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Effect of Vermicomposts from Wastes of the Wine and Alcohol Industries in the Persistence and Distribution of Imidacloprid and Diuron on Agricultural Soils

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The persistence and distribution of diuron (D) and imidacloprid (I) in soils amended or not with winery vermicomposts were recorded for several months. Sandy loam (S1) and silty clay loam (S2) soils with organic carbon contents of <1% were selected. After incubation, around 78% of I remained in the soil and no metabolites were found. Diuron was dissipated more rapidly except in the unamended soil S1 with DT_{50} values of 259 days. The addition of vermicomposts to S1 soil decreased the persistence of D, and high amounts of DPMU (40%) and DPU (20%) metabolites were found. In unamended and amended S2 soils, the persistence of D was lower than in S1 (DT_{50} < 42 days) but only DPMU was determined (up to 5%). Different simulation models from FOCUS guidelines were applied to the experimental data. No relationship between pesticide degradation and soil enzyme activities was found.

KEYWORDS: Diuron; imidacloprid; soil; vermicompost; degradation; modelization; enzyme activity

INTRODUCTION

The problems of pesticide residues in soils and organic waste disposal are currently of great concern in environmental terms and urgently require sustainable solutions. The use of organic agroindustrial wastes, previously stabilized through vermicomposting, as amendments on the plowed layer to modify pesticide dynamics in soils could be a worthwhile solution to these two problems.

Among the different processes that pesticides can go through in soils, degradation is fundamental for reducing pesticide residue levels (*1*). Degradation rates depend on many microbiological, physical, and chemical properties of the soil as well as those of the pesticide. Soil properties are interrelated and may influence these processes in contradictory ways by both stimulating and restricting overall degradation. The predominance of one process over another depends on the soil−pesticide interaction and cannot therefore be generalized. The lack of favorable conditions for microbial degradation is the principal cause of pesticide persistence in soil.

Organic matter (OM) has been reported to be the major controlling component in the sorption, transformation, and transport of most organic pollutants (*2*). Thus, the addition of organic amendments (OA) has specifically led to an increase in the sorption of pesticides (*3*, *4*). It is generally accepted that sorbed chemicals are less accessible to microorganisms and limit their degradation (*5*). However, the degradation of sorbed pesticides, although slower, can be significant, and an increase in sorption does not necessarily give rise to a corresponding reduction in degradation (*6*). Park and co-workers (*7*) concluded that, in some soils under certain conditions, bacteria can access and degrade part of the sorbed pesticide. In addition, OA to soil normally result in increased microbiological activity due to the availability of organic molecules such as sugar and amino acids that could enhance degradation (*8*). Finally, soil amendments incorporate microorganisms such as bacteria, actinomycetes, and fungi (*9*), which are primary agents in the degradation of pesticides (*10*).

The vermicompost from spent grape marc and wine shoots mixed with biosolid vinasse used in this study has been shown to be a highly effective soil amendment for enhancing pesticide sorption capability (*4*, *11*). However, no studies of the role of these vermicomposts in the degradation of pesticides have been carried out until now. The pesticides selected for our study were diuron and imidacloprid. Diuron is a herbicide used to control a wide variety of annual and perennial broadleaf and grassy weeds and is also used in noncrop areas. The main diuron metabolites are 3-(3,4-dichlorophenyl)-1-methylurea (DPMU), 3,4-dichlorophenylurea (DPU), and 3,4-dicholoraniline (DCA). These metabolites have proven to be more toxic than the parent compound (*12*). The imidacloprid insecticide belongs to the chloronicotinyl family of insecticides. The principal imidacloprid metabolites detected in soil include imidacloprid-urea, 6-chloronicotinic (CNA), and 6-hydroxynicotinic acid. Both pesticides are stable in relation to hydrolysis at neutral soil pH values. Biodegradation appeared to be the major cause of diuron degradation (*13*), whereas imidacloprid seems to be very sensitive to both microbial and photodegradation (*14*).

The aim of this study was to analyze the persistence and distribution of these different pesticides in two soil types amended with two vermicomposts from the main types of waste produced by the wine and alcohol industries. To describe the degradation

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kinetics, three FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) kinetic models were selected: the single first-order (SFO), the first-order multicompartment (FOMC), and the hockey-stick models (*15*). The current versions of most tools used to calculate predicted environmental concentrations of pesticides in groundwater, surface water, and sediment within regulatory frameworks are based on the SFO kinetic. However, when SFO kinetics cannot be used to evaluate degradation (*16*), other models based on a biphasic pattern, such as the hockey-stick or FOMC model, also known as the Gustafson and Holden model, should be used.

MATERIALS AND METHODS

Chemicals. Diuron [*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea, D] and its metabolites, DPMU, DPU, and DCA, with purity rates of 99, 97.5, and 99.5%, respectively, were supplied by Dr. Ehrenstorfer (Augsburg, Germany). Diuron has a water solubility of 42 mg L^{-1} and an octanol−water partition constant (K_{ow}) of 700 (*17*). Imidacloprid [1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine, I] with a purity rate of 99% and its metabolite 6-chloronicotinic acid with a purity rate of 99.5% were also supplied by Dr. Ehrenstorfer. The water solubility of this insecticide is 510 mg L^{-1}, and its K_{ow} is 3.72 (*17*).

Amendments. The V1 vermicompost was obtained from spent grape marc and the V2 vermicompost from a 2:1 mixture of biosolid vinasse and wine shoots, which are the principal wastes from the wine and distillery industries. Their properties are shown in Romero et al. (*18*). The vermicomposting process is described elsewhere (*19*).

