

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

3
4 *Rubio-Bellido, M., Morillo, E., Villaverde, J.**

5 *Instituto de Recursos Naturales y Agrobiología (CSIC), Apartado 1052,*
6 *41080, Sevilla, Spain*

7 **Corresponding autor:*

8 *E-mail: j.villaverde@csic.es*

9
10
11 **ABSTRACT**

12
13 The influence of different soil properties on diuron adsorption, and the effect of
14 hydroxypropyl- β -Cyclodextrin (HPBCD) on its desorption, leaching and mineralisation
15 have been investigated. The significant variables on adsorption were organic matter
16 (OM) content, aluminum oxides and clay fraction. Diuron desorption from soils
17 presents negative hysteresis when HPBCD solutions were used as an extractant, in
18 comparison to the positive hysteresis when using $\text{Ca}(\text{NO}_3)_2$ solutions. Therefore, higher
19 amounts of Diuron were desorbed by HPBCD due to the formation of an inclusion
20 complex that causes an increase in its solubility. However, the application of HPBCD in
21 columns of selected soils did not cause increased leaching of the herbicide. Finally, the
22 presence of HPBCD shows clearly an increase in the rate of mineralisation of diuron in
23 contaminated soils and a drastic reduction of DT_{50} values due to its increasing
24 bioavailability. HPBCD is proposed as an efficient tool for Diuron soil bioremediation.

25
26 **KEYWORDS:** Diuron; HPBCD; adsorption-desorption; mobility; contaminated soil;
27 mineralisation.

28 **1. Introduction**

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

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

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

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

69 A large number of papers in which the complexation with CDs is studied using
70 pesticides that present problems can be found, from both agricultural and environmental
71 points of view (Pérez-Martínez et al., 1999; Villaverde et al., 2007; 2013a,b; Morillo et
72 al., 2012; Sánchez-Trujillo et al., 2013, 2014). CDs have the tendency to form inclusion
73 complexes by reversibly incorporating a nonpolar compound into their hydrophobic
74 central cavities (Villaverde et al., 2006). The use of CD solutions as enhancers in
75 pollutant dissipation in soil has been postulated as a promising in situ decontamination
76 tool because it enhances pesticide soil desorption. Previously, our group has reported
77 numerous results using different types of CD solutions for enhancing soil desorption of
78 different herbicides (Villaverde et al., 2005, 2007; Pérez-Martínez et al., 2000). Also, in
79 the presence of the HPBCD solution, almost complete disappearance of diuron was
80 observed at the end of the experiment (only 6.85% of the initial amount remained) when
81 an exogenous bacterial consortium was applied (Villaverde et al., 2012).
82 Therefore, the aim of this work was to gain insight into the fate and behavior of diuron
83 in the soil-water system, regarding its adsorption-desorption, transport and
84 mineralisation in absence or in presence of HPBCD (hydroxypropyl- β -cyclodextrin) to
85 determine if HPBCD can be used for diuron decontamination in soils.

86

87 **2. Materials and Methods**

88

89 *2.1. Materials*

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

102

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(NO₃)₂ as background electrolyte, 200
106 mg L⁻¹ HgCl₂ 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

$$120 \log C_s = \log K_f + n \log C_e$$

121 where C_s ($\mu\text{mol kg}^{-1}$) is the amount of the herbicide sorbed at the equilibrium
122 concentration C_e ($\mu\text{mol l}^{-1}$), 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 ($K_{\text{foc}} = K_f/\text{OC} \times 100$). The organic carbon content (OC) was determined as
126 follows: $\text{OC}\% = 0.58 \times \text{OM} (\%)$. 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(NO₃)₂ solution or HPBCD x10 (HPBCD equivalent to 10
136 times the diuron initially added to soil, 4.29×10^{-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 (2.68×10^{-2} and
155 3.6×10^{-2} mmol. Breakthrough curves (BTCs) were obtained through periodic
156 application of 10 ml of 0.01M Ca(NO₃)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 °C. 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 ¹⁴C-labeled diuron in selected soils was measured (in duplicate)
168 through the evolution of ¹⁴CO₂ produced (Villaverde et al., 2012). The mineralisation
169 assays were carried out in respirometers. All the microcosm components except soils

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 $1 \mu\text{mol l}^{-1}$ 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.

