Effect of addition of HPBCD on Diuron adsorption-desorption, transport and mineralisation in soils with different properties

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ABSTRACT

The influence of different soil properties on diuron adsorption, and the effect of hydroxypropyl-β-Cyclodextrin (HPBCD) on its desorption, leaching and mineralisation have been investigated. The significant variables on adsorption were organic matter (OM) content, aluminum oxides and clay fraction. Diuron desorption from soils presents negative hysteresis when HPBCD solutions were used as an extractant, in comparison to the positive hysteresis when using $Ca(NO_3)_2$ solutions. Therefore, higher amounts of Diuron were desorbed by HPBCD due to the formation of an inclusion complex that causes an increase in its solubility. However, the application of HPBCD in columns of selected soils did not cause increased leaching of the herbicide. Finally, the presence of HPBCD shows clearly an increase in the rate of mineralisation of diuron in contaminated soils and a drastic reduction of DT_{50} values due to its increasing bioavailability. HPBCD is proposed as an efficient tool for Diuron soil bioremediation.

KEYWORDS: Diuron; HPBCD; adsorption-desorption; mobility; contaminated soil; mineralisation.

1. Introduction

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is an herbicide belonging to the phenylamide family and the subclass of phenylurea (Giacomazzi et al., 2004). This substituted urea herbicide inhibits photosynthesis by preventing oxygen production and blocks the electron transfer at the level of photosystem II of photosynthetic micro-organisms plants. This compound is used to control a wide variety of annual and perennial broadleaf and grassy weeds on many agricultural crops such as fruit, cotton, sugar cane, alfalfa and wheat at 0.6-4.8 kg ha^{-1}, as well as on non-crop areas such as

roads, garden paths and railway lines at 3-30 kg ha^{-1} (Tomlin, 2000). Dispersion of this compound in agriculture leads to pollution of the aquatic environment by runoff (Louchart et al., 2001; Thurman et al., 2000) and leaching (Inoue et al., 2014; Langeron et al., 2014). Diuron is considered a Priority Hazardous Substance by the European Commisssion (Directive 2000/60/EC). Understanding the fate of a pesticide in the soil environment requires knowledge of both its adsorption capacity and degradation rate. The processes of adsorption and degradation have often been found to be correlated, since degradation can be restricted to the soil solution and hence sorbed molecules may be protected from microbial attack, what has been proved by several studies for a broad range of chemicals, including pesticides (Kah and Brown, 2007; Quintero et al., 2005) and various organic contaminants (Braida et al., 2004; Stokes et al., 2005).

Environmental microbiologists define bioavailability as "the contaminant fraction which represents the accessibility of a chemical to a living organism for assimilation, degradation and ecotoxicity expression" (Semple et al., 2007). The bioavailability of organic pollutants governs their ecotoxicity and degradation in contaminated soils. This relationship is apparent in soils with high organic matter content, in which easily biodegradable organic compounds persist for long periods even under conditions favorable for biodegradation (Alexander, 1999). Consequently, the bioavailability of contaminants varies with the type, nature of contaminants and environmental factors such as the composition of soil (clay fraction and organic matter content mainly), the residence time of the chemical in soil, the desorption rate and the nature of the microorganisms which influence the bioavailability and the biodegradation and toxicity of sorbed compounds. Biodegradation is by far the best and most likely the only environmentally means of detoxification of organic contaminants in soil and water except when biodegradation leads to the formation of toxic and/or persistent metabolites.

Cyclodextrins (CDs) are cyclic organic compounds obtained by enzymatic transformation of starch. These molecules exhibit a cavity with a hydrophobic character whereas the exterior is strongly hydrophilic. This peculiar structure allows organic molecules to be included in the cavity via non covalent bonds to form inclusion complexes. The complexation of pesticides and other contaminants with CDs may result in considerable improvement of their properties, such as enhancement of solubility and bioavailability (Yu et al., 2015; Bilensoy, 2011; Morillo, 2008; Manolikar et al., 2003).

