SOIL CLAY MODIFICATION WITH SPERMINE AND ITS EFFECT ON THE BEHAVIOR OF THE HERBICIDE FLUOMETURON

B. Gámiz, R. Celis*, M.C. Hermosín, and J. Cornejo

Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina Mercedes 10, P.O. Box 1052, 41080 Sevilla, Spain

* **Corresponding Author:** Dr. Rafael Celis, **Address:** Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina Mercedes 10, P.O. Box 1052, 41080 Sevilla, Spain, **Phone:** +34 954624711, **Fax:** +34 954624002, **E-mail:** rcelis@irnase.csic.es

SOIL CLAY MODIFICATION WITH SPERMINE AND ITS EFFECT ON THE BEHAVIOR OF THE HERBICIDE FLUOMETURON

ABSTRACT

Research on organoclays as sorbents of pesticides has shown the usefulness of these materials both as soil amendments and as pesticide supports, to prolong the efficacy and reduce the environmental impact of soil-applied pesticides. Organoclays can also be formed *in situ* by direct modification of smectite-rich soil clays through treatment with appropriate organic cations. In this work, laboratory experiments were conducted to investigate the effect of the addition to soil of a natural organic cation, spermine, on sorption (batch equilibration procedure), persistence (incubation experiments) and leaching (column experiments) of the herbicide fluometuron. Four soils differing in their clay contents and mineralogy were used. A seven-fold increase in the fluometuron sorption distribution coefficient, K_d, by the presence of spermine was observed for a clay soil rich in smectites (soil 1), whereas the effect on fluometuron sorption was negligible for a soil with similar clay texture but lacking smectites (soil 4) and for a loamy sand soil with very low content in smectites (soil 3). A sandy loam soil (soil 2) with moderate smectite content had an intermediate behavior. The increase in fluometuron sorption was thus related to the smectite content of the soils, strongly indicating that reaction of spermine with soil smectites formed *in situ* an organoclay complex with high affinity for fluometuron. The simultaneous application of fluometuron and spermine to the smectite-rich soil retarded the leaching of the herbicide but, at the same time, increased the total amount of fluometuron leached from 25 to 52%. This was attributed to the fact that spermine, apart from increasing sorption, also prolonged the persistence of fluometuron in the smectite-rich soil. In contrast, the presence of spermine had no effect on the leaching pattern of fluometuron in the clay soil lacking smectites. The results indicated that the application of

25   suitable organic cations to smectitic soils can be a useful strategy to control pesticide retention

26   and persistence in the soil environment.

27

28   **INTRODUCTION**

29   The widespread use of pesticides in modern agricultural production and other activities has

30   resulted in increasing levels of pesticide residues in natural ecosystems, thus becoming an

31   important environmental problem. One main problem of pesticide use relates to the large

32   transport losses that usually affect pesticides applied to agricultural land, which cause offsite

33   movement of the pesticide and diffuse contamination of soil and water systems. In this

34   context, there is a need to develop efficient strategies to prevent and mitigate soil and water

35   contamination by pesticides, and particularly to reduce pesticide mobility in the environment

36   (Hapeman et al., 2003).

37   Organoclays, that is, natural clay minerals with their original inorganic exchangeable

38   cations replaced with organic cations, have been proven to be very good sorbents for different

39   classes of pesticides and, accordingly, have been proposed to prevent and remediate

40   environmental contamination by pesticides (Hermosín et al., 1992; Brixie and Boyd, 1994;

41   Prost and Yaron, 2001; Cornejo et al., 2008). Research on organoclays as sorbents of

42   pesticides has shown the usefulness of these materials both as soil amendments and as

43   pesticide supports, to prolong the efficacy of soil-applied pesticides and to reduce the large

44   transport losses that usually affect pesticides applied in an immediately available form

45   (Carrizosa et al., 2000; El-Nahhal et al., 2001; Trigo et al., 2009; Undabeytia et al., 2010;

46   Gámiz et al., 2010). An alternative approach is the *in situ* formation of organoclay complexes

47   by direct modification of smectite-rich soil clays through treatment with appropriate organic

48   cations. For instance, it has been shown that the addition of alkylammonium cations to soils

49   can enhance the retention of organic pollutants and reduce their leaching through the soil

50   profile (Boyd et al., 1988; Lee et al., 1989; Sánchez et al., 2003; Rodríguez-Cruz et al., 2007).

51   Nevertheless, the existing information on the effects of specific organic cations as soil

52   modifiers on pesticide behavior is still scarce. This is particularly true for naturally-occurring

53   organic cations, since research on organoclays as sorbents of pesticides has focused on

54   synthetic, alkylammonium-type cations of the general form $[(CH_3)_3NR]^+$ or $[(CH_3)_2NR_2]^+$,

55   where R is an aromatic or aliphatic hydrocarbon (Cruz-Guzmán et al., 2004). The use of

56   natural organic cations for clay mineral modification has been stressed as particularly

57   interesting to reduce concern about incorporation of these materials into soil and aquatic

58   environments for practical applications (Cruz-Guzmán et al., 2004; Celis et al., 2007).

59   Fluometuron (Figure 1) is a pre- and early post-emergence phenylurea herbicide widely

60   used for controlling annual grasses and broad-leaved weeds mainly in cotton and olive groves

61   at 1-2 kg ha^{-1}. It has a molecular weight of 232.2 g mol^{-1} and a water solubility of 105 mg L^{-1}

62   at 20 ℃ (Worthing and Hance, 1991). Fluometuron sorption in soil has been related primarily

63   to the soil organic matter content (Savage and Wauchope, 1974; Mueller et al., 1992). In

64   Southern Spain and other regions of the world, cotton and olive crops represent high-risk

65   scenarios of ground and surface water contamination by herbicides; soil properties and

66   climatic conditions favor herbicide leaching and runoff processes and it is therefore important

67   to develop strategies directed to optimize herbicide efficacy and prevent water pollution

68   (Mueller et al., 1992; Trigo et al., 2009; Gámiz et al., 2010, Locke et al., 2011).

69   In previous laboratory experiments, we found that the modification of reference smectites

70   (SWy-2 and SAz-1) with the naturally-occurring organic cation *spermine* (Figure 1) resulted

71   in organoclays with very high affinity for phenylurea herbicides, including fluometuron (Celis

72   et al., 2007; Gámiz et al. 2010). Spermine-exchanged reference smectites were efficient both

73   as soil amendments, to increase the soil retention capacity for such herbicides, and also as

74   herbicide supports, for the development of herbicide slow release formulations (Trigo et al.,

75 2009; Gámiz et al., 2010). The objective of the present work was to investigate the effects of

76 spermine addition to natural soils on sorption, persistence, and leaching of the herbicide

77 fluometuron, and to relate such effects to the soil clay mineralogy. Our hypothesis was that

78 spermine cations should react with soil smectites, increasing the soil retention capacity and

79 retarding herbicide leaching, that is, a sort of "in situ" slow release formulation could be

80 formed in soils by the simultaneous application of the herbicide and spermine. Four soils with

81 different clay contents and mineralogy were selected, characterized, and the influence of

82 spermine on fluometuron behavior (sorption, persistence, and leaching) in the soils assessed

83 by batch sorption, incubation, and column leaching tests.

84

85 **MATERIALS AND METHODS**

86 **Herbicide, organic cation, and soils**

87   Pure analytical fluometuron (purity = 99.5%) purchased from Sigma-Aldrich (Spain) was

88 used to prepare the external standards for fluometuron analysis and the initial solutions used

89 in the batch sorption experiments. Commercial fluometuron (Dinagam Linz, 50% suspension

90 concentrate) supplied by Bayer (Spain) was used in the persistence and column leaching

91 experiments.

92   Spermine (Figure 1) was supplied as a high-purity tetrachloride salt by Sigma-Aldrich

93 (Spain). Spermine is a naturally-occurring polyamine with four pK_a values which lie in the

94 interval 8-11 (Frassineti et al., 1995). Accordingly, the fully protonated form of spermine is

95 supposed to predominate under acidic, neutral, and slightly basic conditions.

