

## Effect of Spent Mushroom Substrate Amendment of Vineyard Soils on the Behavior of Fungicides: 2. Mobility of Penconazole and Metalaxyl in Undisturbed Soil Cores

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Mobility of fungicides penconazole and metalaxyl in unamended and amended vineyard soils with fresh and composted spent mushroom substrates (F-SMS and C-SMS) was studied. Experiments were performed in non-incubated and incubated (outdoors for 77 days) undisturbed soil cores under non-saturated flow conditions. Breakthrough curves (BTC) of metalaxyl leaching were delayed with regard to the tracer ion, and they showed an incomplete leaching in all soil cores after the addition of 2.5–4.5 pore volumes. A decrease of the maximum peak concentration in C-SMS soils with regard to unamended soils (up to 24-fold in Viana soil) and an increase in the fungicide retention by soils (up to 8-fold in the first segment of Viana soil core) was observed. However, a decrease (up to 2.6-fold in Sajazarra soil) or an increase (up to 1.4-fold in Aldeanueva soil) of the maximum peak concentration in F-SMS soils was observed. No leaching of penconazole was observed in all cases. After fungicide incubation in soil cores, the amounts of metalaxyl retained and leached decreased significantly by 1.24–37.8-fold and 1.17–302-fold, respectively, whereas no changes were observed for penconazole. Degradation of metalaxyl occurred in non-incubated soil and increased after incubation (two metalaxyl metabolites were detected in the leachates and the soil extracts), but it was not seen for penconazole in any case. In consequence, the addition of SMS as amendment to soil (especially C-SMS) enhanced adsorption of both fungicides, although metalaxyl could be available for degradation. This effect contributes to prevent groundwater contamination by metalaxyl, but it could contribute to increase the surface water contamination by penconazole, because adsorption protects this fungicide from degradation, increasing its persistence in soils.

**KEYWORDS:** Fungicide; vineyard soil; soil core; spent mushroom substrate; incubation; mobility; degradation

### INTRODUCTION

The use of organic wastes from agricultural and industrial activities as soil amendments and fertilizers is recognized as an alternative method for the ultimate disposal of liquid and solid wastes and, at the same time, as a way of maintaining or increasing soil fertility (1). In this sense, current environmental European Union (EU) legislation is pressing to manage organic wastes more effectively (2). They need to be diverted from landfill, and it could be used in activities different from those where they were generated with no risk for human health or the environment. Mushroom farming is looking for environmental, agricultural, and industrial uses for spent mushroom substrates (SMS) generated on the farm in increasing quantities (3). SMS have been used as biofuel, for animal feeding, for mushroom recultivation, for soil bioremediation or biodegradation of organic pollutants, as a

soil conditioner, amendment, and fertilizer, for remediation of acidic mine wastewater, and as a sorbent for various heavy metals and organic pollutants (4, 5).

In the La Rioja region (northern Spain), the production of mushroom represents the second major activity in this region after vineyard farming. The mushroom industry in this region generated an estimated 325 000 tons of SMS in 2007 from the production of *Agaricus bisporus* (82%), *Pleurotus* spp. (15%), and *Lentinula edodes* or Shiitake (3%) (6). This organic waste, owing to its high organic matter (OM) content and availability of essential plant nutrients, could be exploited as a soil fertilizer and amendment to increase the OM contents of vineyard soils in this region, which are generally low.

It is well-known that dynamics (adsorption, mobility, and degradation) of non-ionic pesticides in soils can be affected by the addition of organic amendments (7). Some studies indicated the reduced leaching of pesticides in amended soils because their adsorption by the amendment OM was increased (8–10), whereas in other studies, the presence of exogenous OM increased

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**Table 1.** Physicochemical Characteristics of Unamended and F-SMS or C-SMS Soils

soil	pH	OM (%)	DOC <sup>a</sup> (mg L <sup>-1</sup> )	CaCO <sub>3</sub> (%)	C/N	sand (%)	silt (%)	clay (%)	clay mineralogy <sup>b</sup>
AL	7.8	1.04	35.7	11.3	8.2	64.4	14.2	21.4	I, K, M, Chl
AL + C-SMS	7.8	1.41	43.1	11.3	7.8	59.1	15.2	25.7	I, K, M, Chl
AL + F-SMS	7.8	1.46	48.5	11.2	8.1	56.3	18.3	25.4	I, K, M, Chl
SA	7.7	1.74	33.2	27.9	9.6	57.9	10.5	31.6	I, K, Chl-I
SA + C-SMS	7.8	1.74	36.8	32.0	9.9	51.3	23.3	25.4	I, K, Chl-I
SA + F-SMS	7.8	1.81	37.2	29.9	9.1	54.7	10.4	34.9	I, K, Chl-I
V	7.8	2.54	65.7	34.6	10	51.8	13.5	34.7	I, K, Chl-I
V + C-SMS	7.7	2.83	66.7	30.7	8.9	50.5	14.3	35.2	I, K, Chl-I
V + F-SMS	7.6	2.96	74.8	30.3	17	48.8	13.9	37.3	I, K, Chl-I

<sup>a</sup>Determined in soil extracts (1:2 ratio) after 24 h of agitation at 20 °C. <sup>b</sup>I, illite; K, kaolinite; M, montmorillonite, Chl-I, chlorite-illite interstratified.

the mobility of pesticides (11, 12). Dissolved organic matter (DOM) derived from the amendments may reduce pesticide adsorption and increase leaching because of the lipophilic interactions between DOM and organic molecules, competition for adsorption sites, or the incomplete interaction of pesticides with soil constituents (7, 11). Modified leaching of pesticides in amended soils may also be due to structural changes in the porosity induced by the higher organic carbon content (13).

The SMS contain high amounts of solid and dissolved OM, which could modify the dynamics of fungicides widely applied in vineyard soils, with implications on their persistence in soil and transport to groundwater. Understanding of fungicide leaching in soils amended with SMS is important and allows assessment of the potential risk of ground and surface water contamination.

Most of the laboratory leaching studies are performed in homogeneous, uniformly packed soil columns under saturated steady-state flow conditions and are therefore not typical of natural field conditions (14). Soil columns taken from the field maintain soil macrostructure undisturbed within the soil profile, and physicochemical and microbiological characteristics of soil are also retained. Use of undisturbed soil columns in pesticide leaching studies has the potential to provide data that are more representative of field conditions (15).

As part of the ongoing project into the evaluation of environmental impact in soil and water of fungicides applied to vineyards previously amended with SMS, studies on adsorption (10.1021/jf902108n) (16) and mobility of fungicides usually applied to vineyards have been carried out. The aim of this work was to study the mobility of the fungicides, metalaxyl and penconazole, with different characteristics, in three unamended and amended with fresh or composted SMS (F-SMS and C-SMS) vineyard soils using undisturbed soil cores. Experiments were performed under non-saturated flow conditions in non-incubated and incubated (outdoors for 77 days) soil cores to study the influence of aging on the mobility of fungicides.