A large number of papers in which the complexation with CDs is studied using pesticides that present problems can be found, from both agricultural and environmental points of view (Pérez-Martínez et al., 1999; Villaverde et al., 2007; 2013a,b; Morillo et al., 2012; Sánchez-Trujillo et al., 2013, 2014). CDs have the tendency to form inclusion complexes by reversibly incorporating a nonpolar compound into their hydrophobic central cavities (Villaverde et al., 2006). The use of CD solutions as enhancers in pollutant dissipation in soil has been postulated as a promising in situ decontamination tool because it enhances pesticide soil desorption. Previously, our group has reported numerous results using different types of CD solutions for enhancing soil desorption of different herbicides (Villaverde et al., 2005, 2007; Pérez-Martinez et al., 2000). Also, in the presence of the HPBCD solution, almost complete disappearance of diuron was observed at the end of the experiment (only 6.85% of the initial amount remained) when an exogenous bacterial consortium was applied (Villaverde et al., 2012).

Therefore, the aim of this work was to gain insight into the fate and behavior of diuron in the soil-water system, regarding its adsorption-desorption, transport and mineralisation in absence or in presence of HPBCD (hydroxypropyl-β-cyclodextrin) to determine if HPBCD can be used for diuron decontamination in soils.

2. Materials and Methods

2.1. Materials

Technical grade (98%) diuron [N-(3,4-cichlorophenyl)-N,N-dimethyl-urea] was provided by PRESMAR S. L. Radiolabelled [ring-U-14C]-diuron was purchased from the Institute of Isotopes (Budapest, Hungary) with specific activity of 1332 MBq mmol^{-1}, chemical purity of 99.9 % and radiochemical purity of 100%. The cyclodextrin (CD) hydroxypropyl-β-CD (HPBCD) was purchased from Cyclolab, Budapest, Hungary. Seven different soils from southwestern Spain were employed in this study. Soil analysis were performed on air-dry soil sieved at 2 mm. They were taken from the superficial horizon (0-20 cm) and analyzed for pH in saturated paste, total carbonate content, particle size distribution and organic matter content. The amorphous and organically bound iron, manganese, and aluminum oxides were determined using ammonium oxalate-oxalic acid (McKeague et al., 1991). These properties are shown in Table 1.

103 *2.2. Diuron adsorption-desorption studies in soils.*

104 Triplicate batch adsorption experiments were performed by mixing 10 g (dry mass) of

105 the soil with 10 ml solution containing 0.01 M $Ca(NO_3)_2$ as background electrolyte, 200

106 mg L^{-1} $HgCl_2$ to prevent bacterial growth, and various concentrations (2, 5, 10, 15 and

107 20 mg l^{-1}) of diuron, in 50 mL polypropylene centrifuge tubes. The samples were

108 shaken for 72 h at 20 ± 1 °C (on an orbital shaker). This time of reaction was chosen

109 from preliminary kinetic studies (not shown) which showed that adsorption had reached

110 pseudoequilibrium. After shaking, the suspensions were centrifuged (12000 g) and the

111 concentration of diuron in the supernatant was determined by using a Shimadzu HPLC

112 equipped with UV detector. The conditions used were as follows: mobile phase,

113 acetonitrile/water (60:40); flow rate, 1.00 ml/min; temperature, 30 °C; chromatographic

114 column, kromasil C18 reverse phase; wavelength, 230 nm and retention time, 2.5 min.

