization and Industrial Quality (Inmetro)<sup>23</sup> and the Brazilian Health Regulatory Agency guidelines (Anvisa),<sup>24</sup> assessing the parameters: linearity and linear range, limit of detection (LOD) and limit of quantification (LOQ), trueness, precision and matrix effect (ME).

The instrumental limit of quantification (IQL) and the instrumental limit of detection (IDL) were obtained by the signal-to-noise method, comparing the analytical signal of samples at low concentrations of analytes with the noise at baseline. The concentration at which the signal-to-noise ratio was observed in the ratio 10:1 and 3:1 for IQL and IDL, respectively, was considered. The quantification limit of the method (LQM) was obtained considering the instrumental limits, the mass of the sample used in the preparation and the extract concentration factor, according to equation 1.

$$LQM (\mu g kg^{-1}) = \frac{IQL (\mu g kg^{-1}) \times extract resuspension volume (L) \times 1000}{sample weight (g)}$$
(1)

Trueness and precision were evaluated by recovery and repeatability assay, respectively, analyzing samples spiked at concentration levels of 3.0, 15.0, and 30.0 µg kg<sup>-1</sup> for both matrices. Prior to fortification, soil and sediment samples were dried under ambient conditions and sieved with a 1.0 mm particle size sieve. For each concentration level, three replicate samples of 10 g each were fortified with a mix of the analytes in methanol and dried for 24 h under ambient conditions. Next, SLE was performed as described in "Sample preparation" sub-section. Provided that both types of soil could present residual levels of the analytes, quantification was performed using the standard addition method. For comparative purposes the extracts were also quantified by internal standardization with curve in the solvent. In order to evaluate the recovery of the method considering the dilution of extracts after SLE, the extracts obtained were diluted (70:30 extract:solvent v/v) with a mixture of 70:30 (v/v) water:methanol with fipronil- $(pyrazole^{-13}C_3, cyano^{-13}C)$  and 2,4-D  $(ring^{-13}C_6)$  (50 µg L<sup>-1</sup> each). Dilute matrix extracts were quantified by internal standardization with curve in the solvent. Trueness and precision were calculated according to equations S1 and S2 (Supplementary Information (SI) section), respectively.

Matrix effect was evaluated by comparison of the calibration curves for the standards in solvent mixture water:methanol 70:30 (v/v) and extracted matrices. Next, the percentage of matrix effect (ME) was calculated using the angular coefficients of calibration curves in the matrix ( $\alpha_{matrix}$ ) and in the solvent ( $\alpha_{solvent}$ ) according to equation 2.

ME (%) = 
$$100 \left( \frac{\alpha_{\text{matrix}}}{\alpha_{\text{solvent}}} - 1 \right)$$
 (2)

For each matrix, three samples of 20 g each were extracted following the procedure presented in "Sample preparation" sub-section, totalizing 4500  $\mu$ L of extract. Calibration levels (10.0; 25.0; 50.0; 100; 200; 300  $\mu$ g L<sup>-1</sup>) were obtained by combining 200  $\mu$ L of extracted matrix with analyte standard solutions. Matrix effect was also evaluated for the diluted extract (70% of matrix) by combining 140  $\mu$ L of matrix extract (100%) with 260  $\mu$ L of analyte standard solutions.

## **Results and Discussion**

#### Method validation

Linearity, linear range, instrumental limits of detection and quantification

Figure S1 (SI section) shows the typical chromatogram of the analytes that were performed in MRM (multiple reaction monitoring) mode with their respective quantification and confirmation ions. Calibration data for each compound was fitted to a linear regression model using software OriginPro 2015<sup>25</sup> by internal standard calibration method. All determination coefficients (R<sup>2</sup>) were higher than 0.99 and linear range was determined to be from their respective IQL to 300 ng mL<sup>-1</sup> (Table 3). IDL ranged from 0.05 to 0.5 ng mL<sup>-1</sup>, whereas IQLs ranged from 0.1 to 5.0 ng mL<sup>-1</sup>.