Soil Samples. Two types of agricultural soil from southeastern Spain were selected: a sandy loam soil (Chromic cambisol) (S1) and a silty clay loam soil (Chromic vertisol) (S2). Soils were sampled from the first 20 cm, air-dried, and sieved (< 2 mm). The soil properties shown in **Table 1** were determined following validated official methods (*20*).

Incubation Experiment. Fractions of 40 g from unamended soils and soils 5% amended with previously ground vermicompost were incubated. Beforehand, they were spiked with a 2 mL solution of acetonitrile containing 60 μg mL^{-1} diuron or imidacloprid and were left to evaporate for 3 h. The final concentration of each pesticide in soil was 3 μg g^{-1} (equivalent to 7.8 kg ha^{-1} taking into account 20 cm deep), which is higher than agricultural dosages to avoid determination problems by HPLC. Incubation was carried out at 20 °C in the dark with soil samples at 80% of their field capacities. The humidity of samples was maintained constant by weighing. Soil samples from each flask at different incubation times (0, 7, 14, 32, 47, 82, and 144 days for S1 and 0, 3, 7, 14, 32, 47, and 61 days for S2) were analyzed in triplicate to determine the pesticide concentration as well as the soil enzyme activity. Different sampling times were used due to the different degradation rates observed in each soil.

Pesticide Analysis. Aliquots of 2.5 g (dry weight) of the samples were placed in 25 mL flasks with 5 mL of distillate water for desorption and with 5 mL of a 60:40 mixture of acetonitrile and water for solvent extraction. They were shaken for 24 h, centrifuged at 1811*g* for 10 min, and filtered through 0.45 μm polytetrafluoroethylene filters for HPLC analysis.

An Agilent series 1100 liquid chromatograph equipped with a diode array detector was used. A Zorbax Rx-C8 2.1 × 150 mm analytical column packed with diisopropyl *n*-octyl (5 μm) and an Eclipse XDB-C8 guard cartridge (2.1 × 12.5 mm i.d.) filled with the same material were used. The operating conditions were as follows: injection volume, 10 μL; flow rate, 0.2 mL min^{-1}; and column temperature, 40 °C. Acetonitrile/sulfuric acid 0.005 M (pH 3) was used for the mobile phase. For diuron and its metabolites, a 40:60 (v/v) ratio and a detection wavelength of 254 nm were used. D, DPMU, DPU, and DCA retention times were 7.94, 6.23, 4.81, and 8.75 min, respectively. For imidacloprid and CNA, the mobile phase ratio was 30:70 (v/v), and the detection wavelengths selected were 270 nm for I and 225 for CNA. The retention times were 4.58 and 3.88 min for imidacloprid and CNA, respectively.

Recovery Studies. Recovery of extractions with the acetonitrile/water mixture was carried out by spiking 2.5 g of dry weight of each treatment with the parent compound and the metabolites at a rate of 3 μg g^{-1}, leaving the acetonitrile to evaporate for 3 h and watering the sample up to 80% field capacity. After 24 h, extraction was carried out with 5 mL of the acetonitrile/water mixture (60:40), which was shaken for 3 and 24 h periods. The rest of the process was carried out as described above.

Table 1. Soil Properties

soil	sand (%)	silt (%)	clay (%)	pH	OC (%)	humic acids (g kg^{-1})	CEC ($cmol_c$ kg^{-1})
S1	69.90	17.01	13.10	6.0	0.36	1.14	12.86
S1 V1 (5%)				6.7	1.92		
S1 V2 (5%)				7.0	1.98		
S2	13.99	50.56	35.45	8.2	0.93	3.75	28.77
S2 V1 (5%)				7.8	2.93		
S2 V2 (5%)				7.9	2.86		

Determination of Dehydrogenase Activity. A 1 g aliquot of a wet soil sample was incubated for 20 h at 25 °C with 0.2 mL of 0.4% 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-tetrazolium chloride (INT). The iodonitrotetrazolium formazan (INTF) produced in the reduction of INT was extracted with acetone/tetrachloroethylene (1.5:1) and measured in a spectrophotometer at 490 nm (*21*). Each sample was determined in triplicate, and assays in soils without INT were simultaneously carried out for control purposes.

Determination of Urease Activity. A 2 mL solution of phosphate buffer (pH 7.1) and 0.5 mL of 1.066 M urea were added to a 2 g wet soil sample, the mixture was incubated at 37 °C for 2 h, and the volume was brought up to 10 mL with distilled water. After centrifugation at 1301*g* for 10 min, the ammonium released from the hydrolytic reaction of urea was measured using an ammonium selective electrode (Orion Research Inc., Beverly, MA) model 95-12. Each sample was determined in triplicate, and controls without urea were carried out for each sample.

Modelization. Modelmaker 4.0 was used for data analysis. Three different degradation diagrams were created according to the metabolites determined in each sample (**Figure 1**). Each diagram is composed of (i) compartment rectangles, which are the parts of the system with defined boundaries where the relevant quantities of pesticide, metabolites, etc., can be stored, and (ii) flows (F*x*), which represent the movement of quantities from one compartment to another.