115 The difference in herbicide concentration between the initial and final equilibrium

116 solutions was assumed to be due to sorption, and the amount of diuron retained by the

117 adsorbent calculated. Sorption isotherms were fitted to the logarithmic form of the

118 Freundlich equation:

119

120 $$\log Cs = \log K_f + n \log Ce$$

121 where Cs (μmol kg^{-1}) is the amount of the herbicide sorbed at the equilibrium

122 concentration Ce (μmol l^{-1}), and K_f and n are constants that characterize the relative

123 sorption capacity and the sorption intensity, respectively, for the herbicide. The

124 normalized distribution coefficient (K_{foc}) of organic carbon (OC) was calculated from

125 the K_f values ($K_{foc} = K_f$/OC x 100). The organic carbon content (OC) was determined as

126 follows: OC% = 0.58 x OM (%). K_{foc} is often used in the discussion of sorption of

127 nonpolar hydrophobic compounds, the concept being compatible with the idea of

128 organic carbon (OC) having the same affinity for a nonpolar compound, independent of

129 the source of OC.

130 Desorption experiments were performed after adsorption equilibrium had been reached

131 for the point corresponding to diuron initial concentration of 10 mg l^{-1} by removing half

132 of the supernatant after centrifugation, replacing it by 5 ml of the extractant solution,

133 allowing equilibration for an additional 24 h period, and after that, operating as in the

134 adsorption experiment. This process was repeated twice. Desorption experiments were

135 carried out using 0.01 M $Ca(NO_3)_2$ solution or HPBCD x10 (HPBCD equivalent to 10

136 times the diuron initially added to soil, 4.29 x 10^{-3} mmol). The percentage of diuron

137 desorbed with respect to that previously adsorbed during adsorption process (%D) was

138 calculated for all the desorption experiments.

139 *2.3. Leaching experiments in soils columns*

140 Leaching experiments were done in duplicate for two selected soils (Soil 1 and soil 7),

141 which were chosen for being the most different in their properties from each other.

142 Homogeneous soil columns were prepared by packing the soil gently and uniformly in

143 16 cm long methacrylate tubes of 3.0 cm internal diameter. The lower end was covered

144 with nylon tissue and padded with a thin layer of glass wool (0.5 g) to hold the soil

145 firmly into the column. Each column was filled with 125 g of soil 1 and 167 g of soil 7

146 to achieve 16 cm occupied by soil. The top of these columns was covered with glass

147 wool to prevent disturbance of the soil by the input liquid.

148 In a preliminary experiment, soil columns were first saturated by capillarity with

149 distilled water to obtain a soil moisture content of 100% of the field capacity. The

150 difference between the weight of the saturated soil column and its dry weight was used

151 to determine the pore volume (35 ml for soil 1, 47 ml for soil 7).

152 Technical diuron equivalent to 5 mg kg^{-1} was applied. 24 h after herbicide application,

153 HPBCD equivalent to 10 times the diuron concentration initially applied to soil was

154 added in solid form on top of the columns selected for this treatment (2.68 x 10^{-2} and

155 3.6 x 10^{-2} mmol. Breakthrough curves (BTCs) were obtained through periodic

156 application of 10 ml of 0.01M Ca(NO$_3$)$_2$ solution until herbicide was no longer detected

157 in the leachates from columns with and without HPBCD. Diuron in the leachates was

158 monitored by HPLC with the conditions described above. After the leaching

159 experiments, the columns were sliced into four 4 cm segments and the herbicide

160 residues that remained adsorbed in the soil were quantified using 2 g of dry soil which

161 were placed in Teflon centrifuge tubes and extracted with 5 ml of methanol. The tubes

162 were placed on an orbital shaker at 100 rpm (New BRUNSWICK Scientific, Edison,

163 N.J.) at 20 ± 1 ºC. After 24 h the tubes were centrifuged at 12000 g and the supernatant

164 was analysed by HPLC after filtration. The extraction was carried out in triplicate.