96   Four agricultural soils from Seville (SW Spain) were used in this study. The soils were

97 selected on the basis of their different clay contents and mineralogy, which resulted in a range

98 of smectite contents in the whole soils. Soil 1 was sampled from a cotton area that had

99 received repeated applications of fluometuron for years as an agricultural practice, although

100 the soil sample used in this study contained no detectable background concentration of

101 fluometuron. Soils 2, 3, and 4 were sampled, respectively, from olive-, cereal-, and citrus-

102 growing plots and had no history of previous applications of fluometuron. All four soils were

103 sampled from the top 0-20 cm layer, air-dried, and sieved (2 mm), prior to use.

104

105 **Soil characterization**

106   The texture of the soils was determined by the hydrometer method (Klute, 1986). The

107 organic carbon contents were determined by dichromate oxidation, the carbonate contents by

108 the pressure calcimeter method, and the cation exchange capacities (CEC) by the 1 M

109 ammonium acetate method (Jackson, 1975; Sparks, 1996). Soil pH values were measured in

110 1:2.5 soil:1 M KCl aqueous suspensions. The variability associated with the measured soil

111 properties was < 10%.

112   A detailed characterization of the pore size distribution of the soils and of their clay

113 mineralogy was also carried out. The soil pore-size distributions (from 40 to 0.004 µm) were

114 determined by mercury intrusion porosimetry using a Carlo Erba 2000 mercury intrusion

115 porosimeter. A soil core of approximately 1 g was sampled and dried at 90 ℃ for 24 hours

116 before the porosity analysis. A value for the surface tension of mercury of $\gamma = 0.48$ N m^{-1} and

117 a mercury solid contact angle of $\theta = 171.3°$ were assumed to use the Washburn (1921)

118 equation for the calculation of the pore size distributions.

119   Carbonates were removed from the soils by treatment with ammonium acetate/acetic acid

120 buffer (pH= 5) before separating the soil clay (< 2 µm) fractions by sedimentation for

121 subsequent mineralogical analysis. The amounts of amorphous Fe and Al in the soil clays

122 were determined by oxalate-extraction (McKeague and Day, 1966), whereas the phyllosilicate

123 mineralogy was determined by X-ray diffraction analysis on oriented specimens of Mg^{2+}- and

124 K^+-saturated clay samples, solvated with liquid ethylene glycol (EG) and calcined at 550 ℃,

125 respectively (Brown, 1961). X-ray diffractograms were obtained in a D-5000 diffractometer

126 (Siemens, Stuttgart) using CuK$_\alpha$ radiation.

127

128 **Sorption experiments**

129 The effect of spermine on fluometuron sorption by the different soils was determined by

130 the batch equilibration procedure using glass centrifuge tubes and a single herbicide initial

131 concentration C_{ini}= 1 mg L^{-1}. This concentration was selected for being representative of field

132 concentrations of fluometuron applied at 1-2 kg ha^{-1}. Triplicate 4 g-soil aliquots were

133 equilibrated by shaking for 24 h at 20 ± 2 °C with 8 mL of aqueous solutions of fluometuron

134 (1 mg L^{-1}), also containing an amount of spermine tetrahydrochloride corresponding to 0, 1, 5,

135 10, 25 or 100% of the CEC of the 4 g-soil aliquot. After equilibration, the suspensions were

136 centrifuged and 4 mL of the supernatant solution was removed, filtered using a glass fiber

137 filter (pore diameter= 0.45 µm), and analyzed by high-performance liquid chromatography

138 (HPLC) to determine the equilibrium concentration, C_e (mg L^{-1}), of fluometuron. The amount

139 of fluometuron sorbed by the soils, C_s (mg kg^{-1}), was calculated from the difference between

140 the initial (C_{ini}) and equilibrium (C_e) solution concentrations. A 1 mg L^{-1} fluometuron solution

141 without soil was also shaken for 24 h and served as a control to rule out the possibility that

142 processes other than sorption to the soils contributed to the decrease in the fluometuron

143 solution concentration during the 24 h equilibration. The percentage of fluometuron sorbed (%

144 Sorbed) for the initial herbicide concentration of 1 mg L^{-1} was calculated by using the

145 following formula: % Sorbed = [(C_{ini}-C_e)/C_{ini}] × 100, whereas sorption distribution

146 coefficients, K_d (L kg^{-1}) were calculated as $K_d = C_s/C_e$. Previous work reported linear sorption

147 of fluometuron on soils at herbicide concentrations around 1 mg L^{-1} and that 24 h was

148 sufficient to reach the soil sorption equilibrium for fluometuron (Rankins et al., 2002).

149

Column leaching experiment

Leaching was studied in 30 cm length x 3.1 cm internal diameter glass columns filled with 160 g (20 cm) of soil (soils 1 and 4), following a procedure similar to that described in Gámiz et al. (2010). The soil columns were saturated with deionized water and allowed to drain for 24 h. The calculated pore volume of the columns after saturation was 63 ± 1 mL for both soils. Once saturated, 6 mL of an aqueous solution containing 0.15 mg of commercial fluometuron, active ingredient (a.i.), was applied to the top of triplicate soil columns to give an herbicide application rate of 2 kg a.i. ha^{-1}. The fluometuron solution added also contained the amount of spermine tetrahydrochloride needed to compensate the CEC of the amount of soil (20 g) present in the top 0-2.5 cm of the column. Daily, 15 mL of deionized water were added to the top of the columns, the leachates were collected, and the concentration of fluometuron was determined by HPLC. Breakthrough curves (BTCs) of fluometuron in soils 1 and 4 were thus obtained and compared with those obtained with control soil columns treated identically but without spermine addition. At the end of the leaching experiment, the soil columns were extracted with methanol to determine the residual amount of fluometuron (Gámiz et al., 2010). All leaching experiments were conducted in triplicate.

The position of the maximum concentration peaks of the BTCs was compared with the predicted retardation factor (*RF*) calculated assuming linear, instantaneous, and reversible sorption conditions using the following equation (Rao et al., 1985):

$$RF = 1 + (\rho/\theta)\, K_{\mathrm{d}} \qquad \text{Eq. (1)}$$

In Eq. 1, ρ is the bulk density of the soil column (g cm^{-3}); θ is the soil column volumetric water content (cm^3 cm^{-3}) and K_{d} (L kg^{-1}) is the sorption distribution coefficient for the herbicide in the soil column. The K_{d} values measured in the batch sorption experiments were

175   assumed for the calculation of the *RF* values using Eq. 1. The *RF* value represents the number

176   of pore volumes at which the maximum concentration of the herbicide is expected to appear

177   in leachates (Flores-Céspedes et al., 2002).

178

179   **Incubation experiment**

180   The effect of spermine on fluometuron dissipation in soil 1 was determined by means of

181   an incubation experiment in which a portion of 100 g of soil was spiked with commercial

182   fluometuron at a rate of 2 mg a.i. kg^{-1}. The aqueous solution of fluometuron (40 mL) used to

183   spike the soil contained either no spermine or the amount of spermine tetrahydrochloride (3.6

184   g) needed to compensate 100% of the CEC of the 100 g-soil aliquot. The spiked soil samples

185   were incubated in glass jars at 20 ± 2ºC for 40 days. The moisture content of the soil was

186   maintained at a constant level ($\sim 30\%$) throughout the experiment by adding distilled water as

187   necessary. Twice a week, triplicate 3 g-soil aliquots were sampled using a sterilized spatula

188   and frozen immediately until analyzed. Fluometuron residues in the 3 g soil-aliquots were

189   determined by extraction with 8 mL of methanol (24 h shaking), followed by centrifugation

190   and analysis of the supernatant by HPLC. Preliminary experiments had shown that this

191   extraction procedure recovered > 95% of the fluometuron freshly applied to the soils (Gámiz

192   et al., 2010). Differences between the amount of fluometuron added to the soil and the

193   amounts extracted with methanol were assumed to be due to degradation and/or formation of

194   strongly-bound herbicide residues (Albarrán et al., 2003).