**Table 3.** Instrumental limit of detection, limit of detection of the method, instrumental limit of quantification, limit of quantification of the method, linear range and determination coefficient ( $R^2$ ) for all compounds

Compound	Unit	LOD	Linear range	$\mathbb{R}^2$	
24.0	(µg L-1)	0.5	5.0-300	0.0007	
2,4-D	(µg kg-1)	0.075	0.75-45	0.9997	
E'	(µg L-1)	0.05	0.1-300	0.9971	
Fipronii	(µg kg-1)	0.0075	0.015-45		
E'	(µg L-1)	0.05	0.1-300	0.0065	
Fipronii-suinde	(µg kg-1)	0.0075	0.015-45	0.9905	
E'	(µg L-1)	0.05	0.1-300	0.0000	
Fipronii-sulfone	(µg kg-1)	0.0075	0.015-45	0.9966	

LOD: limit of detection.

#### Trueness and precision

Trueness and precision were assessed for both matrices using the standard addition calibration method. Undiluted extracts and diluted extracts from the matrix were also quantified by internal standardization with curve in the solvent. Values for recovery (%) and relative standard deviation (%) are presented in Tables S1 and S2 (SI section).

The recovery of extracts (100% of matrix) quantified by standard addition is in the range indicated by the literature (40-120%) for trace analysis for both types of soil.<sup>23</sup> Overall

recoveries for all compounds ranged between 61 and 118%, with a maximum RSD of 4%. 2,4-D recoveries ranged from 77 to 82% in Entisol and 61 to 65% in Oxisol, while fipronil recoveries ranged from 108 to 118% and 94 to 107%, respectively.

The recovery of undiluted extracts quantified by internal standardization with curve in the solvent fell in the expected range of 40-120% for trace analysis,<sup>23</sup> with overall recoveries between 58 and 141%, whereas in the diluted extracts, pesticide recovery mostly agreed with the expected range, with overall recoveries between 56 and 137% (Figures 1 and 2). Precision for undiluted and diluted extracts were according to the expected limit of 20%, with a maximum RSD of 6 and 10%, respectively. The observed values above 120% for fipronil and its degradation products are possibly due to their presence in the matrix in trace levels.



Figure 1. Recovery values for extraction of fortified soil samples (Entisol) in three fortification levels (3, 15 and 30  $\mu$ g kg<sup>-1</sup>), in triplicates each.



**Figure 2.** Recovery values for extraction of fortified soil samples (Oxisol) in three fortification levels (3, 15 and 30  $\mu$ g kg<sup>-1</sup>), in triplicates each.

Firstly, a two-way analysis of variance (ANOVA) with replication test was conducted to examine the effects of level of fortification (3.0, 15.0, and 30.0  $\mu$ g kg<sup>-1</sup>) and matrix dilution (100 and 70% matrix) on pesticide

recovery (measured in percentage). Then, a second test was conducted to examine the effects of level of fortification and type of soil (Oxisol and Entisol). Prior to ANOVA tests, model assumptions were verified. Shapiro-Wilk's test was used to evaluate normality, Grubbs' test was used to detect possible outliers, and Levene's test was used to evaluate the homoscedasticity of data. All tests used a significance level of  $\alpha = 0.05$ .

The first ANOVA test showed that, for all compounds, there was no statistically significant interaction between the independent variables level of fortification and matrix dilution on the dependent variable pesticide recovery (*p*-values > 0.05, Table S3 (SI section)). Also, for all compounds, there was no statistically significant effect of matrix dilution on pesticide recovery for both soil types (*p*-values > 0.05, Table S3). In contrast, there was a statistically significant effect of level fortification on pesticide recovery for all compounds (*p*-values < 0.05, Table S3). For both types of soil, pesticide recovery decreased as the level of fortification increased.

The second ANOVA test also presented no statistically significant interaction between level of fortification and type of soil for any compound (*p*-values > 0.05, Table S4). Equal to the first ANOVA test, level of fortification presented a statistically significant effect for all compounds, while soil type also presented a statistically significant effect (*p*-values < 0.05, Table S4). For all compounds, pesticide recovery was lower in Oxisol than in Entisol, possibly due to the physicochemical properties of the pesticides and the difference in soil composition (Table 2).