Diagram 1 was applied to samples S1, S2, S2 V1, and S2 V2 and diagram 2 to samples S1 V1 and S1 V2. Diagram 3 was applied to all samples spiked with imidacloprid as no metabolites were detected during solvent extraction. The sink compartment includes the percent of pesticide residues that cannot be measured. It includes the metabolites that were not possible to determine, the CO_2 formed during mineralization, and the fraction corresponding to the bound residues. This explains why there are flows from diuron and DPMU to the sink compartment, as we regard the fraction not transformed into a metabolite and not detected in the extraction process as having become bound residue.

Single First-Order Model. The SFO model assumes that the number of pesticide molecules is small relative to the number of degrading microorganisms and soil enzymes. As a result, the rate of change in the pesticide concentration is always directly proportional to the actual concentration remaining in the system (eq 1).

$$\frac{dC}{dt} = -kC \tag{1}$$

By integrating eq 1 and rearranging the terms, a simple exponential equation with only two parameters is obtained (eq 2).

$$C = C_0 \times e^{-kt} \tag{2}$$

C is the pesticide concentration in soil at time *t*, *k* is the degradation rate constant, and C_0 is the initial concentration.

First-Order Multicompartment Model. The soil is regarded as a heterogeneous medium; it is therefore unlikely that degradation occurs at the same rate in the different compartments of the soil studied. This model is more useful than other biphasic models due to the small number of parameters involved (eq 3).

$$\frac{dC}{dt} = -\frac{\alpha}{\beta} C \left(\frac{t}{\beta} + 1\right)^{-1} \tag{3}$$

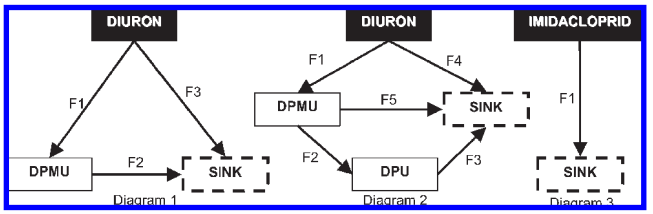

Figure 1. Modelization diagrams for diuron and imidacloprid degradation.

Equation 4 is the integrated form of eq 3.

$$C = \frac{C_0}{\left(\frac{t}{\beta} + 1\right)^{\alpha}} \tag{4}$$

In eq 4, we find two new parameters, where α is the shape formed by the coefficient of variation in k values and β is the location. Dissipation occurs more rapidly for higher α values and lower β values.

Hockey-Stick Model. The hockey-stick model consists of two sequential first-order curves, according to two different first-order kinetic rate constants K_1 and K_2, where K_1 is usually larger than K_2. This model is less applied due to the higher number of parameters. The integrated form of this model is presented in eq 5.

$$C = C_0 \times e^{-K_1 t} \text{ for } t \leq t_b \tag{5}$$

$$C = C_0 \times e^{-K_1 t_b} \times e^{-K_2 (t - t_b)} \text{ for } t > t_b$$

where t_b is the breakpoint, the time at which the rate constant changes.

Half-Life Time. The models enable us to calculate the persistence of pesticides during the half-life time (DT_{50}) for each sample on the basis of eqs 6 and 7 for the SFO and FOMC models, respectively.

$$DT_{50} = \frac{\ln 2}{k} \tag{6}$$

$$DT_{50} = \beta(2^{1/\alpha} - 1) \tag{7}$$

Goodness of Fit. The chi-square test (χ^2) was used to determine the goodness of fit for each model (eq 8). χ^2 represents the deviations between observed and calculated values relative to measurement error, where C is the calculated value, O is the observed value, $\overline{O}$ is the mean of all observed values, and err is the percentage of measurement error.

$$\chi^2 = \sum \frac{(C - O)^2}{\left(\frac{err}{100} \overline{O}\right)^2} \tag{8}$$

To validate the model, the calculated χ^2 values must be less than or equal to the tabulated values ($\chi^2_{m\alpha}$), where m is the degree of freedom (number of measurements minus number of model parameters) and α is the test significance. In this study, α = 0.05 indicates that the model has a 95% probability of being appropriate. Once the data values have been validated and the goodness of the fit has been demonstrated, the scaled error (err_{scaled}) must then be determined on the basis of the error percentage (err, eq 9).

$$err = 100 \times \sqrt{\frac{1}{\chi^2_{tabulated}} \times \sum \frac{(C - O)^2}{\overline{O^2}}} \tag{9}$$

The err_{scaled} is worked out using eq 10. The most suitable model is the one with the lowest err_{scaled} value.

$$err_{scaled} = \frac{err}{100} \times \overline{O} \tag{10}$$

RESULTS

Dehydrogenase and Urease Activities. The dehydrogenase activity occurs in soils as integral parts of intact cells and reflects the total range of the soil microflora's oxidative activities (*22*) and is therefore used as an indicator of microbial activity. On the whole,

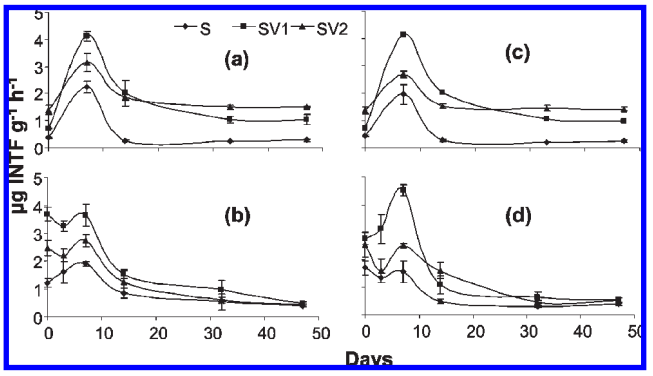

Figure 2. Dehydrogenase activity in the unamended and 5% vermicompost-amended sandy loam soil (S1, S1 V1, and S1 V2) spiked with diuron (**a**) and imidacloprid (**c**) and the corresponding figures for the unamended and 5% vermicompost-amended silty clay loam soil (S2, S2 V1, and S2 V2) spiked with diuron (**b**) and imidacloprid (**d**).