195

196   **Analysis of fluometuron**

197   Fluometuron was determined by high performance liquid chromatography (HPLC) using a

198   Waters 600E chromatograph coupled to a Waters 996 diode-array detector. Details on the

199   chromatographic conditions used for the analysis can be found in Gámiz et al. (2010).

200   Instrumental limit of detection (LOD) was calculated as the lowest observable concentration

201   giving a signal-to-noise (S/N) ratio of 3:1, while instrumental limit of quantification (LOQ)

202   was calculated as the concentration resulting in an S/N ratio of 10:1 (Gámiz et al., 2010). The

203   LOD and LOQ were 0.01 mg L^{-1} and 0.03 mg L^{-1}, respectively.

204

205   **RESULTS AND DISCUSSION**

206   **Soil characterization**

207   Relevant physicochemical characteristics of the soils used in this study are given in Table

208   1. The soils had similar pH (7.0-7.4) and low organic carbon contents (0.62-1.10%), the latter

209   being a typical characteristic of soils from Mediterranean regions. The carbonate content was

210   high for soil 1, moderate for soil 3, and low for soils 2 and 4. The cation exchange capacity

211   (CEC) ranged from 15 to 42 $cmol_c$ kg^{-1} and was found to be highly correlated with the clay

212   content of the soils (r= 0.996, P< 0.01).

213   Figure 2 shows the pore-size distribution of the soils in the range from 40 to 0.004 µm,

214   determined by mercury intrusion porosimetry. Bars indicate the contribution of the

215   corresponding pore-radius interval to the cumulative volume. In agreement with previous

216   work, the mercury pore-size distributions were closely related to soil texture (Cox et al., 1994;

217   Chung and Alexander, 1999; Aichberger et al., 2006). Pores with radii larger than 10 µm

218   predominated in soils 2 and 3, thus reflecting the coarse texture of these soils (Table 1). In

219   contrast, medium- to small-sized pores predominated in soils 1 and 4 (Figure 2). These pores

220   probably resulted from aggregation of fine silt and clay particles, which clearly predominated

221   in soils 1 and 4 (Table 1).

222   The X-ray diffraction patterns of oriented specimens of Mg^{2+}- and K^+-saturated soil clay (<

223   2 µm) fractions are given in Figure 3. The X-ray diffractograms revealed that phyllosilicates

224   dominated the soil clay fractions. The absence of any diffraction peak near 14 Å in the Mg-

225 saturated samples solvated with ethylene glycol (Mg-EG) and in the K-saturated samples

226 calcined at 550 ºC (K-550ºC) indicated absence of chlorite in the soil clay samples (White and

227 Dixon, 2003; Calvaruso et al., 2009). Consequently, the diffractions at 17 Å, 10 Å, and 7 Å in

228 the Mg-EG diffractograms (Figure 3) were attributed to the (001) reflections of smectite-,

229 illite- (or mica-), and kaolinite-type phyllosilicates, respectively. The additional diffractions at

230 5.00 Å (~ 18 º2Θ) and 3.33 Å (~ 27 º2Θ) were attributed to the (002) and (003) illite

231 reflections, whereas that at 3.57 Å (~ 25 º2Θ) was attributed to the (002) kaolinite reflection.

232 It should be noted that although the (101) reflection of quartz could have contributed to the

233 diffraction peak at 3.33 Å, the very low intensity of the (100) quartz reflection at 4.26 Å (~ 21

234 º2Θ) observed in all diffractograms indicated low amounts of quartz in the soil clay samples.

235 The contents in smectite, illite and kaolinite of the soil clay fractions (Table 2) were

236 semiquantitatively estimated from the areas of the 17 Å, 10 Å, and 7 Å diffraction peaks once

237 normalized to the corresponding intensity factors of oriented specimens of the EG-solvated

238 minerals, which were assumed to be 2 for smectite, 0.5 for illite and 1 for kaolinite (Galán and

239 Espinosa, 1974). It was assumed that these phyllosilicates contributed to 100% of the clay

240 fraction of the soils (Kahle et al., 2002). This assumption was based on the lack of significant

241 diffraction peaks corresponding to other crystalline phases in the diffractograms (Figure 3)

242 and the low content of the soils in relevant amorphous materials, such as organic matter and

243 amorphous metal oxides. In this regard, oxalate-extraction indicated that amorphous Fe and

244 Al accounted for less than 2% of the soil clay fractions.

245   The phyllosilicate content in the whole soils, also included in Table 2, was calculated from

246 the composition of the soil clay fractions and the clay content of each soil. Despite smectites

247 predominating in the clay fractions of soils 2 and 3, a low clay content resulted in moderate to

248 low smectite contents in the whole soils. In the case of the clay soils, soil 1 contained 17%

249 smectitic minerals, whereas the absence of any diffraction peak at 17 Å in the Mg-EG

250   diffractogram indicated lack of smectites in soil 4. Thus, the different clay contents and

251   mineralogy of the soils resulted in a range of smectite contents, which decreased in the

252   following order: soil 1(17%) > soil 2 (10%) > soil 3 (3%) > soil 4 (0%).

253

254   **Effect of spermine on fluometuron sorption by the soils**

255   Figure 4 shows the percentage of fluometuron sorbed by the soils at a single herbicide

256   concentration of 1 mg L^{-1} either in the absence of spermine or in the presence of an amount of

257   spermine equal to the CEC of each soil. The corresponding sorption distribution coefficients,

258   K_d (= C_s/C_e), are summarized in Table 3. In the absence of spermine, fluometuron sorption by

259   soils 1 and 4 was considerably greater than that by soils 2 and 3, most likely as a result of their

260   greater clay and organic C contents (Table 1). Predominance of small-sized pores (Figure 2)

261   could have also contributed to enhance the herbicide sorption process in soils 1 and 4 (Cox et

262   al., 1994). Upon spermine addition, a great increase in fluometuron sorption was observed for

263   the clay soil rich in smectites (soil 1), whereas this effect was very small for the clay soil

264   lacking smectites (soil 4) and also for the loamy sand soil (soil 3), with very low smectite

265   content (Figure 4, Table 3). The sandy loam soil (soil 2), with moderate smectite content, had

266   an intermediate behavior. The increase in fluometuron sorption upon spermine addition was

267   thus clearly related to the smectite content of the soils, decreasing in the following order: soil

268   1 > soil 2 > soil 3 ≈ soil 4 (Figure 4, Table 3).

269   Previous work has shown that modification of reference smectites with spermine cations

270   leads to interlayer organoclay complexes with selective affinities for phenylurea herbicides

271   such as diuron and fluometuron (Celis et al., 2007; Gámiz et al., 2010). It appears that the

272   sorption mechanism may involve (i) hydrogen bonding between the C=O group of the

273   herbicide and the $-NH_3^+/-NH_2^+-$ groups of spermine and (ii) hydrophobic interactions between

274   the herbicide and the alkyl chains of spermine (Celis et al., 2007). The surface charge density

275   of the clay mineral and the amount of intercalated spermine appeared to be important factors

276   determining the sorption of diuron and fluometuron by spermine-modified reference

277   smectites, mainly because such variables determine the space available for herbicide sorption

278   within the organoclay structure (Jaynes and Boyd, 1991; Celis et al., 2007, Gámiz et al.,

279   2010). The results reported in Figure 4 and Table 3 show that the presence of spermine

280   enhanced fluometuron sorption also in natural soils, and strongly indicate that this effect was

281   directly related to the presence of expandable (smectitic) clay minerals. As an example, the

282   distribution coefficient, K_d, for fluometuron sorption on soil 1 (containing 17% smectites)

283   increased by a factor of 7.6 by the presence of spermine, whereas an increase in the K_d value

284   as small as 10% was measured for soils 3 and 4, which contained less than 5% smectites

285   (Table 2). This small increase in the K_d value measured for soils 3 and 4 could be attributed to

286   the small amount of smectites present in soil 3 or the presence of some active illitic minerals

287   in soil 4 (Table 2).

