Science of the Total Environment 584–585 (2017) 828–837

Leaching of two fungicides in spent mushroom substrate amended soil: Influence of amendment rate, fungicide ageing and flow condition

Alba Álvarez-Martín, María J. Sánchez-Martín, José M. Ordax, Jesús M. Marín-Benito, M. Sonia Rodríguez-Cruz*

Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Cordel de Merinas 40-52, 37008 Salamanca (Spain)

*Corresponding author.

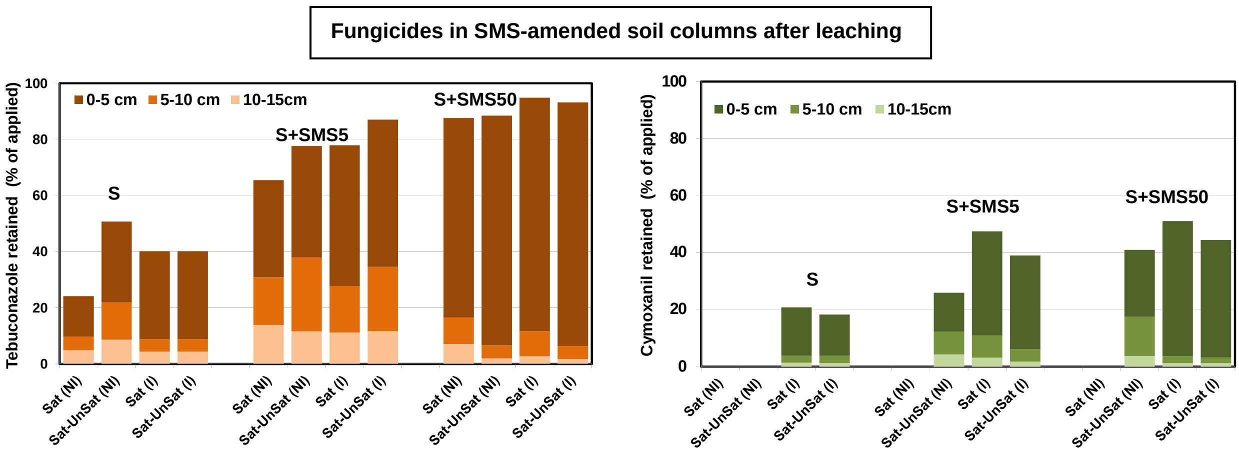
E-mail address: msonia.rodriguez@irnasa.csic.es (M. Sonia Rodríguez-Cruz)

Tel.: +34 923219606. Fax: +34 923219609

Highlights

- Influence of different factors on the leaching of fungicides in a soil was studied.
- Soil amendment decreased leaching of tebuconazole under different flow conditions.
- Leaching of cymoxanil decreased in amended soil under saturated-unsaturated flow.
- Ageing favours fungicide retention decreasing tebuconazole leaching or cymoxanil mineralization.
- Leaching results should be considered when organic amendment is used as a strategy for preventing water contamination.

Graphical Abstract



Abstract

A study has been conducted on the leaching of two fungicides, tebuconazole and cymoxanil, in a soil amended with spent mushroom substrate (SMS), with an evaluation of how different factors influence this process. The objective was based on the potential use of SMS as a biosorbent for immobilizing pesticides in vulnerable soils, and the need to know how it could affect the subsequent transport of these retained compounds. Breakthrough curves (BTCs) for ^{14}C-fungicides, non-incubated and incubated over 30 days, were obtained in columns packed with an unamended soil (S), and this soil amended with SMS at rates of 5% (S+SMS5) and 50% (S+SMS50) under saturated and saturated-unsaturated flows. The highest leaching of tebuconazole (>50% of the total ^{14}C added) was found in S when a saturated water flow was applied to the column, but the percentage of leached fungicide decreased when a saturated-unsaturated flow was applied in both SMS-amended soils. Also a significant decrease in leaching was observed for tebuconazole after incubation in the column, especially in S+SMS50 when both flows were applied. Furthermore, cymoxanil leaching was complete in S and S+SMS when a saturated flow was applied, and maximum peak concentrations were reached at 1 pore volume (PV), although BTCs showed peaks with lower concentrations in S+SMS. The amounts of cymoxanil retained only increased in S+SMS when a saturated-unsaturated flow was applied. A more relevant effect of SMS for reducing the leaching of fungicide was observed when cymoxanil was previously incubated in the column, although mineralization was enhanced in this case. These results are of interest for extending SMS application on the control of the leaching of fungicides with different physicochemical characteristics after different ageing times in the soil and water flow conditions applied.

Keywords: Leaching, Soil column, Tebuconazole, Cymoxanil, Spent mushroom substrate, Water flow

1. Introduction

The application of fungicides is usually intensive in different crops, and the presence of these compounds and their residues has frequently been detected in recent years in waters (Herrero-Hernández et al., 2016; Papadakis et al., 2015; Reilly et al., 2012) and soils (Bermúdez-Couso et al., 2007; Pose-Juan et al., 2015). Therefore, new tools need to be developed to avoid pesticides entering groundwater.

Only strong adsorption (binding) would be an efficient technique for preventing diffuse and/or point water pollution. Because of this, the use of low-cost adsorbent materials for these purposes has recently led researchers to explore the adsorption capacity of organic wastes (Gupta et al., 2009; Kurniawan et al., 2006). Although these wastes are expected to have less adsorption capacity than synthetic adsorbents, their low cost makes them competitive alternatives (Kyzas and Kostoglou, 2014). Some of these cheap biomaterials are sourced from agricultural activities or from industrial activities. They are characterized by a high organic carbon (OC) content and they are used simultaneously both as organic soil amendments to increase agricultural productivity (Courtney and Mullen, 2008; Udom et al., 2016) and as adsorbents of organic contaminants in soils, considering that OC is one of the most important soil factors influencing the adsorption process (Tran et al., 2015; Zolgharnein et al., 2011). The use of different organic residues with the potential to increase the adsorption of pesticides by soils has been reported in the literature (Ahmad et al., 2014; Marín-Benito et al., 2012; Rodríguez-Cruz et al., 2012).

However, adsorption is not the only process controlling the future behavior of pesticides in soils. An understanding of the influence that the organic amendments have on the leaching of adsorbed pesticides in the soil profile is required, as it has been less explored than other processes. Investigations in this direction have frequently involved columns packed with soil after the application of organic amendments such as sewage sludge, grape marc or SMS (Marín-Benito et al., 2013), winery vermicompost (Fernández-Bayo et al., 2015), agro-industrial and composted organic wastes (Fenoll et al., 2015), olive mill waste (Lopez-Piñeiro et al., 2013), biochar (Cabrera et al., 2011; Khorram et al., 2015; Larsbo et al., 2013) or green compost (Kodesova et al., 2012). The leaching of herbicides in general has been addressed in these works, although few have included fungicides. These studies have reported decreasing leaching of pesticides in soils by the effect of organic amendments, although they have usually been conducted under similar flow conditions (saturated or saturated-unsaturated) and ageing state of the pesticide in the amended soil.

Leaching of fungicides under changing conditions could be of interest because these factors together with the characteristics of pesticides determine, to a greater or lesser extent, the mobility of these compounds and the possible contamination of groundwater. In addition, the study of fungicides leaching under these flow conditions is closer to real field conditions than unchangeable flow conditions.

Tebuconazole and cymoxanil are two fungicides that are widely used and are effective against various foliar diseases in grapes, cereals and other field crops. Both fungicides have been approved for use in most European countries, with an expiry date of 2019. They are non-polar (tebuconazole) and polar compounds (cymoxanil) with different chemical structures and properties, but a similar threshold of toxicological concern (high class III) (PPDB, 2015). Tebuconazole is a synthetic triazole, and it is considered a moderate-persistent fungicide with a moderate GUS index (2.85). Cymoxanil is a synthetic cyanoacetamide oxime compound, and it is considered a non-persistent fungicide with a low GUS index (0.34).

Both fungicides enter the soil after their application to plants, and in areas of intense fungicide use in Spain they have been detected in surface and ground waters in higher concentrations than those permitted by EU legislation (0.1 μg L^{-1}) (Herrero-Hernández et al., 2013, 2016), as well as in other areas of the world (Battaglin et al., 2011; De Geronimo et al., 2014; Montagner et al., 2014). These results provided support for the studies on the immobilization and dissipation of both fungicides in soil amended with the organic SMS residue considered by Álvarez-Martín et al. (2016a and 2016b) for proposing a strategy to prevent water contamination by these compounds. A range of SMS doses were used in these studies, and an increase in the adsorption coefficients of up to >20 times (tebuconazole) or >40 times (cymoxanil) was obtained for soils amended with SMS at rates between 2% and 75%. On the other hand, the dissipation of tebuconazole and cymoxanil in SMS-amended soils revealed that SMS reduces the extractable fraction of fungicides through the formation of non-extractable residues. Although the immobilization of these compounds may be the first step to avoid water contamination, other processes such as fungicide leaching should be explored to investigate the time that fungicides remain adsorbed, decreasing their potential for biodegradation and/or their bioavailability in SMS-amended soils.