The phenomenon of analyte retention in soil refers to the capacity of the soil to retain a substrate, which influences the extraction efficiency of such compounds in the matrix. For instance, 2,4-D is an ionizable organic compound of acidic character, highly soluble in water, and with a low tendency to partition into the organic phase.<sup>18,26</sup> Thus, 2,4-D can covalently bond to humic substances in the soil through oxidative fusion (oxidative coupling) due to the presence of the carboxylic group in the molecule. In addition, interactions by ionic bonding can occur between the carboxylic group and the sites with positive charges of Fe and Al oxides, usually found in highly weathered tropical soils, such as Brazilian soils.<sup>27,28</sup> Under pH conditions below  $pK_a$ , 2,4-D molecules are neutral and may interact with soil organic matter by hydrogen bonding.<sup>28</sup>

In contrast, fipronil is a non-ionizable polar organic compound with low water solubility and high lipophilicity.<sup>17,29</sup> Sorption of fipronil and its degradation products fipronil-sulfide and fipronil-sulfone may occur due to dipole-dipole interactions between the highly electronegative atoms present in the insecticide molecule (F,

Cl, O, and N) and the colloidal soil complex formed by clay minerals mixed with SOM that maintains a diffuse double layer of hydrated cations.<sup>30</sup> In addition, such compounds can adsorb to the soil by hydrogen bonds due to oxygen (–O) and hydroxyl radicals (–OH) present in humic substances and clay minerals. As fipronil has low water solubility, the hydrophobic partition can also contribute to the sorption of the insecticide to the soil.<sup>28</sup>

The soils evaluated in the present study are different in composition and physicochemical properties. For instance, Oxisol has a clayey texture, lower acidity (pH 5.9) and higher SOM content (110.6 g dm<sup>-1</sup>), whereas Entisol has a sandy texture, higher acidity (pH 4.5) and lower SOM content (26.7 g dm<sup>-1</sup>).

The extraction efficiency of 2,4-D was more influenced by soil composition (Table S2) than other compounds. The extraction efficiency was lower in the oxysol, possibly due to the greater interaction of 2,4-D with clay minerals and SOM. In contrast, despite the diverse interactions with the soil components, the extraction efficiency of fipronil and its degradation products did not suffer strong interference with the modification of the texture and SOM content of the soils. This result may be associated with a greater tendency of these compounds to partition in the extracting solvent, since fipronil has a higher lipophilic character (log  $K_{ow}$ : 3.75), compared to 2,4-D (log  $K_{ow}$ : -0.82).

#### Matrix effect

Matrix effect can be observed either as a loss in response (ion suppression), resulting in analyte underestimation, or an increase in response (ion enhancement), resulting in analyte overestimation.<sup>31,32</sup> There are several methods to evaluate the matrix effect quantitatively. Herein, the postextraction addition method was adopted. This method is particularly relevant when a blank matrix is unavailable, such as in the case.

Different analyte concentrations were measured in solvent and matrix (100% and 70%) for both types of soil, and obtained data were fitted to linear regression (Figures 3 and 4). All determination coefficients ( $R^2$ ) (Table S5, SI section) were higher than 0.99. Then, the percentage of matrix effect (%ME) was calculated



Figure 3. Linear regression for 2,4-D, fipronil, fipronil-sulfide, and fipronil-sulfone in solvent, 100% matrix, and 70% matrix for Entisol.



Figure 4. Linear regression for 2,4-D, fipronil, fipronil-sulfide, and fipronil-sulfone in solvent, 100% matrix, and 70% matrix for Oxisol.

following equation 2 (Table 4). %ME  $\cong$  0 shows there is no appreciable matrix effect, whereas %ME < 0 represents ion suppression and %ME > 0 represents ion enhancement.

A matrix effect of signal suppression was observed for all analytes in both types of soil, yet the effect was significantly higher in Entisol soil than in Oxisol. Also, matrix effect was more pronounced for 2,4-D than fipronil and its degradation products in both types of soil.

### Application to real samples

The method was applied in the analysis of more than 310 and 280 soil and sediment samples, respectively. Samples were collected during controlled application in mesocosm systems and a real application scenario during conventional Brazilian pasture and sugarcane management on an experimental farm. The concentration of 2,4-D and fipronil determined in the mesocosm sediment after controlled application in semi-field conditions ranged from 1.1 to 228 µg kg<sup>-1</sup> and 0.03 to 44 µg kg<sup>-1</sup>, respectively.

Table 4. Percentage of matrix effect (%ME) for soil and sediment in 100 and 70% matrix

	ME				
Compound	Oxis	ol / %	Entisol / %		
	70% matrix	100% matrix	70% matrix	100% matrix	
2,4-D	-28.1	-31.2	-45.9	-48.7	
Fipronil	-4.5	-4.8	-25.1	-20.0	
Fipronil-sulfide	-7.8	-4.0	-29.0	-21.8	
Fipronil-sulfone	-4.4	-2.4	-21.4	-18.2	

Fipronil sulfone and fipronil sulfide were quantified in 67 and 61% of the samples at concentrations ranging from 0.03 to 14  $\mu$ g kg<sup>-1</sup> and 0.1 to 22  $\mu$ g kg<sup>-1</sup>, respectively.