288   Fluometuron sorption as a function of the amount of spermine added to soil 1 was also

289   determined and the results reported in Figure 5. Fluometuron sorption by soil 1 steadily

290   increased with the amount of spermine added up to an amount of spermine corresponding to

291   25% of the CEC of the soil. Further addition of spermine did not result in additional sorption

292   of fluometuron. Previous work has shown that increasing the amount of organic cation up to

293   the CEC of clay minerals increases the hydrophobicity of the clay mineral surface, but a

294   maximum transformation from hydrophilic to hydrophobic does not always result in a

295   maximum sorption of hydrophobic organic compounds (Carrizosa et al., 2000; Nir et al.,

296   2000; El-Nahhal et al., 1998, 2001; Cruz-Guzmán et al., 2004; Cornejo et al., 2008). Clogging

297   of the interlayer space as a result of large organic cation loadings can give structures which

298   preclude access to the sorption sites (Nir et al., 2000; Cornejo et al., 2008). A similar

299   phenomenon may have operated in soil 1, since the amount of fluometuron sorbed on soil

300 treated with spermine cations at a loading of 25% of the soil CEC was similar to that on soil

301 treated with spermine at a loading up to the CEC (Figure 5). In addition, the possibility exists

302 that not all the cation exchange sites in soil were accessible for spermine cations or that

303 spermine interaction with soil components different from smectites, such as metal oxides or

304 organic matter, produced no effect on fluometuron sorption.

305

306 **Leaching and persistence experiments**

307 Soils 1 and 4 were selected for leaching experiments because, having similar clay textures,

308 their responses to spermine addition with regard to fluometuron sorption were markedly

309 different, as a result of their different smectite contents (Figure 4, Table 3). For the untreated

310 soils, fluometuron concentrations in leachates of soil 4 were considerably greater than those

311 measured in leachates of soil 1, but the maximum concentration of fluometuron appeared at

312 195 mL of water added (3.1 pore volumes) for both soils (Figure 6). This result was in

313 accordance with the expected retention factors (*RF*) for fluometuron in soils 1 and 4,

314 calculated using Eq. 1, which were 2.9 (183 mL water added) for soil 1 and 3.2 (202 mL water

315 added) for soil 4 (Table 3). Thus, linear, instantaneous, and reversible sorption conditions

316 described well the movement of fluometuron in the soil columns.

317 For untreated soils, fluometuron BTCs also revealed a marked difference in the individual

318 leachate concentrations and in turn in the total amount of herbicide leached from soils 1 and 4.

319 Most fluometuron (> 90%) added to soil 4 was recovered in leachates, whereas the total

320 amount of herbicide leached from soil 1 accounted for only 25% of the herbicide applied to

321 the soil column (Figure 6). The amount of herbicide extracted from soil 1 at the end of the

322 column leaching experiment was less than 5% of the amount applied, so that the fluometuron

323 not recovered is likely to correspond to the degradation of the herbicide within the soil column

324 and/or the formation of strongly bound herbicide residues. Taking into account that soil 1 had

325 received repeated applications of fluometuron for years as an agronomic practice for weed

326 control, we believe that the small amount of herbicide leached is likely to be due to rapid

327 degradation of fluometuron in this soil by adapted soil microorganisms (Cox et al., 1996).

328   When spermine was coapplied with fluometuron, herbicide leaching was retarded in soil 1,

329 whereas the effect was negligible in soil 4 (Figure 6). This result can be related to the distinct

330 effect of spermine on the sorption of fluometuron by soils 1 and 4 (Table 3). The position of

331 the maximum concentration peak in the BTC of fluometuron in soil 1 in the presence of

332 spermine was centered at about 250 mL of water added (4.0 pore volumes). This value is close

333 to the value of 275 mL (RF= 4.4) predicted by assuming that spermine interacted with the top

334 0-2.5 cm soil increasing the K_d value of soil 1 from 0.74 to 5.60 L kg^{-1} (Table 3). A similar

335 calculation for soil 4 predicted a negligible effect of spermine on the fluometuron RF value

336 (Table 3).

337   An additional effect of spermine addition to soil 1 was the increase in the total amount of

338 fluometuron leached from 25% to 52% of the amount of herbicide applied to the soil column.

339 This indicated the persistence of the herbicide could have been increased upon spermine

340 coapplication. A soil incubation experiment confirmed longer persistence of fluometuron in

341 soil 1 treated with spermine compared to the untreated soil (Figure 7). The dissipation of

342 fluometuron in spermine-free soil 1 was rapid and the sigmoidal shape of the dissipation curve

343 is typical of the degradation of compounds used as the microbial growth substrate (Gámiz et

344 al., 2010). In the presence of spermine, the dissipation of fluometuron in soil 1 was

345 significantly retarded. Although the enhanced sorption could have protected the herbicide

346 from degradation and/or formation of strongly-bound residues in soil, previous work has

347 indicated that sorption of phenylurea herbicides on spermine-exchanged reference smectites is

348 a reversible process and does not seem to reduce the bioavailability of the herbicide to soil

349 microorganisms (Trigo et al., 2009; Gámiz et al., 2010). Alternatively, spermine cations could

have acted as a readily available carbon and nitrogen source for such bacteria, retarding the degradation of the herbicide. Independent of the cause for the enhanced persistence of the herbicide, our results show that coapplication of spermine could be useful to prolong the persistence of fluometuron in soils where the herbicide is rapidly degraded, for instance due to previous repeated herbicide applications.

CONCLUSIONS

The reaction of organic cations with soil smectites can be useful as a strategy to control pesticide mobility and persistence in soils. The results of this work show that the presence of spermine enhances fluometuron sorption in smectite-containing soils, most likely because reaction between spermine cations and soil smectites forms "in situ" organoclay complexes with high affinity for the herbicide. Sorption experiments with four different soils revealed that the effect of spermine on fluometuron sorption depended on the smectite content of the soil and on the amount of spermine added. The increase in fluometuron sorption by spermine addition to a soil rich in smectites retarded the leaching of the herbicide through soil columns and, interestingly, also prolonged its soil persistence, whereas these effects were negligible for a soil lacking smectites. Therefore, the simultaneous application of fluometuron and spermine could be useful to enhance the herbicide retention process in soil, and to prolong the persistence of the herbicide in scenarios where its rapid degradation could result in loss of herbicidal efficacy. Additional research would be needed to verify adequate weed control of the herbicide under simultaneous application with spermine and to assess potential side-effects of spermine on target and non-target organisms.

375 **REFERENCES**

376

377 Aichberger, H., A.P. Loibner, R. Celis, R. Braun, F. Ottner, and H. Rost. 2006. Assessment of

378 factors governing biodegradability of PAHs in three soils aged under field conditions. Soil

379 Sediment Contam. 15:73-85.

380

381 Albarrán, A., R. Celis, M.C. Hermosín, A. López-Piñeiro, J.J. Ortega-Calvo, and J. Cornejo.

382 2003. Effects of solid olive-mill waste addition to soil on sorption, degradation and leaching

383 of the herbicide simazine. Soil Use Manag. 19:150-156.

384

385 Boyd, S.A., J.F. Lee, and M.M. Mortland. 1988. Attenuating organic contaminant mobility by

386 soil modification. Nature (London) 333:345-347.

387

388 Brixie, J.M., and S.A. Boyd. 1994. Treatment of contaminated soils with organoclays to

389 reduce leachable pentachlorophenol. J. Environ. Qual. 23:1283-1290.

