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

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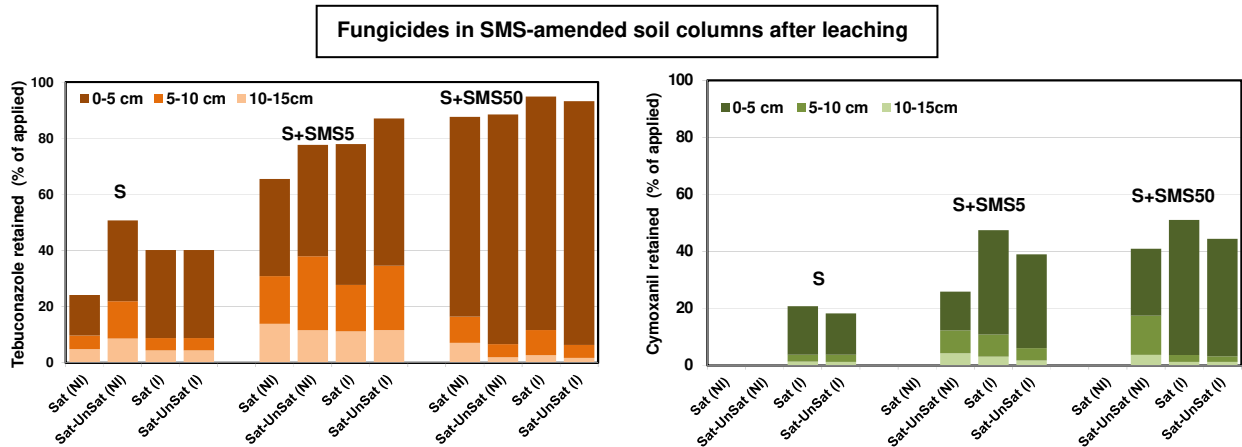
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Highlights

- 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



1 **Abstract**

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

25

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

28

29 **1. Introduction**

30 The application of fungicides is usually intensive in different crops, and the
31 presence of these compounds and their residues has frequently been detected in recent
32 years in waters (Herrero-Hernández et al., 2016; Papadakis et al., 2015; Reilly et al.,
33 2012) and soils (Bermúdez-Couso et al., 2007; Pose-Juan et al., 2015). Therefore, new
34 tools need to be developed to avoid pesticides entering groundwater.

35 Only strong adsorption (binding) would be an efficient technique for preventing
36 diffuse and/or point water pollution. Because of this, the use of low-cost adsorbent
37 materials for these purposes has recently led researchers to explore the adsorption
38 capacity of organic wastes (Gupta et al., 2009; Kurniawan et al., 2006). Although these
39 wastes are expected to have less adsorption capacity than synthetic adsorbents, their low
40 cost makes them competitive alternatives (Kyzas and Kostoglou, 2014). Some of these
41 cheap biomaterials are sourced from agricultural activities or from industrial activities.
42 They are characterized by a high organic carbon (OC) content and they are used
43 simultaneously both as organic soil amendments to increase agricultural productivity
44 (Courtney and Mullen, 2008; Udom et al., 2016) and as adsorbents of organic
45 contaminants in soils, considering that OC is one of the most important soil factors
46 influencing the adsorption process (Tran et al., 2015; Zolgharnein et al., 2011). The use
47 of different organic residues with the potential to increase the adsorption of pesticides
48 by soils has been reported in the literature (Ahmad et al., 2014; Marín-Benito et al.,
49 2012; Rodríguez-Cruz et al., 2012).

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

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

69 Tebuconazole and cymoxanil are two fungicides that are widely used and are
70 effective against various foliar diseases in grapes, cereals and other field crops. Both
71 fungicides have been approved for use in most European countries, with an expiry date
72 of 2019. They are non-polar (tebuconazole) and polar compounds (cymoxanil) with
73 different chemical structures and properties, but a similar threshold of toxicological
74 concern (high class III) (PPDB, 2015). Tebuconazole is a synthetic triazole, and it is

75 considered a moderate-persistent fungicide with a moderate GUS index (2.85).
76 Cymoxanil is a synthetic cyanoacetamide oxime compound, and it is considered a non-
77 persistent fungicide with a low GUS index (0.34).

78 Both fungicides enter the soil after their application to plants, and in areas of
79 intense fungicide use in Spain they have been detected in surface and ground waters in
80 higher concentrations than those permitted by EU legislation ($0.1 \mu\text{g L}^{-1}$) (Herrero-
81 Hernández et al., 2013, 2016), as well as in other areas of the world (Battaglin et al.,
82 2011; De Geronimo et al., 2014; Montagner et al., 2014). These results provided
83 support for the studies on the immobilization and dissipation of both fungicides in soil
84 amended with the organic SMS residue considered by Álvarez-Martín et al. (2016a and
85 2016b) for proposing a strategy to prevent water contamination by these compounds. A
86 range of SMS doses were used in these studies, and an increase in the adsorption
87 coefficients of up to >20 times (tebuconazole) or >40 times (cymoxanil) was obtained
88 for soils amended with SMS at rates between 2% and 75%. On the other hand, the
89 dissipation of tebuconazole and cymoxanil in SMS-amended soils revealed that SMS
90 reduces the extractable fraction of fungicides through the formation of non-extractable
91 residues. Although the immobilization of these compounds may be the first step to
92 avoid water contamination, other processes such as fungicide leaching should be
93 explored to investigate the time that fungicides remain adsorbed, decreasing their
94 potential for biodegradation and/or their bioavailability in SMS-amended soils.

95 Accordingly, the aim of this work was to study the leaching of tebuconazole and
96 cymoxanil in an unamended vineyard soil and in one amended with SMS. The leaching
97 of both fungicides was carried out using packed soil columns and to gain a better
98 understanding of the effect of the proposed soil amendment strategy the following
99 factors were evaluated: i) the rate of SMS applied to the soil, with a low rate (5%) and a

100 high rate (50%) being applied to simulate the application of SMS as a soil amendment
101 or as a barrier; ii) the water flow regime applied; a similar water volume was applied
102 under saturated or steady flow or under intermittent saturated-unsaturated flow; and iii)
103 the incubation (or ageing) time of the fungicide in the soil (1 and 30 days) prior to
104 leaching. The influence of these factors jointly evaluated (adsorbent, water flow regime
105 and ageing of fungicide) on the mobility of the fungicides tebuconazole and cymoxanil,
106 with different characteristics, has been not reported to our knowledge.

