

1 SOILS, SEC # • RESEARCH ARTICLE

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3 **Effect of hydroxypropyl- β -cyclodextrin on diuron desorption and**
4 **mineralisation in soils.**

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19 **Abstract**

20 *Purpose* The herbicide diuron has the unfortunate property of being strongly adsorbed onto
21 soil organic matter particles, and hence, is slowly degraded in the environment because of its
22 reduced bioavailability. The aim of this work was to gain insight into the fate and behaviour
23 of diuron in the soil-water system, and develop and test an environmentally friendly soil
24 decontamination technique that could give rise to an enhancement of diuron mineralisation **by**
25 **sensitive soil endogenous microorganisms**, by means of increasing the bioavailability of the
26 pollutant employing **cyclodextrin (CD) solutions what would represent** an improvement
27 from both economic and environmental standpoints.

28 *Materials and methods* Selected soil colloidal components: montmorillonite, a synthetic
29 humic acid and a synthetic acicular goethite, and two different soils were employed in this
30 study to perform batch adsorption-desorption experiments. Desorption experiments were
31 performed using a 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution with and without **hydroxypropyl- β -**
32 **cyclodextrin (HPBCD)** 50 mM.

33 Assays to study the mineralisation of ^{14}C -labelled diuron were performed in **respirometers**,
34 into which 10 g of Soil I and 50 mL of mineral salts medium (MMK) were placed, obtaining a
35 final concentration of 50 mg kg^{-1} and a radioactivity of approximately 900 Bq per flask.

36 *Results and discussion* **Humic acid could be clearly discerned as the major colloidal**
37 **component responsible for adsorption. HPBCD was used in diuron desorption**
38 **experiments from soil, showing a strong extracting power on its removal.**

39 The mineralisation of diuron in the presence and absence of HPBCD was tested in a soil
40 managed with diuron for several years, involving therefore the presence of microorganisms
41 that have some specificity for diuron. Natural soil attenuation for diuron was improved when
42 a HPBCD solution was used in the presence of micronutrients as a bioavailability enhancer,
43 obtaining 66% of mineralisation in comparison to that obtained with only micronutrients
44 addition (44%).

45 *Conclusions* The use of HPBCD solution at a very low concentration of only 10 times the
46 diuron equimolar concentration in soil, acts as a bioavailability enhancer, accelerating the
47 passage of the diuron-desorbing fraction from the soil particle surface to the soil solution, and
48 hence, improving the microorganisms accessibility to the herbicide. **Diuron mineralisation**
49 **rate and the extent of its mineralisation were improved when the HPBCD solution was**
50 **employed in the presence of micronutrients.**

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53 **Keywords** Bioavailability • Bioremediation • Cyclodextrin • Diuron • Natural attenuation • Soil
54 contamination

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57 **1 Introduction**

58 **Diuron is a non-selective, substituted urea herbicide, widely used for the pre- and**
59 **post-emergence control of weeds in cereal crops and applied at high rates as a non-**
60 **selective herbicide in urban areas and also as an algaecide in antifouling paints. Toxic**
61 **effects on plants have been reported up to 3 years after soil treatment with diuron by Ek**
62 **Sebai et al. (2009). From an environmental point of view, diuron is known to be**
63 **persistent with a tendency to leach (Tomlin 2000). However, biodegradation has been**
64 **described as the primary mechanism for diuron dissipation in soils and waters (Sorensen et al.**
65 **2003; Qureshi et al. 2008). Ross and Tweedy (1973) concluded that its biodegradation is**
66 **enhanced on agricultural soil regularly exposed to this herbicide, increasing significantly**

67 **the possibility of natural attenuation. Also, Rouchaud et al. (2000) revealed the**
68 **capability of natural microbial communities to adapt to phenylurea metabolism.**

69 There is a need to understand fully the fate, behaviour, degradation processes and
70 toxicological characteristics of diuron. One of the main objectives of this work was to
71 determine the effects of the different soil colloidal particles and soils of contrasting textures
72 on diuron adsorption–desorption in relation to its bioavailability and its potential degradation
73 in the soil–water system, where the environmental impact from diuron use and its derived
74 compounds was considered a pollution risk.