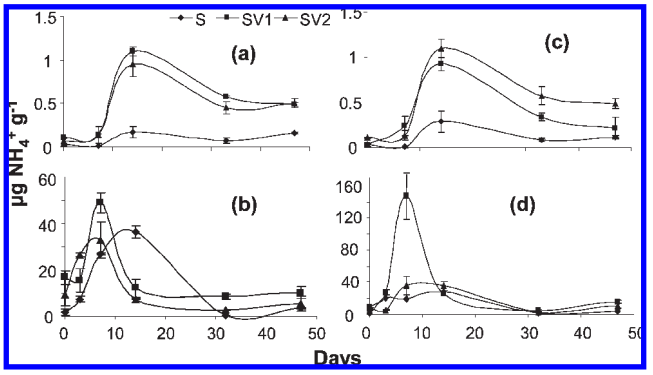

Figure 3. Urease activity in the unamended and 5% vermicompost amended sandy loam soil (S1, S1 V1, and S1 V2) spiked with diuron (**a**) and imidacloprid (**c**), and the corresponding figures for the unamended and 5% vermicompost-amended silty clay loam soil (S2, S2 V1, and S2 V2) spiked with diuron (**b**) and imidacloprid (**d**).

both vermicompost-amended soils presented higher dehydrogenase activity than the unamended soils (**Figure 2**), and this increment was more marked in the presence of vermicompost from spent grape marc (V1). However, in soil S1 (**Figure 2a**,**c**) at the end of the experiment, dehydrogenase activity was greater in S1 V2 than in S1 V1. With respect to soil S2, activity at the end of the experiment did not present any significant differences between treatments (**Figure 2b**,**d**). An initial difference in dehydrogenase activity between diuron and imidacloprid in sample S2 V1 (**Figure 2b**,**d**) was found. In this case, dehydrogenase activity at 0 days was lower and at 7 days higher in the sample spiked with imidacloprid than in the sample spiked with diuron.

Urease is an enzyme of great agricultural importance because it is involved in the decomposition of urea, which is used as a fertilizer (*22*). This enzyme forms complex relationships with soil materials, which protect them from decomposing. It plays an important role in soils by releasing NH_4^+ from the vermicompost. However, its inhibition could reduce the fertilizing effect of these organic amendments. In addition, the diuron molecule presents a urea structure; therefore, it could also be a substrate for this enzyme. Panels **a** and **c** of **Figure 3** show low urease activity in unamended soil S1 when spiked with diuron or imidacloprid, and the addition of the vermicomposts slightly increased this enzyme activity. In general, urease activity in soil S2 was much higher than in S1. This could be due to a high enzyme and clay content in this soil, which protects the enzyme by creating more stable complexes (*23*). As observed with

Table 2. Recoveries for Diuron and Imidacloprid and Their Metabolites in Unamended and Amended Soils S1 and S2 after 3 h of Shaking (Percent ± Standard Deviation)

	diuron	DPMU	DPU	DCA	imidacloprid	CNA
S1	93.54 ± 2.97	90.74 ± 4.04	87.31 ± 4.67	42.86 ± 3.67	97.64 ± 5.05	100.10 ± 0.27
S2	93.70 ± 1.50	85.53 ± 2.81	83.83 ± 0.66	57.15 ± 2.69	99.25 ± 0.62	97.75 ± 2.97
S1 V1	99.56 ± 1.29	96.58 ± 1.14	95.54 ± 2.24	28.41 ± 4.25	93.39 ± 5.45	92.26 ± 4.59
S2 V1	96.55 ± 1.19	89.15 ± 0.37	88.10 ± 4.60	37.04 ± 3.16	95.98 ± 0.22	98.51 ± 3.46
S1 V2	99.99 ± 1.18	95.11 ± 2.08	100.77 ± 3.36	33.82 ± 1.53	92.17 ± 4.62	91.92 ± 2.14
S2 V2	96.48 ± 0.96	88.26 ± 3.02	96.96 ± 5.03	46.09 ± 3.87	95.61 ± 0.53	85.54 ± 5.82

Table 3. Desorption of Diuron (D) and Imidacloprid (I) at Different Incubation Times in Unamended and Amended Soils S1 and S2 (Percent ± Standard Deviation)