390

391 Brown, G. 1961. The X-ray identification and crystal structures of clay minerals.

392 Mineralogical Society, London.

393

394 Calvaruso, C., L. Mareschal, M.P. Turpault, and E. Leclerc. 2009. Rapid clay weathering in

395 the rhizosphere of Norway spruce and oak in an acid forest ecosystem. Soil Sci. Soc. Am. J.

396 73:331-338.

397

398 Carrizosa, M.J., M.J. Calderón, M.C. Hermosín, and J. Cornejo. 2000. Organosmectites as

399 sorbent and carrier of the herbicide bentazone. Sci. Total Environ. 247:285-293.

400

401 Celis, R., C. Trigo, G. Facenda, M.C. Hermosín, and J. Cornejo. 2007. Selective modification

402 of clay minerals for the adsorption of herbicides widely used in olive groves. J. Agric. Food

403 Chem. 55:6650-6658.

404

405 Chung, N., and M. Alexander. 1999. Relationship between nanoporosity and other properties

406 of soil. Soil Sci. 164:726–730.

407

408 Cornejo, J., R. Celis, I. Pavlovic, and M.A. Ulibarri. 2008. Interactions of pesticides with

409 clays and layered double hydroxides: a review. Clay Miner. 43:155-175.

410

411 Cox, L., R. Celis, M.C. Hermosín, and J. Cornejo. 1994. Leaching patterns of pesticides as

412 related to sorption and porosity properties of soils. pp. 1325-1330, In: Proceedings of the

413 BCPC Conference on Pests and Diseases, Vol. 3, BCPC, Brighton, U.K.

414

415 Cox, L., A. Walker, and S.J. Welch. 1996. Evidence for accelerated degradation of

416 isoproturon in soils. Pestic. Sci. 48:253-260.

417

418 Cruz-Guzmán, M., R. Celis, M.C. Hermosín, and J. Cornejo. 2004. Adsorption of the

419 herbicide simazine by montmorillonite modified with natural organic cations. Environ. Sci.

420 Technol. 38:180-186.

421

422 El-Nahhal, Y., S. Nir, T. Polubesova, L. Margulies, and B. Rubin. 1998. Leaching,

423 phytotoxicity, and weed control of new formulations of alachlor. J. Agric. Food Chem.

424 46:3305-3313.

425

426 El-Nahhal, Y., S. Nir, C. Serban, O. Rabinovitz, and B. Rubin. 2001. Organo-clay formulation

427 of acetochlor for reduced movement in soil. J. Agric. Food Chem. 49:5364-5371.

428

429 Flores-Céspedes, F., E. González-Pradas, M. Fernández-Pérez, and M. Villafranca-Sánchez.

430 2002. Effects of dissolved organic carbon on sorption and mobility of imidacloprid in soil. J.

431 Environ. Qual. 31:880-888.

432

433 Frassineti, C., S. Ghelli, P. Gans, A. Sabatini, M.S. Moruzzi, and A. Vacca. 1995. Nuclear

434 magnetic resonance as a tool for determining protonation constants of natural polyprotic bases

435 in solution. Anal. Biochem. 231:374-382.

436

437 Galán, E., and J. Espinosa. 1974. El caolín en España: características, identificación y ensayos

438 cerámicos. Sociedad Española de Cerámica y Vidrio, Madrid.

439

440 Gámiz, B., R. Celis, M.C. Hermosín, and J. Cornejo. 2010. Organoclays as soil amendments

441 to increase the efficacy and reduce the environmental impact of the herbicide fluometuron in

442 agricultural soils. J. Agric. Food Chem. 58:7893-7901.

443

444 Hapeman, C.J., L.L. McConnell, C.P. Rice, A.M. Sadeghi, W.F. Schmidt, G.W. McCarty, J.L.

445 Starr, P.J. Rice, J.T. Angier, and J.A. Harman-Fetcho. 2003. Current United States

446 Department of Agriculture-Agricultural Research Service research on understanding

447 agrochemical fate and transport to prevent and mitigate adverse environmental impacts. Pest

448 Manag. Sci. 59:681-690.

449

450 Hermosín, M.C., M.A. Ulibarri, M. Mansour, and J. Cornejo. 1992. Assaying sorbents for 2,4-

451 dichlorophenoxyacetic acid from water. Fres. Environ. Bull. 1: 472-481.

452

453 Jackson, M.L. 1975. Soil chemical analysis. 2nd edition. University of Wisconsin, Madison,

454 WI.

455

456 Jaynes, W.F., and S.A. Boyd. 1991. Clay mineral type and organic compound sorption by

457 hexadecyltrimethylammonium-exchanged clays. Soil Sci. Soc. Am. J. 55:43-48.

458

459 Kahle, M., M. Kleber, and R. Jahn. 2002. Review of XRD-based quantitative analyses of clay

460 minerals in soils: the suitability of mineral intensity factors. Geoderma 109:191-205.

461

462 Klute, A. 1986. Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd

463 edition. SSSA Book Series Nº 5, Madison, WI.

464

465 Lee, J.-F., J.R. Crum, and S.A. Boyd. 1989. Enhanced retention of organic contaminants by

466 soils exchanged with organic cations. Environ. Sci. Technol. 23:1365-1372.

467

468 Locke, M.A., M.A. Weaver, R.M. Zablotowicz, R.W. Steinriede, C.T. Bryson, and R.F.

469 Cullum. 2011. Constructed wetlands as a component of the agricultural landscape: mitigation

470 of herbicides in simulated runoff from upland drainage areas. Chemosphere 83:1532-1538.

471

472 McKeague, J.A., and J.H. Day. 1966. Dithionite- and oxalate-extractable Fe and Al as aids in

473 differentiating various classes of soils. Can. J. Soil Sci. 46:13-22.

474

475   Mueller, T.C., T.B. Moorman, and C.E. Snipes. 1992. Effect of concentration, sorption, and

476   microbial biomass on degradation of the herbicide fluometuron in surface and subsurface

477   soils. J. Agric. Food Chem. 40:4517-2522.

478

479   Nir, S., T. Undabeytia, D. Yaron-Marcovich, Y. El-Nahhal, T. Polubesova, C. Serban, G.

480   Rytwo, G. Lagaly, and B. Rubin B. 2000. Optimization of adsorption of hydrophobic

481   herbicides on montmorillonite preadsorbed by monovalent organic cations: interaction

482   between phenyl rings. Environ. Sci. Technol. 34:1269-1274.

483

484   Prost, R., and B. Yaron. 2001. Use of modified clays for controlling soil environmental

485   quality. Soil Sci. 166:880-895.

486

487   Rankins Jr., A., D.R. Shaw, and W.L. Kingery. 2002. Comparison of fluometuron sorption to

488   soil from a filter strip and cropped field. Weed Sci. 50:820-823.

489

490   Rao, P.S.C., A.G. Hornsby, and R.E. Jessup. 1985. Indices for ranking the potential for

491   pesticide contamination of groundwater. Proc. Soil Crop Sci. Fla. 44:1-8.

492

493   Rodríguez-Cruz, M.S., M.J. Sánchez-Martín, M.J. Andrades, and M. Sánchez-Camazano.

494   2007. Retention of pesticides in soil columns modified *in situ* with a cationic surfactant. Sci.

495   Total Environ. 378:104-108.

496

497   Sánchez, L., E. Romero, F. Sánchez-Rasero, G. Dios, and A. Peña. 2003. Enhanced soil

498   sorption of methidathion using sewage sludge and surfactants. Pest Manag. Sci. 59:857-864.

499

500 Savage, K.E., and R.D. Wauchope. 1974. Fluometuron adsorption-desorption equilibria in

501 soil. Weed Sci. 22:106-110.

502

503 Sparks, D.L. 1996. Methods of soil analysis. Part 3. Chemical methods. SSSA Book Series Nº

504 5, Madison, WI.

