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Laboratory and Field Studies on Bioremediation of Point-source Contamination in Horticultural Crops Using Biobeds

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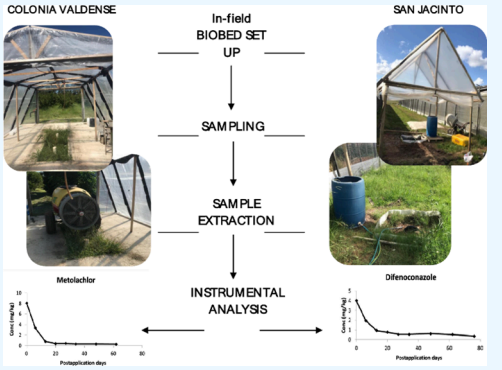
ABSTRACT: Biobeds are technological tools to minimize point-source contamination on productive farms. A four-step workflow for the efficient setting of biobeds in farms and its proof-of-concept is presented: (1) developing a fit-for-purpose pesticide multiresidue analytical method for pesticides in biomatrix; (2) launching biobeds at lab scale for pesticide degradation evaluation; (3) setting up the biobeds in the field; (4) evaluating the pesticide degradation in the biobed during an agricultural year. An ethyl acetate/sodium tetraborate multiresidue method was adapted and validated for 35 pesticides in the biomixture; lab biobeds were installed, and the degradation of 11 pesticides was confirmed. Then, biobeds were installed in two horticultural farms of different productive profiles, considering farmers' conditions, and included in the farmers' routine work. High dissipation rates (∼80%) in both bioreactors were observed for 10 pesticides. This research studies the performance of biobeds in reducing point source contamination and diminishing pesticide concentration from contaminated machinery washings not only at the lab scale but also at in-field experiments performed in productive farms. Moreover, the evaluation of biobeds in actual conditions where different chemical families of pesticides were applied together and the confirmation that repeated applications and accumulation of some compounds throughout the cycle proved biobeds' versatility in diminishing the point of pesticide contamination in farms.



1. INTRODUCTION

Point-source contamination in agricultural areas is mainly generated when handling agrochemicals, and accidental spills can occur. Pesticide formulation, pesticide dilution, and the cleaning of the spraying equipment steps are key hazard points.[1,2] Therefore, large amounts of contaminated water are generated and generally disposed of on the ground, sometimes on a specific farm parcel. There is a lack of regulation about where and how these contaminated waters have to be treated.[3] Among the alternatives to remediate this type of point-source contamination, bioreactors such as biobeds have been developed and assayed to assess their application as a mitigation tool for pesticide wastewater.[4] Biobeds are environmentally friendly, low-cost remediation techniques that effectively degrade contaminants to non-toxic compounds.[5] The first studies reported by del Pilar Castillo et al. showed that biobed efficiency is based on the biomixture composition that gives the system high adsorption capacity and a microbial consortium able to degrade xenobiotics.[6] The first biomixture employed for pesticide degradation using biobeds made of straw, soil, and peat (2:1:1) was able to degrade some of them.[7] Particularly, the straw is the material that allows the growth of basidiomycetes such as white rot fungi, whose enzymatic metabolism is reported to be responsible for the degradation of organic contaminants.[8−10]

In Latin America, climatic conditions are different from those in Sweden. Biobeds have been installed in Guatemala, Chile, Costa Rica, Brazil, and Uruguay, but the original design should be adapted to each country's socioeconomic and climatic conditions.[11−13] Lignocellulosic materials wastes from agricultural productions, rice bran, husk, coconut fiber, and sugar cane were proved to be useful for the degradation of different chemical groups of pesticides.[14−20] Technological packages from different crop productions use a wide range of pesticides from various chemical families. Pesticide physicochemical properties determine the degradation patterns. Biobeds performance is encouraging since it has been proven that some pesticide half-lives in soil are higher than those observed in biomixtures.[18,21] Moreover, an advantage of these

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Table 1. Instrumental Conditions; LC-MS/MS and GC-MS/MS Transitions for Each Compound and Their Retention Times, Declustering Potential (DP), and Collision Energy (CE)

	LC-MS/MS					GC- MS/MS			
pesticide	Rt (min)	precursor ion (m/z)	product ion (m/z)	DP (V)	CE (V)	Rt (min)	precursor ion (m/z)	product ion (m/z)	CE (V)
acetamiprid	15.3	223.2	126.1	55	25				
			99.2		47				
azoxystrobin	20.3	404.1	344	72	31	29.8	344	329.1	24
			372.1		19			183.1	16
benomyl	23	291.2	132.1	41	69				
			160.1		39				
Boscalid	20.9	343.1	139.8	89	24	26.4	342	140.1	14
			112.2		57			112.1	28
carbendazim	13.2	192.2	105.1	61	51				
			132	56	43				
cyprodinil	22.4	226.2	77	76	67	14.8	224.1	208.1	16
			90.9	81	49			197.1	22
chlorantraniliprole	20.1	484.1	286	44	21	21.1	278	249	20
			453		22		280	251	20
difenoconazole (sum)	22.9	406	337	90	21	28.8/28.9	323	265	14
			251.1		37			202	28
epoxiconazole	21.9	330.1	121.3	36	27	20	192	138	14
			101.2		63			111	26
ethion	23.7	385	143	66	33	18.3	230.9	174.9	14
			198.9		13			184.9	12
imidacloprid	14.4	256	209	86	22				
			175		23				
metalaxyl	19.7	280.2	192.1	61	25	12.8	249.2	190.1	8
			220		21			146.1	22
methoxyfenozide	21.2	362.2	313.1	51	11				
			149		21				
metolachlor	22	284.1	176.1	46	35	13.5	238.1	162.1	12
			252		21			133.1	26
metribuzin	18.4	215.2	187.1	66	25	12.5	198.1	82	14
			84.1	61	31			111	10
metsulfuron Me	18.1	382	141.1	60	20				
			199.1	62	31				
propaquizafop	23.7	445	340.9	66	25				
			429.1		17				
pyraclostrobin	22.6	388.1	194.2	67	17	27.7	164	132.1	14
			163.1		39			77	28
pyriproxyfen	23.8	322.2	96.1	50	21	22.7	136.1	78	20
			134.1		33			96	14
spinosyn A	22.3	732.57	142.2	136	43				
			98.3		81				
spinosyn D	22.7	746.5	142.2	66	39				
			98.1		79				
spirotetramat	21.6	374.2	270	56	32				
			358		28				
tebuconazole	22.5	308	70.3	85	40				
			125	128	53				
thiametoxam	12.9	292	211.1	88	15				
			246.1		13				
alachlor						12.7	188.10	160.1	10
								132.1	18
bromacil						13.4	204.9	187.9	14
								162	14
chlorothalonil						11.5	265.9	230.8	14
								168.0	22
chlorpyrifos						13.6	313.9	257.9	14
								285.9	8
cyhalothrin (sum)						22.7	197	141	12

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Table 1. continued

	LC-MS/MS					GC- MS/MS			
pesticide	Rt (min)	precursor ion (m/z)	product ion (m/z)	DP (V)	CE (V)	Rt (min)	precursor ion (m/z)	product ion (m/z)	CE (V)
								161	8
cypermethrin (sum)						26.2/26.5/26.6/26.7	181.1	152.1	22
								127.1	22
fludioxonil						16.7	248	182	14
								154	20
permethrin (sum)						24.6/24.8	183.1	168.1	14
								165.1	14
thiocyclam						8.4	135	71	8
								56	24

bioreactors is that the degradation is performed in an isolated device, avoiding the dissemination of the xenobiotics and their transformation products into the environment.[6]

Although some official GAP farming guidelines advise building biobeds to avoid point-source contamination, few instructions are given on constructing or controlling them.[22] Despite the worldwide use of biobeds, the degradation capacity of each pesticide within the biomixture in each scenario must be proven.