		0 days	7 days	14 days	33 days	47 days	82 days	144 days
S1	D	32.63 ± 0.25	26.21 ± 0.11	24.22 ± 0.07	24.61 ± 1.24	21.21 ± 0.33	16.91 ± 0.20	13.00 ± 0.23
S1 V1	D	18.08 ± 0.20	13.37 ± 0.13	12.13 ± 0.40				
S1 V2	D	13.66 ± 0.12	13.85 ± 0.32	12.58 ± 0.13	8.81 ± 0.26	7.09 ± 0.12		
S1	I	69.04 ± 0.73	49.19 ± 0.36	47.16 ± 1.09	48.98 ± 0.57	49.55 ± 0.98	32.06 ± 0.39	30.75 ± 0.34
S1 V1	I	22.08 ± 0.35	18.42 ± 0.09	16.21 ± 0.36	19.81 ± 0.16	19.58 ± 0.35	12.25 ± 0.04	16.47 ± 0.37
S1 V2	I	28.83 ± 0.44	28.92 ± 0.34	27.92 ± 0.59	28.95 ± 0.26	28.58 ± 0.40	17.88 ± 0.06	21.26 ± 0.05
		0 days	3 days	7 days	14 days	33 days	47 days	62 days
S2	D	16.21 ± 3.05	12.72 ± 0.22	6.65 ± 0.77	6.85 ± 0.32	1.84 ± 0.29		
S2 V1	D	13.25 ± 2.26	9.06 ± 0.08	6.46 ± 0.13	8.21 ± 0.18	4.23 ± 0.63		
S2 V2	D	10.98 ± 1.87	8.95 ± 0.43	6.40 ± 0.23	7.58 ± 0.04	3.80 ± 0.32		
S2	I	33.80 ± 0.63	27.96 ± 1.05	20.81 ± 0.63	22.36 ± 0.36	19.21 ± 0.45	16.76 ± 0.22	24.65 ± 0.38
S2 V1	I	18.69 ± 1.00	16.34 ± 0.31	11.34 ± 0.54	13.88 ± 0.60	11.88 ± 0.12	11.17 ± 0.18	15.92 ± 2.08
S2 V2	I	23.30 ± 0.80	21.86 ± 2.03	14.52 ± 0.53	15.59 ± 0.06	14.20 ± 0.28	13.12 ± 0.13	16.86 ± 3.01

dehydrogenase activity, the vermicompost from spent grape marc (V1) showed the highest increment in urease activity among all of the soil samples (**Figure 3**). Also, in soil S2 V1 spiked with imidacloprid, urease activity was lower at 0 days and considerably higher at 7 days compared with the samples spiked with diuron.

Recoveries. Data relating to the extraction efficiency of imidacloprid and diuron and their metabolites are given in **Table 2**. The recoveries in most of the cases were >90% after 3 h of shaking. DCA showed very low extraction efficiency (**Table 2**).

Several trials with different solvents and microwave-assisted extraction (MAE) were carried out to improve DCA recovery (data not shown). Although the best results were obtained by MAE (up to 70%), chemical degradation of diuron and the other metabolites occurred under these conditions. Low DCA recoveries were also obtained by Polati et al. (*24*). These low recovery rates for the soil samples indicate that a large amount of DCA is retained, suggesting that this pollutant is strongly bound to soil and that much less leaching and potential contamination is to be expected in relation to the chemicals studied.

Desorbed, Extracted, and Remaining Fractions. Table 3 shows the desorbed amounts of diuron and imidacloprid in water during the incubation time. In all samples, the results show that the desorbed amount of diuron decreased significantly as incubation time increased ($P < 0.05$). At the beginning of the incubation, the vermicompost addition to S1 reduced the desorbed amount of diuron from 32.63 to 18.08 and 13.66% for S1 V1 and S1 V2, respectively. After 144 days, the desorbed amount fell to 13% in unamended soil S1. However, in V1 amended soil at 33 days, the desorbed amount was below the limit of determination, whereas in V2 amended soil, this occurred after 47 days. This means that diuron availability decreased more quickly in soil S1 amended with V1 and much more slowly in the unamended soil. In soil S2, the desorbed amount of diuron at the beginning of incubation was almost half that for S1, and at a shorter incubation time (47 days) diuron could not be detected in any of the soil samples. This suggests that the vermicompost had less influence on the amount desorbed in S2 compared with that observed in S1.

Figures 4 and **5** show the three main pesticide fractions (desorbed, extracted, and remaining) of diuron and imidacloprid detected in the experiment. After incubation in sandy soil S1, around 60% of the diuron applied is still in the soil and therefore susceptible to be desorbed and leached. However, the addition of vermicompost considerably reduced the desorbed and extracted fractions. Therefore, after 144 days of incubation, all of the diuron was transformed or considerably sorbed by the amendments. In silty clay loam soil S2, no important differences were observed between unamended and amended samples. After 61 days, only around 10% of diuron remained in the extracted phase and no desorbed fraction was detected. Furthermore, the remaining fraction in S2 increased more rapidly than in S1 for all of the treatments in the first 7 days.

With respect to imidacloprid (**Table 3**), higher values of the desorbed fraction were observed than for diuron. As observed in previous studies, soil S1 had lower sorption capacity of imidacloprid (*4*). The vermicompost addition significantly decreased the availability of this insecticide ($P < 0.05$). The highest reduction in the desorbed fraction occurred with V1, falling from 69.04 to 22.08% in S1 and from 33.80 to 18.68% in S2. With regard to incubation time, we found significant differences in imidacloprid desorption between the beginning and end of the experiment in all of the samples ($P < 0.05$) except for S2 V1. Nevertheless, at the end of the experiment, large amounts of imidacloprid were still determined in water (**Figure 5**). In soil S1, the desorbed fraction was observed to be higher than that for amended samples S1 V1 and S1 V2.