Accordingly, the aim of this work was to study the leaching of tebuconazole and cymoxanil in an unamended vineyard soil and in one amended with SMS. The leaching of both fungicides was carried out using packed soil columns and to gain a better understanding of the effect of the proposed soil amendment strategy the following factors were evaluated: i) the rate of SMS applied to the soil, with a low rate (5%) and a high rate (50%) being applied to simulate the application of SMS as a soil amendment or as a barrier; ii) the water flow regime applied; a similar water volume was applied under saturated or steady flow or under intermittent saturated-unsaturated flow; and iii) the incubation (or ageing) time of the fungicide in the soil (1 and 30 days) prior to leaching. The influence of these factors jointly evaluated (adsorbent, water flow regime and ageing of fungicide) on the mobility of the fungicides tebuconazole and cymoxanil, with different characteristics, has been not reported to our knowledge.

2. Materials and methods

2.1. Chemicals

The fungicides tebuconazole and cymoxanil were used as unlabeled and labeled compounds. The unlabeled compounds (analytical standards of PESTANAL purity >99%) were supplied by Sigma-Aldrich Química S.A. (Madrid, Spain), and the labeled compounds were supplied by IZOTOP Co., Ltd., (Budapest, Hungary). [Triazole-U-^{14}C]-tebuconazole had a specific activity of 4.72 MBq/mg, and a chemical and radiochemical purity of 98% and 95%, respectively, and [acetyl-2-^{14}C]-cymoxanil had a specific activity of 10.08 MBq/mg, and both chemical and radiochemical purity of 98%.

Table 1 shows their physicochemical properties and environmental fate parameters (PPDB, 2015). Tebuconazole is classified as non-polar and immobile with low water solubility (36 mg L^{-1}), while cymoxanil is polar and mobile with high water solubility (780 mg L^{-1}), according to the classification of non-polar when the log K_{ow} value is > 3.0, and as mobile when the log K_{oc} is <2.5 (Delle Site, 2001).

2.2. Soil and amendment

SMS from *Agaricus bisporus* cultivation was supplied by Sustratos de La Rioja S.L. (Pradejón, Spain). Its physicochemical characteristics, as described by Marín-Benito et al. (2012), are pH 6.97, ash content 33.6%, OC content 24.5%, dissolved organic carbon (DOC) content 1.91%, and moisture content 64.5%.

A soil sample was collected from the surface horizon (0-30 cm) in a vineyard located in Sajazarra (42°35′18″N, 2°57′41″W) in Spain's La Rioja region. The soil was air-dried and sieved (<2 mm) to determine its characteristics using standard analytical methods (Sparks, 1996). Soil texture was classified as sandy clay loam (67.0% sand, 11.9% silt, 21.1% clay and 51.0% carbonate content). The soil was amended with SMS at 5% and 50% (w/w) on a dry weight basis. The pH, OC and DOC content were 7.52, 0.67% and <0.01% (unamended soil, S), 7.26, 1.73% and 0.062% (soil amended with SMS at 5%, S+SMS5), and 7.19, 16.3% and 0.439% (soil amended with SMS at 50%, S+SMS50), respectively.

2.3. Soil column setup/leaching studies

Leaching experiments were performed by triplicate for each treatment in glass columns of 3 cm (i.d.) × 25 cm (length) packed with 100 g of soil or S+SMS5, or 80 g in the case of S+SMS50. Each column was oversaturated from the bottom with distilled water to its maximal water holding capacity, and then allowed to drain the excess of water freely for 24 h, so the humidity conditions were equivalent to field capacity. The PV of the packed columns was estimated by the difference in weight between water saturated columns and oven dried columns (mean values obtained were 41.57±1.15, 43.46±1.80 and 46.10±0.50 mL for S, S+SMS5 and S+SMS50, respectively). Fungicides were evenly applied in the top part of the columns by adding 1 mL of a solution of 1 mg mL^{-1} in methanol with a specific activity of 10 kBq mL^{-1}. Chemicals were applied at a dose of 1.4 kg ha^{-1}, and these were 2-5 times higher than the conventional field application rates of tebuconazole and cymoxanil to simulate the worst case scenario of these compounds leaching in soils (Khorram et al., 2015).

Leaching was carried out one day after fungicide application (non-incubated, NI), and after incubation of the fungicide at 20ºC in the dark for 30 days (incubated, I). The columns were then washed by applying 500 mL (70 cm) of 0.01M $CaCl_2$ solution. This solution was used for washing instead of water in order to minimize the disruption of the soil mineral balance.

Two different washing flow regimes were applied: saturated and saturated-unsaturated. Under saturated flow regime, 500 mL of 0.01M $CaCl_2$ solution was continuously pumped in upflow mode for ≈8 h, with the top of the column being in permanent contact with water. Under the saturated-unsaturated flow regime, the fungicides were leached by adding the same total volume of 0.01M $CaCl_2$ solution (500 mL) for 20 days (25 mL per day). The mineralization of the fungicides was also measured over incubation time in the column, or when a saturated-unsaturated flow was applied. A $^{14}CO_2$ trap, consisting of a scintillation vial containing 1M NaOH (1 mL) was firmly fitted onto the top of the column, and $^{14}CO_2$ from mineralized ^{14}C-fungicides was periodically determined.

The leaching flow rate was maintained constant at 1 mL min^{-1} by a Gilson MINIPLUS 3 peristaltic pump (Gilson, Inc., Middleton, WI, USA). Fractions of leaching solution (15 mL) were taken by a Gilson F203 automated fraction collector. After the leaching, the columns were cut into three segments (0-5 cm, 5-10 cm, and 10-15 cm), and the soil contained in each segment was turned over and weighed.

Conservative tracer transport, using chloride as an ion tracer (KCl), was implemented to describe the dispersive characteristics of the columns used for fungicide leaching. An amount of 47 mg of chloride ion was applied per column (1 mL from KCl solution of 100 g L^{-1} in water). Chloride ion leaching was carried out at similar water flow regimes to those used in the fungicide leaching setting (saturated flow and saturated- unsaturated flow).

2.4. Analysis of ^{14}C-fungicide residues

The ^{14}C-fungicide residue concentrations in the leached fractions were determined by triplicate mixing 1 mL of leachate with 4 mL of scintillation liquid (Ecoscint™ A, National Diagnostics, Hessle, UK), then measuring the activity of the fungicide residue in disintegration per minute (dpm) on a Beckman LS6500 Liquid Scintillation Counter (Beckman Instruments Inc., Fullerton, CA, USA). The dpm value recorded was related to the dpm obtained for the aliquots of the respective standards of the fungicide solutions. The limit of quantification for ^{14}C-fungicide residue was determined as the background radioactivity (19-50 dpm) in the $CaCl_2$ solution leached. The range of the coefficient of variation was always between 0.1% and 2%. $^{14}CO_2$ from mineralized ^{14}C-fungicide residue in the scintillation vial containing 1M NaOH (1 mL) was determined by mixing with 4 mL of scintillation cocktail, as previously indicated.

The ^{14}C-fungicide residue retained in the soil after leaching was determined by the combustion of triplicate 1 g dried soil samples from each segment of the column using a Biological Oxidizer OX500 (R.J. Harvey Instrument Corporation, Tappan, NY, USA) under O_2 excess at 900^{o}C. The $^{14}CO_2$ generated was trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxysolve C-400, Zinsser Analytic, Berkshire, UK, 15 mL), and determined as indicated above. The oxidizer's efficiency was calculated prior to sample combustion from the ratio of ^{14}C-standard activity applied in an inert material (mannitol) after combustion in oven and ^{14}C-standard activity without combustion. The ^{14}C-fungicide recovery was always >98%.

Chloride ion concentrations were determined using a Metrohm Ion Chromatograph (Metrohm Ltd., Switzerland) with a conductivity detector. Chloride ion concentrations were determined following the method described by Rodríguez-Cruz et al. (2011).

2.5. Data analysis

The retardation factors, R, were determined as indicators of the maximum peak shifts of the BTC for fungicide leaching relative to chloride tracer in the unamended and amended soil columns. These factors were estimated according to the expression $R = 1 + K \rho / \theta$ (Marín-Benito et al., 2013), assuming that sorption–desorption isotherms are linear and reversible. In this expression, ρ is the bulk density of the soil (g cm^{-3}), θ is the volumetric water content or PV in the packed column divided by the total volume (cm^3 cm^{-3}), and K is the distribution coefficient for the linear adsorption of fungicides by soil (mL g^{-1}). K were determined from linearized data by Álvarez-Martín et al. (2016a), where the adsorption isotherms were obtained for both fungicides in the soils used here. The parameters of the soil columns and the calculated values of adsorption constants (K) and retardation factors (R) are included in Table 2 and Table 3 for tebuconazole and cymoxanil, respectively.

Standard deviation (SD) was used to indicate variability in the leached or retained amounts of pesticides among replicates. Leached and retained amounts of pesticides were subjected to an analysis of variance (ANOVA) to measure the effects of different soil treatments. IBM SPSS 22.0 statistical software was used.