This work aimed to implement and evaluate biobeds for the first time in Uruguayan fields with local farmers while building up a general workflow to optimize biobed settings and performance. Before their installation, the degradation of the selected pesticides allowed for use in horticultural production[22,23] was evaluated in laboratory experiments using a fit for purpose multiresidue analytical method. The hypothesis of this work was to demonstrate that biobeds can be a complementary tool for Good Agricultural Practices using two different agricultural models in field conditions for the very first time.

2. MATERIALS AND METHODS

2.1. Chemicals, Materials, and Standards. Acetonitrile (MeCN), methanol (MeOH), and ethyl acetate (EtOAc) of HPLC quality were provided by J.T. Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Deionized water was obtained using a Thermo Scientific (Marietta, OH, USA) EASYpure RoDi Ultrapure water purification system. J.T. Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA) provided the salts: magnesium sulfate anhydrous p.p.a. ($MgSO_4$), and neutral alumina p.a. (Al_2O_3). Disodium tetraborate decahydrate p.p.a. ($Na_2B_4O_7.10H_2O$) was provided by Carlo Erba (Milan, Italy). Formic acid (>98%) and ammonium formate were provided by Sigma-Aldrich (Steinheim, Germany and India, respectively).

Pure analytical standards of acetamiprid, azoxystrobin, benomyl, carbendazim, cyprodinil, chlorantraniliprole, epoxiconazole, ethion, imidacloprid, methoxyfenozide, pyraclostrobin, pyriproxyfen, thiamethoxam, alachlor, bromacil, chlorothalonil, cyhalothrin, cypermethrin, fludioxonil, permethrin, thiocyclam, and tebuconazole were from Dr. Ehrenstorfer (Augsburg, Germany); and abamectin, boscalid, chlorpyrifos, difenoconazole, metalaxyl, metolachlor, metribuzin, propaquizafop, spirotetramat, and spinosad (sum of spinosyns A and D) were from HPC standards GmbH (Borsdorf, Germany). Stock solutions of each pesticide were prepared in either EtOAc or MeCN between 1000 and 2000 $\mu g\ mL^{-1}$ and stored at −40 °C.

2.2. Biomixture Composition and Preparation. A blank biomixture was prepared for analytical validation. Its composition consists of a representative mixture of topsoil, peat, and rice bran at a ratio of 1:1:2 (w/w) homogenized with an industrial blender. The selected topsoil was collected from the 20 cm top of a field used for agriculture (34°32′38.2″ S, 55°52′04.7″ W). The prepared biomixture was stored at −18 °C and freeze-dried before performing the analytical adjustment.

2.3. Analytical Method. The method was adapted from Rivero et al.[19,20] with the particularity that 35 pesticides were validated. Two g of lyophilized biomixture were weighed and then 5 mL of 10% NaCl w/v in distilled water were added and vortexed for 1 min at 360 g. Ten mL of EtOAc were added and vortexed for 1 min at 360 g. For the salting-out step, 1 g of $Na_2B_4O_7{\cdot}10H_2O$ was poured and manually shaken for 5 min. Tubes were placed in an ultrasonic bath for 3 cycles of 15 min, manually shaken between each cycle for 30 s, and then centrifuged at 633 g for 6 min. For the cleanup step, 2 mL of the supernatant was taken and transferred to a 15 mL tube containing 80 mg of Al_2O_3 and 300 mg of anhydrous $MgSO_4$. Subsequently, the tubes were vortexed for 1 min and centrifuged at 633 g for 4 min. A 50 μL aliquot of the supernatant was dried under a N_2 stream at 45 °C, redissolved in 1 mL of MeCN, and filtered through PDVF of 0.45 mm for the HPLC-MS/MS analysis. A 150 μL aliquot was diluted in 850 μL of EtOAc to GC-MS/MS.

3. INSTRUMENTATION

3.1. LC-MS/MS Analysis. An HPLC Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) coupled to a mass spectrometer model 4000 QTRAP (AB SCIEX) was employed in multiple reaction monitoring (MRM) acquisition mode using electrospray ionization in positive mode. A ZORBAX Eclipse XDB-C18 (150 × 4.6 mm, 5 μm) column (Agilent Technologies) was used. The column oven temperature was set at 20 °C. The injection volume in the LC system was 5 μL, and the flow rate was 0.6 mL min^{-1}. Mobile phases were water with 5 mM HCO_2NH_4, 20% methanol, and 0.1% HCOOH (A) and methanol with five mM HCO_2NH_4, 20% water, and 0.1% HCOOH (B). The elution program was as follows: 10% B was held for 3 min, increased to 100% B over 20 min, held at 100% B for 5 min, returned to 10% B over 3 min, and equilibrated at 10% B for 7 min for a total run of 33 min.

Mass conditions were as follows: ionization voltage of 5000 V, nebulizer and curtain gases N_2 at 50 psi each, and solvent evaporation in the source was assisted by a drying gas (heated nitrogen at 500 °C at 50 psi).

3.2. GC-MS/MS Analysis. A GC 2010 Ultra coupled to a TQ8050 triple quadrupole mass spectrometer (Shimadzu)

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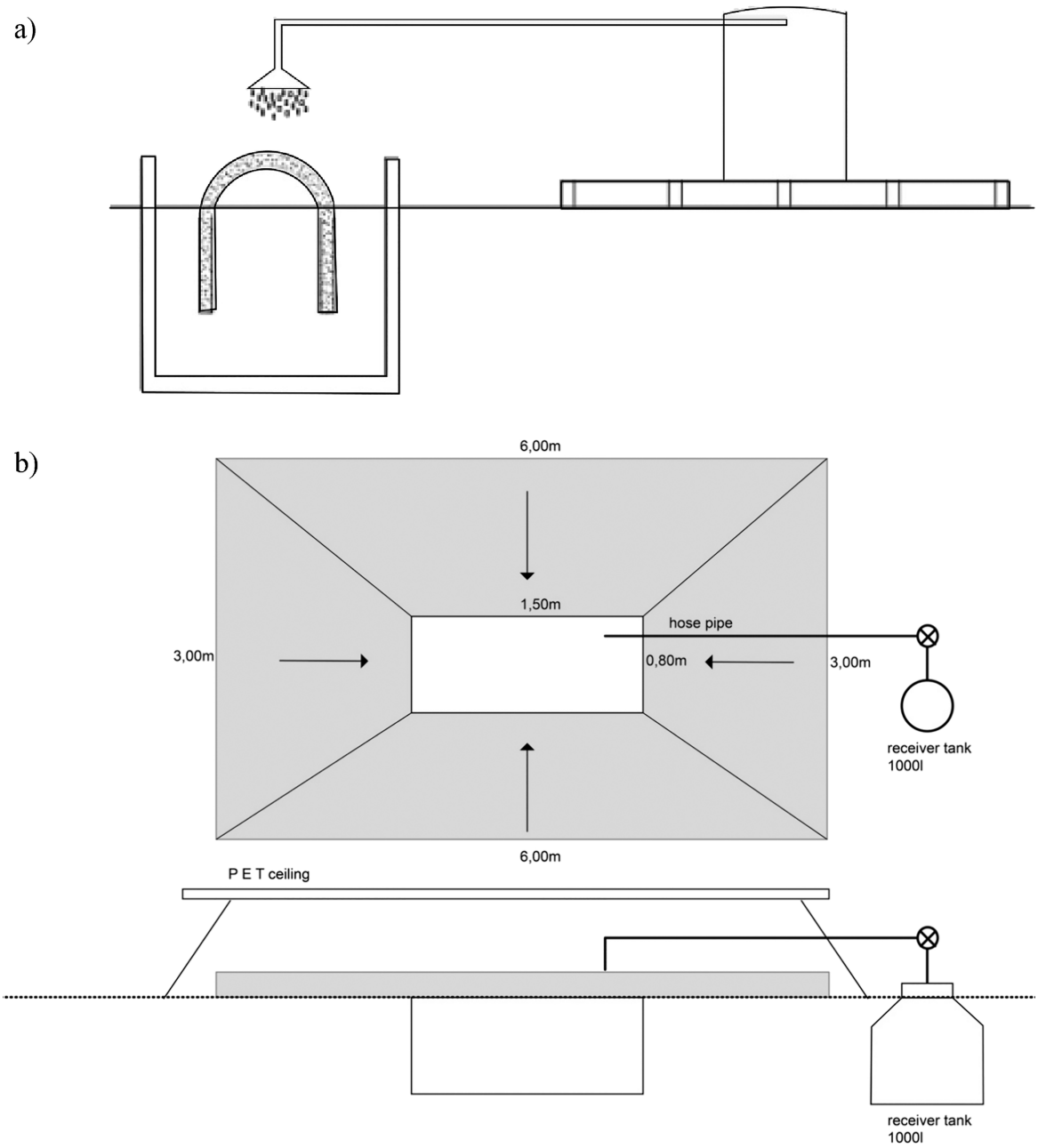