107

108 **2. Materials and methods**

109 *2.1. Chemicals*

110 The fungicides tebuconazole and cymoxanil were used as unlabeled and labeled
111 compounds. The unlabeled compounds (analytical standards of PESTANAL purity
112 >99%) were supplied by Sigma-Aldrich Química S.A. (Madrid, Spain), and the labeled
113 compounds were supplied by IZOTOP Co., Ltd., (Budapest, Hungary). [Triazole-U-
114 ¹⁴C]-tebuconazole had a specific activity of 4.72 MBq/mg, and a chemical and
115 radiochemical purity of 98% and 95%, respectively, and [acetyl-2-¹⁴C]-cymoxanil had a
116 specific activity of 10.08 MBq/mg, and both chemical and radiochemical purity of 98%.

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

122

123 *2.2. Soil and amendment*

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

128 A soil sample was collected from the surface horizon (0-30 cm) in a vineyard
129 located in Sajazarra (42°35'18"N, 2°57'41"W) in Spain's La Rioja region. The soil was
130 air-dried and sieved (<2 mm) to determine its characteristics using standard analytical
131 methods (Sparks, 1996). Soil texture was classified as sandy clay loam (67.0% sand,
132 11.9% silt, 21.1% clay and 51.0% carbonate content). The soil was amended with SMS
133 at 5% and 50% (w/w) on a dry weight basis. The pH, OC and DOC content were 7.52,
134 0.67% and <0.01% (unamended soil, S), 7.26, 1.73% and 0.062% (soil amended with
135 SMS at 5%, S+SMS5), and 7.19, 16.3% and 0.439% (soil amended with SMS at 50%,
136 S+SMS50), respectively.

137

138 2.3. Soil column setup/leaching studies

139 Leaching experiments were performed by triplicate for each treatment in glass
140 columns of 3 cm (i.d.) × 25 cm (length) packed with 100 g of soil or S+SMS5, or 80 g
141 in the case of S+SMS50. Each column was oversaturated from the bottom with distilled
142 water to its maximal water holding capacity, and then allowed to drain the excess of
143 water freely for 24 h, so the humidity conditions were equivalent to field capacity. The
144 PV of the packed columns was estimated by the difference in weight between water
145 saturated columns and oven dried columns (mean values obtained were 41.57±1.15,
146 43.46±1.80 and 46.10±0.50 mL for S, S+SMS5 and S+SMS50, respectively).
147 Fungicides were evenly applied in the top part of the columns by adding 1 mL of a
148 solution of 1 mg mL⁻¹ in methanol with a specific activity of 10 kBq mL⁻¹. Chemicals

149 were applied at a dose of 1.4 kg ha^{-1} , and these were 2-5 times higher than the
150 conventional field application rates of tebuconazole and cymoxanil to simulate the
151 worst case scenario of these compounds leaching in soils (Khorram et al., 2015).

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

157 Two different washing flow regimes were applied: saturated and saturated-
158 unsaturated. Under saturated flow regime, 500 mL of 0.01M CaCl_2 solution was
159 continuously pumped in upflow mode for ≈ 8 h, with the top of the column being in
160 permanent contact with water. Under the saturated-unsaturated flow regime, the
161 fungicides were leached by adding the same total volume of 0.01M CaCl_2 solution (500
162 mL) for 20 days (25 mL per day). The mineralization of the fungicides was also
163 measured over incubation time in the column, or when a saturated-unsaturated flow was
164 applied. A $^{14}\text{CO}_2$ trap, consisting of a scintillation vial containing 1M NaOH (1 mL)
165 was firmly fitted onto the top of the column, and $^{14}\text{CO}_2$ from mineralized ^{14}C -fungicides
166 was periodically determined.

167 The leaching flow rate was maintained constant at 1 mL min^{-1} by a Gilson
168 MINIPLUS 3 peristaltic pump (Gilson, Inc., Middleton, WI, USA). Fractions of
169 leaching solution (15 mL) were taken by a Gilson F203 automated fraction collector.
170 After the leaching, the columns were cut into three segments (0-5 cm, 5-10 cm, and 10-
171 15 cm), and the soil contained in each segment was turned over and weighed.

172 Conservative tracer transport, using chloride as an ion tracer (KCl), was
173 implemented to describe the dispersive characteristics of the columns used for fungicide

174 leaching. An amount of 47 mg of chloride ion was applied per column (1 mL from KCl
175 solution of 100 g L⁻¹ in water). Chloride ion leaching was carried out at similar water
176 flow regimes to those used in the fungicide leaching setting (saturated flow and
177 saturated- unsaturated flow).

178

179 *2.4. Analysis of ¹⁴C-fungicide residues*

180 The ¹⁴C-fungicide residue concentrations in the leached fractions were
181 determined by triplicate mixing 1 mL of leachate with 4 mL of scintillation liquid
182 (EcoscintTM A, National Diagnostics, Hesse, UK), then measuring the activity of the
183 fungicide residue in disintegration per minute (dpm) on a Beckman LS6500 Liquid
184 Scintillation Counter (Beckman Instruments Inc., Fullerton, CA, USA). The dpm value
185 recorded was related to the dpm obtained for the aliquots of the respective standards of
186 the fungicide solutions. The limit of quantification for ¹⁴C-fungicide residue was
187 determined as the background radioactivity (19-50 dpm) in the CaCl₂ solution leached.
188 The range of the coefficient of variation was always between 0.1% and 2%. ¹⁴CO₂ from
189 mineralized ¹⁴C-fungicide residue in the scintillation vial containing 1M NaOH (1 mL)
190 was determined by mixing with 4 mL of scintillation cocktail, as previously indicated.