Some studies on the mobility of these compounds in soils indicated high soil organic carbon/water partition coefficient ( $K_{oc}$ ) values and low mobility for penconazole, whereas metalaxyl presented low  $K_{oc}$  values and higher leaching and, therefore, could be considered as a potential pollutant for aquifer contamination (17–19). However, a literature search revealed that no studies have been performed on the transport behavior of these fungicides in the presence of SMS.

## MATERIALS AND METHODS

**Experimental Field Sites.** Experimental field sites were located in three different vineyard areas named Aldeanueva (AL) (42°14'0"N latitude and 1°53'0"W longitude), Sajazarra (SA) (42°35'0"N latitude and 2°57'0"W longitude), and Viana (V) (42°30'0"N latitude and 2°20'0"W longitude), all of them in the La Rioja region (northern Spain). Each field site was divided in three field subsites with different treatments

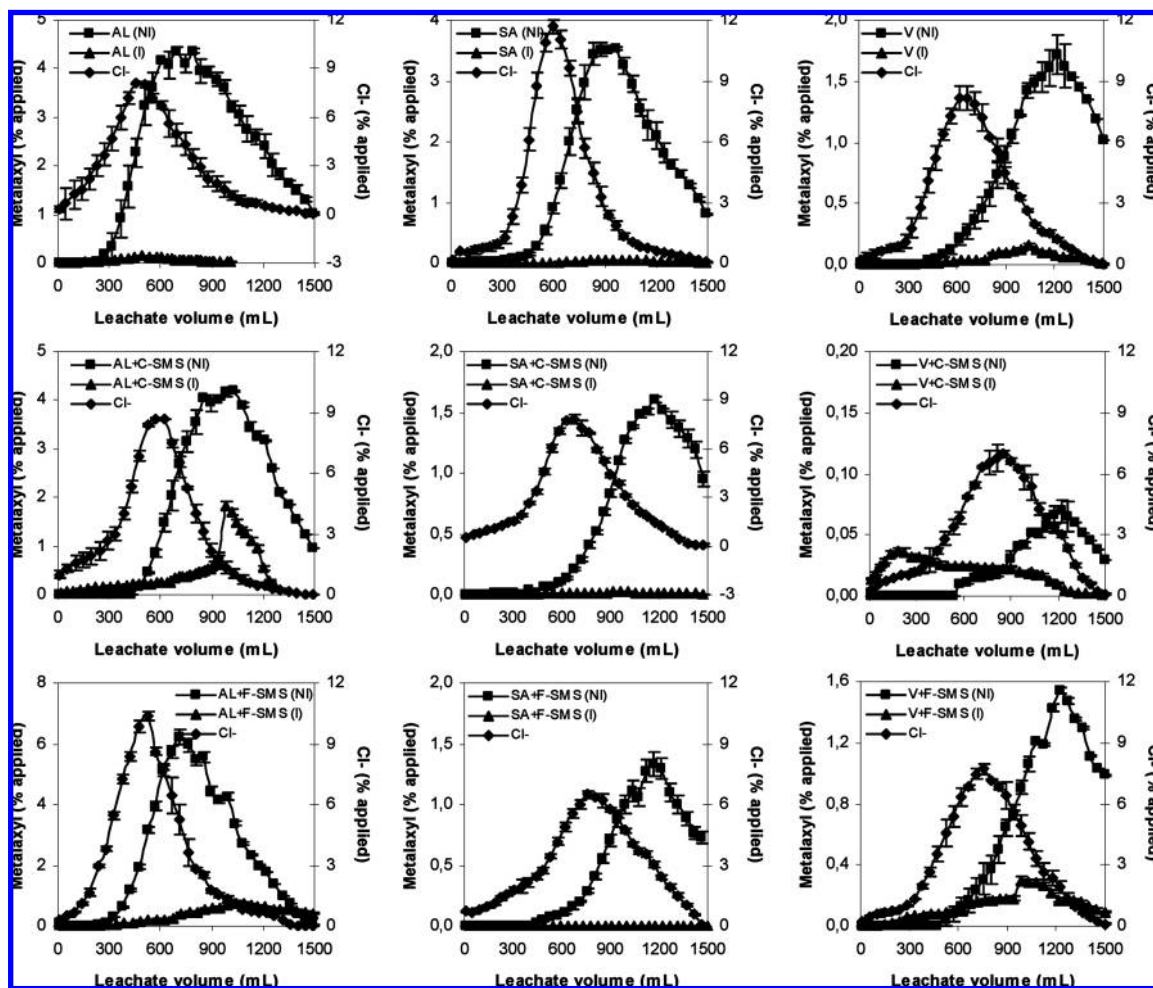
(unamended soil, amended soil with F-SMS, and amended soil with C-SMS). The amendment was homogeneously applied at the agronomic rate of 25 tons ha<sup>-1</sup> and incorporated to the 0–30 cm soil layer. Soils were classified as Fluventic Haplocambids (AL), Typic Calcixerepts (SA), and Typic Xerorthents (V) (20) and were sandy clay loam. Their characteristics determined by usual methods of soil analysis (21) are included in **Table 1**. Dissolved organic carbon (DOC) in a 1:2 (w/v) water extract was determined by a Shimadzu 5050 (Shimadzu, Columbia, MD) organic carbon analyzer. Clay minerals were qualitatively identified by the X-ray diffraction technique using a Philips PW 1710 diffractometer (Eindhoven, The Netherlands).

**SMS.** F-SMS and C-SMS from *A. bisporus* cultivation were supplied by Intraval, TRADEBE Environmental Group, S.L. (La Rioja, Spain) and applied as explained above. SMS is a pasteurized mixture of cereal straw and poultry litter, ammonium nitrate, urea, and minerals (gypsum and/or calcium carbonate). F-SMS is obtained immediately after it is removed from mushroom houses. Alternatively, SMS is placed in compost piles (2.5 m high) and further composted for several weeks under aerobic conditions to obtain C-SMS. Previously, the material is mixed with woodchips to favor its aeration, and it is turned periodically to allow its maturation and decomposition, which increase the uniformity and stability level of the C-SMS. DOC in a 1:100 (w/v) water extract was determined as previously indicated. The total organic carbon (OC) and DOC (expressed as a percentage of the total OC) were 28.8 and 13.3% for F-SMS and 27.4 and 4.34% for C-SMS, respectively. Other characteristics of these residues have been presented elsewhere (10.1021/jf902108n) (16).

**Chemicals.** Fungicides metalaxyl and penconazole (>99% purity) were supplied by Novartis Crop Protection AG (Basel, Switzerland). Metalaxyl metabolites CGA62826 (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine) and CGA67868 (*N*-methoxyacetyl-2,6-dimethyl-aniline) were kindly supplied by Syngenta Crop Protection (Basel, Switzerland). Penconazole is a solid compound with a water solubility of 73 mg L<sup>-1</sup> (20 °C) and a log  $K_{ow}$  of 3.72 (pH 5.72, 25 °C), while metalaxyl is a solid compound with a water solubility of 8400 mg L<sup>-1</sup> (22 °C) and a log  $K_{ow}$  of 1.75 (25 °C) (22).