Figure 1. Biobeds at the field scale. (a) Biobed installed in farm 1 in Canelones. (b) Biobed installed in farm 2 in Colonia.

equipped with an AOC 20 i+s autosampler. A RXi-5MS Sil capillary column (5% diphenyl-95% dimethyl polysiloxane, 30 m; 0.25 mm inner diameter; 0.25 μm film) from Restek (Bellefonte, PA, USA) was used. Injection parameters were as follows: splitless mode with 1 μL of injection volume, injector temperature of 290 °C, and the gas carrier was high purity helium at a flow of 1 mL min^{-1}. The elution program was as follows: first 80 °C for 2 min, ramped 20 °C min^{-1} until 180 °C, and then ramped 5 °C min^{-1} until 300 °C, which was held for 3 min for a total run time of 34 min.

The chromatographic and mass spectrometer conditions are listed in Table 1.

4. METHOD VALIDATION

The evaluated parameters for the analytical method validation followed the guidelines of the current version of the document on pesticides: SANTE/11312/2021: recoveries, repeatability (RSD_r), within-laboratory reproducibility (RSD_{wr}), limits of quantification (LOQs), linearity, and matrix effect. All tests were performed at four levels with five replicates each.[24] For the recovery study, there were selected four levels of concentration: 1, 2, 5, and 10 mg kg^{-1}. Recoveries percentages obtained for the studied pesticides (n = 5) were averaged to estimate the method's trueness and RSD_r; the criteria guidelines for recoveries values range from 70 to 120% and RSD_r less than 20%. These criteria were used to establish the values of LOQs as the minimum value with recoveries between 70 and 120% and RSD < 20%. For calculating, interday precision evaluations were made with interanalyst variations by quintuplicate on different days. Linearity was evaluated with the back-calculated concentration (BCC) obtained from the calibration curves; the acceptance criteria is BCC < ± 20%.

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Matrix effect percentage (ME%) was calculated by comparing the solvent and matrix curves. Matrix-matched external calibration curves were used to quantify the studied compounds' concentration.

$$\%ME = \frac{\text{matrix matched calibration curve slope} - \text{solvent calibration curve slope}}{\text{solvent calibration curve slope}} \times 100$$

5. SELECTION OF PESTICIDES

The selected pesticides for the laboratory experiment were pyriproxyfen, spirotetramat, azoxystrobin, chlorothalonil, difenoconazole, metribuzin, propaquizafop, spinosad, chlorpyrifos, cypermethrin, and metolachlor, based on local horticultural farmers' practices. However, method validation was performed for a wide range of pesticides that are recommended for the evaluated crops by Guía Sata.[23] Finally, the method was validated for 35 pesticides.

6. EXPERIMENT DESIGN AT LABORATORY SCALE

A laboratory-scale experiment was carried out using the described biomixture to study the degradation of the selected pesticides. Five replicates and one blank were installed using plastic trays (48 × 25 × 8 cm) with 1 kg of biomixture each. Each experimental biobed was contaminated by spraying it with a mixture of commercially available formulations of the selected pesticides between 10 and 50 mg kg^{-1} (expressed per kg of biomixture).

The formulations used were OVIS (pyriproxyfen 100 g L^{-1}), MOVENTO (spirotetramat 15% w/w), QUADRIS (azoxystrobin 22.5% w/w), Cloronil 50 (chlorothalonil 50% w/w), SCORE (difenoconazole 24% w/w), TRIBUNE 70 WG (metribuzin 70% w/w), AGIL 700 (propaquizafop 10.5% w/w), TRACER (spinosad 44.2% w/w), PROQUIMUR (Chlorpyrifos 48% w/w), Twister 25ec (cypermethrin 250 g L^{-1}), and Dual Gold (metolachlor 87.3% w/w).

Sampling was performed once a week during the first 2 weeks, followed by once every 15 days until day 76. A composite sample was taken every time from five different points using a commercial soil sampler and homogenized in a blender. The samples were stored in a freezer at −18 °C and lyophilized before the analysis; each sample was analyzed in duplicate. The humidity was controlled using a thermoscale and adjusted once a week at 40% (w/w).

7. EXPERIMENT DESIGNS IN THE FIELD

To verify the biobed performance under field conditions, two farmers from different locations in Uruguay with different crop production and application machinery were selected: Canelones (farm 1) (34°32′38.2″ S 55°52′04.7′′ W) and Colonia (farm 2) (34°21′09.5″ S 57°15′47.0″ W). The design was adapted to the financial capability, the amount of wastewater produced, and the application equipment. The biomixture used for their construction contained the same components as the one used in the laboratory experiment. The only difference was that the soil employed was taken from each farm. The different profiles of soil depend on the region of the country where the farms are located. The predominant soil in farm 1 is Eutric Brunisol and Luvic Ruptic Vertisol (45% silt, 33% clay, 22% sand), and the predominant soil in farm 2 is Typical Eutric Brunosol (48% silt, 27% clay, 24% sand).

Farm 1 cultivates tomatoes, peppers, and onions in greenhouse production. The application equipment was a backpack sprayer (30 L). The washings generated in farm 1 are less than 500 L because it is a small-scale production. The selected design for the biobed was a 1000 L water tank (1 × 1 × 1 m) buried at ground level. The backpack washes were collected in an adjacent tank. The washing collection tank is above the soil surface. It has a tap at the bottom, and gravity flows the water. The operation was done manually with a hosepipe, covering the surface of the biobed evenly and spraying the contaminated water over it, regulating the flow to the bioreactor to prevent floodings. (Figure 1).