237 The role of hydrophobic bonds on adsorption of nonpolar hydrophobic herbicides in soil
238 can be studied by calculating the organic carbon normalised distribution coefficient,
239 K_{foc} . Its concept is based on organic carbon exhibiting the same affinity for a nonpolar
240 compound, independent of the source of OC. The more hydrophobic a molecule is, the
241 greater the probability for partition from the aqueous phase to the organic phase. K_{foc}
242 can be used as a measure of this property in soils. When hydrophobic bonds are
243 principally responsible for herbicide adsorption, K_{foc} values should be in a range
244 relatively similar for the different soils studied. In this work, K_{foc} values ranged from
245 320 to 1738 (Table 2) and the values obtained for soils 1, 3, 4, 5 and 7 were in the range
246 found by other authors for Diuron sorption on soils (Ahangar et al., 2008; López-Piñero
247 et al., 2010), indicating clearly that OM played a fundamental role in diuron retention
248 (Smernik and Kookan, 2015). However, for the soils 2 and 6 the K_{foc} values were very
249 high, 1112 and 1738. These results lead us to believe that diuron adsorption will not
250 only depend on the SOM content of these soils. Differences in K_{foc} values were also
251 determined in different studies. Inoue et al. (2006) observed values of K_{foc} of 531 and
252 1567, concluding that another factor than SOM such as clay content was implicated in
253 diuron sorption. Carbo et al. (2007) confirmed that diuron sorption was not only related
254 with SOM content and that other mineral components such as oxides surfaces (Fe and
255 Al amorphous sesquioxides) and clay minerals were involved. Ghafoor et al. (2012)
256 concluded that in soils with a low SOM content (<2%), inorganic components such as
257 clay minerals, iron and aluminum oxides, in addition to organic carbon, are needed for
258 accurate prediction of pesticide sorption. The role of amorphous oxides in the retention
259 of pesticides has been suggested to be potentially greater than crystalline oxides, due to
260 their extensive specific surface area, high degree of hydration, and highly reactive
261 surface functional groups (Clausen and Fabricius, 2001; Hanna, 2007). Liu et al. (2013)
262 observed that amorphous oxides could act as mediators of the active sites of organic
263 matter and improve the adsorption of butachlor herbicide in soil, as in the case of
264 diuron. On the contrary, Morillo et al. (2004) observed that amorphous oxides acted as
265 coating materials on the OM of soils decreasing norflurazon sorption due to blocking of
266 specific 5 sorption sites. Also Ahaungar et al. (2008) observed a substantial depression
267 of K_{oc} due to the presence of minerals in soils. In conclusion, the presence of amorphous
268 oxides could act in a different manner depending on the pesticide.
269

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 adsorption, 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:

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

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 K_f 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:

$$304 \quad K_f = -0,373 + 4,032 \text{ OM} (\%)$$

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 $\text{Ca}(\text{NO}_3)_2$ 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

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

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

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

378 3.3. Mineralisation experiments

379 Figure 2 shows the kinetics of diuron mineralised by the soil endogenous flora after
380 application of nutrients solution (SNs) and HPBCD x10 in 4 selected soils (Soil 4, 5, 6
381 and 7). The ability to remediate a contaminated soil from highly persistent herbicides
382 depends mainly on the presence of microorganisms that have the capacity to biodegrade
383 them (Caracciolo et al., 2013), but in most cases they require a small activation
384 (bioestimulation) to achieve an accelerated mineralisation of the pollutant (Alexander,
385 1999; Aburto-Medina et al., 2012).

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

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

395

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

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

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

424

425

426 **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.

435

436 **Acknowledgments**

437 The authors are indebted to Presmar S.L. for providing the technical diuron, This work
438 was supported by the Spanish Ministry of Economy and Competitiveness (co-funded
439 by the Fondo Europeo de Desarrollo Regional, FEDER), under the research project
440 CTM2013-42599-R and Junta de Andalucía Project RNM 894.

441

442 **Appendix A. Supplementary data**

443

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-desorption
447 isotherms on soils, using $\text{Ca}(\text{NO}_3)_2$ 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
449 HPBCD.

450

451 **Reference**

452 Aburto-Medina, A., Adetutu, E.M., Aler, S., Weber, J., Patil, S.S., Sheppard, P.J., Ball,
453 A.S., Juhasz, A.L., 2012. Comparison of indigenous and exogenous microbial
454 populations during slurry phase biodegradation of long-term hydrocarbon-contaminated
455 soil. *Biodegradation*. 23, 813-822.