165 *2.4. Mineralisation experiments*

166

167 Mineralisation of ^{14}C-labeled diuron in selected soils was measured (in duplicate)

168 through the evolution of [14]CO$_2$ produced (Villaverde et al., 2012). The mineralisation

169 assays were carried out in respirometers. All the microcosm components except soils

were sterilized before assays, using an autoclave Auster-G, P-Selecta with three cycles at 121 °C, inlet pressure of 103 kPa, during 20 min. In modified 250 mL Erlenmeyers 10 g of dry soil and 50 mL of mineral salts medium (MSM) were placed, and ^{14}C-ring-labelled and unlabelled diuron added to obtain a final concentration of 10 mg L^{-1} (10 kg ha^{-1}) and a radioactivity of approximately 450 Bq per flask. Two different treatments were performed: addition of nutrients solution (SNs) (Appendix Table A-1) (Treatment 1) and SNs +HPBCD x10 (Treatment 2). Respirometers were closed with Teflon-lined stoppers and incubated at 20 ± 1 °C for the time necessary to reach a mineralisation plateau. Sterile controls were also prepared, and no mineralisation was detected (data not shown). Production of $^{14}CO_2$ was measured as radioactivity appearing in the alkali trap of the biometer flasks, which contained 1 mL of 0.5 M NaOH. Periodically, the solution was removed from the trap and replaced with fresh alkali. The NaOH solution was mixed with 5 mL of liquid scintillation cocktail (Ready safe from Perkin Elmer, Inc., USA) and the mixture kept in darkness for about 24 h for dissipation of chemiluminescence. Radioactivity was measured with a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.;model LS5000TD).

For the calculation of the kinetic parameters, mineralisation curves were modelled according to the instructions of the FOCUS guide (2006), using the least squares method with the SOLVER of Microsoft Office Excel 2007 mathematical program. Mineralisation kinetics were fitted to two models: a simple first-order (SFO) model, and a first-order sequential model (Hockey-Stick, HS), according to the following equations:

$$[C]_t = [C]_0 \, e^{-kt} \qquad DT50 = ln2/k \qquad\qquad (SFO)$$

$$[C]_t = [C]_0 \, e^{-k1tb} \, e^{-k2(t-tb)} \quad DT50 = ln2/k1 \qquad if \; DT50 \le t_b \quad (HS)$$
$$DT50 = (ln2 - k1t_b)/k2 \qquad if \; DT50 \ge t_b \quad (HS)$$

where $[C]_t$ and $[C]_0$ are the concentrations of mineralised diuron at time t and just after spiking the soil, respectively (mg kg^{-1}), k is the rate constant of mineralisation (day^{-1}). In the HS model, k1 and k2 are the rate constants of mineralisation for the fast and the slow fractions, respectively, and tb is the time at which rate constant changes. These models have been selected because of their relative simplicity and their potential to

203 better fit the measured dissipation kinetics data sets for diuron that appear to be
204 monophasic or biphasic (Sarmah and Rhoan, 2011; Beulke et al., 2005).

205

206 *2.5. Statistical analysis*

207 Relationships between adsorption coefficients and properties of soils and pesticides
208 were investigated using the statistical analysis program SPSS for windows v. 21
209 (Villaverde et al., 2008). The soil properties given in Table 1 were evaluated as
210 predictors for adsorption process.

211
212 **3. Results and discussion**

213

214 *3.1. Herbicide adsorption-desorption in soils*

215 Diuron adsorption isotherms in the studied soils are shown in Figure 1. In all cases the
216 isotherms fit the Freundlich equation, as confirmed by R^2 values (Table 2). The sorption
217 isotherms were compared using the K_f parameter of the Freundlich equation. The
218 constant K_f is the amount of pesticide sorbed for 1 μmol l^{-1} equilibrium concentration,
219 and hence, under our conditions, it represents adsorption at low adsorbate concentration.
220 The K_f ranged between 0.60 and 14.3 for the soils 7 and 6, respectively, indicating a
221 strong influence of soil properties on diuron adsorption.