Farm 2 has a large production of peaches and plums, and an agricultural atomizer (500 L) is used for application. The biobed design selected was an epoxy waterproofed painted concrete pool built at the central bottom of a concrete basement with a down gradient toward the center. The biobed dimensions (2.5 m × 0.75 m × 0.75 m) were according to the size of the sprayer tank and the volumes of water generated. The machine washings are collected directly in the biobed.[6] They lixiviate through the biobed and are collected in a buried receiver tank (1100 L). The washings are pumped from the receiver tank during the period without application and sprayed manually over the biobed with a hosepipe as in farm 1. These operations were done over time. In both cases, the humidity was controlled using a thermoscale and adjusted once a week at 40% (w/w). The biobeds were refilled with the biomixture whenever it was needed.

The pesticides applied in each field were selected among those advised to protect the different crops cultured on each farm. In farm 1, the degradation of abamectin, azoxystrobin, boscalid, cyprodinil, metalaxyl, metolachlor, and pyriproxyfen was monitored. In farm 2, the degradation of carbendazim, chlorantraniliprole, pyriproxyfen, spinosad (A and D spinosyn isomers), and tebuconazole was monitored.

In each biobed, sampling was performed for one year, and each farmer completed a notebook of the pesticides applied during this period. Farm 1 was installed in September 2018 and sampled until September 2019. Farm 2 was installed in October 2018 and sampled until September 2019. Both experiments included ten sampling dates. Each sample from each biobed was a composite one, made after sampling five different points using a soil sampler (80 × 25 cm). The five points where the biobed was sampled differed from those taken previously for each sampling date. Each time, a dice scheme was followed, with the aim of covering the mass of the biomixture representatively. The individual samples of approximately 0.5 kg were combined. Then, the whole biomixture was homogenized in a blender and stored at −18 °C. Before the analysis, all samples were lyophilized and homogenized again. Two analytical portions (10 g) were taken after quartering. Each sample was analyzed by duplicate.

8. DATA TREATMENT

The evaluation of the degradation was performed by using a first-order kinetic equation:

$$Ct = Coe^{-kt} \quad (1)$$

Co is the concentration of the pesticide at time 0 after application; *k* is the dissipation rate constant ($days^{-1}$), and *Ct* is the pesticide concentration at time *t* (days).

The following equation was used to estimate the half-life of each compound:

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Table 2. Recovery (%) and RSD (%) Values for Each Pesticide at 1, 2, 5, and 10 mg kg^{-1}, LOQ (mg kg^{-1}), ME (%) values, and the Linear Range for LC and GC-MS/MS

	LC-MS/MS										
	1 mg kg^{-1}		2 mg kg^{-1}		5 mg kg^{-1}		10 mg kg^{-1}				
pesticide	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	LOQ (mg kg^{-1})	linear range (mg kg^{-1})	ME (%)
acetamiprid	118	3	92	4	95	8	83	3	1	0.5−12	−9
azoxystrobin	123	4	101	5	107	10	88	5	1	0.5−12	−11
boscalid	89	5	93	10	108	11	85	7	1	0.5−12	−9
carbendazim	85	10	74	4	68	7	96	8	1	0.5−12	10
cyprodinil	78	2	107	4	107	9	87	5	1	0.5−12	−10
chlorantraniliprole	89	18	119	5	65	9	84	5	1	0.5−12	−6
difenoconazole	113	5	77	6	72	7	82	8	1	0.5−12	−10
epoxiconazole	105	7	100	5	105	8	82	3	1	0.5−12	−12
ethion	96	7	102	6	109	9	79	6	1	0.5−12	−15
imidacloprid	73	3	67	4	83	9	80	6	1	0.5−12	−9
metalaxyl	85	5	89	2	92	10	76	6	1	0.5−12	−10
methoxyfenozide	79	4	88	5	90	11	86	5	1	0.5−12	−11
metolachlor	120	5	107	7	116	11	85	5	1	0.5−12	−10
metribuzin	79	2	93	5	96	11	83	5	1	0.5−12	−12
propaquizafop	85	3	69	4	62	11	84	5	1	0.5−12	−18
pyraclostrobin	83	10	100	7	98	10	97	6	1	0.5−12	−12
pyriproxyfen	122	3	107	6	109	10	82	4	1	0.5−12	−13
spinosyn A	75	2	80	4	79	9	78	3	1	0.5−12	−12
spinosyn D	114	3	107	8	113	10	72	7	1	0.5−12	−11
spirotetramat	113	4	112	7	101	8	68	9	1	0.5−12	−10
tebuconazole	80	5	86	6	91	14	55	7	1	0.5−12	−0.2
thiametoxam	124	6	97	7	105	12	81	5	1	0.5−12	−7
	GC-MS/MS										
alachlor	88	2	76	8	74	4	88	4	1	1−12	101
azoxystrobin	87	2	70	13	75	3	87	6	1	1−12	202
boscalid	82	2	69	13	75	2	86	5	1	1−12	294
bromacil	87	6	78	4	76	4	89	5	1	1−12	294
chlorantraniliprole	81	9	81	14	65	3	76	4	1	1−12	113
chlorothalonil	79	8	60	11	66	4	73	3	1	1−12	258
chlorpyrifos	85	2	72	10	74	3	85	3	1	1−12	123
cyhalothrin	87	4	73	13	73	5	88	3	1	1−12	93
cypermethrin	86	4	73	14	73	4	89	6	1	1−12	126
cyprodinil	84	2	70	14	76	4	87	5	1	1−12	93
difenoconazole	85	2	68	13	79	3	86	6	1	1−12	184
epoxiconazole	84	3	71	14	60	56	85	4	1	1−12	106
ethion	85	2	72	11	75	4	86	4	1	1−12	218
fludioxonil	84	2	70	14	75	4	85	4	1	1−12	142
metalaxyl	84	3	77	4	76	3	85	3	1	1−12	105
metolachlor	85	1	74	6	75	2	86	3	1	1−12	114
metribuzin	91	4	80	3	77	4	88	4	1	1−12	141
permethrin	85	3	74	12	72	3	87	4	1	1−12	180
pyraclostrobin	81	25	82	25	106	15	95	13	1	1−12	197
pyriproxyfen	86	3	76	10	73	5	89	4	1	1−12	62
thiocyclam	64	6	58	9	56	10	69	4		1−12	82

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k} \quad (2)$$

A Student t test was performed at $\alpha = 0.05$ to evaluate the difference between the assayed biobeds−mixtures.

9. RESULTS AND DISCUSSION

The designed workflow has three specific steps. First, a suitable multiresidue analytical method must be developed to assess pesticide degradation in biobeds. The biomixture as an analytical matrix is extremely complex. The difficulties of soil analysis and the chemical characteristics of cereal bran and peat add more complexity to the method development, according to the compounds to be analyzed. The method proved to be suitable for the intended purpose. The pH of the media was adjusted with sodium borate to free the basic pesticides and avoid interactions with the humic acids in the soil and the peat. The second step was the study at the laboratory scale of the biobeds' suitability as pesticide-degrading agents. In the third, the pesticide degradation in biobeds constructed in farms with the characteristics of each selected production was also evaluated.