3. Results

3.1. Leaching of chloride ion in unamended and SMS-amended soil columns

The chloride ion BTCs (included in Fig. 1 and 2) were obtained for all the soils under the two different flow conditions in order to explain whether the changes in the leaching of both fungicides are related to flow conditions. They were symmetric, and recorded peak concentrations (maximum concentration of leached ion obtained in the BTCs during the leaching experiment) up to 47% (saturated flow), or up to 77% (saturated-unsaturated flow) of the applied compound in the unamended and amended soils. These peaks were obtained for a water volume close to 1 PV under both flow conditions. This indicates that tracer ion leaching is not affected by the water flow regime applied and the absence of preferential flows throughout the soil columns. The retardation factor R for chloride ion was close to 1 under all the conditions studied. This is the case for conservative ions, which do not undergo retention or degradation in soils, and so an amount of chloride ion close to 100% was recovered at the end of leaching process. A reduction in the maximum peak concentration was observed for S+SMS, together with some broadening of the BTCs relative to the unamended soil, especially when a saturated flow was applied. Chloride ions were present in S+SMS in amounts ~ 2-10% regarding the amount of chloride ions added, and they could have different leaching behavior from ions added. This was also observed by Marín-Benito et al. (2013) in soils amended with different organic residues.

3.2. Leaching of fungicides in unamended and SMS-amended soil columns under different conditions

Fig. 1 and 2 include experimental BTCs corresponding to the leaching of tebuconazole and cymoxanil in unamended soil columns (A,D) and in columns of soil amended with SMS at two rates (B,C,E,F) under two different flow conditions (saturated and saturated-unsaturated) and fungicide incubation time (one day and 30 days of fungicide ageing in soil before leaching).

The BTCs obtained for tebuconazole were asymmetrical, and they were very different to those of the non-reactive tracer, although they all recorded a rapid initial leaching of ^{14}C applied to the column for a PV < 2. In all the systems, there was a maximum concentration peak in unamended soil at a water volume in the range 1.14-1.41 PV, similar to that shown for the chloride ion, although with a lower concentration. This peak represents 3.52%-4.92% of the total ^{14}C applied to the column for the non-incubated fungicide (Table 4, Fig. 1A,D). However, the BTCs of cymoxanil leaching were symmetric in the unamended soil and similar to those of a non-reactive tracer when the fungicide was non-incubated. They were different under other conditions, although all of them recorded a rapid initial leaching of ^{14}C applied in the column (Fig. 2A,D). Peaks were obtained for a PV close to 1 (range between 0.97-1.56 PV) when leaching was carried out with saturated or saturated-unsaturated flows (Table 5), but peak concentrations were different for the non-incubated and incubated fungicide in the column. These concentrations reached ≈39% of the total ^{14}C applied for the non-incubated fungicide in the column and decreased by up to 12.9%-23.1% of the total ^{14}C applied when the fungicide was previously incubated in the column for 30 days. Complete leaching of the compound was reached at ≈3 PV (Fig. 2A,D) under both flow conditions.

In the amended soils, the BTCs for tebuconazole also recorded early peaks for an initial water volume between 1.06 and 1.35 PV (S+SMS5) (Fig. 1B,E) and between 1.08 and 2.15 PV (S+SMS50) (Fig. 1C,F) (Table 4), similar to those indicated for the unamended soil and conservative ion, although peak concentrations were lower. These concentrations were in the range 1.15%-3.95% (S+SMS5) and 0.96%-1.77% (S+SMS50) of the total ^{14}C applied to the column (Table 4). The BTC patterns for the leaching of tebuconazole in S+SMS were similar under all the conditions studied, although peak concentrations decreased after the incubation of the fungicide in the column (Fig. 1B,C,E,F). The BTCs shown in Fig. 1 indicate that after the early peaks, the mobility of tebuconazole was very low. The leaching of ^{14}C was continuously determined up to 12 PV of $CaCl_2$ solution was pumped under saturated or saturated-unsaturated flow conditions, and low ^{14}C concentrations were always measured in the leachates, with no more peaks in the BTCs.

The BTCs obtained for cymoxanil in amended soils were less symmetric than those obtained in the unamended one. They featured tails, but a continuous leaching of ^{14}C was found when up to 12 PV or 6 PV of $CaCl_2$ solution was pumped under saturated or saturated-unsaturated flow conditions, respectively, suggesting different leaching kinetics and a slower leaching process than in the unamended soil (Fig. 2B,C,E,F). The BTCs recorded peaks at an initial water volume ranging between 1.36 and 2.84 PV (Table 5), and the peak concentrations were in the range 13.4%-22.3% (S+SMS5) and 8.46%-10.8% (S+SMS50) of the total ^{14}C applied to the column for the non-incubated compound. The peak concentrations decreased after fungicide incubation in the column by up to 2.42%-5.94% (S+SMS5) and 2.89%-6.76% (S+SMS50).

The total amounts leached and retained of fungicides in the columns expressed as percentages of the total ^{14}C amount initially added to the column are included in Tables 4 and 5. The leached amounts of non-incubated tebuconazole were in the range 32.9%-58.7% after the application of 12 PV to the unamended soil column under both flow conditions (Table 4). They were higher than those in the amended soils (21.6%-27.4% (S+SMS5) and 16.5%-16.7% (S+SMS50)), and are consistent with the adsorption constants of tebuconazole by the soils (Table 2). These leached amounts decreased when the fungicide was previously incubated in the column for 30 days, especially in amended soils. These amounts were in the range 38.4%-46.7% in the unamended soil, and decreased 2.5-5 times or 2.3-3.3 times in the SMS-amended soils under a saturated flow or a saturated-unsaturated flow, respectively. The leaching of non-incubated cymoxanil in the unamended and amended soils was ≈100% after the application of 12 PV to the column under saturated flow, indicating that no fungicide was retained in the column. Fungicide leaching was lower (91.9% (S), 72.5% (S+SMS5) and 36.8% (S+SMS50)) when a saturated-unsaturated flow was applied to the column, and a higher reduction of these amounts was noted when the fungicide was incubated in the soil column. After incubation, the leached amounts were 60.5%-68.2% (S), 17.2%-27.2% (S+SMS5), and 23.4%-27.2% (S+SMS50) under a saturated or saturated-unsaturated flow, being lower than the leaching of the non-incubated fungicide.

The total amounts leached are consistent with the total amounts retained in the columns (Tables 4 and 5), with the total balance of ^{14}C-tebuconazole corresponding to retained, leached and mineralized amounts being determined >83% in S, >93% in S+SMS5, and >100% in S+SMS50. In the unamended soil, the retained amounts varied in the range 24.2%-50.7%, and they were lower than those in the amended soils (65.4%-86.9% in S+SMS5 and 87.6%-94.9% in S+SMS50). The retained amounts were close to 80% in S+SMS5, and close to 95% in S+SMS50 in the worst case studied (Table 4). Fig. 3 (A,B,C) shows the distribution of ^{14}C retained in the different segments of the columns as a percentage of the total ^{14}C applied under the different conditions studied. Tebuconazole was retained mainly in the first segment of the soil column, >50% of the amount in the column, under all the conditions studied. In S+SMS50, an amount >70%

or >80% was retained in the first segment of the soil column when the compound was non-incubated or incubated, respectively (Fig. 3C). The total balance of ^{14}C-cymoxanil was >94% in S, >76% in S+SMS5, and >82% in S+SMS50 (Table 5). The non-incubated cymoxanil was not retained by the unamended and SMS-amended soils under a saturated flow, and the retained amounts in the S+SMS5 and S+SMS50 columns were 25.9% and 40.9% under a saturated-unsaturated flow. After incubation, the retention of cymoxanil increased in the S (20.8%-18.3%), S+SMS5 (47.5%-39.0%), and S+SMS50 (51.0%-44.4%) columns and these amounts were retained mainly in the first segment of the columns (Fig. 3D,E,F).

The retardation factors, R, frequently correlated with the PV corresponding to BTC peaks were calculated (Tables 2 and 3). They varied in the ranges 4.90-5.44 (S), 10.8-11.3 (S+SMS5) and 56.1-62.2 (S+SMS50) for tebuconazole leaching and in the ranges 1.22-1.23 (S), 1.74-1.76 (S+SMS5), and 8.77-9.55 (S+SMS50) for cymoxanil leaching under the different conditions.