222 As commented in previous published works (Villaverde et al., 2012; Kasozi et al., 2010;
223 Wang and Keller, 2009; Giacomazzi and Cochet, 2004) soil organic matter (SOM)
224 content can be considered as the most important soil property to assess Diuron potential
225 adsorption capacity. In general, a positive correlation between SOM content and
226 calculated K_f was observed. However, the soil that showed the highest diuron adsorption
227 capacity, soil 6 (K_f, 14,3), did not have the highest SOM content (1.41%). Soil 1, with
228 the highest SOM content (3.37%) had not the highest K_f value (13.0), being very similar
229 to those observed for soil 2 and soil 3 (13.8 and 13.2) with a SOM content of 2.13% and
230 1.76%, respectively. SOM is a very complex and heterogeneous mixture of humic acids,
231 lignin, polysaccharides, proteins, organic acids, inter alia. (Sheng et al., 2001), and
232 hence different types of interactions between sorbent and adsorbate can be observed
233 depending on SOM composition (Morillo et al., 2014). Inoue et al. (2006) observed
234 diuron K_f values ranging from 5.99 to 9.33 for 6 soils with different properties, and
235 sorption was correlated positively with the organic carbon and clay content of the soil
236 samples.

The role of hydrophobic bonds on adsorption of nonpolar hydrophobic herbicides in soil can be studied by calculating the organic carbon normalised distribution coefficient, K_{foc}. Its concept is based on organic carbon exhibiting the same affinity for a nonpolar compound, independent of the source of OC. The more hydrophobic a molecule is, the greater the probability for partition from the aqueous phase to the organic phase. K_{foc} can be used as a measure of this property in soils. When hydrophobic bonds are principally responsible for herbicide adsorption, K_{foc} values should be in a range relatively similar for the different soils studied. In this work, K_{foc} values ranged from 320 to 1738 (Table 2) and the values obtained for soils 1, 3, 4, 5 and 7 were in the range found by other authors for Diuron sorption on soils (Ahangar et al., 2008; López-Piñero et al., 2010), indicating clearly that OM played a fundamental role in diuron retention (Smernik and Kookan, 2015). However, for the soils 2 and 6 the K_{foc} values were very high, 1112 and 1738. These results lead us to believe that diuron adsorption will not only depend on the SOM content of these soils. Differences in K_{foc} values were also determined in different studies. Inoue et al. (2006) observed values of K_{foc} of 531 and 1567, concluding that another factor than SOM such as clay content was implicated in diuron sorption. Carbo et al. (2007) confirmed that diuron sorption was not only related with SOM content and that other mineral components such as oxides surfaces (Fe and Al amorphous sesquioxides) and clay minerals were involved. Ghafoor et al. (2012) concluded that in soils with a low SOM content (<2%), inorganic components such as clay minerals, iron and aluminum oxides, in addition to organic carbon, are needed for accurate prediction of pesticide sorption. The role of amorphous oxides in the retention of pesticides has been suggested to be potentially greater than crystalline oxides, due to their extensive specific surface area, high degree of hydration, and highly reactive surface functional groups (Clausen and Fabricius, 2001; Hanna, 2007). Liu et al. (2013) observed that amorphous oxides could act as mediators of the active sites of organic matter and improve the adsorption of butachlor herbicide in soil, as in the case of diuron. On the contrary, Morillo et al. (2004) observed that amorphous oxides acted as coating materials on the OM of soils decreasing norflurazon sorption due to blocking of specific 5 sorption sites. Also Ahaungar et al. (2008) observed a substantial depression of K_{oc} due to the presence of minerals in soils. In conclusion, the presence of amorphous oxides could act in a different manner depending on the pesticide.

270   To confirm the most relevant parameters to predict diuron adsorption, including

271   amorphous oxides presented in Table 1, statistical approach studies were carried out.

272   Initially, the selected 7 soils were included in the prediction model, and after that, only

273   5 soils were studied, excluding soils 2 and 6, that is, those soils with the highest

274   amorphous oxides content.

275   Table 3 gives statistical correlations between K_f and selected soil properties of the seven

276   investigated soils (a) and with only five soils (b), excluding soils 2 and 6, to know

277   which soil properties influenced diuron adsorption. When all soils are considered only

278   amorphous Al_2O_3 content was observed to correlate significantly ($P < 0.05$) with K_f.