75 The bioavailability of organic pollutants governs their ecotoxicology and degradation in
76 contaminated soils. This relationship is apparent in soils with high organic matter, in which
77 easily biodegradable organic compounds persist for long periods even under conditions
78 favourable for biodegradation. The lack of biodegradation in such soils has been attributed to
79 the low bioavailability of the contaminants. Environmental microbiologists define
80 bioavailability as “*the contaminant fraction which represents the accessibility of a chemical*
81 *to a living organism for assimilation, degradation and ecotoxicology expression*” (Semple et
82 al. 2007). Consequently, the bioavailability of contaminants varies with soil type, the nature
83 of the contaminants and environmental factors. Factors such as sorbent type, the residence
84 time, the desorption rate and the nature of the microorganisms influence bioavailability, and
85 consequently, the biodegradation and toxicity of sorbed compounds. Biodegradation is by far
86 the best and most likely the only environmentally means of detoxification of organic
87 contaminants in soil and water.

88 **In the case of pesticides, their repeated use in a specific agricultural site often results in**
89 **significant increases in pesticide biodegradation rate through acclimation of specific soil**
90 **microorganisms. This enhanced biodegradation can lead to important reductions in**
91 **pesticide biological efficacy, but from an environmental point of view, it can provide**
92 **benefits by removing unwanted contaminant residues at an accelerated rate (Alexander,**
93 **1999). Even in this specific scenario that favour pesticide degradation, this is often**
94 **limited by its nonavailability in the aqueous phase.** Numerous studies suggest that only
95 pollutants present in the aqueous phase are instantaneously available for degradation and that
96 the sorbed phase must first desorb into the aqueous phase for any significant degradation
97 (Jacobsen et al. 2001; Shchegolikhina et al. 2012; Song et al. 2011). Sorption appears to be
98 one of the crucial factors that limits the degradation of pesticides.

99 In this work, studies were also conducted to establish procedures that might result in an
100 increase in the bioavailability of diuron in contaminated soil through an increase in its

101 solubility using biodegradable molecules. These molecules are cyclodextrins (CDs), which
102 are cyclic oligosaccharides that contain 6 (α -CD), 7 (β -CD) or 8 (γ -CD) R-(1,4)-linked
103 glucose units and are formed from the enzymatic degradation of starch by bacteria. The most
104 important structural feature of these compounds is their toroidal shape with a hydrophobic
105 interior cavity and hydrophilic faces (Szejtli et al. 1992). They are well known to be capable
106 of forming inclusion compounds both in solution and in the solid state with a variety of guest
107 molecules placed in their hydrophobic interior cavity (Villaverde et al. 2004). The application
108 of CDs as solubility-enhancing agents has been investigated. The literature contains a large
109 number of papers in which CDs have been complexed with pesticides that present problems
110 from both agricultural and environmental points of view. Most pesticide–CD complexes have
111 been prepared to improve their solubility in water (Ginés et al., 1996; Pérez-Martínez et al.
112 2000; Morillo et al. 2001; Villaverde et al. 2005a, 2005b, 2007). **However, no works have**
113 **been reported with the aim of finding correlations between this increase in solubility,**
114 **their desorption percentage from soil, and their bioavailability by means of mineralising**
115 **assays that confirm the complete degradation in a real pesticide contaminated soil**
116 **without exogenous microorganisms. Only Fava et al. (1998) studied the effect of two CDs**
117 **on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil, but**
118 **no mineralisation experiments were carried out, and hence, the complete dissipation of**
119 **the pollutant could not be confirmed. The complete dissipation of diuron is specially**
120 **important, since its principal metabolite (3,4-dichloroaniline) is more persistent and**
121 **toxic than the parent compound (Villaverde et al., 2012). Others authors have**
122 **investigated the relationship between the CD enhanced solubility and biodegradation of**
123 **different pollutants using specific degraders (bioaugmentation) (Cai et al. 2006; Allan et**
124 **al. 2007; Wang et al. 2005; Wang et al. 1998) but the natural attenuation was not**
125 **evaluated (Cai et al., 2006;). Therefore, the aim of this work was to gain insight into the**
126 **fate and behaviour of diuron in the soil-water system, and to develop and test an**
127 **environmentally friendly soil decontamination technique that could give rise to an**
128 **enhancement of diuron mineralisation by means of increasing its bioavailability in real**
129 **soils employing HPBCD solutions, what would represent an improvement from both**
130 **economic and environmental standpoints.**