**Soil Core Sampling and Leaching Experiments.** Stainless-steel tubes of 40 cm (length) × 9 cm (inner diameter) were used for sampling undisturbed soil cores. Four replicate of soil cores were taken from each field subsite (36 cores in total) in October 2007 by gently pushing the holding tubes into the soil. After removal from the field, the tubes were sealed at the base and top with a steel lid and they were stored at 4 °C until leaching. Before the experiment was started, the base was sealed with a 1.0 mm nylon mesh held in place with waterproof tape and a filter paper was placed between the mesh and soil to prevent the soil from falling out of the core. The cores were then fixed at their base to 9 cm diameter polypropylene filter funnels with silicone. The outlet from the filter funnels fed directly into separate 125 mL glass bottles. The cores were over-saturated with water and allowed to drain the excess of water freely for 24 h, so that humidity conditions were equivalent to field capacity. The pore volume (PV) of the intact cores was determined by the weight difference of water-saturated cores versus dry cores.

The fungicides were applied (10 mg of each) evenly across the surface by pipet as a mixture of the standard fungicides in 10 mL methanol/water (46:54) to give a concentration on the soil close to 2.5 mg kg<sup>-1</sup> penconazole or metalaxyl. Conservative tracer leaching using chloride as an ion tracer (KCl) was implemented to describe the dispersive characteristics of the



**Figure 1.** BTCs for metalaxyl and the ion chloride leaching in non-incubated (NI) and incubated (I) soils (AL, SA, and V) unamended or amended with C-SMS or F-SMS. Error bars represent the standard error of the mean value ( $n = 2$ ).

cores for the fungicide leaching studies. The amount of chloride ion applied was 250 mg (5 mL from a KCl solution of 50 g L<sup>-1</sup> in water).

A total of 18 cores were mounted indoors, and the leaching experiment was started 24 h after adding the fungicides. The other 18 cores were mounted outdoors, exposed to natural weather conditions for 77 days before starting the leaching experiment to study the influence of aging on the mobility of fungicides in soil. In this period, temperatures ranged between -3.6 and 27.7 °C (mean value of 10.6 °C) and relative humidity ranged between 46 and 91% (mean value of 69.7%).

The cores were leached with 50 mL (equivalent to 0.8 cm) of water every day (unsaturated flow) up to a total of 1500 mL (2.5–4.5 PV), and leachate samples were collected in glass bottles. Glass bottles were replaced every day. The volume of drainage water from each core was recorded, and the leachate samples were kept at 4 °C until analyzed (usually within 3 days). Each water sample was centrifuged at 5045 g and filtered through a 0.45 μm GHP acrodiscs filter (Waters Corporation).

After draining for 30 days, the experiment was stopped. The cores were cut breadthwise into five segments at 8 cm intervals. The soil contained in each segment was turned over, weighed, and sieved by 2 mm. Duplicate 5 g soil samples were taken from each segment and shaken for 24 h with 10 mL of methanol to determine the fungicide content. The recoveries of the method were determined by spiking 5 g of soil with a standard mixture containing metalaxyl, its two metabolites, and penconazole to a final concentration of 1 mg kg<sup>-1</sup> and performing the extraction procedure as depicted above. The recoveries depended upon the soil and soil amendment and ranged between 94 and 97%, 93 and 98%, and 97 and 99% for metalaxyl, its metabolites, and penconazole, respectively.

**Analysis of Leachate Samples and Soil Extracts.** In the leachate samples and soil extracts, fungicides were quantified by high-performance liquid chromatography/diode array detector/mass spectrometry (HPLC/

DAD/MS) using Waters equipment (Waters Association, Milford, MA). The column used was Waters Symmetry C-18 (75 × 4.6 mm inner diameter and 3.5 μm) at ambient temperature and an isocratic mobile phase flow rate of 0.4 mL min<sup>-1</sup>. The mobile phase was acetonitrile/water/formic acid (30:70:0.1, v/v/v), and the injection volume was 20 μL. Detection by HPLC/DAD was at 194.0 nm for metalaxyl and its metabolites and 201.7 nm for penconazole. Quantification of these compounds by HPLC/MS was carried out by monitoring the positive molecular ion ( $m/z$ ) 280.2 for metalaxyl, 266.2 and 194.2 for metalaxyl metabolites 1 and 2, respectively, and 284.1 for penconazole. The retention times of metalaxyl metabolites 1 and 2, metalaxyl, and penconazole were 2.8, 2.9, 3.4, and 5.6 min, respectively.

The chloride ion concentration was determined using a Metrohm ion chromatograph (Metrohm Ltd., Switzerland).

**Determination of Retardation Factors.** Theoretical retardation factors,  $R$ , were determined as indicators of the shifts of breakthrough curve (BTC) maximum peaks for pesticide leaching in unamended and amended soil cores. These factors were calculated according to the expression  $R = 1 + K_f \rho / \theta$  (23), where  $R$  is the retardation factor,  $\rho$  is the density of the soil (g cm<sup>-3</sup>),  $\theta$  is the volumetric water content, and  $K_f$  is the adsorption constant of pesticides by the core soil.  $K_f$  values taken from our previous work (10.1021/jf902108n) (16) were obtained for the same soils, although in disturbed and sieved (fraction < 2 mm) form (24).

## RESULTS AND DISCUSSION

**Metalaxyl Leaching in Soil Cores.** BTCs of metalaxyl with water from cores of unamended and F-SMS or C-SMS soils (AL, SA, and V), non-incubated and incubated, are shown in **Figure 1**. This figure also includes BTCs of the chloride tracer ion obtained in the non-incubated soil cores.



**Table 2.** Pore Volume and Concentration (% of Applied) of the Maximum Peak for the Chloride Conservative Ion and Metalaxyl in Non-incubated and Incubated Cores of Unamended and F-SMS or C-SMS Soils