456

457 Ahangar, A.E., Smernik, R.J., kookama, R.S., Chittleborough, D.J., 2008. Clear effects
458 of soil organic matter chemistry as determined by NMR spectroscopy and the sorption
459 of Diuron. *Chemosphere*. 70, 1156-1160.

460

461 Alexander, M., 1999. Biodegradation and Bioremediation, Second Edition, San Diego,
462 California 92101-4495, USA. ISBN-13: 978-0-12-049861-1.
463

464 Beulke, S., van Beinum, W., Brown, C.D., Mitchell, M., Walker, A., 2005. Evaluation
465 of Simplifying Assumptions on Pesticide Degradation in Soil. *J. Environ. Qual.* 34,
466 1933–43.
467

468 **Bilensoy, E., 2011. Cyclodextrins in Pharmaceuticals, Cosmetics, and Biomedicine:
469 Current and Future Industrial Applications. John Wiley & Sons.**
470

471 Braida, W.J., White, J., Pignatello, J.J., 2004. Indices for bioavailability and
472 biotransformation potential of contaminants in soils. *Environ. Toxicol. Chem.* 23, 1585–
473 1591.
474

475 Caracciolo, A.B., Bottoni, P., Grenni, P., 2013. Microcosm studies to evaluate microbial
476 potential to degrade pollutants in soil and water ecosystems. *Microchemical*, 107, 126-
477 130.
478

479 Carbo, L., Martins, E.L., Does, E.F.G.C., Spadotto, C.A., Weber, O.L.S., De-
480 Lamonica-Freire, E.M., 2007. Acetamiprid, carbendazim, diuron and thiamethoxam
481 sorption in two brazilian tropical soils. *J. Environ. Sci. Health Part B.* 42, 499-507.
482

483 Clausen, L., Fabricius, I., 2001. Atrazine, isoproturon, mecoprop, 2,4-D, and bentazone
484 adsorption onto iron oxides *J. Environ. Qual.* 30, 858-869.

485 Directive 2000/60/EC. Decision No. 2455/2001/EC of the European Parliament and of
486 the Council of 20 November 2001 Establishing the List of Priority Substances in the
487 Field of Water Policy and Amending Directive 2000/60/EC; *Official Journal of the*
488 *European Communities*, 15.12.2001.

489 Fenyvesi, E., Gruiz, K., Verstichel, S., De Wilde, B., Leitgib, L., Csabai, K., Szaniszló,
490 N., 2005. Biodegradation of cyclodextrins in soil. *Chemosphere.* 60, 1001-1008.

491 FOCUS, Guidance Document on Estimating Persistence and Degradation Kinetics from
492 Environmental Fate Studies on Pesticides in EU Registration, 2006. Report of the

493 FOCUS Work Group on Degradation Kinetics, EC Document Reference
494 Sanco/10058/2005 version 2.0, 434 pp.
495
496 Ghafoor, A., Jarvis, N.J., Stenstrom, J., 2012. Modelling pesticide sorption in the
497 surface and subsurface soils of an agricultural catchment. *Pest Manag. Sci.* 69, 919-929.
498
499 Giacomazzi, S., Cochet, N., 2004. Environmental impact of diuron transformation: a
500 review, *Chemosphere.* 56, 1021-1032.
501
502 Hanna, H., Carteret, C., 2007. Sorption of 1-hydroxy-2-naphthoic acid to goethite,
503 lepidocrocite and ferrihydrite: Batch experiments and infrared study. *Chemosphere*, 70,
504 178-186.
505
506 Inoue, M.H., Oliveira, R.S., Regitano, J.B., Tormena, C.A., Constantin, J., Tornisielo,
507 V.L., 2006. Sorption-desorption of atrazine and diuron in soils from southern Brazil, J.
508 *Environ. Sci. Health. Part B.* 41, 605-621.
509
510 Inoue, M.H., Possamai, A.C.S., Mendes, K.F., Ben, R., de Matos, A.K.A., dos Santos,
511 E.G., 2014. Potential of leaching of herbicide used in sugar cane in soils contrasting
512 *Biosci. J.* 30, 659-665.
513
514 Kah, M., Brown, C.D., 2007. Factors influencing degradation of pesticides in soil. J.
515 *Agric. Food Chem.* 55, 4487-4492.
516
517 Kasozi, G.N., Nkedi-Kizza, P., Agyin-Birikorang, S., Zimmerman, A.R., 2010.
518 Characterization of adsorption and degradation of diuron in carbonatic and
519 noncarbocatic soils. *J. Agricul. Food Chem.* 58, 1055-1061.
520
521 Langeron, J., Sayen, S., Couderchet, M., Guillon, E., 2014. Leaching potential of
522 phenylurea herbicides in a calcareous soil: comparison of column elution and batch
523 studies. *Environ. Sci. Pollut. Research.* 21, 4906-4913.
524
525 Lewartz, B., Louchart, X., 2007. Effect of drying on the desorption of diuron and
526 terbutylazine from natural soils. *Environ. Pollut.* 146, 180-187.