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135 2 Materials and methods

136 2.1 Materials

137 Technical grade (98%) diuron [*N*-(3,4-dichlorophenyl)-*N,N*-dimethylurea] was provided by
138 PRESMAR S.L. (Spain) Radiolabeled [ring-U-¹⁴C]-diuron was purchased from the Institute
139 of Isotopes (Budapest, Hungary) with specific activity of 36 mCi mmol⁻¹, chemical purity of
140 99.9% and radiochemical purity of 100%. **The CD employed was hydroxypropyl-β-CD**
141 **(HPBCD) from Cyclolab (Budapest, Hungary) and with a chemical purity of 97%.**

142 The different soil colloidal components used were a montmorillonite (SWy-2) obtained from
143 Source Clay Minerals Repository (Missouri, USA); a synthetic humic acid (supplied by
144 Fluka, Spain) and a synthetic acicular goethite with a specific surface area of 43 m²g⁻¹ and a
145 point of zero charge of 8.2 (Villaverde et al. 2007).

146 Two different soils with significantly different properties were employed in this study (Table
147 1). They were taken from the superficial horizon (0-20 cm), and both are from southwestern
148 Spain; they are named here as Soil I and Soil II. The soils were air-dried for 24 h to enable
149 them to be sieved through 2 mm mesh to remove stones and plant material and to facilitate
150 mixing. The soils were analysed for particle size distribution, which was measured on a
151 Bouyoucos densimeter; organic matter, which was measured by K₂Cr₂O₇ oxidation; pH,
152 which was determined in the 1:2.5 soil/water extract; and total carbonate content, which was
153 measured using the manometric method. Soil I had been managed with diuron for several
154 years.

155

156 2.2 Methods

157 2.2.1 Diuron adsorption–desorption on colloidal components and soils

158 Triplicate batch adsorption experiments were performed by mixing 5 g of soil in 50-mL
159 polypropylene centrifuge tubes with 10 mL of a 0.01 M Ca(NO₃)₂ solution that contained
160 various concentrations of diuron (0-15 mg L⁻¹). The samples were shaken on an orbital shaker
161 for 24 h at 20 ± 1°C. This time of reaction was chosen on the basis of preliminary kinetic
162 studies (not shown) that indicated that adsorption had reached pseudoequilibrium. After
163 shaking, the suspensions were centrifuged, and the concentration of diuron in the supernatant
164 was determined using a Shimadzu HPLC equipped with UV detector (wavelength 230 nm);
165 the conditions were as follows: mobile phase, acetonitrile/water (60:40); flow rate, 0.6
166 mL/min; temperature, 30°C; chromatographic column, Kromasil C18 reverse phase. The
167 difference in herbicide concentration between the initial and final equilibrium solutions was
168 assumed to be due to sorption, and the amount of diuron retained by the adsorbent was

169 calculated. Soil sorption isotherms were fitted to the logarithmic form of the Freundlich
170 equation:

171

$$172 \quad \log C_s = \log K_f + n \log C_e \quad (1)$$

173

174 where C_s ($\mu\text{mol kg}^{-1}$) is the amount of herbicide sorbed at the equilibrium concentration C_e
175 ($\mu\text{mol L}^{-1}$) and K_f and n are constants that characterise the relative sorption capacity and the
176 sorption intensity, respectively, for the herbicide. The normalised distribution coefficient
177 ($K_{f_{oc}}$) of organic carbon (OC) was calculated from the K_f values ($K_{f_{oc}} = K_f/OC \times 100$). The
178 coefficient $K_{f_{oc}}$ is often used to characterise the sorption of nonpolar hydrophobic
179 compounds; the concept is based on organic carbon exhibiting the same affinity for a
180 nonpolar compound, independent of the source of OC.

181 Desorption experiments were performed after adsorption equilibrium had been reached for the
182 different diuron initial concentrations by removing half of the supernatant after centrifugation,
183 replacing it with 5 mL of the extractant solution, allowing equilibration for an additional 24 h
184 period, and then following the procedure used in the adsorption experiment. This process was
185 repeated twice more. Desorption experiments were performed using a 0.01 M $\text{Ca}(\text{NO}_3)_2$
186 solution with and without HPBCD 50 mM. The percentage of diuron desorbed with respect to
187 that previously adsorbed during the adsorption process (%D) was calculated for all of the
188 desorption experiments.