279   This fact suggests that some of the soils alter the correlation between adsorption and

280   OM content influenced by other soil properties. If soils 2 and 6, which have the highest

281   total amorphous oxides content (TAO) are excluded, K_f is positively correlated with

282   SOM content. For these two soils, TAO promotes diuron adsoption, and hence, a higher

283   adsorption capacity is observed than that expected according to their OM contents.

284   The automated linear regression equation obtained by applying the selection step

285   forward method and using the criterion value input $P < 0.05$, including the analyzed

286   properties of the 7 soils investigated for K_f, was as follows:

287                $K_f = 0.654 + 0.880\ Al_2O_3\ (\%) + 0.156\ \text{clay fraction}\ (\%)$

288   K_f appears to be related not only with Al_2O_3 content, but also with clay fraction despite

289   no significant relationship with Kf was found (Table 3). Other authors confirm these

290   results about the role of clay fraction in Diuron adsorption. Inoue et al. (2006) observed

291   that clay content significantly affected K_f values. Wang and Keller (2009) reviewed the

292   strong adsorption of diuron on the clay fraction, concluding that in soils with clay

293   minerals/OM contents ratios >60, herbicide adsorption could be on both SOM and clay

294   minerals surfaces. Villaverde et al. (2008) observed a similar result for 4 herbicides,

295   2,4-D, dicamba and two sulfonylureas, on soils from southern Spain, with a content of

296   clay minerals 30 times greater than SOM content. Smernik and Kookana (2015)

297   investigated potential causes of variation in diuron K_{oc} based on OM-mineral

298   interactions attributing the K_{oc} increase after treatment with hydrofluoric acid to the

299   elimination of the mineral fraction, which was blocking the OM sorption sites.

300   However, as far as we know, no references exist dealing with the adsorption of diuron

301   specifically on amorphous oxides.

302 The linear regression equation was also obtained for only 5 soils using the same
303 procedure as previously indicated:

$$K_f = -0,373 + 4,032 \, OM \, (\%)$$

305 In this case, the only significant variable was the OM content. From results of soil
306 diuron adsorption it can be concluded that it depends not only on the SOM content, but
307 also on the amorphous oxides and on the clay fraction of soils.

308 Diuron desorption isotherms when using a $Ca(NO_3)_2$ 0.01 M solution as extractant are
309 shown in Appendix (Fig. 1) and the results are summarised in Table 4. Diuron
310 desorption results deviated markedly from those corresponding to the adsorption
311 isotherms showing positive hysteresis in all cases and indicating that diuron soil
312 adsorption was not completely reversible. Lewartz and Louchart (2007) also observed
313 positive hysteresis for Diuron, but Liu et al. (2013) only observed it on 3 of the 6 soils
314 studied. Total percentages of diuron desorbed (%D) for an initial concentration of 10
315 mg L^{-1} without HPBCD are shown in Table 4. The percentages of desorption observed
316 for each soil are, in general, in agreement with the values obtained for sorption (Table
317 2), that is, the higher the K_f, the lower the percentage of diuron removed from the soil.
318 The only exception was soil 2 that had the highest TAO percentage and especially in
319 large amounts of amorphous Al_2O_3 (Table 1).

320 Diuron desorption isotherms using a solution of HPBCD was carried out using 10 times
321 the molar concentration of the herbicide initially added (HPBCD x10) as extractant,
322 according to the study previously carried out by Villaverde et al. (2005) (Table 4, Figure
323 A-1). In all cases hysteresis was observed, since the desorption results deviated
324 markedly from the adsorption isotherm, but in this case it was a negative hysteresis,
325 indicating that a higher amount of diuron was desorbed than expected from the
326 adsorption isotherm. The behavior observed indicated that the herbicide has a greater
327 tendency to be incorporated into the soil solution in the presence of HPBCD. Similar
328 results were obtained by Pérez-Martínez et al. (2000) using CDs extractants in
329 desorption studies of herbicide 2,4-D.