527

528 Liu, Z., He, Y., Xu, J., Zeng, F., 2013. How do amorphous sesquioxides affect and
529 contribute to butachlor retention in soils?. *J. Soils Sedim.* 13, 617-628.

530

531 López-Piñero, A., Albarrán, A., Peña, A., 2010. Accumulative and residual effects of de
532 oiled two phase olive mill waste application to soil on diuron sorption, leaching,
533 degradation and persistence. *Chemosphere.* 78, 139-146.

534

535 Louchart, X., Voltz, M., Andrieux, P., Moussa, R., 2001. Herbicide transport to surface
536 waters at field and watershed scales in a mediterranean vineyard area. *J. Environ. Qual.*
537 248, 189-200.

538

539 Manolikas, M.K., and Sawant, M.R., 2003. Study of solubility of isoproturon by its
540 complexation with α -cyclodextrin. *Chemosphere.* 51, 811-816.

541

542 McKeague, J.A., Brydon, J.E., Miles, N.M., 1971. Differentiation of forms of
543 extractable iron and aluminium in soils. *Soil Sci. Soc. Am. Proc.* 35, 33-38.

544

545 Morillo, E., Undabeytia, T., Cabrera, A., Villaverde, J., Maqueda, C., 2004. Effect of
546 soil type on adsorption-desorption, mobility, and activity of the herbicide norflurazon. *J.*
547 *Agricul. Food Chem.* 52, 884-890.

548

549 **Morillo, E., 2008. Application of cyclodextrins in agrochemistry. Cyclodextrins and**
550 **Their Complexes. Edited by: Dodziuk, Helena. Wiley-VCH Verlag GmbH & Co.**
551 **KGaA: Weinheim, Germany, 459-467.**

552

553 Morillo, E., Sánchez-Trujillo, M.A., Moyano, J.R., Villaverde, J., Gómez-Pantoja,
554 M.E., Pérez-Martínez, J.E., 2012. Enhanced Solubilisation of Six PAHs by Three
555 Synthetic Cyclodextrins for Remediation Applications: Molecular Modelling of the
556 Inclusion Complexes. *PLoS ONE.* 7(9): e44137.

557

558 Morillo, E., Sánchez-Trujillo, M.A., Villaverde, J., Madrid, F., Undabeytia, T., 2014.
559 Effect of contact time and the use hydroxypropyl- β -cyclodextrin in the removal of
560 fluorine and fluoranthene from contaminated soils. *Sci.Total Environm.* 496, 144-154.
561

562 Navarro, S., Hernández-Bastida, J., Cazaña, G., Pérez-Lucas, G., Fenoll, J., 2012.
563 Assessment of the leaching potential of 12 substituted phenylurea herbicides in two
564 agricultural soils under laboratory conditions. *J. Agricul. Food Chem.* 60 (2012), 5279-
565 5286.
566

567 Pérez-Martínez, J.I., Ginés, J.M., Morillo, E., Moyano, J.R., 2000. ^1H - Nuclear
568 magnetic resonance and phase solubility studies of the stoichiometries in 2,4-D: α - and
569 β -cyclodextrins inclusion complexes. *J. Incl. Phenomena and Macrocyclic Chem.* 37,
570 171-178.
571

572 Pérez-Martínez, J.I., Morillo, E., Ginés, J.M., 1999. β -CD effect on 2,4-D soil
573 adsorption. *Chemosphere.* 39, 2047-2056.
574

575 Quintero, J.C., Moreira, M.T., Feijoo, G., Lema, L.M., 2005. Effect of surfactants on
576 the soil desorption of hexachlorocyclohexane (HCH) isomers and their anaerobic
577 biodegradation. *J. Chem. Technol. Biot.* 80, 1005–1015.
578