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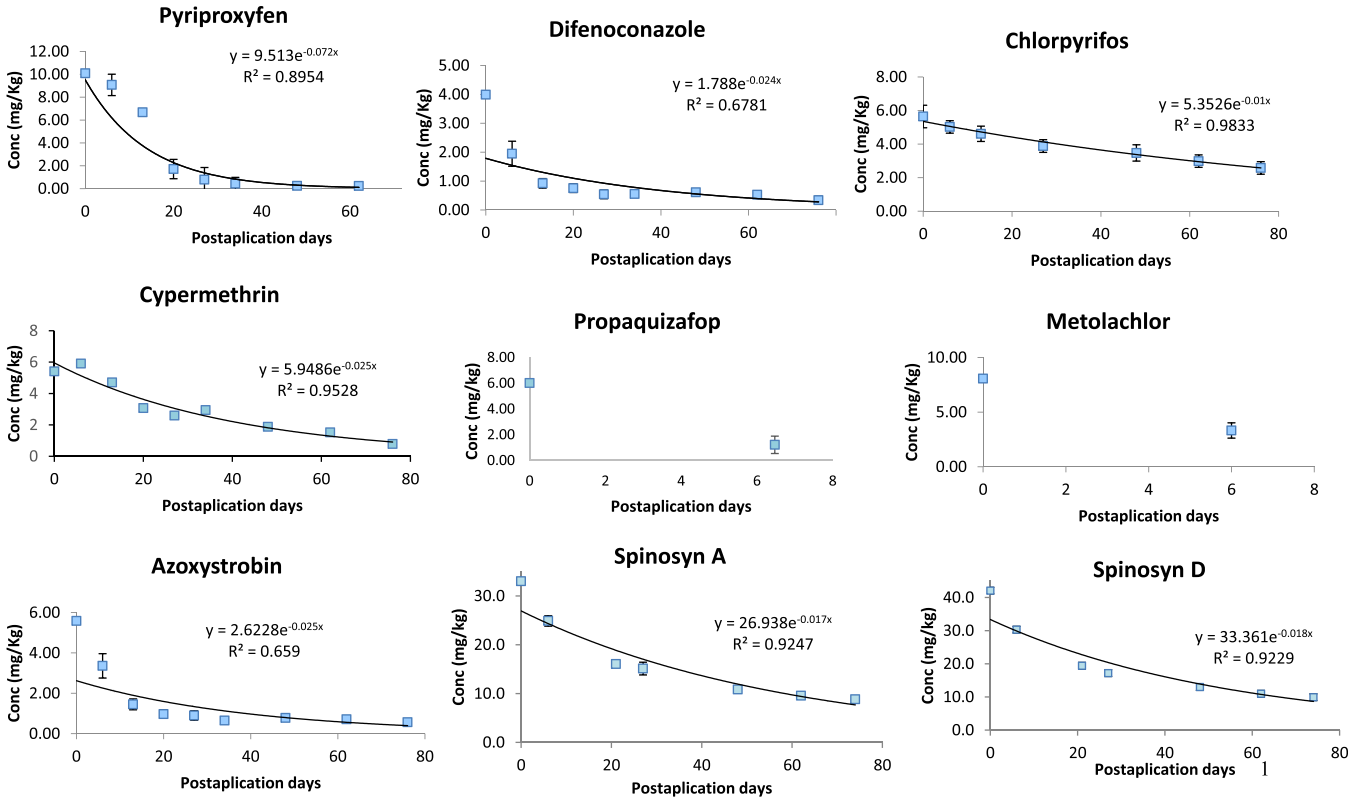

Figure 2. Laboratory experiment and degradation curves for each compound.

9.1. Method Validation by LC-MS/MS. For the method validation, a blank sample of biomixture was spiked at 1, 2, 5, and 10 mg kg^{-1} to evaluate accuracy and repeatability (% RSD). The recoveries obtained are shown in Table 2, ranging between 62 and 123%, and RSD% ranged from 2 to 20%. Metribuzin and spinosyn D showed the lowest recoveries. However, the RSDs for both analytes were low (<20 and 9%, respectively).

For all the analytes, the linear range reached by the instrument was 5−100 $\mu g\ L^{-1}$ and the LOQ was 1 mg kg^{-1}. Since the concentration decreases to values lower than 1 mg kg^{-1} for azoxystrobin, cyprodinil, chlorantraniliprole, difenoconazole, ethion, methoxyfenozide, pyraclostrobin, pyriproxyfen, spinosyn A, and spirotetramat were also validated at 0.1 mg kg^{-1}, reaching recoveries between 70 and 120% and RSD% of <20%.

Matrix effects were low or moderate in all cases, varying between −28 and 10%. All pesticides presented signal suppression, as was expected for the LC-MS/MS with ESI ionization.[25]

9.2. Method Validation by GC-MS/MS. The validation of the same concentration levels was studied with GC-MS/MS (Table 2). The recoveries for most of the compounds were between 70 and 120%, with an RSD of <20%. Thiocyclam recoveries at all concentration levels were between 56 and 69%, with %RSD values of <10%. Recoveries of boscalid, chlorantraniliprole, chlorothalonil, difenoconazole, and epoxiconazole were also between 56 and 69% at 2 and 5 mg kg^{-1}. However, the %RSD obtained for these compounds was <13%. Pyraclostrobin recoveries at 1 and 2 mg kg^{-1} were 81 and 82%, respectively, but the %RSDs for both levels were 25%.

The linear range and LOQs for all pesticides were the same as those for LC (5−100 $\mu g\ L^{-1}$, 1 mg kg^{-1}).

The ME values for all compounds were high, and they showed a high signal enhancement, as expected in GC-MS;[26] therefore, all determinations were performed using matrix-matched calibration curves.

9.3. Biobeds at Laboratory Scale. Normally, in farms, the contaminated waters from machinery washing are collected in one tank and disposed of in the environment. When a biobed is settled, wastewater is discharged into it. The assays on the lab scale were performed first to assess the biobed behavior. To simulate this situation, commercial pesticides were added as a mixture to the little biobeds built for the evaluation. The experimental half-lives obtained after the assay were compared with the DT_{50} of the compounds in soils taken from the literature. The degradation behavior of the pesticide mixture in a biobed is quite different from the degradation of a single pesticide in the soil under laboratory conditions (normal degradation experiments presented by regulatory bodies and databases). For that reason, the situation for the biobed is unfavorably compared with the degradation in soils reported. The mixture of compounds may also have different influences on the microbiota responsible for pesticide degradation, for example, fungicides can inhibit the development of fungi, which are acknowledged as the main responsible for xenobiotic degradation.[27]

The validated method was used to evaluate the pesticide degradation in the biomixture. Each biobed replica was analyzed by duplicate. The degradation curves are presented in Figure 2, which were built until the concentration reached the LOQ value of the validated method for all the assayed analytes. Degradation percentages and $t_{1/2}$ were calculated for each pesticide. The laboratory degradation results are listed in Table 3. The results were compared with DT_{50} values in the soil at a lab scale reported by the Pesticides Properties

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Table 3. Lab Scale Degradation (%) for Each Pesticide, Their Calculated $t_{1/2}$ and Their DT_{50} in Soil and Classification Adapted from PPDB[29]

pesticide	degradation lab scale (%)	calculated $t_{1/2}$ (days)	DT50 soil (days)	classification
azoxystrobin	90	28	78	moderately persistent
chlorpyrifos	54	69	386	very persistent
cypermethrin	85	28	22	nonpersistent
difenoconazole	92	29	130	persistent
metolachlor	59	5	90	moderately persistent
propaquizafop	81	3	2	nonpersistent
pyriproxyfen	99	10	10	nonpersistent
spinosyn A	73	41	24	nonpersistent
spinosyn D	76	38	45	moderately persistent
spirotetramat			0.2	nonpersistent

Database (PPDB), a lab scale reported by the PPDB as one of the main databases that include chemical structure, physicochemical data, human health data, and ecotoxicological data.