soil	chloride maximum peak		metalaxyl maximum peak	
	pore volume	concentration	pore volume	concentration
Non-incubated Soil Cores				
AL	1.06 ± 0.06	8.08 ± 0.05	1.60 ± 0.05	4.37 ± 0.13
AL + C-SMS	1.08 ± 0.06	8.64 ± 0.09	1.96 ± 0.06	4.19 ± 0.03
AL + F-SMS	1.13 ± 0.08	10.4 ± 0.09	1.53 ± 0.05	6.21 ± 0.78
SA	1.83 ± 0.03	11.7 ± 0.43	3.12 ± 0.06	3.53 ± 0.04
SA + C-SMS	1.77 ± 0.14	7.82 ± 0.42	3.08 ± 0.25	1.60 ± 0.35
SA + F-SMS	2.11 ± 0.07	6.50 ± 0.24	3.47 ± 0.11	1.33 ± 0.14
V	0.95 ± 0.05	8.19 ± 1.83	1.88 ± 0.05	1.71 ± 0.23
V + C-SMS	1.43 ± 0.04	7.02 ± 1.17	2.04 ± 0.07	0.07 ± 0.01
V + F-SMS	1.48 ± 0.02	7.70 ± 0.38	2.40 ± 0.04	1.54 ± 0.14
Incubated Soil Cores				
AL	0.81 ± 0.03	5.65 ± 0.06	0.81 ± 0.04	0.15 ± 0.05
AL + C-SMS	0.80 ± 0.02	5.32 ± 0.09	1.94 ± 0.09	1.83 ± 0.08
AL + F-SMS	0.70 ± 0.03	8.27 ± 0.15	2.48 ± 0.10	0.74 ± 0.03
SA	1.41 ± 0.05	7.55 ± 0.13	2.75 ± 0.03	0.06 ± 0.01
SA + C-SMS	1.34 ± 0.03	6.25 ± 0.08	3.32 ± 0.12	0.02 ± 0.00
SA + F-SMS	1.46 ± 0.04	8.60 ± 0.23	2.82 ± 0.05	0.01 ± 0.00
V	0.99 ± 0.03	6.07 ± 0.18	1.94 ± 0.04	0.16 ± 0.02
V + C-SMS	1.00 ± 0.02	6.01 ± 0.31		
V + F-SMS	2.19 ± 0.08	6.02 ± 0.07	3.23 ± 0.04	0.30 ± 0.04

Tracer ions are known to travel water-like through the soil profile, and differences in the leaching of fungicides with respect to the ion tracer leaching should not be related to water flow (24). According to this, BTCs of the chloride ion should reach a maximum at about 1 PV, because it is generally expected to occur in the percolation of conservative ions, which does not undergo retention or degradation in soils. However, BTCs of the chloride ion in our nondisturbed soil cores showed differences in peak position and chloride profile shape for the three soils assayed. The maximum peak PV ranged between 0.95 and 2.19, being higher for the amended soils (Table 2). These effects indicated that water movement under non-saturated conditions was not totally regular in all soil cores studied. Differences in the transport of the tracer ion in soil cores have already been indicated by other authors. Vincent et al. (23) suggested a slight degree of physical non-equilibrium transport to explain differences in the transport of the tracer ion Br<sup>-</sup> in undisturbed soil cores sampled in forest or cultivated soils. On the other hand, Sharma and Taniguchi (25) indicated a hysteresis effect of the Br<sup>-</sup> tracer ion to justify the observed differences for the tracer ion movement in a loam and sand soil column under a water flow in intermittent conditions.

The BTCs obtained for leaching of metalaxyl in all soil cores under a non-saturated water flow had an asymmetrical shape with a final tail, indicating a slow and incomplete leaching of fungicide at the end of the experiment. These curves were delayed with regard to tracer ion BTCs in all soils (0.61–1.36 and 0–1.98 PV in non-incubated and incubated soil cores) and showed a greater degree of asymmetry than those of the chloride ion. The peak of the maximum concentration was found for a water volume (expressed as PV) of 1.60 (AL), 3.12 (SA), and 1.88 (V) (Table 2) in unamended soils. Similar curves are also produced for leaching of other pesticides in structured soils with a different pore system when a certain degree of pesticide–soil interaction is established (26). The maximum concentration of metalaxyl leached represented between 1.71 and 4.37% of the fungicide amount applied to the cores.

**Table 3.** Parameters of Soil Cores, Adsorption Constants ( $K_f$ ), and Retardation Factors ( $R$ ) for Metalaxyl in Unamended and F-SMS or C-SMS Soils

core	soil (g)	PV (mL)	$\rho$ (g cm <sup>-3</sup> )	$\theta$	$K_f^a$	$R$
AL	3583	435	1.408	0.171	0.26	3.14
AL + C-SMS	3548	527	1.395	0.207	0.29	2.95
AL + F-SMS	3703	465	1.456	0.183	0.34	3.71
SA	3401	310	1.337	0.122	0.36	4.95
SA + C-SMS	3456	385	1.359	0.151	0.47	5.22
SA + F-SMS	3568	355	1.403	0.140	0.40	5.01
V	3013	650	1.184	0.256	0.54	3.50
V + C-SMS	2561	600	1.007	0.235	0.67	3.86
V + F-SMS	3053	510	1.200	0.200	0.60	4.59

<sup>a</sup>Data from Marín-Benito et al. (10.1021/jf902108n) (16).

In amended soils, the BTC maximum concentration peaks of metalaxyl leaching appeared for a similar or higher volume of water than in unamended soils and the maximum concentrations ranged between 0.07 and 6.21% of the amount applied initially (Table 2). C-SMS soils decreased the maximum peak concentration with regard to unamended soils (up to 24-fold in V soil). However, F-SMS soils only decreased the leached maximum concentration in V and SA soils, whereas in AL, this concentration increased 1.4-fold after the addition of F-SMS.

These results are in agreement with the adsorption increase of metalaxyl by SMS amended soils as indicated by the adsorption constant  $K_f$  (Table 3) obtained in our previous work (10.1021/jf902108n) (16). Results also indicated that F-SMS increased fungicide leaching in soil cores with respect to C-SMS. DOC content in F-SMS was higher than in C-SMS and can interact in solution with metalaxyl (highly soluble in water). This might favor the fungicide leaching (11, 27), although this effect was not observed in the adsorption study by the batch equilibrium technique (10.1021/jf902108n) (16).