4. Discussion

4.1. Leaching of tebuconazole in unamended and SMS-amended soil columns under different conditions

The BTCs corresponding to the leaching of tebuconazole recorded a long tail with shoulders (saturated flow) (Fig. 1A) or peaks (saturated-unsaturated flow) (Fig. 1D), indicating the steady leaching of the fungicide up to 500 mL (~12 PV) of $CaCl_2$ solution was pumped under two flow conditions. This asymmetrical shape was also observed for other pesticides in unamended or amended soils (Rodríguez-Cruz et al., 2011), and recorded a time-dependent interaction between tebuconazole and soil components due to non-equilibrium adsorption (Brusseau et al., 1989). The equilibrium adsorption is probably more difficult to reach when the water flow is continuous relative to batch studies when steady-state conditions are attained (Dousset et al., 2010). The BTC pattern was similar for the leaching of tebuconazole after 30-day incubation in the soil column, although peaks of lower concentrations (3.41%-4.17% of the total ^{14}C applied to the column) than those for non-incubated fungicide were found (Fig. 1A,D). Similar BTCs were also obtained for compounds with high adsorption by unamended or amended soils (Marín-Benito et al., 2013; Okada et al., 2016).

The BTCs obtained in S+SMS were less asymmetric than in the unamended soil, pointing to an apparent slower leaching kinetics of tebuconazole in the unamended soil than in the amended ones, and suggesting a stronger interaction between the fungicide and the unamended soil. However, this is not consistent with the higher adsorption constants obtained for tebuconazole by the amended soils than by the unamended one (Table 2). The leaching pattern of tebuconazole in the amended soils was similar to a non-reactive ion or compound not retained by the soil because it was leached in the first PV. This pattern does not match the leaching of tebuconazole according to its reported behavior (moderate to persistent) in soil (EFSA, 2008). An explanation for these BTCs may be the leaching of tebuconazole bound to DOC from the amended soil, as reported in a field-scale dissipation study of this fungicide in a vineyard soil amended with SMS (Herrero-Hernández et al., 2011). The DOC in the soils could govern the initial behavior of tebuconazole in the amended soils, where it is produced in greater amounts according to its higher content, as indicated above. The DOC may be adsorbed or leached, depending on the pH of the soil. At pH > 7 of this soil, DOC adsorption would be low leading to a high degree of leaching (Herrero-Hernández et al., 2011). So, the interaction of tebuconazole with the DOC derived from the SMS might have helped to promote the mobility of a small amount of the fungicide in the soil, as indicated for other compounds (Cabrera et al., 2011). This initial effect could also correspond to ^{14}C leached from a degradation product present as an impurity with ^{14}C-tebuconazole (radiochemical purity of 95%) which may be easily leachable.

The total leached amounts decreased 2-3.5 times or 1.5-2 times when a saturated flow or a saturated-unsaturated flow was applied in the S+SMS5 and S+SMS50 soils. Dousset et al. (2010) also obtained low percentages of leached tebuconazole (<10%) in undisturbed columns involving bare and grass-covered soils under laboratory conditions, and Singh (2003) reported the scarce mobility of triazole fungicides in soil.

On the other hand, these leached amounts decreased when the fungicide was previously incubated in the column for 30 days, especially in amended soils. This is consistent with the increased adsorption of tebuconazole by the unamended and amended soils over time, as indicated in a previous study by Álvarez-Martín et al. (2016b) on the dissipation of tebuconazole. That study found that after a one-month incubation period the fraction extracted with a 0.01M $CaCl_2$ water solution decreased by up to 10%, and was lower in S+SMS50 than in S+SMS5 and S. The non-extractable fraction of tebuconazole increased over time. This is usually attributed to the diffusion of the compound to less accessible adsorption sites (Koskinen et al., 2002). As a result of the ageing process a redistribution of the chemical from weaker to stronger adsorption sites may occur (Gevao et al., 2000) and probably physicochemical interactions of high binding energy were established between tebuconazole (non-polar and hydrophobic compound) and soil components as reported for other compounds (Alonso et al., 2015). The increase of this fraction was higher in S+SMS50 than in S and S+SMS5 and it could explain the lower amount of leached tebuconazole after incubation and the decreased mobility of the fungicide.

The concentration of this non-polar fungicide in the top of the column is in agreement with its capacity to form bound residues indicating a low leaching capacity of these residues (Barriuso et al., 2008). Fenoll et al. (2010) have also determined the higher percentage of tebuconazole remaining in the upper layer of a clay loam soil column after the application of a saturated-unsaturated leaching flow. Furthermore, no mineralization of tebuconazole was detected in any one of the systems studied, indicating the scarce bioavailability of tebuconazole for degradation, and the high capacity for remaining adsorbed under different flow conditions and fungicide ageing in the soil column.

The R factors, are not in agreement with the PV values corresponding to the maximum peaks, although the higher R values obtained in S+SMS indicate the lower mobility of tebuconazole in the amended soil owing to its greater adsorption. The adsorption of tebuconazole by the unamended soil doubled, and then increased 13 times when this soil was amended with SMS at 5% and 50%, respectively, due to the increase in soil OC provided by the SMS (Table 2). A significant correlation between adsorption coefficients and the OC content of SMS-amended soils has already been reported (Álvarez-Martín et al., 2016a). Accordingly, the choice of the right SMS dose is crucial since the application to soil of SMS at high rates could be used as a tool to decrease the leaching of this fungicide, whereas that low SMS rates would be adequate for avoiding the enhanced retention and persistence of fungicides in an amended soil as bound residues.

4.2. Leaching of cymoxanil in unamended and SMS-amended soil columns under different conditions

The shape of BTCs for cymoxanil in the unamended soil with short final tails was considered similar to that found for the conservative ion, and indicates a rapid leaching kinetics due to the weak interaction between this polar fungicide and the unamended soil. Similar BTCs have been obtained for the leaching of other polar fungicides, herbicides (Rodríguez-Cruz et al., 2011) or insecticides (Kurwadkar et al., 2014) in soils of different characteristics. The adsorption of polar compounds by soils is usually low, and it could decrease in column leaching experiments compared to batch adsorption experiments. On the other hand, the BTCs obtained in amended soils were less symmetric than those obtained in the unamended one. They featured tails, but a continuous leaching of ^{14}C was found under saturated or saturated-unsaturated flow conditions, respectively, suggesting different leaching kinetics and a slower leaching process than in the unamended soil.

The peak concentrations were always higher after the application of a saturated-unsaturated flow than after a saturated flow was applied, and the leaching kinetics was more rapid. This behavior is consistent with the evolution of cymoxanil adsorbed in non-extractable form or mineralized when a saturated-unsaturated flow was applied (Álvarez-Martín et al., 2016b), and it is consistent with the amounts leached, retained, and mineralized under these conditions (Table 5).

The leaching results are in agreement with the increase in fungicide adsorption by the amended soils relative to the unamended one. The adsorption constants included in Table 3 were twice (S+SMS5) and 20 times (S+SMS50) higher than the adsorption constant of cymoxanil by the unamended soil (Álvarez-Martín et al., 2016a). However, the adsorbed compound could be bioavailable, as mineralized amounts up to ≈18% (S), ≈12% (S+SMS5) and ≈10% (S+SMS50) were detected under both flows (Table 5). The dissipation mechanism of cymoxanil in SMS-amended soils studied by Álvarez-Martín et al. (2016b) indicated that the mineralization of cymoxanil in unamended and SMS-amended soils increased over time, reaching an amount >50% in S and S+SMS5 after one-month incubation, and >25% in S+SMS50. These authors have reported a decrease in the water extractable cymoxanil fraction over time, being lower in S+SMS50 than in S and S+SMS5, while the non-extractable fraction of cymoxanil increased over time. These processes could explain the lower leached amounts found for the non-incubated cymoxanil under a saturated-unsaturated flow, and the incubated cymoxanil under both flow conditions. The results suggest that cymoxanil residues converted into bound residues or mineralized may help to decrease the leaching of this fungicide when it remains in the soil over time, especially with medium-high OM content (Alonso et al., 2015).

The retention of cymoxanil by the SMS-amended soils was higher than that found for other polar compounds such as metalaxyl (Rodríguez-Cruz et al., 2011) or some phenylureas (Fenoll et al., 2015) in soils amended with other organic residues. The increase in the retention of cymoxanil in the unamended and amended soil columns after incubation was in step with the increases in the adsorption constants of cymoxanil by the SMS-amended soils with regard to the unamended one (Álvarez-Martín et al., 2016a).

A certain relationship between R values and the PV values corresponding to the peaks was noted in S and S+SMS5. The higher R values obtained in S+SMS50 revealed the lower mobility of cymoxanil, as previously indicated. According to this, SMS should facilitate the retention of the fungicide by the amended soil, while allowing its biodegradation.

5. Conclusions

The results show the different effects of SMS applied to soil for the leaching of the fungicides non-polar tebuconazole and polar cymoxanil with different characteristics. The leached amounts of tebuconazole in the unamended soil decreased by up to 2 or 3.5 times in S+SMS5 and S+SMS50, respectively, when a water volume corresponding to 12 PV was applied as a saturated or saturated-unsaturated flow. The decrease was greater (up to 2.5 and 5 times in S+SMS5 and S+SMS50, respectively) when the leaching experiment was conducted after the 30-day incubation of the fungicide in the soil column. These effects are in agreement with the amounts retained in the SMS-amended soil under the prevailing environmental conditions. The leaching of polar cymoxanil was complete in the unamended and SMS-amended soils under saturated flow conditions. However, the leaching of this fungicide fell by 1.3 and 2.5 times in S+SMS5 and S+SMS50, respectively, when the saturated-unsaturated flow was applied. The leached amounts decreased whilst the retained and mineralized amounts increased similarly in both amended soils after the incubation of the fungicide in the soil column with regard to the unamended soil. In conclusion, the doses of SMS can be adjusted as a soil amendment when their purpose is to decrease the leaching of both fungicides studied. A high dose decreases the leaching of fungicides, but may increase their adsorption in a non-extractable form over time, decreasing their bioavailability (non-polar fungicide) or mineralization (polar fungicide). Both effects should be taken into account when SMS is used as a strategy for preventing water contamination by different types of pesticides.