189 These adsorption–desorption experiments were also performed with the different soil
190 colloidal components using the same procedure previously described; however, in this case, a
191 different soil:solution ratio was employed: 50 mg of the colloidal component/10 mL of the
192 herbicide solution.

193

194 *2.2.2 Mineralisation assay.*

195 **Assays to study the mineralisation of ^{14}C -labelled diuron were performed in**
196 **respirometers (modified 250 mL Erlenmeyer flasks), into which 0.25 mL of a 2000 mg kg $^{-1}$**
197 **diuron stock solution in acetone, which also contained ^{14}C -labelled diuron was initially**
198 **added to 2.5 g of Soil I (25% of the total soil used, 10 g). The respirometers were closed**
199 **for 5 min to let the solvent disperse. Thereafter, the solvent was evaporated for 16 h. The**
200 **remaining 75% was added and mixed to avoid damaging to all the indigenous**
201 **microorganisms.** After that, 50 mL of mineral salts medium (MMK) were added (Brinch et
202 al. 2002; Reid et al. 1998), obtaining a final concentration of 50 mg kg $^{-1}$ and a radioactivity of

203 approximately 900 Bq per flask. The flasks were closed with Teflon-lined stoppers and
204 incubated at $20 \pm 1^\circ\text{C}$ under shaking. The production of $^{14}\text{CO}_2$ was measured as the
205 radioactivity appearing in the alkali trap of the biometer flasks. The trap contained 1 mL of
206 0.5 M NaOH. Periodically, the solution was removed from the trap and replaced with fresh
207 alkali solution. The NaOH solution was mixed with 5 mL of liquid scintillation cocktail
208 (Ready Safe from Perkin Elmer, USA), and the mixture was stored in darkness for
209 approximately 24 h for the dissipation of chemiluminescence. Radioactivity was measured as
210 described by Posada-Baquero et al. (2008). 1 mL of a micronutrient solution containing trace
211 elements ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$, KBr,
212 KCl, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was also added in mineralisation
213 experiments (Fenlon et al. 2011).

214 **Mineralisation experiments were also performed in the presence of HPBCD (10 times**
215 **the millimoles of diuron added to Soil I). The CD solution was added 17 days after the**
216 **diuron addition with the aim of observing its effect after an aging period.**

217

218 *2.2.3 Model of mineralisation kinetics.*

219 Mineralisation data (expressed as the percentage [P] of the initial activity converted to $^{14}\text{CO}_2$
220 as a function of time [t]) were fitted to a first-order equation of the following form (Gerin and
221 Boyd 1992):

$$222 \quad P = P_{max}(1 - e^{-kt}) \quad (3)$$

223 A nonlinear regression analysis (Sigmaplot v. 8.0) was used to estimate the parameters P_{max}
224 (overall extent of ^{14}C mineralisation) and k (first-order mineralisation rate).

225

226 **3 Results and discussion**

227

228 3.1 Diuron adsorption–desorption on selected soils and representative soil colloidal
229 components

230 Figure 1a shows diuron adsorption isotherms on humic acids, montmorillonite and goethite.
231 In this figure, the humic acid component could be clearly discerned as the major component
232 responsible for adsorption, followed by the clay mineral montmorillonite. In the case of
233 goethite, herbicide sorption was negligible. In this case, it can be concluded that attention
234 must be paid to the diuron sorbed especially on soil organic matter and clay minerals, which
235 could be potentially mobilised under field conditions and thereby lead to widespread
236 contamination, or could decrease the herbicide fraction available for biodegradation in the soil

237 solution. The amount of diuron adsorbed is shown in Table 2, indicating that adsorption is 8-
238 11 times higher in humic acid than in montmorillonite.

239 Diuron desorption experiments were performed on the soil colloidal components only in cases
240 where adsorption could be measured (HA and Montmorillonite). In all cases, the desorption
241 behaviour deviated markedly from those corresponding to the adsorption isotherms (figures
242 not shown), which indicates that diuron adsorption on these colloidal components was not
243 completely reversible and it would be a persistent chemical in soils with a high organic matter
244 or clay minerals content.

