Presence of Pesticides and Transformation Products and Associated Risk Assessment in Groundwater of a Region with an Intensive Agricultural Activity

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Material and methods

The study area

The aquifer lithology consists of continental detrital sediments of Tertiary age (sand-gravel assemblages, arkosic materials, and local clay intercalations) resulting in

moderate permeability. Recharge occurs predominantly through rainfall infiltration and interaction with surface water courses. Specifically, the study area is part of the Tertiary detrital aquifer system of Las Villas (Salamanca), characterized by moderately permeable sand and gravel formations with localized clay intercalations that may reduce vertical flow.

Sample collection

Samples were collected manually in the case of spring and wells, or by pumping in the case of deep wells. They were collected in duplicate in 1 L amber glass bottles to avoid alterations caused by light and transported to the laboratory in refrigerated boxes. The samples' physicochemical parameters (pH, electrical conductivity) were determined immediately using a portable measuring device (Crison PH 25+ and CM 35+) and are included in Table 1. Within four days, the samples were filtered through nitrocellulose filters with 0.45 μm pore size membranes (Millipore) and kept refrigerated at 4°C in the dark before extracting. The extracts were analyzed within two weeks of collection.

Table S1. Common names and physicochemical properties of pesticides selected for the study (data taken from PPDB, 2019). Chemicals with letters in brackets correspond to degradation compounds of parent compounds with the same letter as superscripts.

Pesticide	Field of use	Water Solubility (mg L^{-1})[1]	GUS index[2]	Log Kow[3]	DT50 (days)[4]
Metalaxyl [a]	Fungicide	8400	2.11	1.65	45
CGA 92370 (a)	Degr Prod.	-	-	-	-
Azoxystrobin	Fungicide	6.0	2.53	2.5	131
Dimethomorph	Fungicide	28.95	2.56	2.68	57
Tebuconazole	Fungicide	36.0	2.0	3.7	63
Benalaxyl	Fungicide	28.6	0.51	3.54	33.2
Flutriafol	Fungicide	95	5.29	2.3	1358
Thiamethoxam	Insecticide	4100	3.58	-0.13	50
Imidacloprid	Insecticide	610	3.76	0.57	191
Dimethoate	Insecticide	39800	1.05	0.70	2.6
Chlorpyrifos	Insecticide	1.05	0.15	4.7	76
Cypermethrin	Insecticide	0.009	-2.12	5.3	60
Pyrimidinol	Degr Prod.	-	6.81	0.7	-
Ethofumesate	Herbicide	50	3.38	2.7	21.6
Chloridazon	Herbicide	422	2.54	1.19	31
Metamitron	Herbicide	1770	3.09	0.85	30
Tribenuron-methyl	Herbicide	2483	1.39	2.63	9.1
Mesotrione	Herbicide	1500	1.45	0.11	19.6
Atrazine [b,c]	Herbicide	35	3.30	2.7	42
DIA (b)	Degr Prod.	670	-	-	-
DEA (c)	Degr Prod.	3200	3.54	-	-
Chlorotoluron [j]	Herbicide	74	2.79	2.5	63.6
Diuron	Herbicide	35.6	1.83	2.87	75.5
Terbuthylazine [d,e]	Herbicide	6.6	3.07	3.4	75.1
DET (d)	Degr Prod.	327.1	3.54	-	-
HT (e)	Degr Prod.	7.19	4.59	-	-
Metolachlor [f,g]	Herbicide	530	3.49	3.4	90
ESA-Metolachlor (f)	Degr Prod.	212461	7.22	-1.89	400
OA-Metolachlor (g)	Degr Prod.	360000	6.88	-	325
Prosulfocarb	Herbicide	13.2	0.76	4.48	11.9
Fluazifop-butyl	Herbicide	1	0.61	4.5	21
Linuron	Herbicide	63.8	2.03	3.0	57.6
Pendimethaline	Herbicide	0.33	-0.32	5.4	182.3
Oxyfluorfen	Herbicide	0.116	0.26	4.5	138
Metazachlor	Herbicide	450	1.75	2.49	8.6
Metribuzin	Herbicide	1165	2.57	1.65	11.5
Bentazone	Herbicide	7112	1.95	-0.46	20
2,4-D	Herbicide	24300	3.82	-0.82	4.4
MCPA	Herbicide	250000	3.13	-0.81	12.07

[1]Solubility in water at 20 ºC. [2]DT50, Half-life in soil. [3]logK_{ow}, octanol/water partition coefficient at pH 7 and 20 ºC. [4]GUS index, indicator of potential pollution that allows classify pesticides in leaching (GUS > 2.8), non-leaching (GUS < 1.8) and transition (1.8 < GUS > 2.8). (PPDB. Pesticide Properties DataBase, 2019).