579 Sánchez-Trujillo, M.A., Lacorte, S., Villaverde, J., Barata, C., Morillo, E., 2014.
580 Decontamination of polycyclic aromatic hydrocarbons and nonylphenol from sewage
581 sludge using hydroxypropyl- β -cyclodextrin and evaluation of the toxicity of leachates.
582 *Environ. Sci. Pollut. Res.* 21,507-517.
583

584 Sánchez-Trujillo, M.A., Morillo, E., Villaverde, J., Lacorte, S., 2013. Comparative
585 effects of several cyclodextrins on the extraction of PAHs from an aged contaminated
586 soil. *Environ. Pollut.* 178, 52-58.
587

588 Sarmah, A.K., Rohan, M., 2011. Evaluation of four mathematical models to describe
589 dissipation kinetics of 4-n-nonylphenol and bisphenol-A in groundwater–
590 aquifer material slurry. *J. Environ. Monit.* 13, 157-66.
591

592 Semple, K.T., Doick, L.J., Wick, L.Y., Harms, H., 2007. Microbial interactions with
593 organic contaminants in soil: Definitions, processes and measurement. *Environ. Pollut.*
594 150, 166-176.
595

596 Sheng, G., Johnston, C.T., Teppen, B.J., Boyd, S.A., 2001. Potential contributions of
597 smectite clays and organic matter to pesticide retention in soils. *J. Agric. Food Chem.*
598 49, 2899-2907.
599

600 Smernik, R.J. and Kookana, R.S., 2015. The effects of organic matter-mineral
601 interactions and organic matter chemistry on diuron sorption across a diverse range of
602 soils. *Chemosphere*. 119, 99-104.
603

604 Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C., Semple, K.T., 2005. Prediction of
605 polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous
606 hydroxypropylbeta-cyclodextrin extraction technique. *Environ. Toxicol. Chem.* 24,
607 1325–1330.
608

609 Thurman, E.M., Bastian, K.C., Mollhagen, T., 2000. Occurrence of cotton herbicides
610 and insecticides in playa lakes of the High Plains of West Texas. *Sci. Total. Environ.*
611 248, 189-200.
612

613 Tomlin, C.D.S., 2000. Ed. Diuron. *The pesticide manual Handbook* 12th ed. British
614 Crop Protection Council. Surrey, UK. 276-277.
615

616 Villaverde, J., 2007. Time-dependent sorption of norflurazon in four different soils: Use
617 of beta-cyclodextrin solutions for remediation of pesticide-contaminated soils. *J.*
618 *Hazard. Mater.* 142, 184-190.
619

620 Villaverde, J., Kah, M., Brown, C.D., 2008. Adsorption and degradation of four acidic
621 herbicides in soils from southern Spain. *Pest Manag. Sci.* 64, 703-710.
622

623 Villaverde, J., Maqueda, C., Morillo, E., 2005. Improvement of the desorption of the
624 herbicide norflurazon from soils via complexation with β -cyclodextrin, *J. Agric. Food*
625 *Chem.* 53, 5366 – 5372.

626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659

Villaverde, J., Maqueda, C., Morillo, E., 2006. Effect of the simultaneous addition of β -cyclodextrin and the herbicide norflurazon on its adsorption and movement in soils. *J. Agricul. Food Chem.* 54, 4766-4772.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M., Laiz, L., Saiz-Jimenez, C., Sánchez-Trujillo, M.A., Morillo, E., 2012. Enhanced mineralization of diuron using a cyclodextrin-based bioremediation technology. *J. Agricul. Food Chem.* 60, 9941-9947.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M., Morillo, E., 2013a. Effect of hydroxypropyl- β -cyclodextrin on diuron desorption and mineralisation in soils. *J. Soil Sedim.* 13, 1075-1083.

Villaverde, J., Rubio-Bellido, M., Posada-Baquero, R., Madrid, F., Morillo, E., 2013b. Hydroxypropyl- β -cyclodextrin-based extraction for diuron bioaccessibility in an artificially contaminated soil. *Internat. J. Environm. Analyt. Chem.* 93, 1620-1627.

Wang, P., Keller, A.A., 2009. Sorption and desorption of atrazine and diuron onto water dispersable soil primary size fractions. *Wat. Res.* 43, 1448-1456.

Yu, C., Fan, T., Guo, X., Wu, X., 2015. Inclusion complexes of γ -cyclodextrin with pendimethalin. *Gaodeng Xuexiao Huaxue Xuebao/Chemical Journal of Chinese Universities.* 6, 306-309.

660

661

662

663

664

665