191 The ¹⁴C-fungicide residue retained in the soil after leaching was determined by
192 the combustion of triplicate 1 g dried soil samples from each segment of the column
193 using a Biological Oxidizer OX500 (R.J. Harvey Instrument Corporation, Tappan, NY,
194 USA) under O₂ excess at 900°C. The ¹⁴CO₂ generated was trapped in a mixture of
195 ethanolamine (1 mL) and scintillation cocktail (Oxysolve C-400, Zinsser Analytic,
196 Berkshire, UK, 15 mL), and determined as indicated above. The oxidizer's efficiency
197 was calculated prior to sample combustion from the ratio of ¹⁴C-standard activity

198 applied in an inert material (mannitol) after combustion in oven and ^{14}C -standard
199 activity without combustion. The ^{14}C -fungicide recovery was always >98%.

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

204

205 2.5. Data analysis

206 The retardation factors, R , were determined as indicators of the maximum peak
207 shifts of the BTC for fungicide leaching relative to chloride tracer in the unamended and
208 amended soil columns. These factors were estimated according to the expression $R = 1$
209 $+ K \rho / \theta$ (Marín-Benito et al., 2013), assuming that sorption–desorption isotherms are
210 linear and reversible. In this expression, ρ is the bulk density of the soil (g cm^{-3}), θ is the
211 volumetric water content or PV in the packed column divided by the total volume (cm^3
212 cm^{-3}), and K is the distribution coefficient for the linear adsorption of fungicides by soil
213 (mL g^{-1}). K were determined from linearized data by Álvarez-Martín et al. (2016a),
214 where the adsorption isotherms were obtained for both fungicides in the soils used here.
215 The parameters of the soil columns and the calculated values of adsorption constants
216 (K) and retardation factors (R) are included in Table 2 and Table 3 for tebuconazole and
217 cymoxanil, respectively.

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

222

223 3. Results

224 3.1. Leaching of chloride ion in unamended and SMS-amended soil columns

225 The chloride ion BTCs (included in Fig. 1 and 2) were obtained for all the soils
226 under the two different flow conditions in order to explain whether the changes in the
227 leaching of both fungicides are related to flow conditions. They were symmetric, and
228 recorded peak concentrations (maximum concentration of leached ion obtained in the
229 BTCs during the leaching experiment) up to 47% (saturated flow), or up to 77%
230 (saturated-unsaturated flow) of the applied compound in the unamended and amended
231 soils. These peaks were obtained for a water volume close to 1 PV under both flow
232 conditions. This indicates that tracer ion leaching is not affected by the water flow
233 regime applied and the absence of preferential flows throughout the soil columns. The
234 retardation factor R for chloride ion was close to 1 under all the conditions studied. This
235 is the case for conservative ions, which do not undergo retention or degradation in soils,
236 and so an amount of chloride ion close to 100% was recovered at the end of leaching
237 process. A reduction in the maximum peak concentration was observed for S+SMS,
238 together with some broadening of the BTCs relative to the unamended soil, especially
239 when a saturated flow was applied. Chloride ions were present in S+SMS in amounts ~
240 2-10% regarding the amount of chloride ions added, and they could have different
241 leaching behavior from ions added. This was also observed by Marín-Benito et al.
242 (2013) in soils amended with different organic residues.

243

244 3.2. Leaching of fungicides in unamended and SMS-amended soil columns under 245 different conditions

246 Fig. 1 and 2 include experimental BTCs corresponding to the leaching of
247 tebuconazole and cymoxanil in unamended soil columns (A,D) and in columns of soil

248 amended with SMS at two rates (B,C,E,F) under two different flow conditions
249 (saturated and saturated-unsaturated) and fungicide incubation time (one day and 30
250 days of fungicide ageing in soil before leaching).

251 The BTCs obtained for tebuconazole were asymmetrical, and they were very
252 different to those of the non-reactive tracer, although they all recorded a rapid initial
253 leaching of ^{14}C applied to the column for a PV < 2. In all the systems, there was a
254 maximum concentration peak in unamended soil at a water volume in the range 1.14-
255 1.41 PV, similar to that shown for the chloride ion, although with a lower concentration.
256 This peak represents 3.52%-4.92% of the total ^{14}C applied to the column for the non-
257 incubated fungicide (Table 4, Fig. 1A,D). However, the BTCs of cymoxanil leaching
258 were symmetric in the unamended soil and similar to those of a non-reactive tracer
259 when the fungicide was non-incubated. They were different under other conditions,
260 although all of them recorded a rapid initial leaching of ^{14}C applied in the column (Fig.
261 2A,D). Peaks were obtained for a PV close to 1 (range between 0.97-1.56 PV) when
262 leaching was carried out with saturated or saturated-unsaturated flows (Table 5), but
263 peak concentrations were different for the non-incubated and incubated fungicide in the
264 column. These concentrations reached $\approx 39\%$ of the total ^{14}C applied for the non-
265 incubated fungicide in the column and decreased by up to 12.9%-23.1% of the total ^{14}C
266 applied when the fungicide was previously incubated in the column for 30 days.
267 Complete leaching of the compound was reached at ≈ 3 PV (Fig. 2A,D) under both flow
268 conditions.

269 In the amended soils, the BTCs for tebuconazole also recorded early peaks for
270 an initial water volume between 1.06 and 1.35 PV (S+SMS5) (Fig. 1B,E) and between
271 1.08 and 2.15 PV (S+SMS50) (Fig. 1C,F) (Table 4), similar to those indicated for the
272 unamended soil and conservative ion, although peak concentrations were lower. These

273 concentrations were in the range 1.15%-3.95% (S+SMS5) and 0.96%-1.77%
274 (S+SMS50) of the total ^{14}C applied to the column (Table 4). The BTC patterns for the
275 leaching of tebuconazole in S+SMS were similar under all the conditions studied,
276 although peak concentrations decreased after the incubation of the fungicide in the
277 column (Fig. 1B,C,E,F). The BTCs shown in Fig. 1 indicate that after the early peaks,
278 the mobility of tebuconazole was very low. The leaching of ^{14}C was continuously
279 determined up to 12 PV of CaCl_2 solution was pumped under saturated or saturated-
280 unsaturated flow conditions, and low ^{14}C concentrations were always measured in the
281 leachates, with no more peaks in the BTCs.