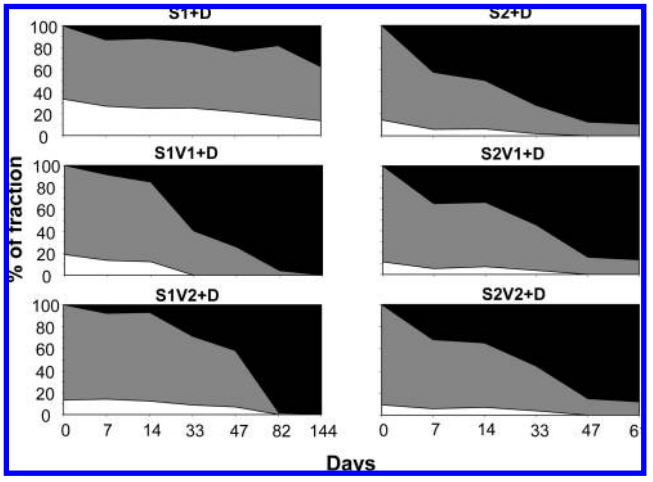

Figure 4. Desorbed (white), extracted (gray), and remaining (black) fractions during the incubation of diuron in unamended and amended soils S1 and S2.

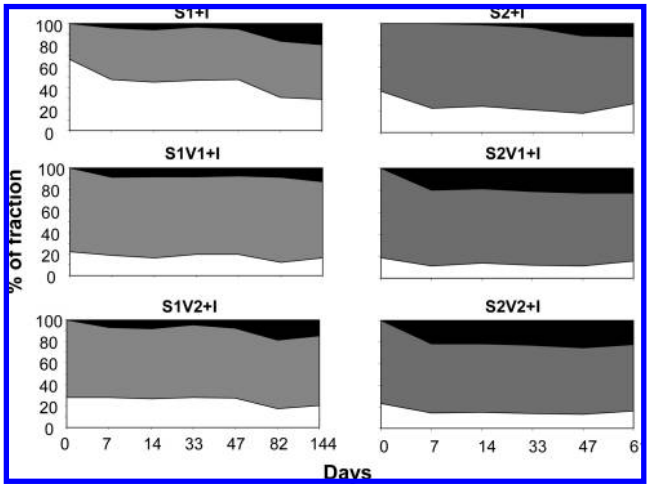

Figure 5. Desorbed (white), extracted (gray), and remaining (black) fractions during the incubation of imidacloprid in unamended and amended soils S1 and S2.

However, after incubation, the amount susceptible to leaching was around 80% in all samples. Again, in S2, the desorbed fraction is higher in the unamended soils than in the amended soils, although no major differences were observed between both vermicomposts. After incubation, >78% of imidacloprid is potentially available for lixiviation, which was also true for S1. Although the remaining fraction of imidacloprid was similar for unamended and amended samples from S1, it was greater and more constant in soil S2 amended with vermicompost (**Figure 5**).

Modelization. *Diuron*. Diuron degradation in all of the samples closely conforms to the SFO kinetic, presenting values of $R^2 > 0.94$ and χ^2_{calcd} values equal to χ^2 tabulated values ($\chi^2_{5,0.05}$ $\chi^2_{calcd} = 11.07$). In unamended soil S1, the degradation kinetic showed the lowest degradation rate (k) with a DT_{50} of 259 days and DT_{90} of 862 days (**Table 4**). The only metabolite susceptible to determination was DPMU. However, in amended S1 samples, the degradation rate was considerably higher, with the DT_{50} reduced to 25 and 41 days for S1 V1 and S1 V2, respectively. In these amended samples, DPMU and DPU metabolites were found, but no DCA was detected. Although DCA was probably produced, it was not detected due to its low recovery rate. This hypothesis can be confirmed by the results obtained previously under similar conditions with ^{14}C-diuron in samples S1, S1 V1, and S1 V2 (*11*), where a small amount of $^{14}CO_2$ was detected after 77 days.

Table 4. Parameters of Diuron Dissipation Based on the SFO Model for Unamended and Vermicompost-Amended Soils S1 and S2

	$C_i \pm SD^a$ (%)	C_i^b (%)	k ($\times 10^{-3}$ days^{-1})	R^2	DT_{50} (days)	DT_{90} (days)	err_{scaled}
S1	97.89 ± 0.53	91.70	2.67	0.99	259	862	3.13
S1 V1	97.83 ± 1.73	104.89	27.5	0.94	25	83	4.87
S1 V2	100.67 ± 1.49	108.48	16.8	0.94	41	137	9.82
S2	112.08 ± 6.05	100.93	42.1	0.98	16	55	5.24
S2 V1	115.23 ± 2.26	107.92	31.5	0.98	22	73	5.93
S2 V2	114.21 ± 2.16	111.36	33.2	0.98	21	69	5.20

[a] Experimental C_i values. [b] C_i values worked out with the model (optimized by ordinary least-squares).

In soil S2, the diuron degradation rate was faster and its DT_{50} of 16.5 days was the lowest (**Table 4**). However, only a small amount of DPMU was determined. When vermicomposts were added to the soil, slight differences were observed. As degradation rates decreased, the DT_{50} rose slightly to 22 and 21 days for S2 V1 and S2 V2, respectively, and DT_{90} values were always <80 days. Due to the high degradation rate and the limited presence of DPMU in S2, S2 V1, and S2 V2, the sink fraction was considerably higher than in S1, S1 V1, and S1 V2.