In soil samples collected during conventional management of sugarcane and pastures, 2,4-D and fipronil concentration ranged from 1.1 to 282  $\mu$ g kg<sup>-1</sup> and 0.1 to 1145  $\mu$ g kg<sup>-1</sup>, respectively. Fipronil sulfide and fipronil sulfone were quantified in 72 and 97% of samples collected from areas where the insecticide was applied, in

concentrations ranging from 0.1 to 27  $\mu g$  kg^-1, and from 0.1 to 1065  $\mu g$  kg^-1, respectively.

Furthermore, the limits of quantification of the analytical method were lower than the Predicted No-Effect Concentration (PNEC) values for the compounds under study, thus allowing the use of occurrence data to carry out the environmental risk assessment.

## Conclusions

The analytical method developed and validated in this study using SLE-LC-MS/MS allowed the simultaneous determination of pesticides and their degradation products in different types of soil from Brazil (Oxisol and Entisol). The method showed acceptable values of recovery and precision and has a wide linear range of applicability. A matrix effect of signal suppression was observed for all analytes in both soil types, yet the effect was significantly higher in Entisol than in Oxisol. The extraction efficiency of 2,4-D was more influenced by soil composition than other target compounds, possibly due to a more significant interaction of 2,4-D with clay minerals and SOM. The method was used to quantify hundreds of real soil and sediment samples and the data obtained helped to predict the fate, persistence and toxicity of 2,4-D and fipronil in the environment.

### Supplementary Information

Supplementary data (values for recovery, relative standard deviation, *P*-values for a two-way ANOVA and determination coefficients) are available free of charge at http://jbcs.sbq.org.br as a PDF file.

## Acknowledgments

The authors thank the financial support from the São Paulo State Research Support Foundation (FAPESP, Proc. No. 2015/18790-3). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil 2015/18790-3 (CAPES)-Finance Code 001.

## References

- Kalsi, N. K.; Kaur, P.; *Ecotoxicol. Environ. Saf.* 2019, *170*, 375. [Crossref]
- Silva, V.; Montanarella, L.; Jones, A.; Fernández-Ugalde, O.; Mol, H. G. J.; Ritsema, C. J.; Geissen, V.; *Sci. Total Environ.* 2018, 621, 1352. [Crossref]
- Nunes, A.; Schmitz, C.; Moura, S.; Maraschin, M.; *Braz. J. Dev.* 2021, 7, 37885. [Crossref]