282 The BTCs obtained for cymoxanil in amended soils were less symmetric than
283 those obtained in the unamended one. They featured tails, but a continuous leaching of
284 ^{14}C was found when up to 12 PV or 6 PV of CaCl_2 solution was pumped under saturated
285 or saturated-unsaturated flow conditions, respectively, suggesting different leaching
286 kinetics and a slower leaching process than in the unamended soil (Fig. 2B,C,E,F). The
287 BTCs recorded peaks at an initial water volume ranging between 1.36 and 2.84 PV
288 (Table 5), and the peak concentrations were in the range 13.4%-22.3% (S+SMS5) and
289 8.46%-10.8% (S+SMS50) of the total ^{14}C applied to the column for the non-incubated
290 compound. The peak concentrations decreased after fungicide incubation in the column
291 by up to 2.42%-5.94% (S+SMS5) and 2.89%-6.76% (S+SMS50).

292 The total amounts leached and retained of fungicides in the columns expressed
293 as percentages of the total ^{14}C amount initially added to the column are included in
294 Tables 4 and 5. The leached amounts of non-incubated tebuconazole were in the range
295 32.9%-58.7% after the application of 12 PV to the unamended soil column under both
296 flow conditions (Table 4). They were higher than those in the amended soils (21.6%-
297 27.4% (S+SMS5) and 16.5%-16.7% (S+SMS50)), and are consistent with the

298 adsorption constants of tebuconazole by the soils (Table 2). These leached amounts
299 decreased when the fungicide was previously incubated in the column for 30 days,
300 especially in amended soils. These amounts were in the range 38.4%-46.7% in the
301 unamended soil, and decreased 2.5-5 times or 2.3-3.3 times in the SMS-amended soils
302 under a saturated flow or a saturated-unsaturated flow, respectively. The leaching of
303 non-incubated cymoxanil in the unamended and amended soils was $\approx 100\%$ after the
304 application of 12 PV to the column under saturated flow, indicating that no fungicide
305 was retained in the column. Fungicide leaching was lower (91.9% (S), 72.5%
306 (S+SMS5) and 36.8% (S+SMS50)) when a saturated-unsaturated flow was applied to
307 the column, and a higher reduction of these amounts was noted when the fungicide was
308 incubated in the soil column. After incubation, the leached amounts were 60.5%-68.2%
309 (S), 17.2%-27.2% (S+SMS5), and 23.4%-27.2% (S+SMS50) under a saturated or
310 saturated-unsaturated flow, being lower than the leaching of the non-incubated
311 fungicide.

312 The total amounts leached are consistent with the total amounts retained in the
313 columns (Tables 4 and 5), with the total balance of ^{14}C -tebuconazole corresponding to
314 retained, leached and mineralized amounts being determined $>83\%$ in S, $>93\%$ in
315 S+SMS5, and $>100\%$ in S+SMS50. In the unamended soil, the retained amounts varied
316 in the range 24.2%-50.7%, and they were lower than those in the amended soils (65.4%-
317 86.9% in S+SMS5 and 87.6%-94.9% in S+SMS50). The retained amounts were close to
318 80% in S+SMS5, and close to 95% in S+SMS50 in the worst case studied (Table 4).
319 Fig. 3 (A,B,C) shows the distribution of ^{14}C retained in the different segments of the
320 columns as a percentage of the total ^{14}C applied under the different conditions studied.
321 Tebuconazole was retained mainly in the first segment of the soil column, $>50\%$ of the
322 amount in the column, under all the conditions studied. In S+SMS50, an amount $>70\%$

323 or >80% was retained in the first segment of the soil column when the compound was
324 non-incubated or incubated, respectively (Fig. 3C). The total balance of ¹⁴C-cymoxanil
325 was >94% in S, >76% in S+SMS5, and >82% in S+SMS50 (Table 5). The non-
326 incubated cymoxanil was not retained by the unamended and SMS-amended soils under
327 a saturated flow, and the retained amounts in the S+SMS5 and S+SMS50 columns were
328 25.9% and 40.9% under a saturated-unsaturated flow. After incubation, the retention of
329 cymoxanil increased in the S (20.8%-18.3%), S+SMS5 (47.5%-39.0%), and S+SMS50
330 (51.0%-44.4%) columns and these amounts were retained mainly in the first segment of
331 the columns (Fig. 3D,E,F).

332 The retardation factors, R, frequently correlated with the PV corresponding to
333 BTC peaks were calculated (Tables 2 and 3). They varied in the ranges 4.90-5.44 (S),
334 10.8-11.3 (S+SMS5) and 56.1-62.2 (S+SMS50) for tebuconazole leaching and in the
335 ranges 1.22-1.23 (S), 1.74-1.76 (S+SMS5), and 8.77-9.55 (S+SMS50) for cymoxanil
336 leaching under the different conditions.

337

338 **4. Discussion**

339 *4.1. Leaching of tebuconazole in unamended and SMS-amended soil columns under*
340 *different conditions*

341 The BTCs corresponding to the leaching of tebuconazole recorded a long tail
342 with shoulders (saturated flow) (Fig. 1A) or peaks (saturated-unsaturated flow) (Fig.
343 1D), indicating the steady leaching of the fungicide up to 500 mL (~12 PV) of CaCl₂
344 solution was pumped under two flow conditions. This asymmetrical shape was also
345 observed for other pesticides in unamended or amended soils (Rodríguez-Cruz et al.,
346 2011), and recorded a time-dependent interaction between tebuconazole and soil
347 components due to non-equilibrium adsorption (Brusseau et al., 1989). The equilibrium

348 adsorption is probably more difficult to reach when the water flow is continuous relative
349 to batch studies when steady-state conditions are attained (Dousset et al., 2010). The
350 BTC pattern was similar for the leaching of tebuconazole after 30-day incubation in the
351 soil column, although peaks of lower concentrations (3.41%-4.17% of the total ¹⁴C
352 applied to the column) than those for non-incubated fungicide were found (Fig. 1A,D).
353 Similar BTCs were also obtained for compounds with high adsorption by unamended or
354 amended soils (Marín-Benito et al., 2013; Okada et al., 2016).