Table S2. Quality control parameters of the SPE-LC-MS method applied to the analysis of pesticides in groundwaters.

Pesticide	m/z [1]	ESI	V_{cone} [2]	t_R [3] (min)	Recovery %	RSD [4] (%)	r^2 [5]	LOD [6] (µg L^{-1})	LOQ [7] (µg L^{-1})
Thiamethoxam	292.2	+	15	5.8	61	12	0.997	0.012	0.034
Pyrimidinol	153.2	+	25	6.1-8.6	79.4	4	0.990	0.024	0.059
DIA	174.2	+	25	6.3	63.7	9	0.996	0.021	0.064
Imidacloprid	256.2	+	15	6.5.-9.3	95.0	18	0.984	0.027	0.08
Chloridazon	222.1	+	30	6.8	84.9	14	0.999	0.026	0.071
Dimethoate	230.2	+	15	6.9	84.8	16	0.993	0.012	0.032
Metamitron	203.2	+	25	7.0	47.0	8	0.986	0.015	0.04
DEA	188.1	+	25	7.2	87.8	8	0.992	0.013	0.034
Tribenuron-methyl	396.2	+	20	7.3	67.1	12	0.999	0.028	0.073
CGA 92370	194.2	+	25	7.5	88.0	14	0.996	0.021	0.064
DETbz	202.2	+	20	8.8	100.4	9	0.998	0.024	0.058
Flutriafol	302.2	+	20	9.3	96.7	16	0.997	0.022	0.062
Mesotrione	340.3	+	20	9.6	50.6	17	0.991	0.038	0.094
HTbz	212.2	+	25	10.1	82.7	7	0.996	0.011	0.038
Metalaxyl	280.2	+	25	10.3	102.7	5	0.993	0.011	0.037
Atrazine	216.1	+	30	10.3	60.1	11	0.990	0.021	0.057
Chlorotoluron	213	+	20	10.3	72.2	7	0.995	0.026	0.066
Azoxystrobin	404.2	+	25	11.1	68.4	13	0.993	0.013	0.035
Diuron	233.2	+	25	11.5	90.7	20	0.995	0.016	0.043
Terbuthylazine	230.2	+	25	11.6	73.0	10	0.993	0.023	0.055
Ethofumesate	287.2	+	20	11.7	88.7	19	0.998	0.024	0.063
Dimethomorph	388.2	+	25	12.3	73.9	15	0.995	0.017	0.063
Metolachlor	284.2	+	15	12.7	92.4	9	0.999	0.017	0.052
Benalaxyl	326.2	+	25	13.5	84.0	3	0.996	0.012	0.036
Tebuconazole	308	+	25	13.8	92.1	14	0.998	0.015	0.05
Prosulfocarb	252.1	+		14.4	79.1	17	0.994	0.007	0.024
Fluazifop-butyl	384.3	+	20	15.9	59.2	21	0.991	0.019	0.032
Chlorpyrifos[l]	350.1	+	20	16.0	49.8	27	0.988	0.033	0.064
Linuron	250.1	+	20	16.8	87.9	13	0.983	0.021	0.037
Pendimethalin	282	+	25	17.6	21.0	14	0.993	0.026	0.068
Oxyfluorfen	362	+	20	17.7	94.5	10	0.996	0.013	0.048
Cypermethrin	416.2	+	20	18.3	19.6	18	0.983	0.083	0.185
Metazachlor	259.1	+	20	20.1	67.9	12	0.997	0.019	0.037
Metribuzin	215.1	+	20	21.7	82.6	6	0.997	0.031	0.079
ESA-Metolachlor	328	-	20	5.3	91.3	7	0.998	0.013	0.036
OA-Metolachlor	278.1	-	20	6.4	91.7	10	0.994	0.023	0.062
Bentazone	239.2	-	15	7.1	80.6	14	0.998	0.034	0.104
2,4-D	220.2	-	20	8.7	90.8	12	0.991	0.028	0.068
MCPA	199.3	-	20	9.5	60.6	25	0.995	0.022	0.049