330 In conclusion, desorption of the herbicide diuron was always more effective in the
331 presence of HPBCD x10. Diuron tends to form an inclusion complex with HPBCD
332 (Villaverde et al., 2012), which acts as an extractant towards the diuron adsorbed in soil.
333 That is an advantage of HPBCD to decontaminate soils.

334 *3.2. Herbicide mobility in soils*

335 Although the study of adsorption-desorption allows the evaluation of adsorption
336 mechanisms, these results cannot be directly extrapolated to the dynamic flow regime
337 that occurs through the soil, due, among other reasons, to the nonequilibrium conditions
338 and to the highly different soil/solution ratios in soil columns. For these reasons,
339 experiments using soil columns have been carried out to emulate more closely the real
340 conditions in soil and the effect of HPBCD addition in soil properties in the leaching
341 process.

342 Two soils (1 and 7) were selected, due to the differences in soil properties to study the
343 effect of HPBCD addition on the leaching. The results obtained have been represented
344 in the form of breakthrough curves (BTCs). Appendix Figure A-2 shows the BTCs for
345 diuron leaching through these soils in the absence or presence of HPBCD, and Table 5
346 shows the cumulative amount of diuron eluted in the leachates, as well as the amount of
347 diuron remaining at different depths in the soil columns.

348 The low amount of herbicide eluted in the case of soil 1 is worth mentioning; the
349 percentage of the herbicide eluted was only 6.65% or 7.71% in the absence or presence
350 of HPBCD, respectively. On the contrary, the percentage of diuron eluted from soil 7
351 was much higher (83.1% and 73.2%, without and with HPBCD). These results are in
352 agreement with those obtained in the adsorption experiments, where the K_f value
353 obtained for soil 1 was much higher (13.0) than that obtained for soil 7 (0.60). Langeron
354 et al. (2014) studied the mobility in soil columns of 11 herbicides belonging to the
355 phenylureas family, diuron included, in a soil with similar properties to soil 1, with a
356 high OM content, but they observed that the percentage of diuron eluted was 66%. This
357 percentage was explained because this soil had a high content of calcite (81.3%), which
358 caused a blockage in the adsorption sites of the OM. In our case this did not happen
359 because soil 1 had only 1.60% carbonates (Table 1). Navarro et al. (2012) studied the
360 leaching of herbicides belonging to the phenylurea family in two soils with different
361 physico-chemical properties and leaching of diuron was only 5% of the amount applied
362 in the soil columns with the highest OM content (6.6%), a similar behaviour to that
363 observed in soil 1.

364 Table 5 also shows the distribution of diuron residues in the soil columns as a function
365 of column depth, after completion of leaching assay. Diuron residues extracted from

soil 7 at each soil depth were low due to the large amounts previously eluted, both in the absence and the presence of HPBCD.

In soil 1, a high amount of diuron residues was extracted from the upper two segments and it was not detected at depths below 8 cm, indicating a high retention of the herbicide on the surface horizons of this soil, according to the high Kf obtained in the adsorption experiment (Table 2). The amounts recovered after extraction from the 4 rings of the columns were 96.5% and 90.9% of that initially applied in the absence and presence of HPBCD, respectively (Table 5).

These results indicate that the HPBCD did not cause an increasing leaching of the herbicide in soils of very different properties, and it is considered an advantage because there will be a decrease in the risks of groundwater contamination by leaching when using the appropriate concentration of HPBCD in bioremediation processes.

3.3. Mineralisation experiments

Figure 2 shows the kinetics of diuron mineralised by the soil endogenous flora after application of nutrients solution (SNs) and HPBCD x10 in 4 selected soils (Soil 4, 5, 6 and 7). The ability to remediate a contaminated soil from highly persistent herbicides depends mainly on the presence of microorganisms that have the capacity to biodegrade them (Caracciolo et al., 2013), but in most cases they require a small activation (bioestimulation) to achieve an accelerated mineralisation of the pollutant (Alexander, 1999; Aburto-Medina et al., 2012).