355 The BTCs obtained in S+SMS were less asymmetric than in the unamended soil,
356 pointing to an apparent slower leaching kinetics of tebuconazole in the unamended soil
357 than in the amended ones, and suggesting a stronger interaction between the fungicide
358 and the unamended soil. However, this is not consistent with the higher adsorption
359 constants obtained for tebuconazole by the amended soils than by the unamended one
360 (Table 2). The leaching pattern of tebuconazole in the amended soils was similar to a
361 non-reactive ion or compound not retained by the soil because it was leached in the first
362 PV. This pattern does not match the leaching of tebuconazole according to its reported
363 behavior (moderate to persistent) in soil (EFSA, 2008). An explanation for these BTCs
364 may be the leaching of tebuconazole bound to DOC from the amended soil, as reported
365 in a field-scale dissipation study of this fungicide in a vineyard soil amended with SMS
366 (Herrero-Hernández et al., 2011). The DOC in the soils could govern the initial behavior
367 of tebuconazole in the amended soils, where it is produced in greater amounts according
368 to its higher content, as indicated above. The DOC may be adsorbed or leached,
369 depending on the pH of the soil. At pH > 7 of this soil, DOC adsorption would be low
370 leading to a high degree of leaching (Herrero-Hernández et al., 2011). So, the
371 interaction of tebuconazole with the DOC derived from the SMS might have helped to
372 promote the mobility of a small amount of the fungicide in the soil, as indicated for

373 other compounds (Cabrera et al., 2011). This initial effect could also correspond to ¹⁴C
374 leached from a degradation product present as an impurity with ¹⁴C-tebuconazole
375 (radiochemical purity of 95%) which may be easily leachable.

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

381 On the other hand, these leached amounts decreased when the fungicide was
382 previously incubated in the column for 30 days, especially in amended soils. This is
383 consistent with the increased adsorption of tebuconazole by the unamended and
384 amended soils over time, as indicated in a previous study by Álvarez-Martín et al.
385 (2016b) on the dissipation of tebuconazole. That study found that after a one-month
386 incubation period the fraction extracted with a 0.01M CaCl₂ water solution decreased by
387 up to 10%, and was lower in S+SMS50 than in S+SMS5 and S. The non-extractable
388 fraction of tebuconazole increased over time. This is usually attributed to the diffusion
389 of the compound to less accessible adsorption sites (Koskinen et al., 2002). As a result
390 of the ageing process a redistribution of the chemical from weaker to stronger
391 adsorption sites may occur (Gevao et al., 2000) and probably physicochemical
392 interactions of high binding energy were established between tebuconazole (non-polar
393 and hydrophobic compound) and soil components as reported for other compounds
394 (Alonso et al., 2015). The increase of this fraction was higher in S+SMS50 than in S
395 and S+SMS5 and it could explain the lower amount of leached tebuconazole after
396 incubation and the decreased mobility of the fungicide.

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

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

418

419 *4.2. Leaching of cymoxanil in unamended and SMS-amended soil columns under*
420 *different conditions*

421 The shape of BTCs for cymoxanil in the unamended soil with short final tails
422 was considered similar to that found for the conservative ion, and indicates a rapid
423 leaching kinetics due to the weak interaction between this polar fungicide and the
424 unamended soil. Similar BTCs have been obtained for the leaching of other polar
425 fungicides, herbicides (Rodríguez-Cruz et al., 2011) or insecticides (Kurwadkar et al.,
426 2014) in soils of different characteristics. The adsorption of polar compounds by soils is
427 usually low, and it could decrease in column leaching experiments compared to batch
428 adsorption experiments. On the other hand, the BTCs obtained in amended soils were
429 less symmetric than those obtained in the unamended one. They featured tails, but a
430 continuous leaching of ^{14}C was found under saturated or saturated-unsaturated flow
431 conditions, respectively, suggesting different leaching kinetics and a slower leaching
432 process than in the unamended soil.

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

439 The leaching results are in agreement with the increase in fungicide adsorption
440 by the amended soils relative to the unamended one. The adsorption constants included
441 in Table 3 were twice (S+SMS5) and 20 times (S+SMS50) higher than the adsorption
442 constant of cymoxanil by the unamended soil (Álvarez-Martín et al., 2016a). However,
443 the adsorbed compound could be bioavailable, as mineralized amounts up to $\approx 18\%$ (S),
444 $\approx 12\%$ (S+SMS5) and $\approx 10\%$ (S+SMS50) were detected under both flows (Table 5). The
445 dissipation mechanism of cymoxanil in SMS-amended soils studied by Álvarez-Martín

446 et al. (2016b) indicated that the mineralization of cymoxanil in unamended and SMS-
447 amended soils increased over time, reaching an amount >50% in S and S+SMS5 after
448 one-month incubation, and >25% in S+SMS50. These authors have reported a decrease
449 in the water extractable cymoxanil fraction over time, being lower in S+SMS50 than in
450 S and S+SMS5, while the non-extractable fraction of cymoxanil increased over time.
451 These processes could explain the lower leached amounts found for the non-incubated
452 cymoxanil under a saturated-unsaturated flow, and the incubated cymoxanil under both
453 flow conditions. The results suggest that cymoxanil residues converted into bound
454 residues or mineralized may help to decrease the leaching of this fungicide when it
455 remains in the soil over time, especially with medium-high OM content (Alonso et al.,
456 2015).

457 The retention of cymoxanil by the SMS-amended soils was higher than that
458 found for other polar compounds such as metalaxyl (Rodríguez-Cruz et al., 2011) or
459 some phenylureas (Fenoll et al., 2015) in soils amended with other organic residues.
460 The increase in the retention of cymoxanil in the unamended and amended soil columns
461 after incubation was in step with the increases in the adsorption constants of cymoxanil
462 by the SMS-amended soils with regard to the unamended one (Álvarez-Martín et al.,
463 2016a).

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

469

470 **5. Conclusions**

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

492

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498

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500

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638

639 **Figure Legends**

640

641 **Fig. 1.** Breakthrough curves for the leaching of conservative chloride ion and
642 tebuconazole (% of the initially added ¹⁴C-fungicide applied) without incubation (NI)
643 and after incubation (I) in unamended soil columns (S) (A,C) and in soil columns
644 amended with spent mushroom substrate (SMS) at rates of 5% (S+SMS5) (B,E) and
645 50% (S+SMS50) (C,F) under different flow regimes.