The degradation percentage of most pesticides under study was greater than 80%. The comparison between the calculated and reported $t_{1/2}$ in soil showed mostly faster degradation in the biomixture than in soil for the persistent compounds and some of the compounds reported as moderately persistent. Most of the studied pesticides showed an exponential decay in the biobeds. The insecticides cypermethrin, pyriproxyfen, chlorpyrifos, spinosyns A and D showed an R^2 of >0.9 fitting to an exponential model, the degradation of the fungicides azoxystrobin and difenoconazole showed a regular fit of the exponential decay model (R^2 = 0,6 and 0.7, respectively), hinting for other underlying mechanisms. Axozystrobin reached a degradation percentage of 90% with a $t_{1/2}$ of 28 days when its DT_{50} in the soil is reported to be 78 days. In contrast, difenoconazole degradation was 92% with a calculated $t_{1/2}$ of 29 days, although this compound is reported as persistent with a DT_{50} in soil of 130 days. For the herbicides metolachlor and propaquizafop, only two detections were possible as their degradation was very fast. Metolachlor is a moderately persistent pesticide that showed a $t_{1/2}$ of 5 days in the biobed, while in the soil, 90 days. Propaquizafop was degraded by 81% with a $t_{1/2}$ of 3 days, which is similar to the one reported in soil (2 days). Pyriproxyfen showed the highest degradation percentage (99%) with a $t_{1/2}$ of 10 days, corresponding to the one reported in soil (10 days). The most recalcitrant pesticide studied was chlorpyrifos. Its degradation only reached 54% with a $t_{1/2}$ of 69 days; this result was lower than previous reports,[13] although it showed a lower $t_{1/2}$ compared to data reported in soil (386 days). Spinosad isomers showed similar degradation rates: 73 and 76% and half-lives of 41 and 38 days. Particularly, the isomer spinosyn A showed a higher $t_{1/2}$ than the one reported in soil (24 days) and cataloged as a nonpersistent pesticide, whereas the isomer D is reported as a moderately persistent compound and the $t_{1/2}$ obtained was lower than the one in soil (45 days). Some reports showed spinosad dissipation at the lab scale with soils under different moisture regimes of more than 90% in 30 days.[28] Spirotetramat is reported as a nonpersistent pesticide with a DT_{50} in the soil of 0.2 days. In this experiment, it was only detected on the first sampling day, being consistent with our findings at lab-scale and other reports which showed degradation ranges between 0.3 and 1.0 days in the field.[29]

9.4. Biobeds in the Farms. Both infield biobed designs were customized for farmers due to the differences in their application systems and generated wastewater volumes. Farmers in farm 1 employed manual application machinery. The volume of pesticide applied was controlled. The washings of the backpack sprayer were done in a 500 L independent tank, and the collected rinses were used to irrigate the biobed in a controlled way, aiming to maintain the moisture content within an acceptable range (40−70% v/v). On the other hand, in farm 2, pesticides were applied using a sprayer tank with a 500 L capacity, and the water was applied directly into the biobed because it was washed over the biobed. The water volumes obtained during the machinery washing on farm 2 were higher than those on farm 1. Therefore, an independent collection tank was required for the lixiviate to recirculate to the biobed using a pump.

The validated analytical method was additionally used to study the degradation of pesticides in the biobeds located in the production areas. The evaluated pesticides were those reported by the producer to be applied. In farm 1, seven pesticides were evaluated (abamectin, azoxystrobin, cyprodinil, metalaxyl, metolachlor, and pyriproxyfen), while in farm 2, six of them (carbendazim, chlorantraniliprole, pyriproxyfen, spinosad (A + D isomers), and tebuconazole) were used. The degradation curves are presented in Figure 3 a,b. Degradation percentages in the experiment were calculated between the maximum concentration level obtained after the application and the lowest concentration level that reached the LOQ values.

In general, the degradation of the studied compounds behaved differently in each scenario (lab experiment, farm 1 and farm 2); the main reasons could be explained by the diverse biota present in the soils employed in each biomixture and the different operational protocols: in farm 1, the machinery washings were poured regularly. In farm 2, the first washings were drained directly in the biobeds, and afterward, the lixiviates were collected and recirculated. Other factors could additionally affect the degradation ratio, such as the peculiar pesticide combinations in each case with differential effect on the biota or their physicochemical properties (Table 4). In a lab-scale experiment, the biomixture was prepared with soil from a natural prairie where sheep are raised, and the others came from the soil of each farm previously exposed to pesticides. Table 5 summarizes which pesticides were applied in each farm production with their corresponding degradation percentage compared with the ones obtained in the lab-scale experiment and correlated with the calculated $t_{1/2}$ value in the field and the ones reported in the field by the PPDB.

In this case, the DT_{50} in-field reported values were chosen for comparison with the experimentally calculated in-field value $t_{1/2}$.

In farm 1, azoxystrobin showed a degradation of 74% with a calculated $t_{1/2}$ of 38 days which is slightly higher compared with the experimental $t_{1/2}$ from the lab assay (28 days). However, the reported DT_{50} value in the field is 181 days, and the one reported in soils is 78 days. Both values are significantly higher than the ones observed experimentally within the biobeds. In the case of cyprodinil, the calculated $t_{1/2}$ (38 days) was similar to that reported in the field (37 days),

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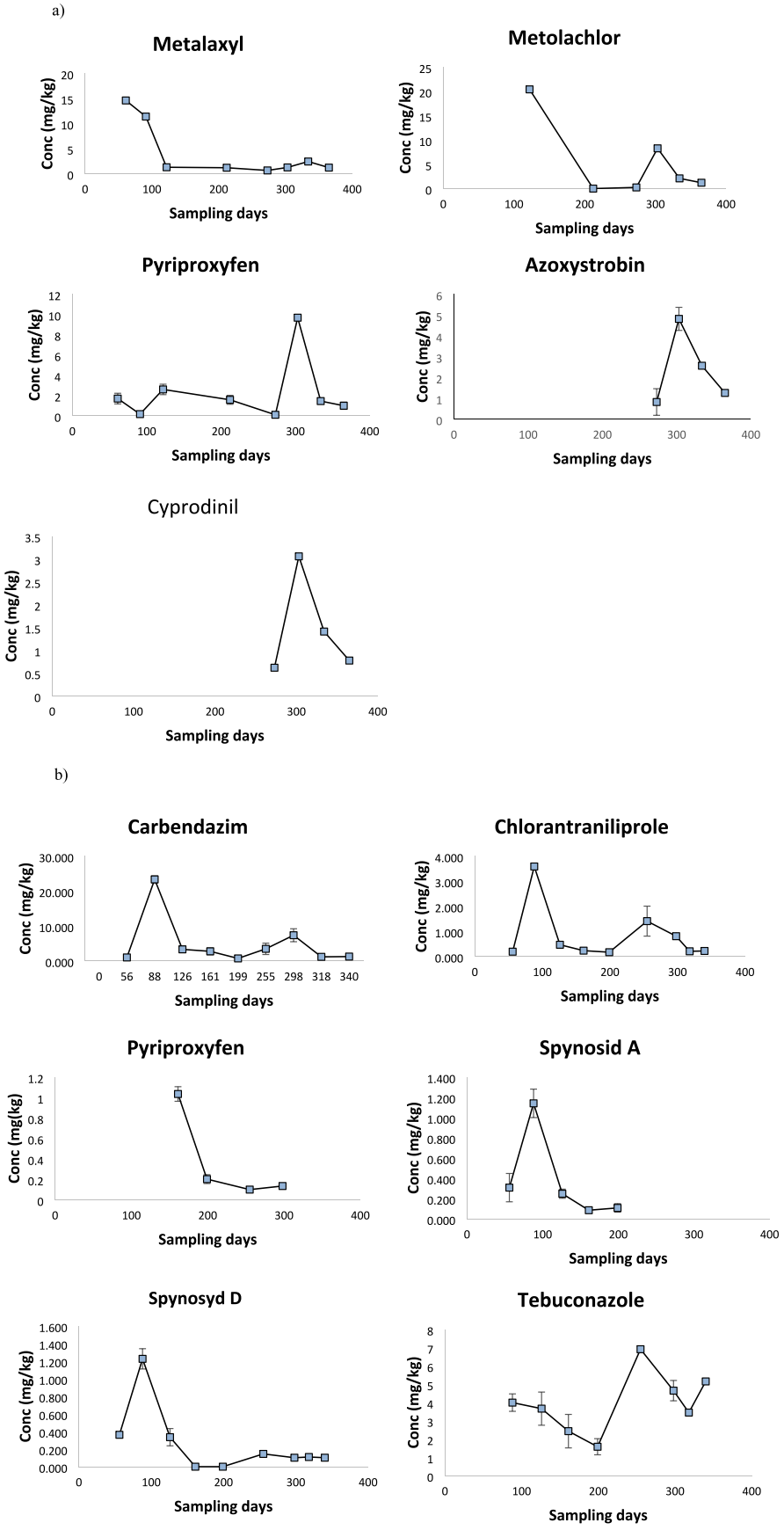