245 In Table 2, the percentage of diuron desorbed (%D) using an aqueous solution of 0.01 Ca
246 $(\text{NO}_3)_2$ is also summarised. In general, diuron adsorption on selected soil colloids is slightly
247 more irreversible (lower %D) at lower concentrations of adsorbed diuron (desorption from an
248 initial concentration of 5 mg L^{-1}); i.e., diuron molecules are more strongly sorbed at low
249 surface coverage, and their desorption is therefore more difficult, **as previously observed for**
250 **other pesticides (Morillo et al. 2002; Undabeytia et al. 2004)**. In spite of the much higher
251 amount of diuron adsorbed on humic acids, its desorption is quite difficult (only about 30-
252 36% of that adsorbed). On the contrary, a great amount was desorbed from montmorillonite,
253 reaching almost 100% of that previously adsorbed for the higher concentrations. It indicates
254 that in soils with low OM content and high presence of clay minerals diuron can be easily lost
255 and mobilised increasing the environmental contamination.

256 Because of its low volatility and its high adsorption capacity on the organic matter particles,
257 diuron is found in the solid phase rather than in the gaseous or liquid phase. Sorption studies
258 of diuron have shown that the proportion of organic matter in soil directly influences the
259 amount of adsorbed diuron (Inoue et al. 2009; Kasozi et al. 2010). Wang and Keller (2009),
260 conducted studies on the sorption and desorption of diuron onto water-dispersible soil primary
261 size fractions (clay, silt and sand) and concluded that pesticide sorption onto the different size
262 fractions reproduces well the total amount of the pesticide sorbed onto the bulk soils and that,
263 because of their higher soil organic carbon content, the clay fractions were much more
264 effective sorbents for the herbicide than the bulk soils, silt, and sand fractions.

265 Diuron adsorption isotherms on the studied soils are shown in Figure 1b. The sorption data
266 were well described by the Freundlich equation (Table 3). The constant K_f is the amount of
267 pesticide sorbed for an equilibrium concentration of $1 \text{ } \mu\text{mol L}^{-1}$ and therefore represents
268 adsorption at a low adsorbate concentration, but K_f values can be used to compare the
269 adsorption capacity of the different soil samples toward diuron. The K_f values varied from
270 4.34 (Soil I) to $92.47 \text{ } \mu\text{mol kg}^{-1}$ (Soil II), which indicates the strong influence of soil

271 properties on diuron adsorption, mainly the organic matter content. The role of hydrophobic
272 bonds on the adsorption of nonpolar hydrophobic herbicides on soils can be compared by
273 normalising the Freundlich adsorption parameter to the percentage of organic carbon ($K_{f_{oc}}$) in
274 the different samples: a more hydrophobic molecule results in a greater probability of
275 partitioning from the aqueous phase to the organic phase. In order to compare among different
276 pesticides, the $K_{f_{oc}}$ parameter can be used as a measure of this property in soils, and it is
277 independent of other soil properties. For a specific pesticide, when hydrophobic bonds are the
278 only factor responsible for its adsorption, the $K_{f_{oc}}$ values should be relatively constant among
279 the different soils. However, the values obtained in this study are quite different, 749 and
280 1138, and indicates that the sorption mechanism is perhaps related to some other soil
281 properties in addition to the OC content (Sheng et al. 2011; Villaverde et al. 2003; Morillo et
282 al. 2002). A similar result was observed by Undabeytia et al. (2011) after using four soils with
283 different characteristics, concluding that other properties influenced the diuron soil sorption,
284 including the clay content. They concluded that despite the larger affinity of diuron for
285 organic matter, the fraction of diuron sorbed onto clay minerals can be important in soils with
286 high clay content. These results corroborated the previously discussed sorption of diuron onto
287 colloidal components, where the sorption onto the clay mineral was also observed.
288 The total percentages of diuron desorbed from the soils used (%D) for each concentration
289 under study are also shown in Table 2. In general, diuron adsorption on soils was more
290 irreversible (lower %D) at low adsorbed concentrations (desorption from an initial
291 concentration of 5 mg L^{-1}) (Morillo et al. 2004), the same as occurred with colloidal particles,
292 but this behaviour was more pronounced in soil I, due to its low OM content. The percentages
293 of desorption observed for each soil are in agreement with the values obtained for the sorption
294 capacity parameter: the higher the K_f value, the lower the percentage of diuron removed from
295 the soil, being remarkable the 87.2 % of desorbed diuron in Soil I regarding to the 4.26% for
296 the Soil II at an initial concentration of 15 mg L^{-1} .