646

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

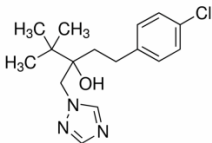
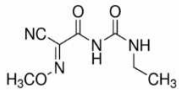
652

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

657

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 (<i>RS</i>)-1- <i>p</i> -chlorophenyl-4,4-dimethyl-3-(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)pentan-3-ol		36	3.7	769	63-365	2.85
Cymoxanil 1-[(<i>EZ</i>)-2-cyano-2-methoxyiminoacetyl]-3-ethylurea		780	0.67	43.6	0.7-3.5	0.34

WS water solubility at 20 °C, Kow octanol/water partition coefficient at pH 7 and 20°C, Koc adsorption coefficient normalized to organic carbon content, DT₅₀ 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 \pm SD ^a (mL g ⁻¹)	PV \pm SD (mL)	ρ \pm SD (g cm ⁻³)	θ \pm SD (cm ³ cm ⁻³)	R \pm SD
Soil (S)		1.67 \pm 0.01				
Sat ^b	Non-incubated		42.60 \pm 0.59	1.21 \pm 0.02	0.52 \pm 0.00	4.90 \pm 0.05
Sat-Unsat ^c	Non-incubated		39.89 \pm 1.14	1.20 \pm 0.01	0.48 \pm 0.02	5.16 \pm 0.12
Sat	Incubated		37.49 \pm 3.28	1.18 \pm 0.01	0.44 \pm 0.04	5.44 \pm 0.39
Sat-Unsat	Incubated		38.45 \pm 0.04	1.20 \pm 0.01	0.46 \pm 0.00	5.32 \pm 0.00
S+SMS5		4.38 \pm 0.30				
Sat	Non-incubated		44.48 \pm 1.50	1.19 \pm 0.02	0.53 \pm 0.03	10.8 \pm 0.33
Sat-Unsat	Non-incubated		42.41 \pm 0.25	1.17 \pm 0.02	0.50 \pm 0.01	11.3 \pm 0.06
Sat	Incubated		43.49 \pm 2.64	1.18 \pm 0.01	0.51 \pm 0.03	11.1 \pm 0.61
Sat-Unsat	Incubated		43.47 \pm 0.01	1.20 \pm 0.01	0.52 \pm 0.01	11.1 \pm 0.00
S+SMS50		32.8 \pm 0.49				
Sat	Non-incubated		42.95 \pm 1.59	0.65 \pm 0.01	0.35 \pm 0.02	62.2 \pm 2.25
Sat-Unsat	Non-incubated		46.79 \pm 5.53	0.71 \pm 0.10	0.42 \pm 0.11	57.6 \pm 6.60
Sat	Incubated		46.73 \pm 4.05	0.68 \pm 0.08	0.40 \pm 0.08	57.5 \pm 4.85
Sat-Unsat	Incubated		47.94 \pm 4.81	0.69 \pm 0.08	0.42 \pm 0.09	56.1 \pm 5.50

^aStandard deviation of replicates (n=3). ^bSaturated. ^cSaturated-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 \pm SD ^a (mL g ⁻¹)	PV \pm SD (mL)	ρ \pm SD (g cm ⁻³)	θ \pm SD (cm ³ cm ⁻³)	R \pm SD
Soil (S)		0.11 \pm 0.01				
Sat	Non-incubated		42.47 \pm 0.38	1.15 \pm 0.02	0.48 \pm 0.01	1.22 \pm 0.00
Sat-Unsat	Non-incubated		39.97 \pm 1.19	1.15 \pm 0.00	0.46 \pm 0.01	1.23 \pm 0.01
Sat	Incubated		41.71 \pm 2.14	1.23 \pm 0.09	0.51 \pm 0.01	1.22 \pm 0.03
Sat-Unsat	Incubated		42.13 \pm 0.92	1.15 \pm 0.00	0.48 \pm 0.01	1.22 \pm 0.01
S+SMS5		0.33 \pm 0.06				
Sat	Non-incubated		44.06 \pm 0.40	1.11 \pm 0.01	0.49 \pm 0.01	1.75 \pm 0.01
Sat-Unsat	Non-incubated		43.41 \pm 0.41	1.13 \pm 0.01	0.49 \pm 0.00	1.76 \pm 0.01
Sat	Incubated		44.48 \pm 0.59	1.12 \pm 0.00	0.50 \pm 0.01	1.74 \pm 0.02
Sat-Unsat	Incubated		44.90 \pm 0.58	1.11 \pm 0.01	0.50 \pm 0.00	1.74 \pm 0.01
S+SMS50		3.28 \pm 0.06				
Sat	Non-incubated		38.36 \pm 1.81	0.81 \pm 0.03	0.31 \pm 0.02	9.55 \pm 0.40
Sat-Unsat	Non-incubated		40.66 \pm 0.41	0.88 \pm 0.02	0.36 \pm 0.01	9.06 \pm 0.08
Sat	Incubated		38.72 \pm 0.26	0.84 \pm 0.04	0.33 \pm 0.02	9.46 \pm 0.06
Sat-Unsat	Incubated		42.16 \pm 0.57	0.82 \pm 0.01	0.35 \pm 0.00	8.77 \pm 0.11

^aStandard deviation of replicates (n=3). ^bSaturated. ^cSaturated-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	4.17 ± 0.57	3.41 ± 0.10
PV	1.41 ± 0.07	2.90 ± 0.50	1.35 ± 0.34	1.19 ± 0.04
Total retained	24.2 ± 0.30	1.14 ± 0.02	40.2 ± 4.08	49.6 ± 13.8
Total leached	58.7 ± 2.96	3.17 ± 0.04	46.7 ± 0.92	38.4 ± 4.46
Total column	82.8 ± 3.26	50.7 ± 12.4	86.9 ± 5.00	87.9 ± 9.31
S+SMS5				
Max. Peak	2.25 ± 0.18	3.95 ± 0.02	1.15 ± 0.12	3.19 ± 1.08
PV	1.35 ± 0.05	1.12 ± 0.03	2.12 ± 0.83	1.09 ± 0.01
Total retained	65.4 ± 4.41	77.7 ± 1.58	1.06 ± 0.01	86.9 ± 5.78
Total leached	27.4 ± 1.71	21.6 ± 3.40	1.77 ± 0.04	16.8 ± 1.22
Total column	92.9 ± 2.70	99.2 ± 4.90	77.9 ± 1.80	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

^aStandard 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	10.8 ± 1.04	2.89 ± 0.06	6.76 ± 0.03
	7.01 ± 0.21			
PV	1.68 ± 0.22	1.57 ± 0.40	1.28 ± 0.28	1.93 ± 0.37
	3.28 ± 0.31			
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

^aStandard deviation of replicates (n=3).