505

506 Trigo, C., R. Celis, M.C. Hermosín, and J. Cornejo. 2009. Organoclay-based formulations to

507 reduce the environmental impact of the herbicide diuron in olive groves. Soil Sci. Soc. Am. J.

508 73:1652-1657.

509

510 Undabeytia, T., F. Sopeña, T. Sánchez-Verdejo, J. Villaverde, S. Nir, E. Morillo, and C.

511 Maqueda. 2010. Performance of slow-release formulations of alachlor. Soil Sci. Soc. Am. J.

512 74:898-905.

513

514 Washburn, E.W. 1921. Note on a method of determining the distribution of pore sizes in a

515 porous material. Proc. Nat. Acad. Sci. USA 7:115-116.

516

517 White, G.N., and J.B. Dixon. 2003. Soil mineralogy laboratory manual, 9th edition,

518 Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas.

519

520 Worthing, C.R., and R.J. Hance. 1991. The pesticide manual. British Crop Protection Council,

521 Surey, U.K.

522

523

524

LEGENDS FOR FIGURES

525

526

527 Figure 1. Molecular structures of fluometuron and spermine.

528

529 Figure 2. Relative (bars) and cumulative (curves) pore-size distributions of the soils

530 determined by mercury intrusion porosimetry. Error bars correspond to the standard error of

531 triplicate measurements.

532

533 Figure 3. X-ray diffractograms of oriented specimens of soil clay fractions: Mg^{2+}-saturated

534 (Mg), Mg^{2+}-saturated followed by solvation with ethylene glycol (Mg-EG), K^+-saturated (K),

535 and K^+-saturated, then calcined at 550 ºC (K-550ºC).

536

537 Figure 4. Percentages of fluometuron sorbed by the soils in the absence of spermine and in the

538 presence of an amount of spermine equal to the CEC of each soil. The experiment was

539 conducted at an initial fluometuron concentration 1 mg L^{-1} and a soil:solution ratio 4 g:8 mL.

540

541 Figure 5. Percentages of fluometuron sorbed by soil 1 in the presence of an amount of

542 spermine equal to 0, 1, 5, 10, 25, and 100% of the CEC of the soil. The experiment was

543 conducted at an initial fluometuron concentration 1 mg L^{-1} and a soil:solution ratio 4 g:8 mL.

544

545 Figure 6. Relative and cumulative breakthrough curves (BTCs) of fluometuron in soils 1 and 4

546 in the absence and in the presence of spermine.

547

548 Figure 7. Dissipation curves of fluometuron in soil 1 in the absence and in the presence of

549 spermine.

Table 1. Physicochemical characteristics of the soils used.

Soil	Texture	Sand	Silt	Clay	CaCO$_3$	Organic C	CEC	pH
		——————— % ———————					— cmol$_c$ kg^{-1} —	
Soil 1	Clay	7	34	59	17.2	0.73	42	7.2
Soil 2	Sandy loam	73	8	19	1.0	0.52	20	7.2
Soil 3	Loamy sand	83	7	10	6.5	0.54	15	7.4
Soil 4	Clay	22	33	45	2.8	1.10	32	7.0

Table 2. Smectite, illite and kaolinite contents of the soil clay fractions and the whole soils.

Soil	Soil clay (< 2 μm) fraction			Whole soil		
	Smectite	**Illite**	**Kaolinite**	**Smectite**	**Illite**	**Kaolinite**
	———————— % ————————			———————— % ————————		
Soil 1	28	57	15	17	34	9
Soil 2	52	19	29	10	4	6
Soil 3	34	46	20	3	5	2
Soil 4	0	88	12	0	40	5

Table 3. Distribution coefficients, K_d, for fluometuron sorption on untreated and spermine-treated soils and predicted retardation factors (RF) for fluometuron leaching through soil columns calculated assuming linear, instantaneous, and reversible sorption conditions (Eq. 1).

Soil	Untreated soil		Spermine-treated soil	
	K_d	RF	$K_d^{\dagger}$	$RF^{\ddagger}$
	—— L kg^{-1} ——		—— L kg^{-1} ——	
Soil 1	$0.74 \pm 0.01^{\S}$	2.9	5.60 ± 0.20	4.4
Soil 2	0.39 ± 0.02	2.0	0.69 ± 0.03	2.1
Soil 3	0.36 ± 0.01	1.9	0.39 ± 0.01	1.9
Soil 4	0.87 ± 0.02	3.2	0.96 ± 0.02	3.2

[†] K_d values for soils treated with an amount of spermine equal to their CEC.

[‡] It was assumed that the spermine added reacted with the top 0-2.5 cm of the soil column.

[§] Mean ± standard error.

Fluometuron
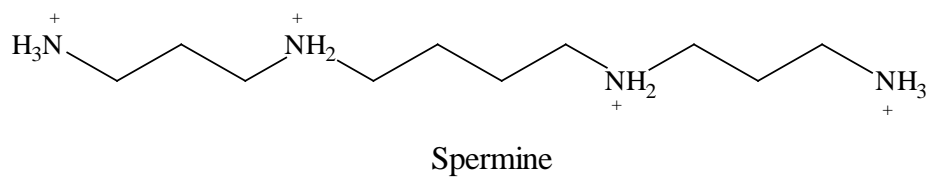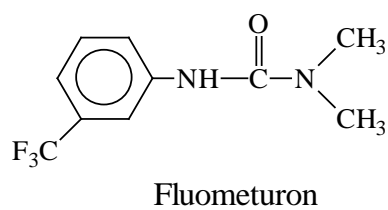
Spermine