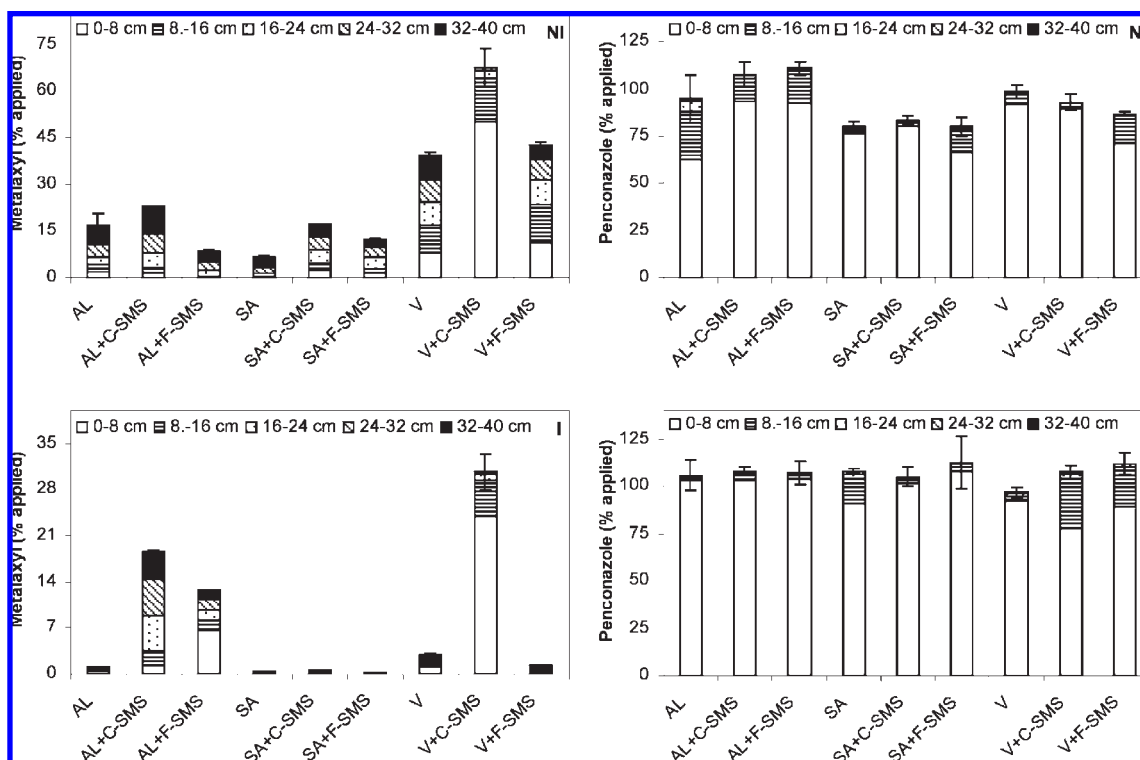
The  $R$  factor values calculated for metalaxyl leaching in unamended and amended soils (Table 3) were in agreement with PV values corresponding to the maximum peaks ( $r = 0.90$ ,  $p < 0.001$ ). The highest  $R$  values were found for leaching of metalaxyl in amended soils with a greater OM content and adsorption capacity. However, there is not a significant relationship between the  $R$  factors and the adsorption constants  $K_f$  or the OM content, indicating that other factors besides the OM content related to the structure of soils must influence the mobility of metalaxyl.

The total amounts of pesticide leached and the residual amounts in the cores after leaching, expressed as percentages of the amount initially added, are included in Table 4. Leaching of metalaxyl after the addition of a water volume of 1500 mL was not complete in any soil, but the highest leached amounts were, in general, in unamended soils (AL > SA > V) and in the F-SMS amended soils (AL > V > SA). Higher total residual amounts of fungicide were found in C-SMS amended soils (V + C-SMS > AL + C-SMS > SA + C-SMS). The more uniform nature of C-SMS with respect to F-SMS (28) would give rise to a lesser variability in adsorption by C-SMS soils than by F-SMS soils. This observation, together with the higher DOC content of F-SMS, which contributes to increase fungicide leaching, may explain the obtained results.

Distribution of metalaxyl in the unamended and amended soil cores is shown in Figure 2. Viana soil presented higher fungicide retention on the top of the core (0–8 cm), especially in C-SMS soils (8-fold higher than in unamended soil), whereas amounts of fungicide retained in SA and AL soils increased with soil depth. This is in agreement with the low maximum peak concentration found for unamended and amended V soil and indicates again the importance of the adsorption process on the fungicide leaching, with both processes being inversely related (29). Other authors

**Table 4.** Total Amounts (Retained and Leached) of Metalaxyl (% of Applied) and Its Metabolites (mg) in Non-incubated and Incubated Cores of Unamended and F-SMS or C-SMS Soils