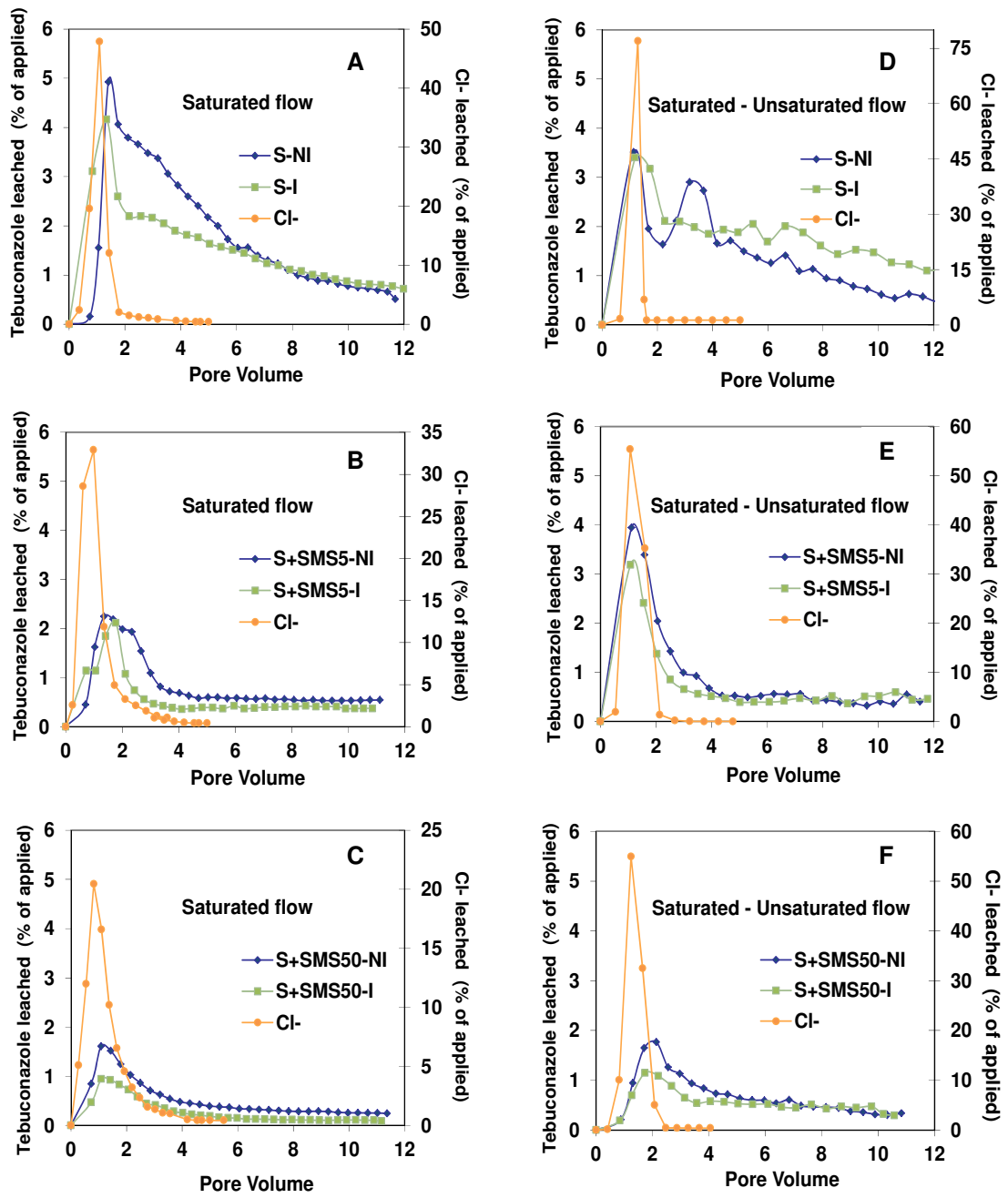


Figure 1.