Figure 1. Molecular structures of fluometuron and spermine.
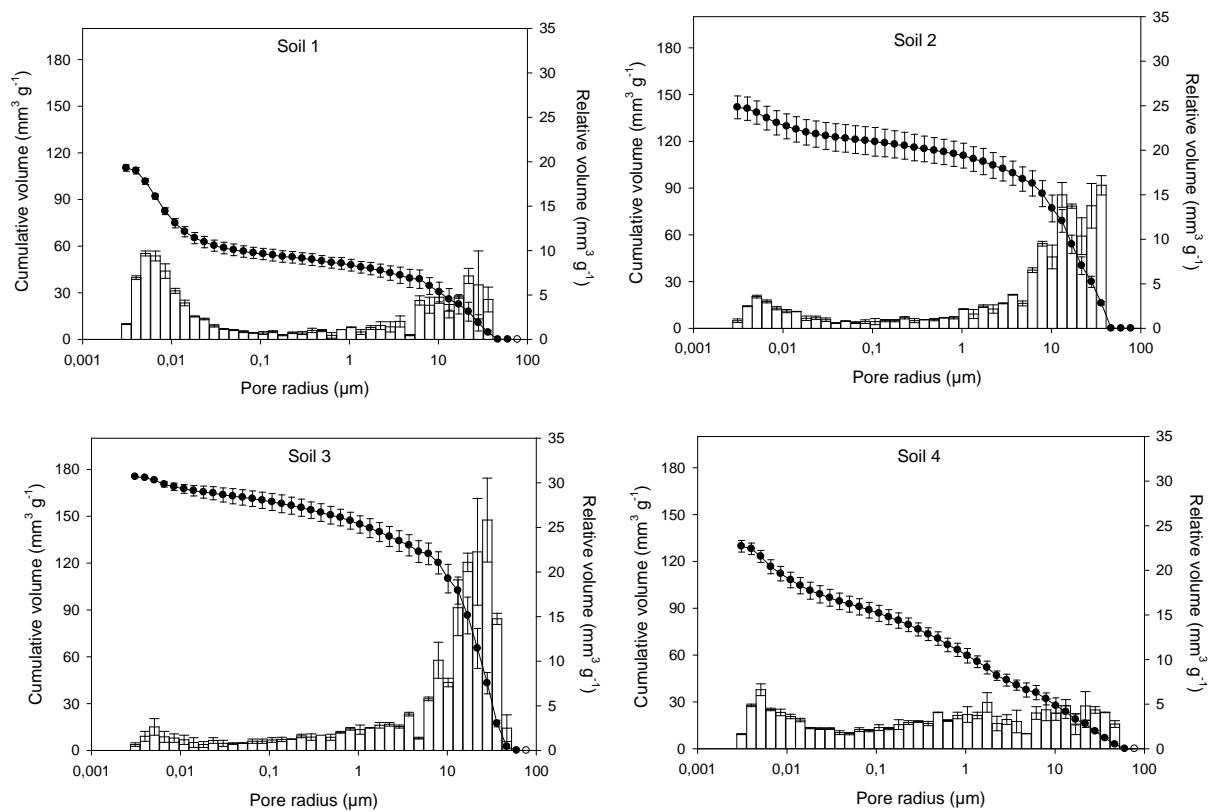
Figure 2. Relative (bars) and cumulative (curves) pore-size distributions of the soils determined by mercury intrusion porosimetry. Error bars correspond to the standard error of triplicate measurements.
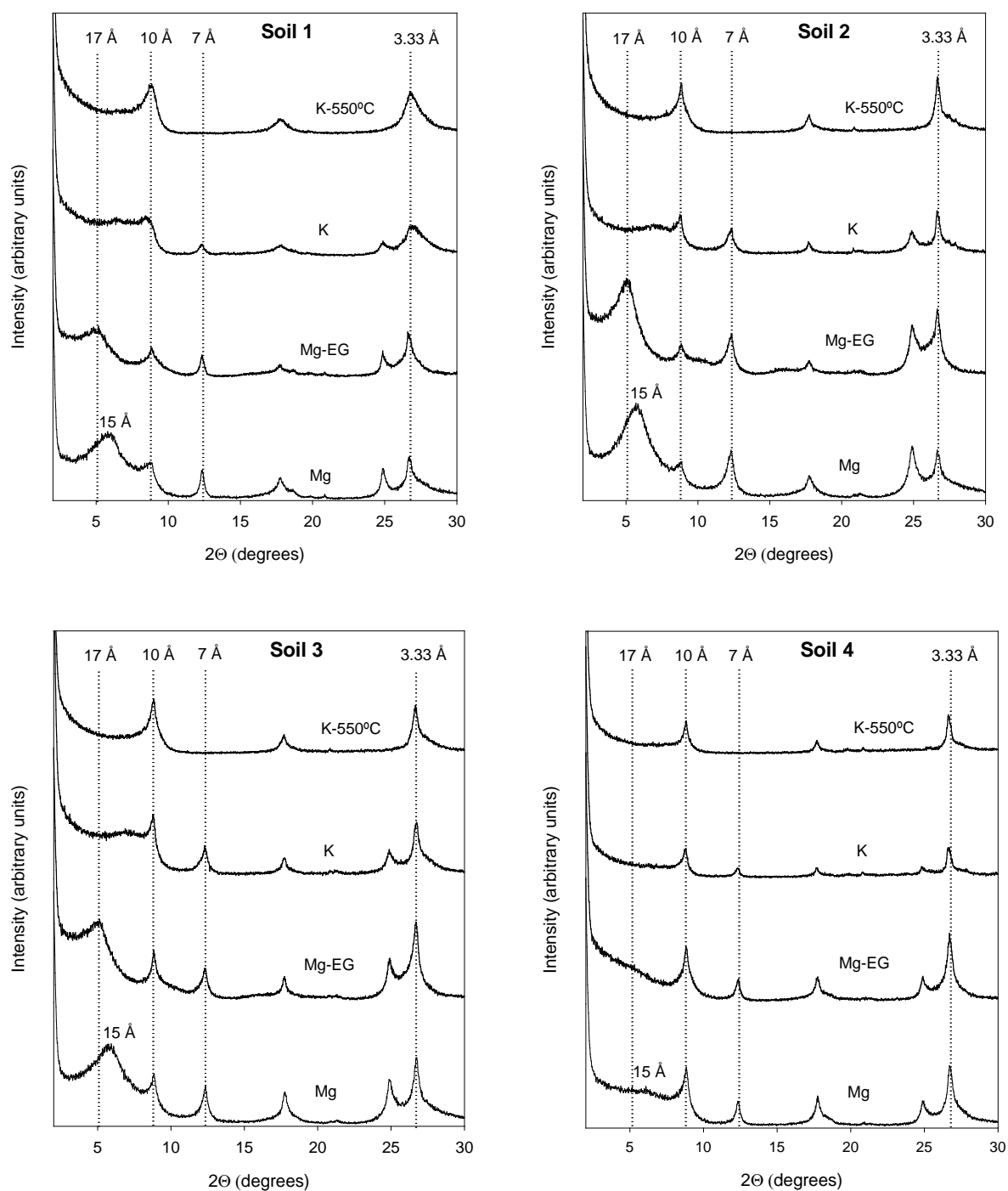
Figure 3. X-ray diffractograms of oriented specimens of soil clay fractions: Mg^{2+}-saturated (Mg), Mg^{2+}-saturated followed by solvation with ethylene glycol (Mg-EG), K^+-saturated (K), and K^+-saturated, then calcined at 550 ºC (K-550ºC).
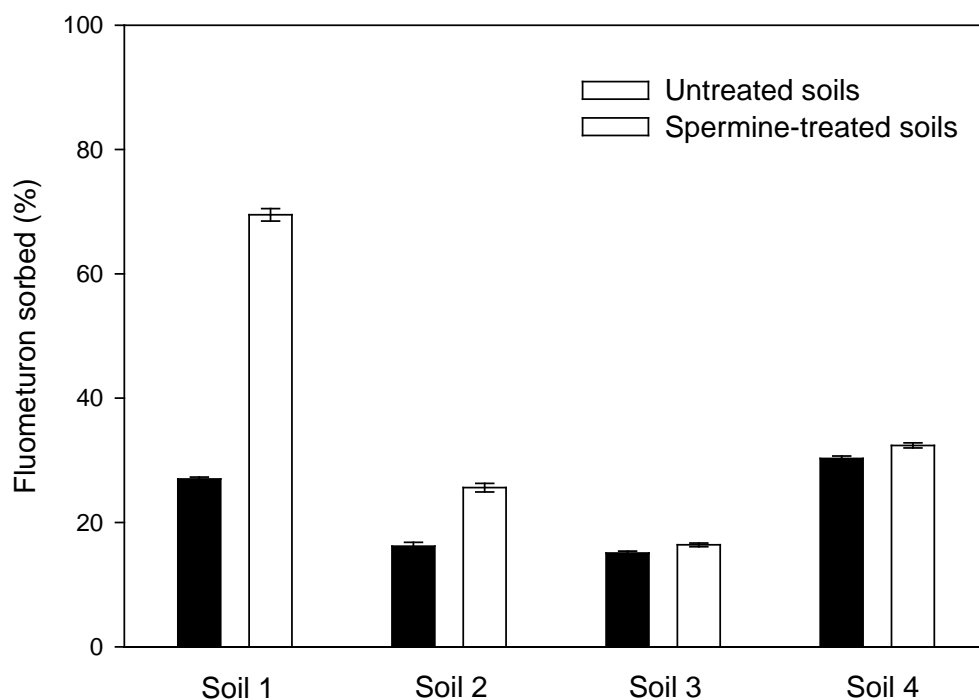
Figure 4. Percentages of fluometuron sorbed by the soils in the absence of spermine and in the presence of an amount of spermine equal to the