Imidacloprid. Because the degradation of imidacloprid is much slower than that for diuron, its persistence in soil is longer. CNA was not found in any of the samples. In general, the SFO kinetic model did not show good R^2 values, although data were validated with the χ^2 test ($\chi^2_{5,0.05} = \chi^2_{calcd} = 11.07$). It can be seen (**Figure 6**) that some cases showed a fast initial dissipation phase followed by a slower degradation phase. Imidacloprid degradation was also therefore modelized using two different biphasic models. The FOMC and hockey-stick models were validated through the χ^2 square test ($\chi^2_{3,0.05} = \chi^2_{calcd} = 9.49$ and $\chi^2_{4,0.05} = \chi^2_{calcd} = 7.81$, respectively). The unamended soil S1 presented the lowest err_{scaled} for the SFO kinetic model; however R^2 and DT_{50} were similar in all of the models assayed. S1 V1 and S1 V2 experimental values were better adjusted to the hockey-stick model (**Table 5**). In unamended soil S2, degradation presented the best fit ($R^2 = 0.92$) for the FOMC model, and DT_{50} was 232 days and DT_{90} was 1223 days. The degradation process in amended soils S2 V1 and S2 V2 differed, marked by a rapid dissipation in the first week followed by a much slower second phase. The hockey-stick model fits better with these samples ($R^2 > 0.84$). The fast reduction in the amount of insecticide found in the first week of incubation may be due to sorption processes rather than degradation. In fact, addition of these vermicomposts to these soils increased significantly the sorption of imidacloprid (data partially published in ref (*4*)).

DISCUSSION

In general, the addition of vermicompost increased dehydrogenase activity, and there was a direct relationship between OC content and dehydrogenase activity at 0 days ($R^2 = 0.70$; $P < 0.01$) in the amended and unamended soil samples. No direct relationship could be observed between pesticide degradation and enzymatic activities, and neither diuron nor imidacloprid appeared to have any significant effect on enzymatic activities. The toxic effects of diuron on soil microbiota following degradation are reported in the literature (*25*, *26*). However, this possible toxicity was not reflected in the enzymatic activity analyzed in our study. Imidacloprid appeared to have only a slight toxic effect in S2 V1 at the beginning of the incubation process. Imidacloprid initially inhibited dehydrogenase activity and, then, after a certain period of time, enzymatic activity increased again (**Figure 2d**). The increment was more pronounced in urease activity (**Figure 3d**).

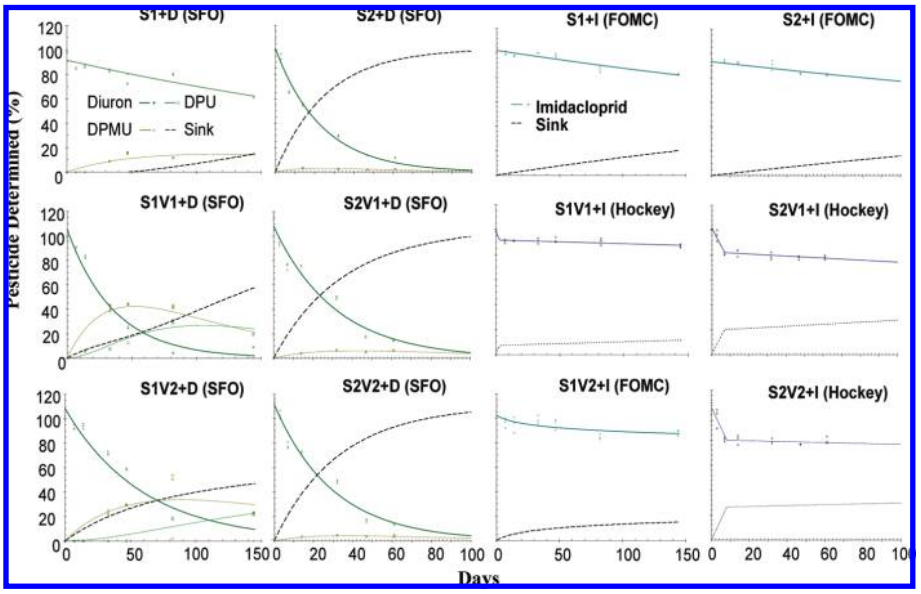

Figure 6. Modelization of diuron (D) and imidacloprid (I) dissipation (SFO, single first-order kinetic; FOMC, first-order multicompartment kinetic; Hockey, hockey-stick model).

Table 5. Parameters of Imidacloprid Dissipation Based on the SFO, FOMC, and Hockey-Stick Models for Unamended and Vermicompost-Amended Soils S1 and S2

Single First-Order (SFO) Model

	$C_i \pm$ SD[a] (%)	C_i[b] (%)	k ($\times 10^{-3}$ day^{-1})	R^2	DT_{50} (days)	DT_{90} (days)	err_{scaled}
S1	103.71 ± 2.51	102.03	1.52	0.87	455	1510	1.89
S1 V1	99.04 ± 1.39	93.23	0.51	0.40	1363	4527	1.99
S1 V2	103.61 ± 3.33	98.82	1.02	0.45	680	2258	2.90
S2	89.48 ± 3.07	93.10	1.89	0.63	366	1217	1.78
S2 V1	100.61 ± 0.38	91.96	3.56	0.52	195	646	4.58
S2 V2	102.75 ± 7.36	92.98	3.84	0.43	180	598	6.14

First-Order Multicompartment (FOMC) Model

	C_i[b] (%)	α	β	R^2	DT_{50} (days)	DT_{90} (dyas)	err_{scaled}
S1	102.41	4.07	2604.38	0.89	478	1979	2.03
S1 V1	–	–	–	–	–	–	–
S1 V2	102.35	0.06	8.98	0.69	$>10^4$	$>10^4$	2.82
S2	93.10	195.40	103246.50	0.92	233	1223	1.96
S2 V1	100.97	0.06	0.54	0.87	$>10^4$	$>10^4$	2.63
S2 V2	103.23	0.07	0.58	0.77	7989	$>10^4$	4.50