Acknowledgements

This work was funded by the Ministry of Science and Innovation (MINECO/FEDER UE) (Project AGL2010-15976/AGR). AAM thanks the Spanish

Ministry of Economy and Competitiveness (MINECO) for her FPI-predoctoral fellowship (BES-2011-047811).

Conflicts of Interest: The authors declare no conflict of interest.

References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere 99, 19–33.

Alonso, D.G., Oliveira, R.S.Jr., Hall, K.E., Koskinen, W.C., Constantin, J., Mislankar, S., 2015 Changes in sorption of indaziflam and three transformation products in soil with aging. Geoderma 239–240, 250–256.

Álvarez-Martín, A., Rodríguez-Cruz, M.S., Andrades, M.S., Sánchez-Martín, M.J., 2016a. Application of a biosorbent to soil: A useful method for controlling water pollution by pesticides. Environ. Sci. Pollut. Res. 23, 9192–9203.

Álvarez-Martín, A., Sánchez-Martín, M.J., Pose-Juan, E., Rodríguez-Cruz, M.S., 2016b. Effect of different rates of spent mushroom substrate on the dissipation and bioavailability of cymoxanil and tebuconazole in an agricultural soil. Sci. Total Environ. 550, 495–503.

Barriuso, E., Benoit, P., Dubus, I.G., 2008. Formation of pesticide nonextractable (bound) residues in soil: Magnitude, controlling factors and reversibility. Environ. Sci. Technol. 42, 1845–1854.

Battaglin, W.A., Sandstrom, M.W., Kuivila, K.M., Kolpin, D.W., Meyer, M.T., 2011. Occurrence of azoxystrobin, propiconazole, and selected other fungicides in US streams 2005–2006. Water Air Soil Pollut. 218, 307–322.

Bermúdez-Couso, A., Arias-Estévez, M., Nóvoa-Muñoz, J.C., López-Periago, E., Soto-González, B., Simal-Gándara, J., 2007. Seasonal distributions of fungicides in soils and sediments of a small river basin partially devoted to vineyards. Water Res. 41, 4515–4525.

Brusseau, M.L., Jessup, R.E., Rao, P.S.C., 1989. Modeling the transport of solutes influenced by multiprocess nonequilibrium. Water. Resour. Res. 25, 1971–1988.

Cabrera, A., Cox, L., Spokas, K.A., Celis, R., Hermosin, M.C., Cornejo, J., Koskinen, W.C., 2011. Comparative sorption and leaching study of the herbicides fluometuron and 4-chloro-2-methylphenoxyacetic acid (MCPA) in a soil amended with biochars and other sorbents. J. Agric. Food Chem. 59, 12550–12560.

Courtney, R.G., Mullen, G.J., 2008. Soil quality and barley growth as influenced by the land application of two compost types. Bioresource Technol. 99, 2913–2918.

De Gerónimo, E., Aparicio, V.C., Bárbaro, S., Portocarrero, R., Jaime, S., Costa, J.L., 2014. Presence of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107, 423–431.

Delle Site, A., 2001. Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. J. Phys. Chem. 30,187–439.

Dousset, S., Thévenot, M., Schrack, D., Gouy, V., Carluer, N., 2010. Effect of grass cover on water and pesticide transport through undisturbed soil columns, comparison with field study (Morcille watershed, Beaujolais). Environ. Pollut. 158, 2446–2453.

EFSA, European Food Safety Authority, 2008. Conclusion on the peer review of tebuconazole. EFSA Sci. Rep. 176, 1–109.

Fenoll, J., Garrido, I., Hellin, P., Flores, P., Vela, N., Navarro, S., 2015. Use of different organic wastes as strategy to mitigate the leaching potential of phenylurea herbicides through the soil. Environ. Sci. Pollut. Res. 22, 4336–4349.

Fenoll, J., Ruiz, E., Flores, P., Hellin, P., Navarro, S., 2010. Leaching potential of several insecticides and fungicides through disturbed clay-loam soil columns. Int. J. Environ. Anal. Chem. 90, 276–285.

Fernández-Bayo, J.D., Nogales, R., Romero, E., 2015. Winery vermicomposts to control the leaching of diuron, imidacloprid and their metabolites: Role of dissolved organic carbon content. J. Environ. Sci. Health B. 50, 190–200.

Gevao, B., Semple, K.T., Jones, K.C., 2000. Bound pesticide residues in soils: a review. Environ. Pollut. 108, 3-14.

Gupta, V. K., Carrott, P. J. M., Ribeiro Carrott, M.M.L., Suhas, 2009. Low-Cost Adsorbents: Growing Approach to Wastewater Treatment-a Review. Crit. Rev. Env. Sci. Tec. 39, 783–842.

Herrero-Hernández, E., Andrades, M.S., Marín-Benito, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2011. Field-scale dissipation of tebuconazole in a vineyard soil amended with spent mushroom substrate and its potential environmental impact. Ecotox. Environ. Safe. 74, 1480–1488.

Herrero-Hernández, E., Andrades, M.S., Álvarez-Martín, A., Pose-Juan, E., Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2013. Occurrence of pesticides and some of their degradation products in waters in a Spanish wine region. J. Hydrol. 486, 234–245.

Herrero-Hernández, E., Pose-Juan, E., Sánchez-Martín, M.J., Andrades, S.M., Rodríguez-Cruz, M.S., 2016. Intra-annual trends of fungicide residues in waters from a vineyard areas in La Rioja region of northern Spain. Environ. Sci. Pollut. Res. 23, 22924–22936.

Khorram, M.S., Wang, Y., Jin, X., Fang, H., Yu, Y., 2015. Reduced mobility of fomesafen through enhanced adsorption in biochard-amended soil. Environ. Toxicol. Chem. 34, 1258–1266.

Kodesova, R., Kocarek, M., Hajkova, T., Hybler, M., Drabek, O., Kodes, V., 2012. Chlorotoluron mobility in compost amended soil. Soil Till. Res. 118, 88–96.

Koskinen, W.C., Rice, P.J., Anhalt, J.A., Sakaliene, O., Moorman, T.B., Arthur, E.L., 2002. Sorption–desorption of "aged" sulfonylaminocarbonyltriazolinone herbicides in soil. J. Agric. Food Chem. 50, 5368–5372.

Kurniawan, T.A., Chan, G.Y.S., Lo W.H., Babel, S., 2006. Comparisons of low-cost adsorbents for treating wastewaters laden with heavy metals. Sci. Total Environ. 366, 409–426.

Kurwadkar, S., Wheat, R., McGahan, D.G., Mitchell, F., 2014. Evaluation of leaching potential of three systemic neonicotinoid insecticides in vineyard soil. J. Contam. Hydrol. 170, 86–94.

Kyzas, G.K., Kostoglou, M., 2014. Green adsorbents for wastewaters: A critical review. Materials 7, 333–364.

Larsbo, M., Löfstrand, E., de Veer, D.V., Ulén, B., 2013. Pesticide leaching from two Swedish top soils of contrasting texture amended with biochar. J. Contam. Hydrol. 147, 73–81.

Lopez-Piñeiro, J.A., Peña, D., Albarrán, A., Sánchez-Llerena, J., Becerra, D., 2013. Behavior of MCPA in four intensive cropping soils amended with fresh, composted, and aged olive mill waste. J. Contam. Hydrol. 152, 137–146.

Marín-Benito, J.M., Andrades, M.S, Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2012 Changes in the sorption-desorption of fungicides over time in an amended sandy clay loam soil under laboratory conditions. J. Soils Sediments 12, 1111–1123.

Marín-Benito, J.M., Brown, C.D., Herrero-Hernández, E., Aienzo, M. Rodríguez-Cruz, M.S., 2013. Use of raw or incubated organic wastes as amendments in reducing pesticide leaching through soil columns. Sci. Total Environ. 463, 589–599.

Montagner, C.C., Vidal, C., Acayaba, R.D., Jardim, W.F., Jardim, I.C.S.F., Umbuzeiro, G.A., 2014. Trace analysis of pesticides and an assessment of their occurrence in surface and drinking waters from the State of Sao Paulo (Brazil). Anal. Methods. 6, 6668–6677.

Okada, E., Costa, J.L., Bedmar, F., 2016. Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage. Geoderma 263, 78–85.