The aim of this study was to determine the potential capacity of soil natural attenuation for the pollutant diuron, concluding that the soil was not able to mineralise the herbicide without being assisted by the addition of biostimulants.

Figure 2 shows two different treatments: Treatment 1 (without HPBCD), SNs; Treatment 2 (with HPBCD), SNs + HPBCD. The kinetic parameters obtained following the recommendations included in the FOCUS guide (2006) are shown in Table 6. All mineralisation curves were fitted to a biphasic first order kinetic model (Hockey- Stick, HS), except mineralisation in soil 5, treatment 1 (endogenous flora + SNs), which was better fitted to a simple first-order kinetic model (SFO).

When treatment 1 (without HPBCD) was applied the extent of diuron mineralisation ranged from 1.81% to 29.4% (soils 5 and 4, respectively). In the case of soil 5 endogenous flora was only able only to slightly degrade the herbicide (1.81%). Natural attenuation assisted only by nutrients solution (SNs) needed a significant time for removing from the soil the bioavailable fraction of diuron, giving as a result high values for DT_{50} in all the investigated soils (from 665 to 5887 days). For the studied soils no clear correlation between soil properties and mineralisation could be determined, because there were many factors that influenced the mineralisation process. Among others, we can mention the different kinds of adsorption surfaces on the soil, including the OM, not only due to its content, but also due to the diversity in the quality of its adsorption points which influence the desorption process and, therefore, the pesticide bioavailability, the extremely high diversity depending on the type of soil and the more or less favourable conditions that they need to perform the biodegradation process (Caracciolo et al., 2013).

When treatment 2 was applied (with HPBCD) a significant enhancement in diuron mineralisation was observed, and the overall extent of mineralisation in all the studied soils was greater than after only nutrients application, especially in the case of soil 5, where 46.7% mineralisation was reached compared to 1.81% in the absence of HPBCD (Table 6). DT_{50} was also drastically reduced in all cases, being remarkable also the case of soil 5, which showed a 25 times lower value regarding to the single addition of SNs (Table 6). The lag phase was significantly reduced when using HPBCD in soil 4, but in soils 6 and 7 an inverse effect was observed, which can be explained by competition between the herbicide and HPBCD as carbon sources for the soil flora, due to the biodegradability of HPBCD (Fenyvesi et al., 2005).

In conclusion, these results showed that the application of HPBCD leads to an increase in the rate of mineralisation of Diuron in contaminated soils, thanks to the formation of an inclusion complex **(Villaverde et al., 2012)** that causes an increase in the apparent solubility of the herbicide and thus a greater bioavailability.

4. Conclusions

427 Soil desorption of the herbicide diuron has always been more effective in the presence
428 of HPBCD because diuron tends to form an inclusion complex with this cyclodextrin,
429 which acts as an extractant towards the diuron adsorbed in soils. HPBCD did not cause
430 an increasing soil leaching of the herbicide, which is considered key for deciding on the
431 use of HPBCD in soil bioremediation. Finally, its application led to an increase in the
432 rate of mineralisation, thanks to an increase in the apparent solubility of the herbicide,
433 and thus its greater bioavailability. These results showed that the application of HPBCD
434 was used for decontamination of diuron-polluted soils with different properties.

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441

442 **Appendix A. Supplementary data**
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444 Supplementary data associated with this article can be found in the online version.
445 These data include one table with the Solution nutrients (SNs). Additionally, two
446 figures have been also included. One of them with diuron adsorption-desoption
447 isotherms on soils, using $Ca(NO_3)_2$ or HPBCD as extractant solutions. And the other
448 with Diuron break through curves in soils 7 and 1 in the presence or absence of
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