Hockey-Stick Model

	C_i[b] (%)	t_b (days)	K_1 (day^{-1})	K_2 ($\times 10^{-3}$ day^{-1})	R^2	DT_{50} (days)	DT_{90} (days)	err_{scaled}
S1	103.70	2.23	0.01	1.3	0.90	518	1762	2.28
S1 V1	99.04	2.55	0.03	0.3	0.76	2075	7473	0.80
S1 V2	103.61	2.00	0.03	0.8	0.73	834	2956	2.96
S2	–	–	–	–	–	–	–	–
S2 V1	101.93	7.00	0.03	0.8	0.93	626	2632	4.69
S2 V2	104.65	7.67	0.03	0.3	0.84	1380	6218	7.60

[a] Experimental C_i values. [b] C_i values worked out with the model (optimized by ordinary least-squares). [c] –, the model did not fit the data.

This could be explained by the release of easily degraded material from dead cells due to the initial toxic effect of imidacloprid at the beginning of the incubation process. As a consequence, the imidacloprid degradation rate may be affected. Nevertheless, no references to this phenomenon have been found in the literature, and further studies should be done.

We found a higher degradation rate for diuron and smaller amounts of metabolites in unamended and amended soil S2, which could be explained by the higher level of urease activity detected in these samples compared with S1. Because urease enzymes are said to attack the carboxyl group in the urea compounds (*22*), a rapid transformation of diuron, DPMU,

and DPU can occur, thus releasing DCA, which is then strongly adsorbed. Some studies also report that phenylurea herbicides sorb less in soils than aniline metabolites (*26*).

Figure 4 shows that the dissipation rate for diuron in the aqueous phase is lower than the incremental rate in the remaining phase. This is explained by the reduction in the desorbed phase of pesticides due to the rate of degradation, whereas the increment in the remaining fraction is attributed to degradation and the aging process (*27*). This aging of pesticides is related to diffusion to less accessible sorption sites. Although additions of vermicompost enhance diuron's dissipation rate in S1 in the aqueous phase, previous studies have shown that they do not enhance the aging process (*11*). The dissipation of diuron was more marked in unamended and amended soil S2 than in S1. The presence of clay in S2 contributes to the formation of micropores where diuron can diffuse. This characteristic together with diuron's high rate of dissipation and the small amount of metabolites proves that so-called bound residues are formed. Also, it is possible that part of a pesticide's sorbed fraction is degraded by microorganisms (*6*).

Imidacloprid behaved differently (**Figure 5**). As previous studies have shown (*4*, *11*, *18*, *28*), imidacloprid is less sorbed than diuron due to its higher solubility, and the addition of vermicompost significantly increases the sorption capacity of both soils and both pesticide residues (**Table 3**). However, this greater availability of imidacloprid does not lead to a higher rate of degradation, as degradation depends not only on the availability of the pesticide. The apparent aging effect is very low compared with diuron. As mentioned by other authors (*14*) the larger size of the imidacloprid molecule may prevent it from entering the clay's interlayer and the soil's micropores. This possible effect and the higher solubility of imidacloprid may limit the formation of aging residues.

As the experiments were carried out in the dark and hydrolysis of both pesticides is negligible at the pH and temperature levels studied (*14*, *29*), we can therefore state that the degradation measured was mainly microbiological in nature. Microbial degradation has been reported to be the main cause of diuron dissipation in soils (*13*). Diuron-degrading microorganisms have actually been isolated in laboratory cultures (*30*). However, it is believed that the degradation of diuron is caused by a bacterial consortium rather than by single strains (*31*). Some research has indicated that the addition of exogenous amendments can enhance pesticide degradation due to the introduction of exogenous microorganisms (*32*), and dehydrogenase results show that microbial activity is stimulated by vermicompost additions, possibly explaining the lower DT_{50} in S1 amended samples (**Table 4**). Other researchers have found that pesticides are less available for degradation due to the higher sorption of the amended samples (*33*). This could explain the slight increase in diuron's DT_{50} in amended soils S2 in relation to the unamended soil (**Table 4**). In all of our experiments, the half-life of diuron is in line with that found in the literature (*13*).

The DT_{50} values for imidacloprid reported in the literature range from 33 to 129 days (*3*, *34*, *35*), which are much lower than in our experiments (**Table 5**). Many studies have demonstrated imidacloprid's sensitivity to photodegradation and hydrolysis at alkaline pH values, which reduce its half-life considerably (*3*, *14*, *36*). The absence of photodegradation and the pH values of our samples (**Table 1**) contribute to the stability of imidacloprid molecules in these soils. In line with our study, other research has found a slow dissipation rate for imidacloprid in Mediterranean soils (*37*). In addition, the absence of microbial activation as well as the slight toxic effect observed in sample S2 V1 and the low degradation rate of imidacloprid indicate the absence of a biotic component capable of degradation. Both arguments may explain the long half-life presented by this insecticide, even though other studies have indicated that organic amendments enhance imidacloprid degradation (*38*). Either vermicompost additions from wine waste protect the compound from degradation, as already observed by Rouchaud et al. (*34*), or the microorganisms tend to degrade the more labile organic fractions in the amendments rather than the target pesticide (*38*).

In general, the addition of vermicompost enhanced diuron dissipation in the sandy loam soil, whereas it only reduced desorption in the silty clay loam soil. In the case of imidacloprid, the addition of vermicompost increased its sorption slightly, although the vermicomposts did not enhance the dissipation of this molecule.

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