Papadakis, E-N., Tsaboula, A., Kotopoulou, A., Kintzikoglou, K., Vryzas, Z., Papadopoulou-Mourkidou, E., 2015. Pesticides in the surface waters of Lake Vistonis Basin, Greece: Occurrence and environmental risk assessment. Sci. Total Environ. 536, 793–802.

Pose-Juan, E., Sánchez-Martín, M.J., Andrades, M.S., Rodríguez-Cruz, M.S., Herrero-Hernández, E., 2015. Pesticide residues in vineyard soils from Spain: Spatial and temporal distributions. Sci. Total Environ. 514, 351–358.

PPDB, Pesticide Properties DataBase, 2015. University of Hertfordshire. Publishing in http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm. Accessed October 2016.

Reilly, T.J., Smalling, K.L., Orlando, J.L., Kuivila, K.M., 2012. Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States. Chemosphere 89, 228–234.

Rodríguez-Cruz, M.S., Ordax, J.M., Arienzo, M., Sánchez-Martín, M.J., 2011. Enhanced retention of linuron, alachlor and metalaxyl in sandy soil columns intercalated with wood barriers. Chemosphere 82, 1415–1421.

Rodríguez-Cruz, M.S., Herrero-Hernández, E., Ordax, J.M., Marín-Benito, J.M., Draoui, K., Sánchez-Martín, M.J., 2012. Adsorption of pesticides by sewage sludge, grape marc, spent mushroom substrate and by amended soils. Int. J. Environ. Anal. Chem. 92, 933–948.

Singh, N., 2003. Organic manure and urea effect on metolachlor transport through packed soil columns. J. Environ. Qual. 32, 1743–1749.

Sparks, D.L., 1996. Methods of Soil Analysis. Part 3-Chemical Methods. Soil Science Society of America, Inc., Madison, WI.

Tran V.S., Ngo H.H., Guo W., Zhang J., Liang S., Ton-That C., Zhang X., 2015. Typical low cost biosorbents for adsorptive removal of specific organic pollutants from water. Bioresource Technol. 182, 353–363.

Udom, B.E., Nuga, B.O., Adesodun, J.K., 2016 Water-stable aggregates and aggregate-associated organic carbon and nitrogen after three annual applications of poultry manure and spent mushroom wastes. Appl. Soil Ecol. 101, 5–10.

Zolgharnein, J., Shahmoradi, A., Ghasemi, J., 2011. Pesticides removal using conventional and low-cost adsorbents: a review. Clean Soil Air Water 39, 1105–1119.

Figure Legends

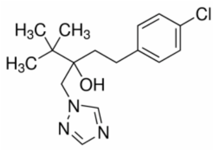
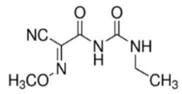
Fig. 1. Breakthrough curves for the leaching of conservative chloride ion and tebuconazole (% of the initially added ^{14}C-fungicide applied) without incubation (NI) and after incubation (I) in unamended soil columns (S) (A,C) and in soil columns amended with spent mushroom substrate (SMS) at rates of 5% (S+SMS5) (B,E) and 50% (S+SMS50) (C,F) under different flow regimes.

Fig. 2. Breakthrough curves for the leaching of conservative chloride ion and cymoxanil (% of the initially added ^{14}C-fungicide applied) without incubation (NI) and after incubation (I) in unamended soil columns (S) (A,D) and in soil columns amended with spent mushroom substrate (SMS) at rates of 5% (S+SMS5) (B,E) and 50% (S+SMS50) (C,F) under different flow regimes.

Fig. 3. Amounts of tebuconazole (left, A,B,C) and cymoxanil (right, D,E,F) without incubation (NI) and after incubation (I) retained in unamended soil columns (S) and in soil columns amended with spent mushroom substrate (SMS) at rates of 5% (S+SMS5) and 50% (S+SMS50) under different flow regimes.

Table 1

Chemical structure and physicochemical properties of fungicides studied.

Common name IUPAC name	Chemical structure	WS (mg L^{-1})	Log K_{ow}	Koc (mL g^{-1})	DT_{50} (days)	GUS index
Tebuconazole (*RS*)-1-p-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol		36	3.7	769	63-365	2.85
Cymoxanil 1-[(*EZ*)-2-cyano-2-methoxyiminoacetyl]-3-ethylurea		780	0.67	43.6	0.7-3.5	0.34

WS water solubility at 20 ºC, Kow octanol/water partition coefficient at pH 7 and 20ºC, Koc adsorption coefficient normalized to organic carbon content, DT_{50} degradation half-life time, GUS leaching potential index (PPDB, 2015).

Table 2
Distribution coefficients (K), parameters of soil columns (pore volume (PV), density (ρ) and porosity (θ)) and retardation factor (R) for tebuconazole in unamended (S) or amended soils (S+SMS5 and S+SMS50).

Column/Flow	Fungicide treatment	K ± SD[a] (mL g^{-1})	PV ± SD (mL)	ρ ± SD (g cm^{-3})	θ ± SD (cm^3 cm^{-3})	R ± SD
Soil (S)		1.67±0.01				
Sat[b]	Non-incubated		42.60 ± 0.59	1.21 ± 0.02	0.52 ± 0.00	4.90±0.05
Sat-Unsat[c]	Non-incubated		39.89 ± 1.14	1.20 ± 0.01	0.48 ± 0.02	5.16±0.12
Sat	Incubated		37.49 ± 3.28	1.18 ± 0.01	0.44 ± 0.04	5.44±0.39
Sat-Unsat	Incubated		38.45 ± 0.04	1.20 ± 0.01	0.46 ± 0.00	5.32±0.00
S+SMS5		4.38±0.30				
Sat	Non-incubated		44.48 ± 1.50	1.19 ± 0.02	0.53 ± 0.03	10.8±0.33
Sat-Unsat	Non-incubated		42.41 ± 0.25	1.17 ± 0.02	0.50 ± 0.01	11.3±0.06
Sat	Incubated		43.49 ± 2.64	1.18 ± 0.01	0.51 ± 0.03	11.1±0.61
Sat-Unsat	Incubated		43.47 ± 0.01	1.20 ± 0.01	0.52 ± 0.01	11.1±0.00
S+SMS50		32.8±0.49				
Sat	Non-incubated		42.95 ± 1.59	0.65 ± 0.01	0.35 ± 0.02	62.2±2.25
Sat-Unsat	Non-incubated		46.79 ± 5.53	0.71 ± 0.10	0.42 ± 0.11	57.6 ±6.60
Sat	Incubated		46.73 ± 4.05	0.68 ± 0.08	0.40 ± 0.08	57.5±4.85
Sat-Unsat	Incubated		47.94 ± 4.81	0.69 ± 0.08	0.42 ± 0.09	56.1±5.50

[a]Standard deviation of replicates (n=3). [b]Saturated. [c]Saturated-unsaturated.

Table 3
Distribution coefficients (K), parameters of soil columns (pore volume (PV), density (ρ) and porosity (θ)) and retardation factor (R) for cymoxanil in unamended (S) or amended soils (S+SMS5 and S+SMS50).

Column/Flow	Fungicide treatment	K ± SD[a] (mL g^{-1})	PV ± SD (mL)	ρ ± SD (g cm^{-3})	θ ± SD (cm^3 cm^{-3})	R ± SD
Soil (S)		0.11±0.01				
Sat	Non-incubated		42.47 ± 0.38	1.15 ± 0.02	0.48 ± 0.01	1.22 ±0.00
Sat-Unsat	Non-incubated		39.97 ± 1.19	1.15 ± 0.00	0.46 ± 0.01	1.23 ±0.01
Sat	Incubated		41.71 ± 2.14	1.23 ± 0.09	0.51 ± 0.01	1.22 ±0.03
Sat-Unsat	Incubated		42.13 ± 0.92	1.15 ± 0.00	0.48 ± 0.01	1.22 ±0.01
S+SMS5		0.33±0.06				
Sat	Non-incubated		44.06 ± 0.40	1.11 ± 0.01	0.49 ± 0.01	1.75 ±0.01
Sat-Unsat	Non-incubated		43.41 ± 0.41	1.13 ± 0.01	0.49 ± 0.00	1.76 ±0.01
Sat	Incubated		44.48 ± 0.59	1.12 ± 0.00	0.50 ± 0.01	1.74 ±0.02
Sat-Unsat	Incubated		44.90 ± 0.58	1.11 ± 0.01	0.50 ± 0.00	1.74 ±0.01
S+SMS50		3.28±0.06				
Sat	Non-incubated		38.36 ± 1.81	0.81 ± 0.03	0.31 ± 0.02	9.55 ±0.40
Sat-Unsat	Non-incubated		40.66 ± 0.41	0.88 ± 0.02	0.36 ± 0.01	9.06 ±0.08
Sat	Incubated		38.72 ± 0.26	0.84 ± 0.04	0.33 ± 0.02	9.46 ±0.06
Sat-Unsat	Incubated		42.16 ± 0.57	0.82 ± 0.01	0.35 ± 0.00	8.77 ±0.11

[a]Standard deviation of replicates (n=3). [b]Saturated. [c]Saturated-unsaturated

Table 4

Total amounts of retained and leached tebuconazole (% of ^{14}C applied) in unamended and amended soil columns at different flow conditions (saturated and saturated-unsaturated) and incubation time of fungicide before leaching.