[1]Mass/charge relation. [2]Volume of cone. [3]$t_{R,}$ retention time. [4]Standard relative deviation, calculated from the replicated analysis (n = 5) of spiked (0.1 µg L^{-1}) groundwater samples. [5] r^2 in the linear calibration range 0.05-1.50µg L^{-1}. [6]Limit of Detection. [7]Limit of Quantitation.

Analytical methodology

Stock standard solutions (1000 or 500 μg mL^{-1}) for each of the selected analytes (Table S1) and an intermediate standard solution at a concentration of 10 μg mL^{-1} of all the target analytes were prepared in methanol and stored in the dark at 4 ºC. The intermediate standard solution was used as spiking solution for the aqueous calibration standards. The organic solvents (acetonitrile, methanol, and acetone) were of HPLC grade and supplied by Fischer Scientific (Loughborough, UK), being used as received. Ultra-high quality (UHQ) water was obtained with a Milli-Q water purification system (Millipore, Milford, MA, USA).

The concentration of pesticides in the aqueous phase was determined in the samples preconcentrated using the methodology proposed by Herrero-Hernández et al. (2013), Oasis HLB cartridges were used as SPE sorbents. A volume of 500 mL of water was preconcentrated in triplicate with a Gilson Minipuls 2 HP 8 peristaltic pump at a constant flow of 7 mL min^{-1}. Each cartridge was previously conditioned with 5 mL of acetone, 5 mL of acetonitrile, and 10 mL of UHQ water. After the passage of the sample, the cartridges were vacuum-dried under an air stream (at -20 mm Hg) for five minutes. Elution was performed with 4 mL of acetone and 4 mL of acetonitrile. The solvent was evaporated to dryness under a nitrogen stream at 37ºC, and the residue was redissolved in 500 μL of methanol/water mixture (1:1) for analysis by LC-MS.

LC–MS analysis was carried out using a Waters (Milford, MA, USA) system with an ESI interface. The compounds were separated in a Luna PFP2 analytical column. Following the methodology proposed by Herrero-Hernández et al (2020) albeit slightly modified to include the new pesticides used in this region. The mobile phase was methanol (solvent A) – 5 mM ammonium formate at pH = 5 (solvent B). The elution gradient was as follows: the mobile phase started with 65% of methanol, which

was increased linearly to 75% in 5 min, then raised to 100% in 8 min., and kept constant for 6 min. Finally, it was returned to the initial state in 1 min. The column was equilibrated for 5 min, and the flow rate was 0.3 mL min^{-1}. The volume injected was 20 µL. Two injections per sample were performed, first in the + ion mode and the second in the – ion mode. In this second injection a resumed gradient was used: the mobile phase started with 65% of methanol, which was increased linearly to 100% in 7 min. and then it was returned to the initial state in 1 min. with an equilibration time of 5 min.

Optimization of the preconcentration procedure was carried out in a previous work, where SPE sorbent and elution conditions were optimized (Herrero-Hernández et al., 2013). Quantification was performed by external calibration using matrix-matched standards, which were managed in a similar way to collected water samples. Calibration curves were obtained by plotting analyte peak areas (obtained from the total ion chromatogram (TIC) in SIM mode versus concentration. The proposed methodology was validated for each of the compounds by studying different analytical parameters such as, the accuracy (average recovery), precision (reproducibility and repeatability) at the level of concentration established by EU legislation, the linearity parameters and the limits of detection (LOD) and quantification (LOQ) of the complete method.

The accuracy and precision of the proposed method were determined by recovery experiments and the relative standard deviations (RSDs) of the signals or peak areas obtained for each analyte corresponding to five groundwater samples spiked with 0.1 µg L^{-1} for each analyte. To estimate linearity, LOD, and LOQ, eight matrix-matched standards were spiked with all the analytes in the 0.05-1.50 µg L^{-1} concentration range. The LODs and LOQs were estimated as the analyte concentration with a signal-to-noise ratio of 3 and 10, respectively. Quality control parameters of the proposed methodology are summarized in Table S2.