297

298 3.2 Proposal of a decontamination technique using cyclodextrin solutions as diuron
299 bioavailability enhancers

300

301 *3.2.1 Diuron desorption using HPBCD as an extractant solution.*

302 **Diuron phase solubility assays in the presence of different CDs were previously reported**
303 **by Villaverde et al. (2012). HPBCD showed the best complexation parameters for the**
304 **formation of CD-diuron water-soluble inclusion complexes, concluding that this CD was**

305 **the best candidate to obtain the best results for diuron soil decontamination. The**
306 **stoichiometry of the complex obtained was 1:1, its formation constant was 207.70 ± 3.55**
307 **M^{-1} and the solubilisation increase obtained when using 100 mM HPBCD solution was**
308 **23.27 ± 1.33 . These results are in the same order of magnitude than those observed by**
309 **Mahedero et al. (2002) using a different analytical technique based on photochemically-**
310 **induced fluorescence properties of diuron and its photoproducts (K_c , $474 M^{-1}$). The**
311 **desorption percentages (%D) obtained for the two soils under study when the HPBCD was**
312 **employed as extractant solution are shown in Table 2. The same behaviour described**
313 **previously for the relative irreversibility of the adsorbed diuron is also observed because**
314 **diuron adsorption was more irreversible at the lowest diuron concentration, but only in Soil II**
315 **(18.95% desorbed for 5 mg L^{-1} , and 35.48% for 10 mg L^{-1}), since in Soil I almost 100 % of**
316 **diuron previously adsorbed was desorbed using HPBCD at all initial concentrations used. In**
317 **general, %D for Soil II were 2.5-5 times higher using HPBCD than when the electrolyte**
318 **solution was used for any diuron initial concentrations. These results indicate the high**
319 **extracting power of HPBCD towards the herbicide previously adsorbed onto the soils in**
320 **comparison to the percentages extracted with $\text{Ca}(\text{NO}_3)_2$ solution because of the formation of**
321 **water-soluble inclusion complexes between diuron and HPBCD. Similar results have been**
322 **reported by other authors who used HPBCD and BCD as extractant solutions for the**
323 **herbicides 2,4-D and norflurazon from soil (Pérez-Martínez et al. 2000; Morillo et al. 2001;**
324 **Villaverde et al. 2005a; 2005b; 2006; 2007). In general, low-polarity pesticides have a strong**
325 **tendency to be adsorbed onto organic soil surfaces, which leads to their inactivation and to**
326 **low bioavailability. If these pesticides are able to form inclusion complexes with CDs, and as**
327 **a consequence, increase their solubility, the application of CD solutions to soils that contain a**
328 **high concentration of adsorbed pesticide residues can increase their removal and facilitate**
329 **their passage to the soil solution, where they become bioavailable **to be degraded by soil****
330 **microorganisms.**

331

332 *3.2.2 Diuron mineralisation in the presence of HPBCD and micronutrients.*

333 From previous **studies (Villaverde et al., 2012)**, the use of HPBCD appears to be the best
334 choice for application as a decontamination technique in the case of the herbicide diuron. **For**
335 **mineralisation experiments only Soil I was employed, since it was considered as the**
336 **worst scenario for a diuron contaminated soil, because of its lower adsorption capacity,**
337 **and hence, higher possibility to release from soil to other environmental phases. In the Soil II**
338 **with a very high adsorption capacity (14% organic matter content), the contaminant is**

339 **highly retained by the soil particles, and hence, its availability will be drastically**
340 **reduced, representing smaller risk for the environment.** Mineralisation experiments that
341 employed soil suspensions in the absence and in the presence of HPBCD solutions were
342 performed to observe if HPBCD enhanced diuron bioavailability and its subsequent
343 dissipation rate. This process potentially makes the bioavailable herbicide fraction to pass
344 more rapidly to the soil solution, as previously demonstrated when higher desorption
345 percentages of diuron from the soils studied were obtained when the HPBCD solution was
346 used. The ability of soils to release (desorb) pollutants determines the susceptibility of the
347 pollutants to microbial degradation and thereby influences the effectiveness of the
348 bioremediation process. The degradation of sorbed contaminants can presumably occur via
349 microbially mediated desorption of contaminants in the soil solution and, as consequence, the
350 development of a steep gradient between the contaminant present in the solid phase and that
351 in the interfacial layer (Megharaj et al. 2011).