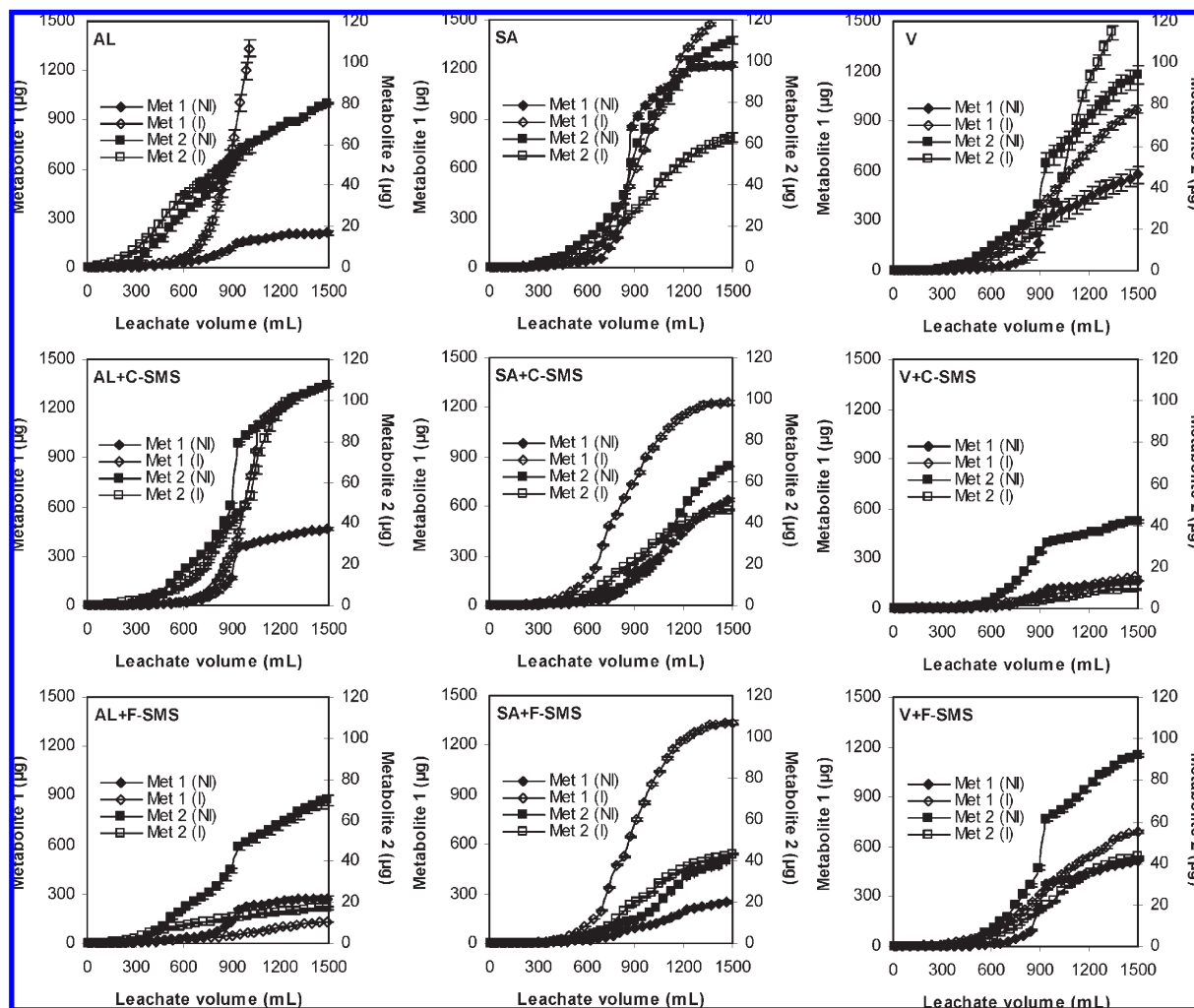
soil	metalaxyl		metabolite 1		metabolite 2	
	retained (%)	leached (%)	retained (mg)	leached (mg)	retained (mg)	leached (mg)
Non-incubated Soil Cores						
AL	17.1 ± 4.94	71.9 ± 3.31	0.02 ± 0.00	0.21 ± 0.02	0.02 ± 0.00	0.08 ± 0.00
AL + C-SMS	23.0 ± 0.30	58.8 ± 2.07	0.02 ± 0.01	0.47 ± 0.14	0.03 ± 0.01	0.11 ± 0.01
AL + F-SMS	8.60 ± 0.25	74.3 ± 2.76	0.06 ± 0.02	0.27 ± 0.01	0.01 ± 0.00	0.07 ± 0.00
SA	6.59 ± 0.59	46.1 ± 0.63	0.03 ± 0.00	1.22 ± 0.24	0.01 ± 0.00	0.11 ± 0.00
SA + C-SMS	17.2 ± 0.61	19.5 ± 4.32	0.05 ± 0.01	0.64 ± 0.07	0.02 ± 0.01	0.07 ± 0.02
SA + F-SMS	12.1 ± 0.68	15.1 ± 1.01	0.05 ± 0.01	0.25 ± 0.01	0.02 ± 0.00	0.04 ± 0.00
V	39.5 ± 1.39	22.0 ± 0.03	0.05 ± 0.01	0.58 ± 0.07	0.01 ± 0.00	0.09 ± 0.01
V + C-SMS	67.6 ± 8.62	0.81 ± 0.09	0.04 ± 0.01	0.17 ± 0.02	0.02 ± 0.00	0.04 ± 0.01
V + F-SMS	42.7 ± 1.61	17.8 ± 0.32	0.05 ± 0.01	0.52 ± 0.12	0.01 ± 0.00	0.09 ± 0.00
Incubated Soil Cores						
AL	1.13 ± 0.05	2.09 ± 0.08	0.03 ± 0.00	1.33 ± 0.21	0.08 ± 0.02	0.06 ± 0.00
AL + C-SMS	18.5 ± 0.21	20.4 ± 0.51	0.06 ± 0.01	1.27 ± 0.16	0.08 ± 0.01	0.10 ± 0.01
AL + F-SMS	12.8 ± 0.07	11.5 ± 0.63	0.00 ± 0.00	0.13 ± 0.03	0.08 ± 0.02	0.02 ± 0.00
SA	0.45 ± 0.03	0.63 ± 0.06	0.03 ± 0.00	1.47 ± 0.09	0.06 ± 0.01	0.06 ± 0.00
SA + C-SMS	0.63 ± 0.06	0.29 ± 0.05	0.00 ± 0.00	1.23 ± 0.14	0.06 ± 0.00	0.05 ± 0.01
SA + F-SMS	0.32 ± 0.01	0.05 ± 0.01	0.00 ± 0.00	1.34 ± 0.06	0.08 ± 0.03	0.04 ± 0.01
V	2.96 ± 0.14	1.58 ± 0.05	0.12 ± 0.02	0.97 ± 0.03	0.11 ± 0.01	0.12 ± 0.02
V + C-SMS	30.7 ± 3.91	0.69 ± 0.02	0.16 ± 0.05	0.19 ± 0.01	0.06 ± 0.01	0.01 ± 0.00
V + F-SMS	1.28 ± 0.02	4.22 ± 0.12	0.12 ± 0.03	0.69 ± 0.21	0.06 ± 0.01	0.04 ± 0.00

**Figure 2.** Amounts of metalaxyl and penconazole retained in non-incubated (NI) and incubated (I) soils (AL, SA, and V) unamended or amended with C-SMS or F-SMS. Error bars represent the standard error of the mean value ( $n = 2$ ).

have also found a decrease in the mobility of metalaxyl in soils amended with peat and organic wastes from wine distillery or from olive oil production industry (27, 30, 31).

**Figure 1** also includes BTCs of metalaxyl leaching in unamended and amended soils after 77 days of incubation. Chloride ion BTCs (no shown) were also obtained in these conditions, and they were almost identical to those of non-incubated soils (**Table 2**). The BTCs of metalaxyl leaching in these soil cores

were more delayed and showed a greater degree of asymmetry with regard to tracer ion BTCs than those in non-incubated soils. The curves indicated a dramatic decrease in the fungicide leaching in all soil cores. The PV values corresponding to maximum peaks were lesser, similar or greater than those in non-incubated soil cores, although maximum peak concentrations always decreased and some BTCs did not show a maximum peak under a non-saturated flow (V + C-SMS soil) (**Table 2**).



**Figure 3.** Cumulative curves for metalaxyl metabolites (metabolite 1 and metabolite 2) leaching in non-incubated (NI) and incubated (I) soils (AL, SA, and V) unamended or amended with C-SMS or F-SMS. Error bars represent the standard error of the mean value ( $n = 2$ ).

The total amounts of metalaxyl leached, expressed as percentages of the amount initially added, decreased considerably with regard to non-incubated soils (Table 4), and the lesser amount leached was always obtained in Sajazarra soil. The residual fungicide amounts in the soil cores after leaching ranged between 0.32 and 30.7%, being higher in C-SMS soils. Incubated V + C-SMS soil presented the highest amount of fungicide retained on top of the core, similar to the non-incubated soil (22-fold higher than in the unamended soil) (Figure 2).

The total amount of fungicide retained and leached was <100% for the unamended and amended soils. This amount ranged between 27.2 and 89.0% in non-incubated soils and decreased up to 0.37–38.9% after incubation, being higher in C-SMS soils (Table 4). This low total balance would indicate that fungicide might be degraded, mineralized, or immobilized in the soil as bound residues during the contact time between fungicide and soils (30 and 107 days for non-incubated and incubated soils, respectively). Some authors have indicated time-dependent adsorption of pesticides by soils (32, 33), and a decrease in the recovery values with the incubation period could occur because of a higher adsorption of the fungicide by the main soil components (OM and clay) forming strongly bound residues (32, 34).