- Popp, J.; Pető, J.; Nagy, J.; Agron. Sustainable Dev. 2013, 33, 243. [Crossref]
- Calderon, M. J.; De Luna, E.; Gomez, J. A.; Hermosin, M. C.; Sci. Total Environ. 2016, 569-570, 416. [Crossref]
- Bleam, W.; Soil and Environmental Chemistry; Elsevier: Madison, 2017.
- Durães, N.; Novo, L. A. B.; Candeias, C.; da Silva, E. F.; Soil Pollution; Elsevier: London, 2018.
- Abraham, J.; Gajendiran, A.; *Pestic. Biochem. Physiol.* 2019, 155, 90. [Crossref]
- Baldaniya, D. M.; Singh, S.; Saini, L. K.; Gandhi, K. D.; *Int. J. Chem. Stud.* 2020, 8, 1524. [Crossref]
- Biswas, S.; Mondal, R.; Mukherjee, A.; Sarkar, M.; Kole, R. K.; *Food Chem.* **2019**, *272*, 559. [Crossref]
- Chawla, S.; Gor, H. N.; Patel, H. K.; Parmar, K. D.; Patel, A. R.; Shukla, V.; Ilyas, M.; Parsai, S. K.; Somashekar; Meena, R. S.; Shah, P. G.; *Environ. Sci. Pollut. Res.* 2018, *25*, 19167. [Crossref]
- de Amarante Jr., O.; Brito, N.; dos Santos, T.; Nunes, G.; Ribeiro, M.; *Talanta* 2003, 60, 115. [Crossref]
- Prado, B.; Strozzi, A. G.; Huerta, E.; Duwig, C.; Zamora, O.; Delmas, P.; Casasola, D.; Márquez, J.; *Geoderma* **2016**, *279*, 87. [Crossref]
- Tomazini, R.; Grosseli, G. M.; Nara Ribeiro de Sousa, D.; Fadini, P. S.; Talarico Saia, F.; Langenhoff, A.; van der Zaan, B.; Mozeto, A. A.; *Anal. Methods* 2020, *12*, 3242. [Crossref]
- Moutinho, M. F.; de Almeida, E. A.; Espíndola, E. L. G.; Daam, M. A.; Schiesari, L.; *Ecotoxicology* 2020, 29, 1043. [Crossref]
- 16. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA); *Relatórios de Comercialização de Agrotóxicos*; https://www.gov.br/ibama/pt-br/assuntos/ quimicos-e-biologicos/agrotoxicos/relatorios-decomercialização-de-agrotoxicos, accessed in July 2023.
- PPDB: Pesticide Properties DataBase, *Fipronil*, http://sitem. herts.ac.uk/aeru/ppdb/en/Reports/316.htm, accessed in July 2023.
- PPDB: Pesticide Properties DataBase, 2,4-D, http://sitem.herts. ac.uk/aeru/ppdb/en/Reports/4.htm, accessed in July 2023.
- Goulart, B. V.; Vizioli, B. D. C.; Espindola, E. L. G.; Montagner, C. C.; *Environ. Monit. Assess.* **2020**, *192*, 797. [Crossref]
- dos Santos, H. G.; Jacomine, P. K. T.; dos Anjos, L. H. C.; de Oliveira, V. A.; Lumbreras, J. F.; Coelho, M. R.; de Almeida, J. A.; de Araujo Filho, J. C.; de Oliveira, J. B.; Cunha, T. J. F.; *Sistema Brasileiro de Classificação de Solos*, 5<sup>th</sup> ed.; EMBRAPA: Brasília, Brazil, 2018.
- Pinto, T. J. S.; Freitas, J. S.; Moreira, R. A.; da Silva, L. C. M.; Yoshii, M. P. C.; Lopes, L. F. P.; Goulart, B. V.; Vanderlei, M. R.; Athayde, D. B.; Fraga, P. D.; Ogura, A. P.; Schiesari, L.; Montagner, C. C.; Daam, M. A.; Espindola, E. L. G.; *Aquat. Toxicol.* **2021**, *231*, 105712. [Crossref]
- 22. Girotto, L.; Freitas, I. B. F.; Yoshii, M. P. C.; Goulart, B. V.;

Montagner, C. C.; Schiesari, L. C.; Espíndola, E. L. G.; Freitas, J. S.; *Environ. Sci. Pollut. Res.* **2023**, *30*, 21010. [Crossref]

Goulart et al.

- Instituto Nacional de Metrologia Qualidade e Tecnologia (Inmetro); Orientação sobre Validação de Métodos Analíticos, http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8\_08.pdf, accessed in July 2023.
- 24. Agência Nacional de Vigilância Sanitária (Anvisa); Resolução da Diretoria Colegiada (RDC) No. 166, de 24 de julho de 2017, *Dispõe sobre a Validação de Métodos Analíticos e dá Outras Providências*; Diário Oficial da União (DOU), No. 141, de 25 de julho de 2017. [Link] accessed in July 2023.
- 25. Origin(Pro), version 2015; OriginLab Corporation, Northampton, USA, 2015.
- 26. Buerge, I. J.; Pavlova, P.; Hanke, I.; Bächli, A.; Poiger, T.; *Environ. Sci. Eur.* **2020**, *32*, 150. [Crossref]
- 27. Bhandari, A.; Xu, F.; *Environ. Sci. Technol.* **2001**, *35*, 3163. [Crossref]

- de Oliveira Jr., R.; Regitano, J.; *Química e Mineralogia do Solo*; Melo, V.; Alleoni, R., eds.; SBCS: Viçosa, 2009.
- Bonmatin, J.-M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. A. D.; Noome, D. A.; Simon-Delso, N.; Tapparo, A.; *Environ. Sci. Pollut. Res.* 2015, *22*, 35. [Crossref]
- Singh, A.; Srivastava, A.; Srivastava, P. C.; *Pest Manage. Sci.* 2016, 72, 1491. [Crossref]
- Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A.; Mass Spectrom. Rev. 2011, 30, 491. [Crossref]
- Cappiello, A.; Famiglini, G.; Palma, P.; Pierini, E.; Termopoli, V.; Trufelli, H.; *Anal. Chem.* 2008, *80*, 9343. [Crossref]

Submitted: February 6, 2023 Published online: August 1, 2023