Figure 3. Field experiment: (a) farm 1 degradation curves and (b) farm 2 degradation curves. *The errors were calculated with the standard deviation of three determinations. Those error bars are not represented in the graphic and cannot be appreciated due to the minimum value in between.

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Table 4. Physicochemical Properties of the Pesticides Used in Field

pesticide	log*P* (o/w partition coefficient)	pKa	DT_{50} soil (days)	solubility in water (mg L^{-1})
azoxystrobin	2.5	not applicable	78	6.7
carbendazim	1.48	4.2	40	8.0
chlorantraniliprole	2.86	10.88	597	0.88
cyprodinil	4.44	4	37	13
metolachlor	3.4	not applicable	90	530
metalaxyl	1.75	0	36	8400
pyriproxyfen	5.37	6.87	10	0.37
spinosyn A	2.8	8.1	24	89.4
spinosyn D		7.8	45	28
tebuconazole	3.7	5.0	63	36

consistent with the classification of a moderately persistent compound. Metolachlor showed a more significant degradation percentage in farm 1 (95%) than in the lab scale experiment (59%), with an experimental $t_{1/2}$ of 28 days, similar to the one reported (21 days). Pyriproxyfen was almost 100% degraded, as in the biobed in the lab.

The last pesticide studied in farm 1, abamectin, was so rapidly degraded that it was not possible to determine its concentration on the first sampling day because it was lower than the LOQ.[27]

The results for overall degradation for the pesticides used in farm 2 were different. For tebuconazole, the degradation was around 92%. Surprisingly, the calculated $t_{1/2}$ for this pesticide in this case is 102 days, which is higher than the one reported by the PPDB in the field (47 days) and is not consistent with moderately persistent classification. However, tebuconazole was not degraded at all in biobeds built in Costa Rica.[21] A possible explanation is that the microbiota degrade tebuconazole slower than other pesticides because tebuconazole is a fungicide CytP450 inhibitor. In the case of carbendazim, a 95% degradation was observed, with a calculated $t_{1/2}$ of 25 days, similar to the one reported in the field (22 days). For chlorantraniliprole, the degradation percentage was 94% with a calculated $t_{1/2}$ of 25 days. Remarkably, this pesticide is classified as very persistent with a reported $t_{1/2}$ in the field for 204 days. In the case of pyriproxyfen, it was used in both farms but applied at different times during the experiment: in farm 1, it was applied in November 2018 (end of spring), and reapplication was done in July 2019 (winter), while in farm 2, it was only applied in March 2019 (late summer−beginning of autumn); as stated above, in farm 1, the overall degradation was 94% and the $t_{1/2}$ was 29 days, while in farm 2, the degradation reached an 84% with an experimental $t_{1/2}$ of 23 days. The calculated $t_{1/2}$ s in both farms were comparable, but the literature value was lower (4 days). Additionally, pyriproxyfen in the laboratory experiment reached a degradation of 99% with a half-life of 10 days, as stated above. For spinosyns A and D, the degradation ratio of the lab scale experiments was only 18 and 31%. However, farm 2 degradation reached values of 65 and 92% with a calculated $t_{1/2}$ of 30 and 53 days, respectively. In this case, there were no DT_{50}s in the field reported in the PPDB to compare with the ones calculated, but the "typically DT_{50}s" reported are 24 for spinosyn A and 45 days for spinosyn D,[29] which are similar values to the ones obtained.

The difference in the degradation of pyriproxyfen and spinosyn A and spinosyn D can be explained due to the different sorption properties of the compounds on the biomixture and the fact that the soil employed in the lab experiments came from a noncultivated prairie. However, the biobed design, the operational protocols, and climate conditions at the open field could play a role in the behavior of the system.

When comparing the degradation curves for all compounds, it is observed that the maximum concentrations obtained correspond to reapplications. This also shows how the biobeds are an additional control to determine if the concentrations and pesticides applied are according to GAPs. After 10 months of experiments, it was observed that the biobeds could degrade greater than 80% of the pesticide concentrations poured into them, although repeated applications and accumulation of some compounds throughout the cycle confirmed biobeds' versatility in diminishing the punctual pesticide contamination in farms. It is noteworthy that biobeds were adaptable to diverse productions, with different designs and application technologies, spraying all of the used compounds simultaneously into the bioreactor. Moreover, it was demonstrated that their efficiency is maintained over time despite the different productive systems where biobeds are installed. The results obtained in this work are of main interest to the state of the art biobed because they will contribute to ameliorating different biobed field designs.

Table 5. Degradation Comparison between Laboratory and Field Experiments

	pesticide	degradation in field (%)	degradation lab scale (%)	calculated $t_{1/2}$ (days)	reported $t_{1/2}$ in field (days)
farm 1	abamectin	not included	not included		
	azoxystrobin	74	90	38	181
	cyprodinil	75	not included	38	37
	metalaxyl	92	not included		
	metolachlor	95	59	28	21
	pyriproxyfen	94	99	29	4
farm 2	carbendazim	95	not included	25	22
	chlorantraniliprole	94	not included	25	204
	pyriproxyfen	84	99	23	4
	spynosin A	65	73	30	
	spynosin D	92	76	53	
	tebuconazole	92	not included	102	47

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Notes

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REFERENCES

(1) Campos, M.; Perruchon, C.; Karas, P. A.; Karavasilis, D.; Diez, M. C.; Karpouzas, D. G. Bioaugmentation and Rhizosphere-Assisted Biodegradation as Strategies for Optimization of the Dissipation Capacity of Biobeds. *J. Environ. Manage* **2017**, *187*, 103−110.

(2) Odukkathil, G.; Vasudevan, N. Bacteria Amended Clay Biochar Composite Biobed System to Treat Agriculture Runoff. *J. Environ. Manage.* **2020**, *269*, No. 110694.

(3) Carniel, L. S. C.; Niemeyer, J. C.; de Oliveira Filho, L. C. I.; Alexandre, D.; Gebler, L.; Klauberg-Filho, O. Are There Any Risks of the Disposal of Pesticide Effluents in Soils? Biobed System Meets Ecotoxicology Ensuring Safety to Soil Fauna. *Ecotoxicology* **2020**, *29* (9), 1409−1421.