The hypothesis of metalaxyl degradation was supported by the detection of two metalaxyl metabolites: *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine (CGA-62826 or metabolite 1) and *N*-methoxyacetyl-2,6-dimethyl-aniline (CGA 67868 or metabolite 2)

in leachate samples and soil extracts. Metalaxyl must be degraded by micro-organisms in soil forming the main acid metabolite CGA-62826 (metabolite 1). A second metabolite, CGA 67868 (metabolite 2), is formed either directly from metalaxyl or the metabolite 1 (35). Cumulative curves for leaching of both metabolites in incubated and non-incubated soil cores are shown in Figure 3. In general, both metabolites had a similar leaching trend, but metabolite 1 was detected at higher amounts than metabolite 2 in both leachate samples and soil extracts. Metabolite 2 behaved similar to metabolite 1, suggesting that metabolite 2 is mainly derived from metabolite 1 degradation. Both metabolites appeared at higher amounts in non-incubated and incubated SA soil, explaining the low total balance of metalaxyl in this soil at the end of the experiment. A certain negative relationship was observed between the total amount of metabolite 1 leached and the volumetric water content of soil cores, and SA soil presented the lowest PV value. It was explained by the high gravel percentage (>30%) in this soil seen after extraction of soil from cores. Fungicide could be more available for degradation in SA soil than in other non-incubated soil cores because of the lesser contact time of fungicide–soil (31). In amended soils, results were conflicting because amendments could stimulate microbial growth and, hence, the biodegradation process, but they could also increase the adsorption and decrease the degradation (Table 4). We observed that amounts of metabolite 1 leached from incubated soil cores were higher than those from non-incubated soil cores,

**Table 5.** Total Amounts (Retained and Leached) of Penconazole (% of Applied) in Non-incubated and Incubated Cores of Unamended and F-SMS or C-SMS Soils

soil	retained (%)	leached (%)
Non-incubated Soil Cores		
AL	94.9 ± 18.0	nd <sup>a</sup>
AL + C-SMS	108 ± 9.19	nd
AL + F-SMS	111 ± 4.90	nd
SA	80.6 ± 2.74	nd
SA + C-SMS	83.5 ± 3.05	nd
SA + F-SMS	80.1 ± 7.51	nd
V	98.9 ± 4.96	nd
V + C-SMS	93.1 ± 5.91	nd
V + F-SMS	86.7 ± 1.51	nd
Incubated Soil Cores		
AL	106 ± 11.3	0.01 ± 0.00
AL + C-SMS	108 ± 2.32	0.08 ± 0.01
AL + F-SMS	107 ± 8.51	0.02 ± 0.00
SA	108 ± 1.86	0.06 ± 0.02
SA + C-SMS	105 ± 6.79	nd
SA + F-SMS	112 ± 19.5	nd
V	96.7 ± 3.55	0.01 ± 0.00
V + C-SMS	107 ± 4.61	0.39 ± 0.11
V + F-SMS	112 ± 8.43	0.33 ± 0.09

<sup>a</sup> nd = no detected.

indicating that the greater pesticide residence time increased the opportunity for biodegradation of slightly adsorbed fungicide in all soils. Results from metalaxyl leaching in soils confirm the influence of adsorption and degradation processes on fungicide mobility in soil cores.

**Penconazole Leaching in Soil Cores.** Penconazole was not detected in leachate samples from amended or unamended soil cores after leaching under a non-saturated flow. Penconazole was only detected at very low concentrations (0.01–0.39% of applied fungicide) in leachate samples from incubated soil cores (Table 5). A high fungicide adsorption by soils is suggested to explain its immobilization. Penconazole immobilization was also indicated in a mobility study of triazole fungicides in Indian soils with low organic carbon content (36).

Distribution of penconazole in the soil cores is shown in Figure 2. Amounts of fungicide retained decreased with soil depth, indicating a higher retention of penconazole in the upper layer (0–8 cm) (>60% in non-incubated soils and >75% in incubated soils of applied fungicide). For the unamended soils, retention in the upper layer followed the order V > SA > AL, according to the OM content of these soils and the adsorption results (10.1021/jf902108n) (16). In non-incubated V + F-SMS and SA + F-SMS soils, amounts of penconazole in the upper layer were lesser than those in unamended soils and higher in deeper layers.

The total amount of fungicide retained in the soils was ≥100% for non-incubated and incubated cores, respectively (Table 5). When the total balance was lower than 100%, fungicide could be as bound residues in soil or could have been mineralized and/or degraded, although degradation products were not detected in the soil extracts. Penconazole retention in soil was not modified with the core incubation, and therefore the adsorbed compound was not bioavailable for microbial degradation.

Results obtained highlighted leaching behavior for the fungicides penconazole and metalaxyl, with different water solubility and hydrophobicity, in unamended and SMS amended soils. This study demonstrated that C-SMS and F-SMS added to soil as

amendment delayed leaching of metalaxyl and increased the amount of fungicide retained in the soil. Similar effects could be observed after fungicide incubation in soil cores for 77 days, although leached and retained fungicide percentages decreased significantly. The degradation/mineralization of metalaxyl or formation of bound residues was suggested to explain this low total balance. SMS soil amendment might have a beneficial effect to prevent metalaxyl groundwater pollution. However, SMS amendment increased penconazole adsorption, and degradation was not seen. Fungicide persistence in topsoil was maintained in non-incubated and incubated soil cores, which could imply a potential risk for surface water contamination by runoff.

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#### LITERATURE CITED

- (1) Cameron, K. C.; Di, H. J.; McLaren, R. G. Is soil an appropriate dumping ground for our wastes? *Aust. J. Soil Res.* **1997**, *35*, 995–1035.
- (2) Council Directive 99/31/EC of April 26, 1999 on the landfill of waste, [http://ec.europa.eu/environment/waste/landfill\\_index.htm](http://ec.europa.eu/environment/waste/landfill_index.htm) (accessed on May, 2009).
- (3) Wuest, P. J.; Levanon, D.; Hadar, Y. *Environmental, Agricultural and Industrial Uses for Spent Mushroom Substrate from Mushroom Farms*; JG Press, Inc.: Emmaus, PA, 1995.
- (4) Chiu, S. W.; Law, S. C.; Ching, M. L.; Cheung, K. W.; Chen, M. J. Themes for mushroom exploitation in the 21st century: Sustainability waste management, and conservation. *J. Gen. Appl. Microbiol.* **2000**, *46*, 269–282.
- (5) Paredes, C.; Medina, E.; Moral, R.; Pérez-Murcia, M. D.; Moreno-Caselles, J.; Bustamante, M. A.; Cecilia, J. A. Characterization of the different organic matter fractions of spent mushroom substrate. *Commun. Soil Sci. Plant Anal.* **2009**, *40*, No. 150161.
- (6) Martín, I.; Larrieta, C.; Martínez, J. M.; Domínguez, N.; Benito, A.; Pérez, E. P.; Peregrina, F.; Pérez, M.; García-Escudero, E. Efecto de la aplicación de Sustrato Postcultivo del Champiñón en las propiedades de un suelo vitícola. *Boletín de la Asociación Española de Cultivadores de Champiñón* **2009**, *57*, 17–28.
- (7) Briceño, G.; Palma, G.; Durán, N. Influence of organic amendment on the biodegradation and movement of pesticides. *Crit. Rev. Environ. Sci. Technol.* **2007**, *37*, 233–271.
- (8) Si, Y.; Zhang, J.; Wang, S.; Zhang, L.; Zhou, D. Influence of organic amendment on the adsorption and leaching of ethametsulfuron-methyl in acidic soils in China. *Geoderma* **2006**, *130*, 66–76.
- (9) Hiller, E.; Fargašová, A.; Zemanová, L.; Bartai, M. Influence of wheat ash on the MCPA immobilization in agricultural soils. *Bull. Environ. Contam. Toxicol.* **2008**, *81*, 285–288.
- (10) Singh, N. Biocompost from sugar distillery effluent: Effect on metribuzin degradation, sorption and mobility. *Pest Manage. Sci.* **2008**, *64*, 1057–1062.
- (11) Li, K.; Xing, B.; Torello, W. Effects of organic fertilizer derived dissolved organic matter on pesticides sorption and leaching. *Environ. Pollut.* **2005**, *134*, 187–194.
- (12) Briceño, G.; Demanet, R.; Mora, M. D.; Palma, G. Effect of liquid cow manure on andisol properties and atrazine adsorption. *J. Environ. Qual.* **2008**, *37*, 1519–1526.
- (13) Cox, L.; Cecchi, A.; Celis, R.; Hermosin, M. C.; Koskinen, W. C.; Cornejo, J. Effect of exogenous carbon on movement of simazine and 2,4-D in soils. *Soil Sci. Soc. Am. J.* **2001**, *65*, 1688–1695.
- (14) Bergström, L.; Shirmohammadi, A. A review of herbicide leaching studies in Sweden: Field, lysimeter, and laboratory measurements.