Parameters	Non-incubated fungicide		Incubated fungicide	
	Saturated	Saturated-Unsaturated	Saturated	Saturated-Unsaturated
		Soil (S)		
Max. Peak	4.92 ± 0.33[a]	3.52 ± 0.61 2.90 ± 0.50	4.17 ± 0.57	3.41 ± 0.10
PV	1.41 ± 0.07	1.14 ± 0.02 3.17 ± 0.04	1.35 ± 0.34	1.19 ± 0.04
Total retained	24.2 ± 0.30	50.7 ± 12.4	40.2 ± 4.08	49.6 ± 13.8
Total leached	58.7 ± 2.96	32.9 ± 13.9	46. 7 ± 0.92	38.4 ± 4.46
Total column	82.8 ± 3.26	83.9 ± 0.98	86.9 ± 5.00	87.9 ± 9.31
		S+SMS5		
Max. Peak	2.25 ± 0.18	3.95 ± 0.02	1.15 ± 0.12 2.12 ± 0.83	3.19 ± 1.08
PV	1.35 ± 0.05	1.12 ± 0.03	1.06 ± 0.01 1.77 ± 0.04	1.09 ± 0.01
Total retained	65.4 ± 4.41	77.7 ± 1.58	77.9 ± 1.80	86.9 ± 5.78
Total leached	27.4 ± 1.71	21.6 ± 3.40	18.0 ± 0.67	16.8 ± 1.22
Total column	92.9 ± 2.70	99.2 ± 4.90	94.9 ± 2.33	103 ± 7.00
		S+SMS50		
Max. Peak	1.61 ± 0.18	1.77 ± 0.26	0.96 ± 0.23	1.15 ± 0.10
PV	1.08 ± 0.24	2.15 ± 0.25	1.08 ± 0.45	1.73 ± 0.19
Total retained	87.6 ± 1.81	88.4 ± 4.37	94.9 ± 2.42	93.2 ± 2.08
Total leached	16.5 ± 1.10	16.7 ± 0.23	9.16 ± 1.04	11.8 ± 0.25
Total column	104 ± 0.70	105 ± 4.14	104 ± 1.38	105 ± 2.33

[a]Standard deviation of replicates (n=3).

Table 5

Total amounts of retained and leached cymoxanil (% of ^{14}C applied) in unamended and amended soil columns at different flow conditions (saturated and saturated-unsaturated) and incubation time of fungicide before leaching.

Parameters	Non-incubated fungicide		Incubated fungicide	
	Saturated	Saturated-Unsaturated	Saturated	Saturated-Unsaturated
		Soil (S)		
Max. Peak	39.5 ± 1.4[a]	39.1 ± 4.58	12.9 ± 5.93	23.1 ±11.9
PV	1.38 ± 0.21	1.56 ± 0.07	1.13 ± 0.13	0.97 ± 0.33
Total retained	0.00	0.00	20.8 ± 0.46	18.3 ± 0.07
Total leached	101 ± 3.13	91.9 ± 1.32	60.5 ± 1.11	68.2 ± 2.62
Total mineralized	0.21 ± 0.03	2.00 ± 0.03	18.8 ± 1.93	18.6 ± 0.43
Total column	101 ± 3.16	93.9 ±1.28	100 ± 3.04	105 ± 2.19
		S+SMS5		
Max. Peak	13.4 ± 2.33	22.3 ± 2.48	2.42 ± 0.99	5.94 ± 2.90
PV	1.36 ± 0.02	1.45 ± 0.38	2.84 ± 1.72	1.41 ± 0.32
Total retained	0.00	25.9 ± 2.24	47.5 ± 6.62	39.0 ± 1.59
Total leached	96.7 ± 3.39	72.5 ± 0.94	17.2 ± 6.24	27.2 ± 2.04
Total mineralized	0.57 ± 0.16	5.80 ± 0.39	11.6 ± 1.35	12.8 ± 2.35
Total column	97.2 ± 3.23	104 ± 2.79	76.3 ± 0.98	79.0 ± 1.89
		S+SMS50		
Max. Peak	8.46 ± 0.51 7.01 ± 0.21	10.8 ± 1.04	2.89 ± 0.06	6.76 ± 0.03
PV	1.68 ± 0.22 3.28 ± 0.31	1.57 ± 0.40	1.28 ± 0.28	1.93 ± 0.37
Total retained	0.00	40.9 ± 1.00	51.0 ± 1.78	44.4 ± 0.61
Total leached	98.1 ± 2.98	36.8 ± 1.19	23.4 ± 0.90	27.2 ± 2.04
Total mineralized	0.16 ± 0.00	9.38 ± 3.29	7.63 ± 1.10	10.5 ± 1.52
Total column	98.3 ± 2.99	87.2 ± 4.48	82.1 ± 1.98	82.1 ± 4.18

[a]Standard deviation of replicates (n=3).

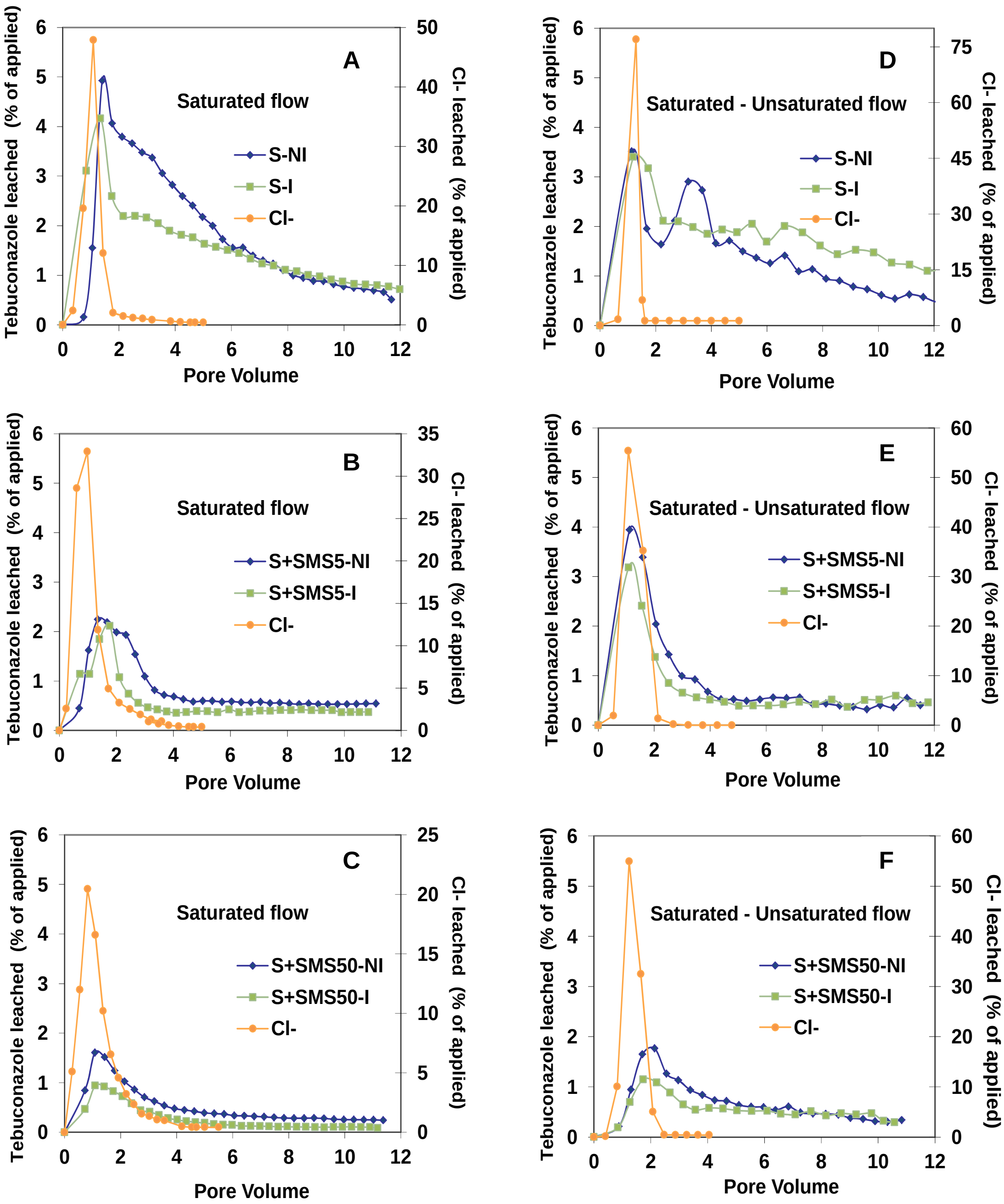

A
Saturated flow
S-NI
S-I
Cl-
Tebuconazole leached (% of applied)
Cl- leached (% of applied)
Pore Volume
D
Saturated - Unsaturated flow
S-NI
S-I
Cl-
B
Saturated flow
S+SMS5-NI
S+SMS5-I
Cl-
E
Saturated - Unsaturated flow
S+SMS5-NI
S+SMS5-I
Cl-
C
Saturated flow
S+SMS50-NI
S+SMS50-I
Cl-
F
Saturated - Unsaturated flow
S+SMS50-NI
S+SMS50-I
Cl-

Figure 1.