CEC of each soil. The experiment was conducted at an initial fluometuron concentration 1 mg L^{-1} and a soil:solution ratio 4 g:8 mL.
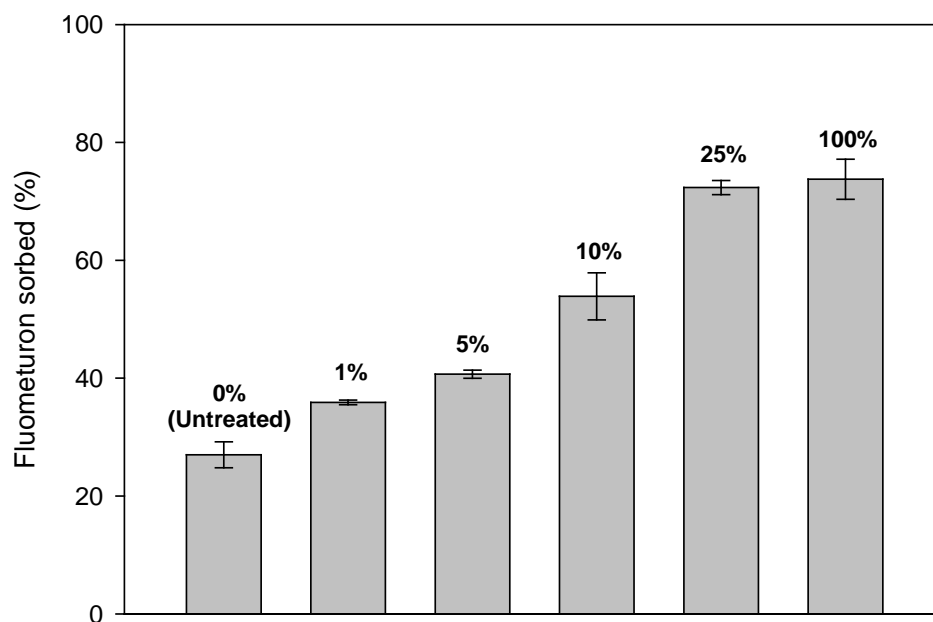
Figure 5. Percentages of fluometuron sorbed by soil 1 in the presence of an amount of spermine equal to 0, 1, 5, 10, 25, and 100% of the CEC of the soil. The experiment was conducted at an initial fluometuron concentration 1 mg L^{-1} and a soil:solution ratio 4 g:8 mL.
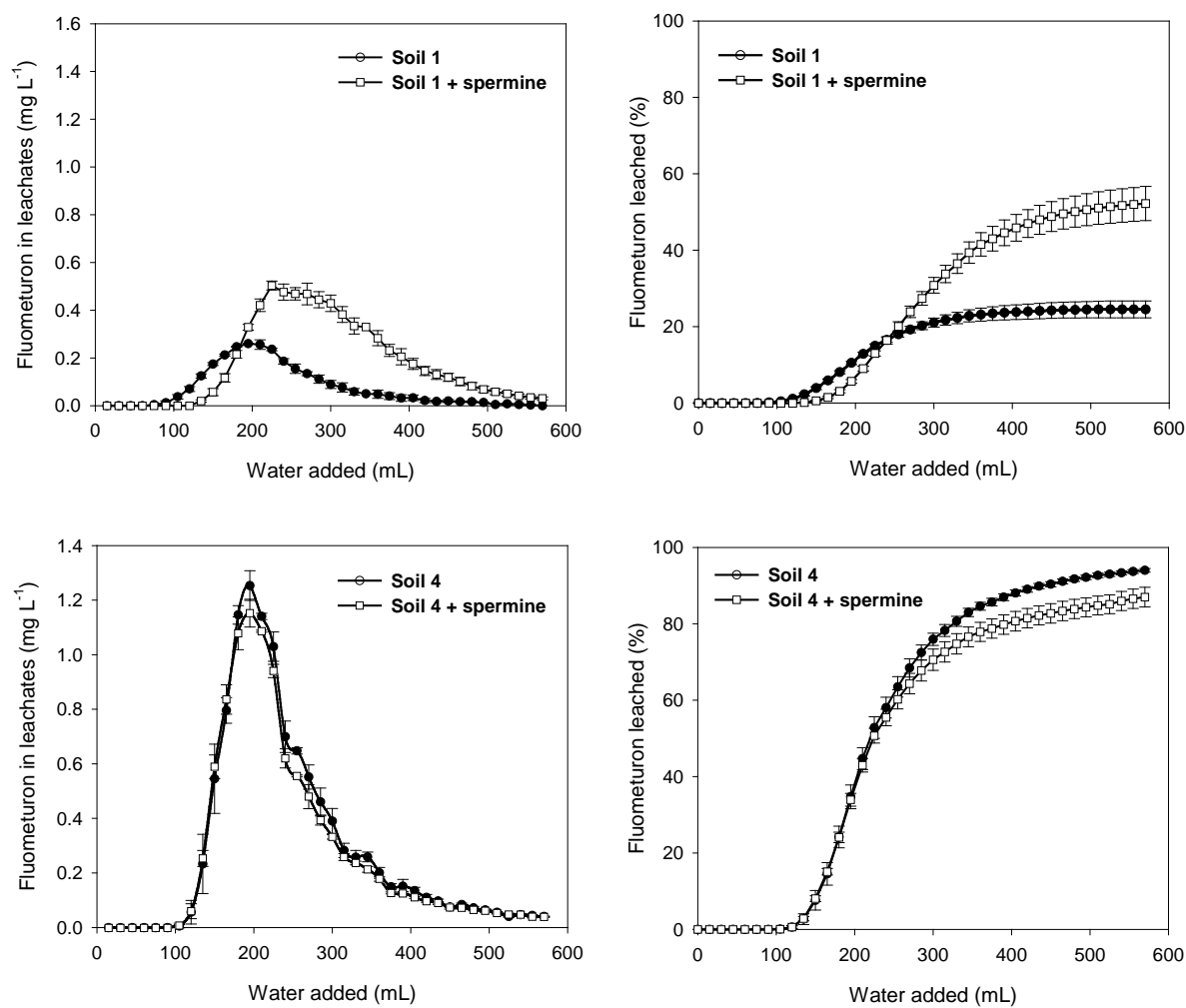
Figure 6. Relative and cumulative breakthrough curves (BTCs) of fluometuron in soils 1 and 4 in the absence and in the presence of spermine.
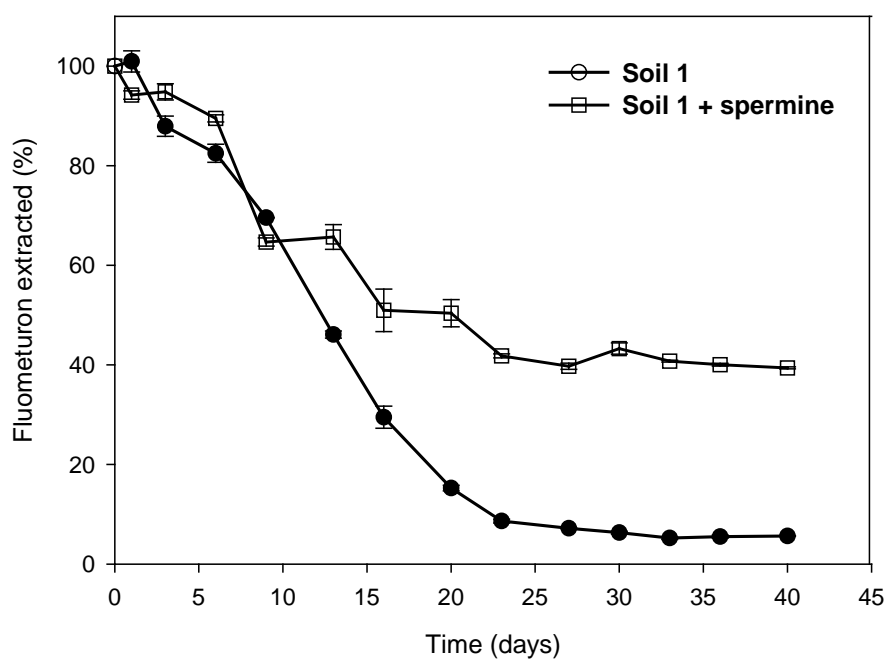
Figure 7. Dissipation curves of fluometuron in soil 1 in the absence and in the presence and of spermine.