(4) Tortella, G. R.; Rubilar, O.; Castillo, M. D. P.; Cea, M.; Mella-Herrera, R.; Diez, M. C. Chlorpyrifos Degradation in a Biomixture of Biobed at Different Maturity Stages. *Chemosphere* **2012**, *88* (2), 224−228.

(5) Andreoni, V.; Gianfreda, L. Bioremediation and Monitoring of Aromatic-Polluted Habitats. *Appl. Microbiol. Biotechnol.* **2007**, *76* (2), 287−308.

(6) del Pilar Castillo, M.; Torstensson, L.; Stenström, J. Biobeds for Environmental Protection from Pesticide Use—A Review. *J. Agric. Food Chem.* **2008**, *56* (15), 6206−6219.

(7) Thorstensen, C. W.; Lode, O. Laboratory Degradation Studies of Bentazone, Dichlorprop, MCPA, and Propiconazole in Norwegian Soils. *J. Environ. Qual.* **2001**, 947−953.

(8) Adak, T.; Mahapatra, B.; Swain, H.; Patil, N. B.; Pandi, G. G. P.; Gowda, G. B.; Annamalai, M.; Pokhare, S. S.; Meena, K. S.; Rath, P. C.; Jena, M. Indigenous Biobed to Limit Point Source Pollution of Imidacloprid in Tropical Countries. *J. Environ. Manage.* **2020**, *272*, No. 111084.

(9) Pinto, A. P.; Lopes, M. E.; Dordio, A.; Castanheiro, J. E. F. Bioaugmentation an Effective Strategy to Improve the Performance of Biobeds: A Review. *Agrochem. Detect., Treat. Rem.* **2020**, 207.

(10) Christian, V.; Shrivastava, R.; Shukla, D.; Modi, H. A.; Vyas, B. R. M. Degradation of Xenobiotic Compounds by Lignin-Degrading White-Rot Fungi: Enzymology and Mechanisms Involved. *Indian J. Exp. Biol.* **2005**, *43* (4), 301−312.

(11) Dias, L. de A.; Gebler, L.; Niemeyer, J. C.; Itako, A. T. Destination of Pesticide Residues on Biobeds: State of the Art and Future Perspectives in Latin America. *Chemosphere* **2020**, *248*, No. 126038.

(12) Chin-Pampillo, J. S.; Ruiz-Hidalgo, K.; Masís-Mora, M.; Carazo-Rojas, E.; Rodríguez-Rodríguez, C. E. Adaptation of Biomixtures for Carbofuran Degradation in On-Farm Biopurification Systems in Tropical Regions. *Environmental Science and Pollution Research* **2015**, *22* (13), 9839−9848.

(13) Rivero, A.; Gérez, N.; Jesús, F.; Niell, S.; Pía Cerdeiras, M.; Heinzen, H.; Cesio, M. V. Unambiguous Evaluation of Chlorpyrifos and TCP Bioremediation in Laboratory and Field Experiments. *Int. J. Environ. Anal. Chem.* **2020**, *102* (18), 6845−6857.

(14) Diez, M. C.; Tortella, G. R.; Briceño, G.; Castillo, M. D. P.; Díaz, J.; Palma, G.; Altamirano, C.; Calderón, C.; Rubilar, O. Influence of Novel Lignocellulosic Residues in a Biobed Biopurification System on the Degradation of Pesticides Applied in Repeatedly High Doses. *Electron. J. Biotechnol.* **2013**, *16* (6), 11.
(15) Urrutia, C.; Rubilar, O.; Tortella, G. R.; Diez, M. C. Degradation of Pesticide Mixture on Modified Matrix of a Biopurification System with Alternatives Lignocellulosic Wastes. *Chemosphere* **2013**, *92* (10), 1361−1366.
(16) Karanasios, E.; Tsiropoulos, N. G.; Karpouzas, D. G.; Menkissoglu-Spiroudi, U. Novel Biomixtures Based on Local Mediterranean Lignocellulosic Materials: Evaluation for Use in Biobed Systems. *Chemosphere* **2010**, *80* (8), 914−921.
(17) Lescano, M. R.; Masin, C. E.; Rodríguez, A. R.; Godoy, J. L.; Zalazar, C. S. Earthworms to Improve Glyphosate Degradation in Biobeds. *Environmental Science and Pollution Research* **2020**, *27* (21), 27023−27031.
(18) Rezende, S.; Cesio, M. V.; Archondo, L.; Russi, C.; Martínez, P.; Rivero, A.; Hladki, R.; Heinzen, H.; Besil, N. Pilot Study of Biobeds Application for the Remediation of Citrus Agro-Industrial Wastewaters. *Int. J. Environ. Anal. Chem.* **2023**, *103*, 9698.
(19) Rivero, A.; Niell, S.; Cesio, V.; Cerdeiras, M. P.; Heinzen, H. Analytical Methodology for the Study of Endosulfan Bioremediation under Controlled Conditions with White Rot Fungi. *J. Chromatogr B Analyt Technol. Biomed Life Sci.* **2012**, *907*, 168−172.
(20) Rivero, A.; Niell, S.; Cerdeiras, M. P.; Heinzen, H.; Cesio, M. V. Development of Analytical Methodologies to Assess Recalcitrant Pesticide Bioremediation in Biobeds at Laboratory Scale. *Talanta* **2016**, *153*, 17.
(21) Masís-Mora, M.; Lizano-Fallas, V.; Tortella, G.; Beita-Sandí, W.; Rodríguez-Rodríguez, C. E. Removal of Triazines, Triazoles and Organophophates in Biomixtures and Application of a Biopurification System for the Treatment of Laboratory Wastewaters. *Chemosphere* **2019**, *233*, 733−743.
(22) Ministerio de Ganadería, Agricultura y Pesca (UY) *Guía de Buenas Practicas Agricolas Para La Producción de Frutas y Hortalizas Frescas En Uruguay*; 2014.
(23) *Guía Sata: Guía Uruguaya Para La Protección y Nutrición Vegetal*; 17th ed.; Sata: 2022.
(24) European Commission *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*. SANTE/12682/2019. 2019, 1−53.
(25) Besil, N.; Cesio, V.; Heinzen, H.; Fernandez-Alba, A. R. Matrix Effects and Interferences of Different Citrus Fruit Coextractives in Pesticide Residue Analysis Using Ultrahigh-Performance Liquid Chromatography−High-Resolution Mass Spectrome. *J. Agric. Food Chem.* **2017**, *65*, 4819.
(26) European Commission *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*. SANTE/11813/2017. 2017, 1−53.
(27) Zubrod, J. P.; Bundschuh, M.; Arts, G.; Brühl, C. A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J. J.; Rohr, J.; Scharmüller, A.; Smalling, K.; Stehle, S.; Schulz, R.; Schäfer, R. B. Fungicides: An Overlooked Pesticide Class? *Environ. Sci. Technol.* **2019**, *53* (7), 3347−3365.
(28) Adak, T.; Mukherjee, I. Investigating Role of Abiotic Factors on Spinosad Dissipation. *Bull. Environ. Contam. Toxicol.* **2016**, *96* (1), 125−129.
(29) Lewis, K. A.; Tzilivakis, J.; Warner, D. J.; Green, A. An International Database for Pesticide Risk Assessments and Management. *Hum. Ecol. Risk Assess.: Int. J.* **2015**, *22*, 1050.

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