352 Diuron mineralisation curves obtained by natural soil attenuation and in the absence and in
353 the presence of micronutrients and HPBCD solution in Soil I are shown in figure 2. A
354 significant increase in the diuron mineralisation rate (up to about 44%) was observed when
355 micronutrients were applied (Table 4), which indicates the presence of diuron-sensitive
356 endogenous microorganisms in Soil I, since it was managed with this herbicide for several
357 years. In spite of this, soil natural attenuation was negligible (fig.2) and even when only
358 HPBCD was applied diuron mineralisation was also negligible (**in these cases**
359 **biodegradation kinetics parameters could not be obtained because 5% of diuron**
360 **mineralisation was not reached**). Nutrient amendments, used as a biostimulating agents for
361 soil bioremediation purposes, have been broadly described in the literature. The use of these
362 amendments results in an increase in microbial activity and/or biomass (Delille and Coulon,
363 2008; Kadian et al. 2012), reaching more than 40% mineralisation at the end of the
364 experiment.

365 However, when the HPBCD solution in the presence of the micronutrients was applied into
366 the soil slurry, a drastic increase in the extent of mineralisation was observed, which reached
367 a value about 66% (**50% higher than that obtained with only micronutrients**). T_{40} was
368 also calculated for both mineralisation curves (Table 4). 40% of diuron mineralised was
369 reached more quickly when HPBCD was present (after 35 days) regarding to that reached
370 when only micronutrients were present (73 days). This latter result is due to the contribution
371 of the CD to make diuron molecules more bioaccessible quickly through the increase of its
372 desorption rate from soil particles, improving its uptake by microorganisms. Although the

373 literature contains numerous reports regarding the effect of other bioavailability enhancers,
374 such as surfactants or biosurfactants, **as far as we know, no works have been published**
375 **regarding the effect of HPBCD solutions on natural attenuation of persistent organic**
376 **pollutants in a contaminated soil.** White et al. (1999) used an anionic surfactant, Alfoinic
377 810-60, to enhance the phenanthrene bioavailability in soil and aquifer material. Shin et al.
378 (2006) examined the combined solubilisation–biodegradation process in a soil contaminated
379 by phenanthrene using a biosurfactant solution composed of rhamnolipids. In general, the use
380 of surfactants is limited by their high capacity to be adsorbed onto soil particle surfaces, and
381 the interaction of microbial cells with the surfactants would modify the function of the cell
382 membrane and enzymes and lead to their inhibition (Katagi, 2008). CDs are considered non-
383 toxic, biodegradable molecules (Fenyvesi et al. 2005; Gould and Scott, 2005; **Wacker**
384 **chemie, 2013**), and it has been reported that HPBCD exhibits a low tendency to adsorb onto
385 soil particles (Badr and Brauer, 2004).

386

387 **4 Conclusions**

388 In conclusion, based on these results, the indigenous microbial communities in a soil managed
389 with diuron over a period of years have the potential to actively and extensively degrade the
390 target herbicide after the enrichment with micronutrients, which is essential for natural soil
391 attenuation activation. The use of HPBCD solution at a very low concentration of only 10
392 times the diuron equimolar concentration in soil acts as a bioavailability enhancer,
393 accelerating the passage of the diuron-desorbing fraction from the soil particle surface to the
394 soil solution, and improving the microorganisms accessibility to the herbicide. These effects
395 induce not only an increase in the percentage of mineralised diuron, but also a shortening of
396 the time required to the herbicide, and, therefore, to reduce the presence of diuron's main
397 toxic and persistent metabolite, 3,4-DCA, in the soil solution.

398

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541 **Figure Captions**

542 **Figure 1.** Diuron adsorption isotherms: a) on soil colloidal components: Humic acid (x);
543 Goethite (●); Montmorillonite (▲), b) on Soil I (◆) and Soil II (■).

544

545 **Figure 2.** Mineralisation of ¹⁴C-labelled diuron in Soil I amended with different treatments:
546 no treatment (◆); HPBCD (x); micronutrients (▲); micronutrients plus HPBCD (■).

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