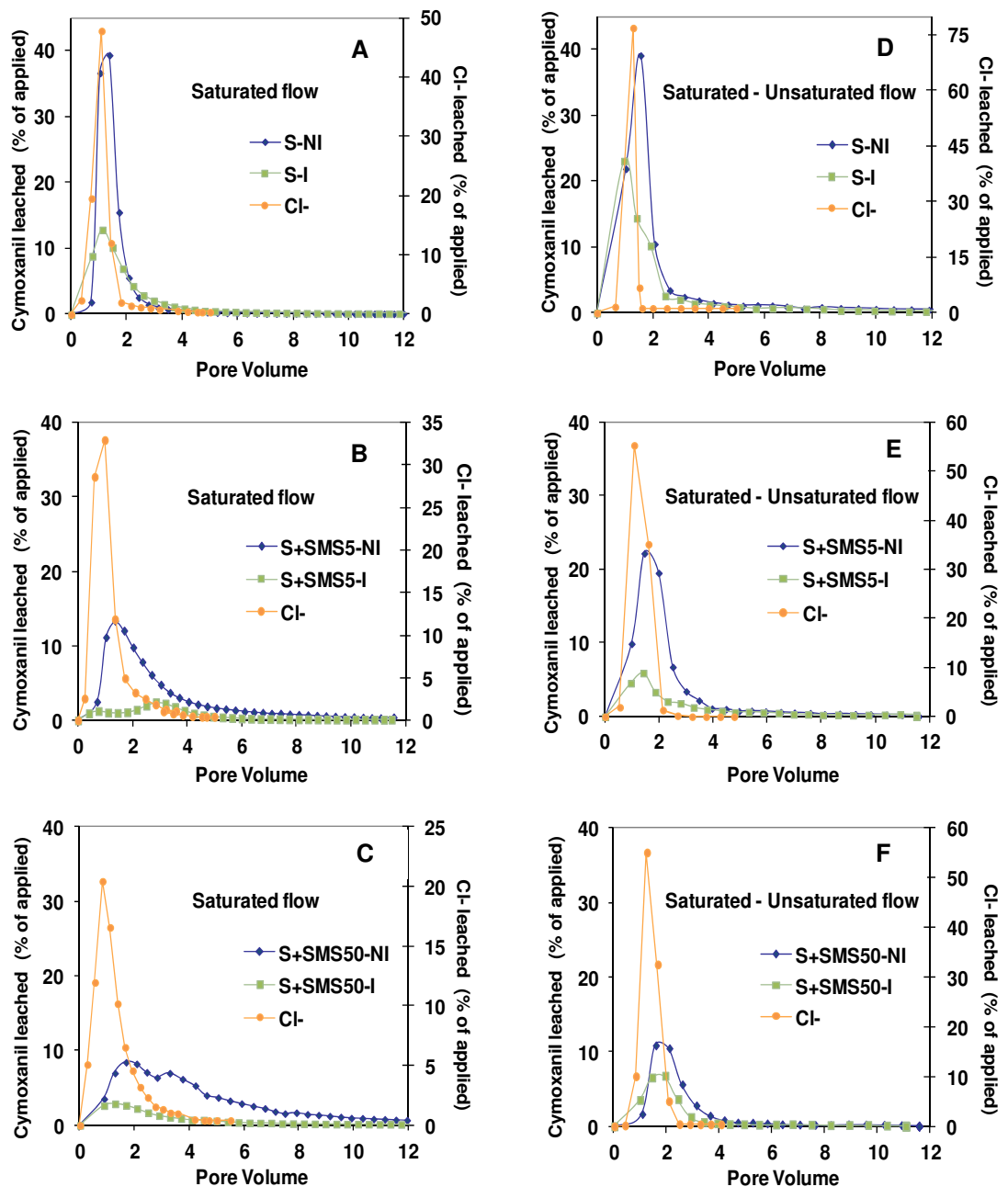


Figure 2.

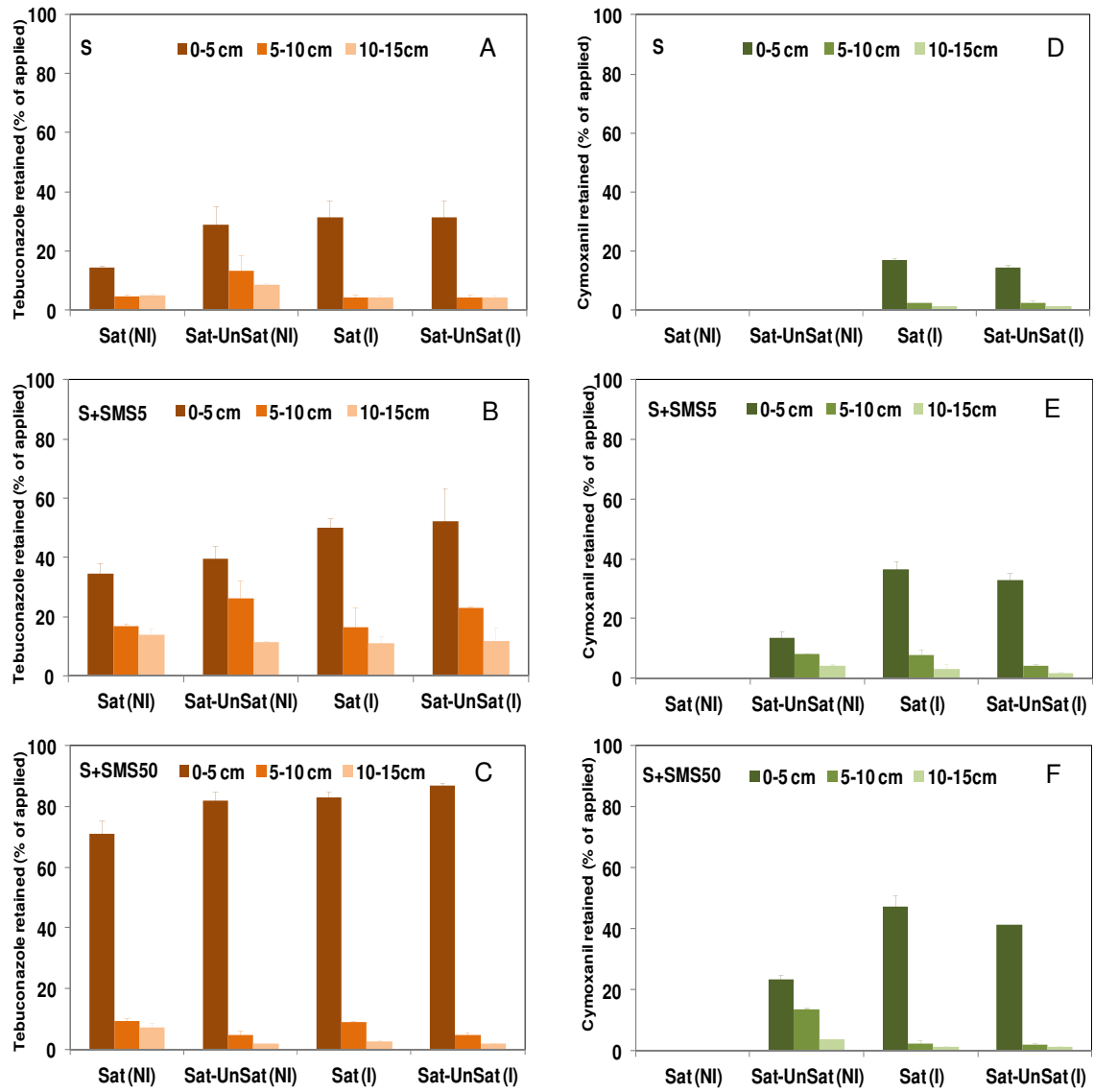


Figure 3.