- In *The Lysimeter Concept: Environmental Behavior of Pesticides*, ACS Symposium Series, Number 699; American Chemical Society: Washington, D.C., 1998; pp 76–87.
- (15) Rodríguez-Cruz, M. S.; Jones, J. E.; Bending, G. D. Study of the spatial variability of the biodegradation rate of the herbicide bentazone with soil depth using contrasting incubation methods. *Chemosphere* **2008**, *73*, 1211–1215.
  - (16) Marín-Benito, J. M.; Sánchez-Martín, M. J.; Andrades, M. S.; Pérez-Clavijo, M.; Rodríguez-Cruz, M. S. Effect of spent mushroom substrate amendment of vineyard soils on the behavior of fungicides: 1. Adsorption–desorption of penconazole and metalaxyl by soils and subsoils. *J. Agric. Food Chem.* **2009**, DOI: 10.1021/jf902108n.
  - (17) Sánchez-Martín, M. J.; Andrades, M. S.; Sánchez-Camazano, M. Soil properties influencing the adsorption and mobility of penconazole in vineyard soils. *Soil Sci.* **2000**, *165*, 951–960.
  - (18) Andrades, M. S.; Sánchez-Martín, M. J.; Sánchez-Camazano, M. Significance of soil properties in the adsorption and mobility of the fungicide metalaxyl in vineyard soils. *J. Agric. Food Chem.* **2001**, *49*, 2363–2369.
  - (19) Fernandes, M. C.; Cox, L.; Hermosín, M. C.; Cornejo, J. Adsorption–desorption of metalaxyl as affecting dissipation and leaching in soils: Role of mineral and organic components. *Pest Manage. Sci.* **2003**, *59*, 545–552.
  - (20) Soil Survey Staff. *Keys to Soil Taxonomy*, 10th ed.; United States Department of Agriculture (USDA)–Natural Resources Conservation Service: Washington, D.C., 2006; p 332.
  - (21) Ministerio de Agricultura, Pesca y Alimentación (MAPA). *Métodos Oficiales de Análisis*; MAPA, Dirección General de Política Alimentaria, Secretaria General Técnica de MAPA: Madrid, Spain, 1986; Vol. 3, pp 93–208.
  - (22) Tomlin, C. D. S. *The Pesticide Manual*; British Crop Protection Council: Cambridge, U.K., 2003.
  - (23) Vincent, A.; Benoit, P.; Pot, V.; Madrigal, I.; Delgado-Moreno, L.; Labat, C. Impact of different land uses on the migration of two herbicides in a silt loam soil: Unsaturated soil column displacement studies. *Eur. J. Soil Sci.* **2007**, *58*, 320–328.
  - (24) Sánchez, L.; Romero, E.; Peña, A. Ability of biosolids and a cationic surfactant to modify methidathion leaching. Modelling with pescol. *Chemosphere* **2003**, *53*, 843–850.
  - (25) Sharma, M. L.; Taniguchi, M. Movement of a non-reactive solute tracer during steady and intermittent leaching. *J. Hydrol.* **1991**, *128*, 323–334.
  - (26) Beck, A. J.; Johnston, A. E. J.; Jones, K. C. Movement of nonionic organic chemicals in agricultural soils. *Crit. Rev. Environ. Sci. Technol.* **1993**, *23*, 219–248.
  - (27) Andrades, M. S.; Rodríguez-Cruz, M. S.; Sánchez-Martín, M. J.; Sánchez-Camazano, M. Effect of the addition of wine distillery wastes to vineyard soils on the adsorption and mobility of fungicides. *J. Agric. Food Chem.* **2004**, *52*, 3022–3029.
  - (28) Asociación Española de Cultivadores de Champiñón. Producción de compost de alta calidad para la agricultura con el sustrato agotado del cultivo de champiñón. *Boletín de la Asociación Española de Cultivadores de Champiñón* **2007**, *50*, 26–31.
  - (29) Sharma, D.; Awasthi, M. D. Adsorption and movement of metalaxyl in soils under unsaturated flow conditions. *Plant Soil* **1997**, *195*, 293–298.
  - (30) Petrovic, A. M.; Barrett, W. C.; Kovach, I. M. L.; Reid, C. M.; Lisk, D. J. The influence of a peat amendment and turf density on downward migration of metalaxyl fungicide in creeping bentgrass sand lysimeters. *Chemosphere* **1996**, *33*, 2335–2340.
  - (31) Fernandes, M. C.; Cox, L.; Hermosín, M. C.; Cornejo, J. Organic amendments affecting sorption, leaching and dissipation of fungicides in soils. *Pest Manage. Sci.* **2006**, *62*, 1207–1215.
  - (32) Walker, A.; Rodríguez-Cruz, M. S.; Mitchell, M. J. Influence of ageing of residues on the availability of herbicides for leaching. *Environ. Pollut.* **2005**, *133*, 43–51.
  - (33) Mamy, L.; Barriuso, E. Desorption and time-dependent sorption of herbicides in soils. *Eur. J. Soil Sci.* **2007**, *58*, 174–187.
  - (34) Semple, K. T.; Doick, K. J.; Burauel, P.; Craven, A.; Harms, H.; Jones, K. C. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environ. Sci. Technol.* **2004**, *38*, 228–231.
  - (35) Pesaro, M.; Nicollier, G.; Zeyer, J.; Widmer, F. Impact of soil drying–rewetting stress on microbial communities and activities on degradation of two crop protection products. *Appl. Environ. Microbiol.* **2004**, *70*, 2577–2587.
  - (36) Singh, N. Mobility of four triazole fungicides in two Indian soils. *Pest Manage. Sci.* **2005**, *61*, 191–196.

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