Results

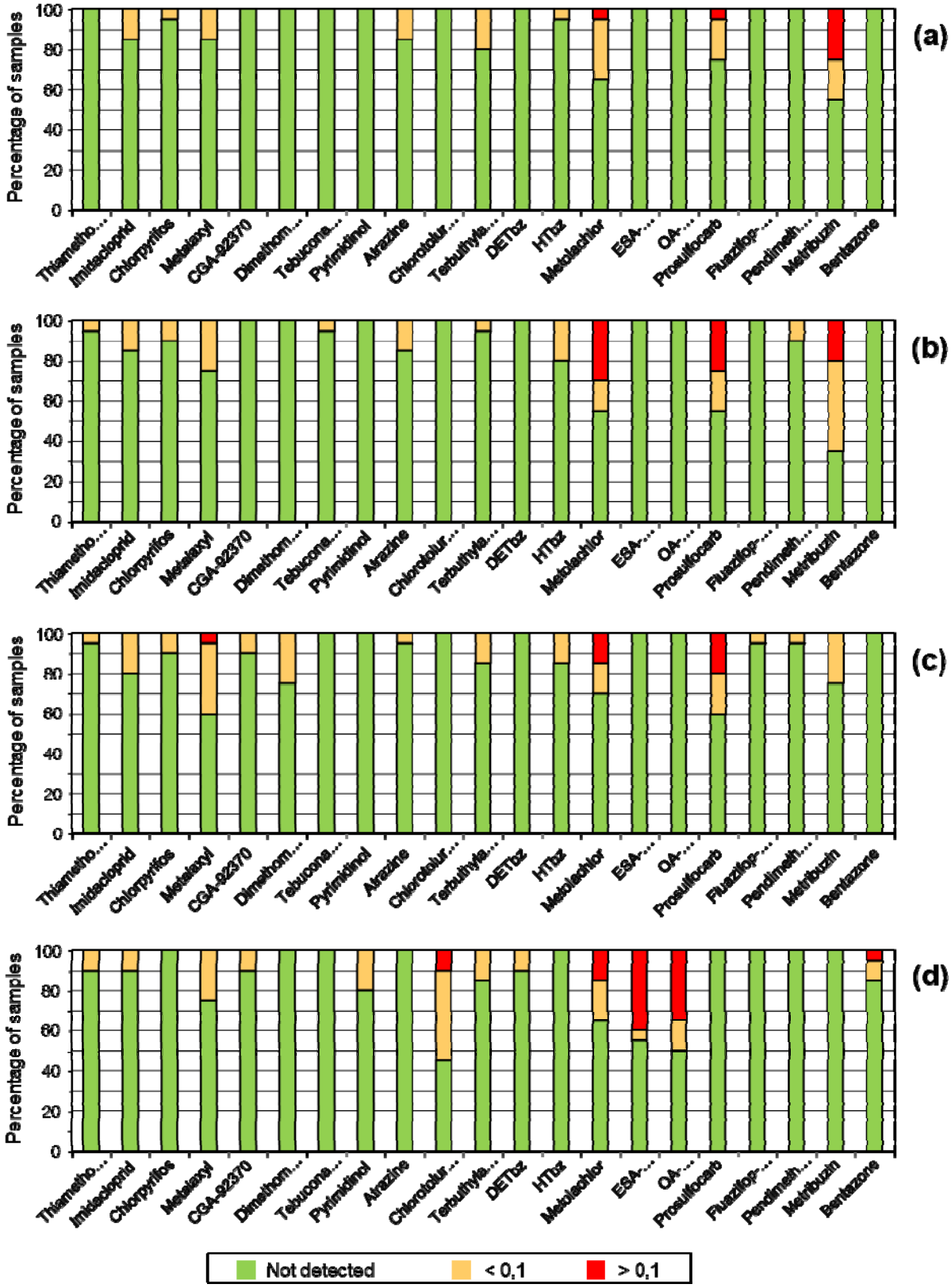


Figure S1. Concentrations of fungicides, insecticides and herbicides detected in the surface and groundwater samples (logarithmic scale).

References

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