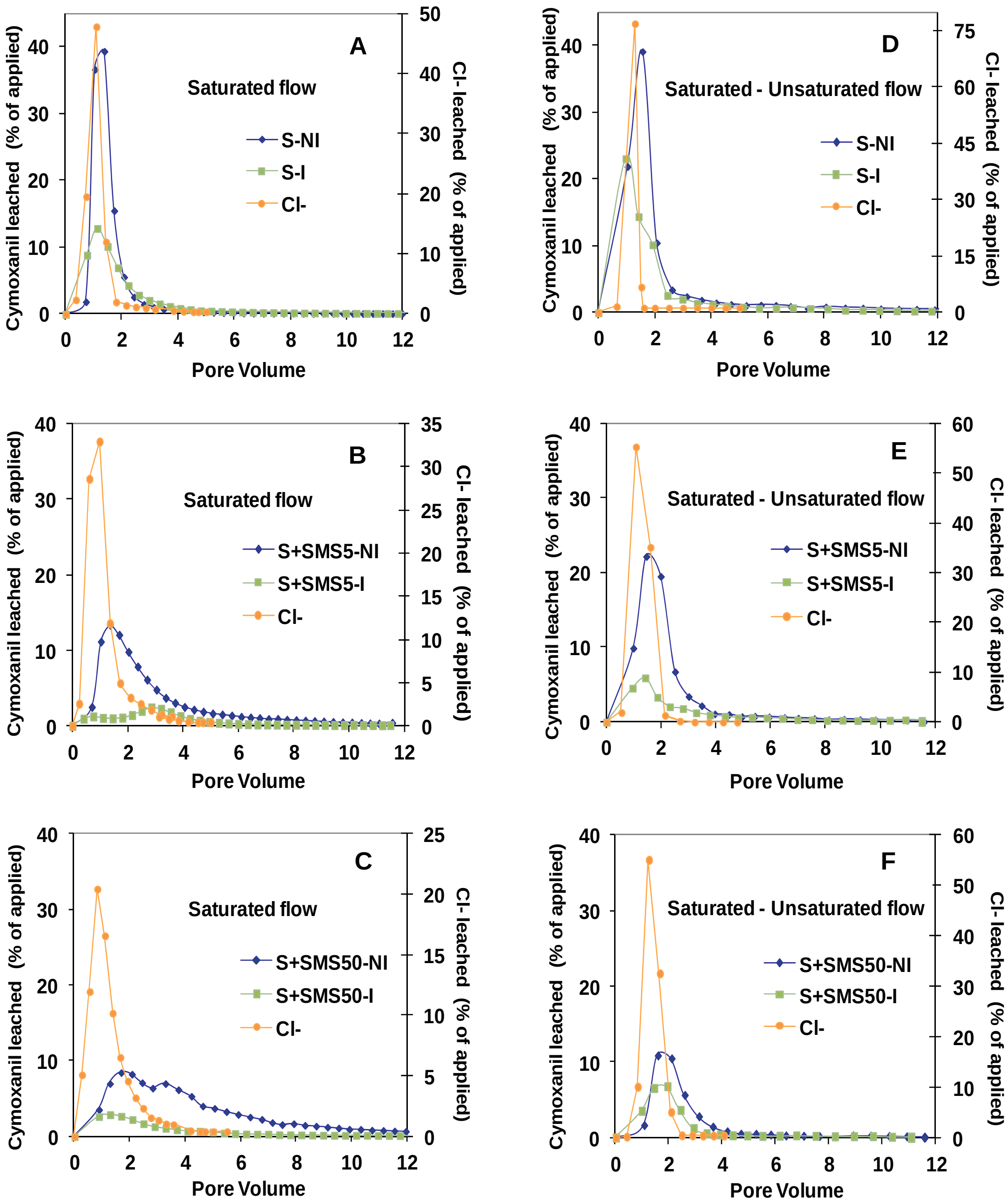

A
Saturated flow
S-NI
S-I
Cl-
Cymoxanil leached (% of applied)
Cl- leached (% of applied)
Pore Volume
D
Saturated - Unsaturated flow
S-NI
S-I
Cl-
B
Saturated flow
S+SMS5-NI
S+SMS5-I
Cl-
E
Saturated - Unsaturated flow
S+SMS5-NI
S+SMS5-I
Cl-
C
Saturated flow
S+SMS50-NI
S+SMS50-I
Cl-
F
Saturated - Unsaturated flow
S+SMS50-NI
S+SMS50-I
Cl-

Figure 2.

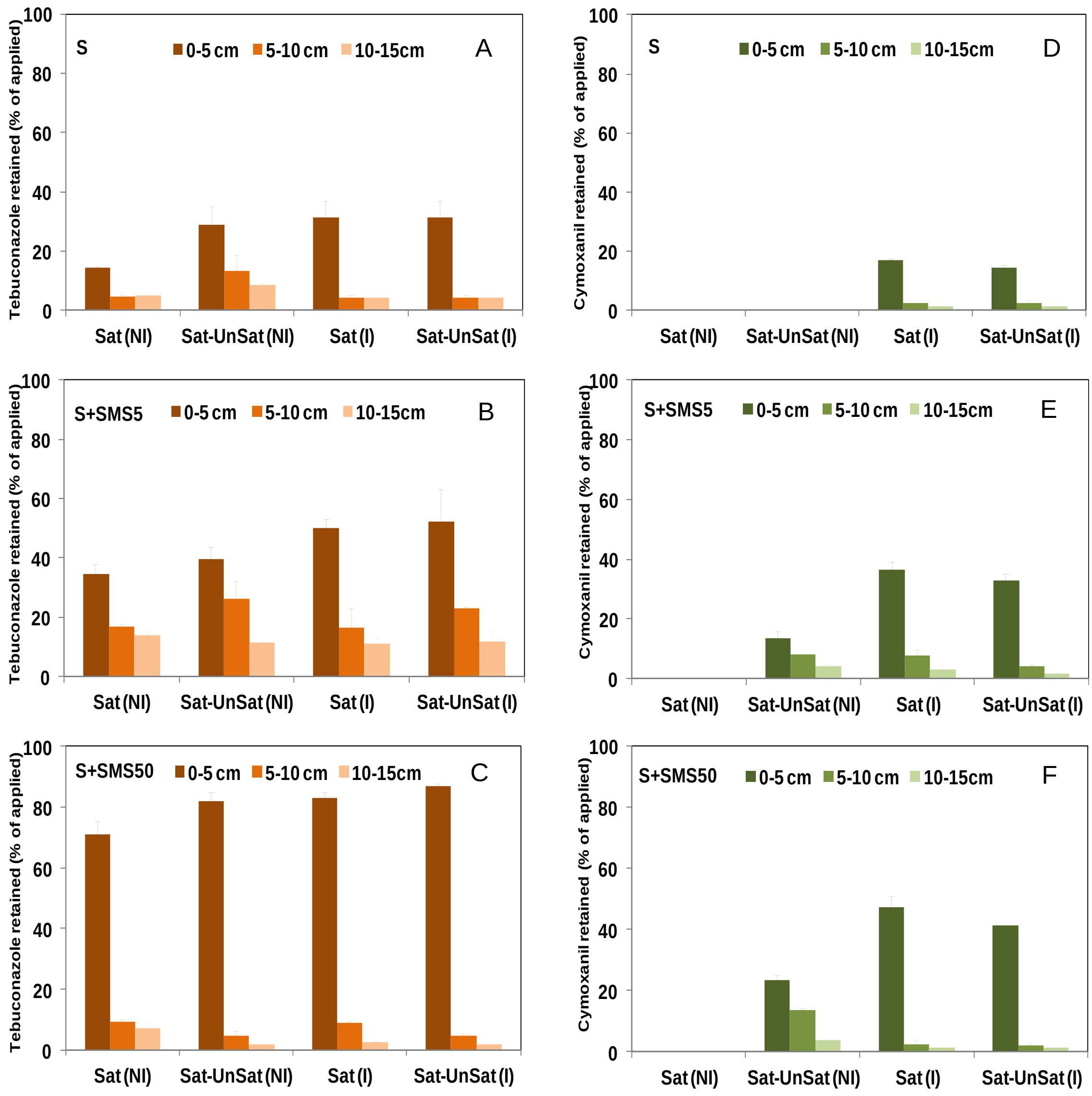

S
0-5 cm
5-10 cm
10-15cm
A
Tebuconazole retained (% of applied)
Sat (NI)
Sat-UnSat (NI)
Sat (I)
Sat-UnSat (I)
S+SMS5
B
S+SMS50
C
Cymoxanil retained (% of applied)
D
E
F

Figure 3.