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(NO₃)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 DT₅₀ 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 microorganisms 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 Commission (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-Martínez 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-chlorophenyl)-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.
2.2. Diuron adsorption-desorption studies in soils.

Triplicate batch adsorption experiments were performed by mixing 10 g (dry mass) of the soil with 10 ml solution containing 0.01 M Ca(NO₃)₂ as background electrolyte, 200 mg L⁻¹ HgCl₂ to prevent bacterial growth, and various concentrations (2, 5, 10, 15 and 20 mg l⁻¹) of diuron, in 50 mL polypropylene centrifuge tubes. The samples were shaken for 72 h at 20 ± 1 °C (on an orbital shaker). This time of reaction was chosen from preliminary kinetic studies (not shown) which showed that adsorption had reached pseudoequilibrium. After shaking, the suspensions were centrifuged (12000 g) and the concentration of diuron in the supernatant was determined by using a Shimadzu HPLC equipped with UV detector. The conditions used were as follows: mobile phase, acetonitrile/water (60:40); flow rate, 1.00 ml/min; temperature, 30 °C; chromatographic column, kromasil C18 reverse phase; wavelength, 230 nm and retention time, 2.5 min.

The difference in herbicide concentration between the initial and final equilibrium solutions was assumed to be due to sorption, and the amount of diuron retained by the adsorbent calculated. Sorption isotherms were fitted to the logarithmic form of the Freundlich equation:

\[
\log C_s = \log K_f + n \log C_e
\]

where \(C_s\) (µmol kg⁻¹) is the amount of the herbicide sorbed at the equilibrium concentration \(C_e\) (µmol l⁻¹), and \(K_f\) and \(n\) are constants that characterize the relative sorption capacity and the sorption intensity, respectively, for the herbicide. The normalized distribution coefficient \((K_{foc})\) of organic carbon (OC) was calculated from the \(K_f\) values \((K_{foc} = K_f/OC \times 100)\). The organic carbon content (OC) was determined as follows: \(OC\% = 0.58 \times OM\%\). \(K_{foc}\) is often used in the discussion of sorption of nonpolar hydrophobic compounds, the concept being compatible with the idea of organic carbon (OC) having the same affinity for a nonpolar compound, independent of the source of OC.

Desorption experiments were performed after adsorption equilibrium had been reached for the point corresponding to diuron initial concentration of 10 mg l⁻¹ by removing half of the supernatant after centrifugation, replacing it by 5 ml of the extractant solution, allowing equilibration for an additional 24 h period, and after that, operating as in the adsorption experiment. This process was repeated twice. Desorption experiments were carried out using 0.01 M Ca(NO₃)₂ solution or HPBCD x10 (HPBCD equivalent to 10 times the diuron initially added to soil, 4.29 x 10⁻³ mmol). The percentage of diuron
desorbed with respect to that previously adsorbed during adsorption process (%D) was calculated for all the desorption experiments.

2.3. Leaching experiments in soils columns

Leaching experiments were done in duplicate for two selected soils (Soil 1 and soil 7), which were chosen for being the most different in their properties from each other. Homogeneous soil columns were prepared by packing the soil gently and uniformly in 16 cm long methacrylate tubes of 3.0 cm internal diameter. The lower end was covered with nylon tissue and padded with a thin layer of glass wool (0.5 g) to hold the soil firmly into the column. Each column was filled with 125 g of soil 1 and 167 g of soil 7 to achieve 16 cm occupied by soil. The top of these columns was covered with glass wool to prevent disturbance of the soil by the input liquid.

In a preliminary experiment, soil columns were first saturated by capillarity with distilled water to obtain a soil moisture content of 100% of the field capacity. The difference between the weight of the saturated soil column and its dry weight was used to determine the pore volume (35 ml for soil 1, 47 ml for soil 7).

Technical diuron equivalent to 5 mg kg\(^{-1}\) was applied. 24 h after herbicide application, HPBCD equivalent to 10 times the diuron concentration initially applied to soil was added in solid form on top of the columns selected for this treatment (2.68 \(\times\) 10\(^{-2}\) and 3.6 \(\times\) 10\(^{-2}\) mmol. Breakthrough curves (BTCs) were obtained through periodic application of 10 ml of 0.01M Ca(NO\(_3\))\(_2\) solution until herbicide was no longer detected in the leachates from columns with and without HPBCD. Diuron in the leachates was monitored by HPLC with the conditions described above. After the leaching experiments, the columns were sliced into four 4 cm segments and the herbicide residues that remained adsorbed in the soil were quantified using 2 g of dry soil which were placed in Teflon centrifuge tubes and extracted with 5 ml of methanol. The tubes were placed on an orbital shaker at 100 rpm (New BRUNSWICK Scientific, Edison, N.J.) at 20 ± 1 ºC. After 24 h the tubes were centrifuged at 12000 g and the supernatant was analysed by HPLC after filtration. The extraction was carried out in triplicate.

2.4. Mineralisation experiments

Mineralisation of \(^{14}\)C-labeled diuron in selected soils was measured (in duplicate) through the evolution of \(^{14}\)CO\(_2\) produced (Villaverde et al., 2012). The mineralisation 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 14C-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 14CO$_2$ 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:

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

\[
[C]_t = [C]_0 e^{-k_{1}t} e^{-k_{2}(t-t_{b})} \quad DT50= \ln2/k_{1} \quad \text{if } DT50 \leq t_{b} \quad \text{(HS)}
\]

\[
DT50= (\ln2-k_{1}t_{b})/k_{2} \quad \text{if } DT50 \geq t_{b} \quad \text{(HS)}
\]

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, \(k_{1}\) and \(k_{2}\) are the rate constants of mineralisation for the fast and the slow fractions, respectively, and \(t_{b}\) is the time at which rate constant changes. These models have been selected because of their relative simplicity and their potential to
better fit the measured dissipation kinetics data sets for diuron that appear to be monophasic or biphasic (Sarmah and Rhoan, 2011; Beulke et al., 2005).

2.5. Statistical analysis

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

3. Results and discussion

3.1. Herbicide adsorption-desorption in soils

Diuron adsorption isotherms in the studied soils are shown in Figure 1. In all cases the isotherms fit the Freundlich equation, as confirmed by R² values (Table 2). The sorption isotherms were compared using the Kᵢ parameter of the Freundlich equation. The constant Kᵢ is the amount of pesticide sorbed for 1 µmol l⁻¹ equilibrium concentration, and hence, under our conditions, it represents adsorption at low adsorbate concentration. The Kᵢ ranged between 0.60 and 14.3 for the soils 7 and 6, respectively, indicating a strong influence of soil properties on diuron adsorption.

As commented in previous published works (Villaverde et al., 2012; Kasozi et al., 2010; Wang and Keller, 2009; Giacomazzi and Cochet, 2004) soil organic matter (SOM) content can be considered as the most important soil property to assess Diuron potential adsorption capacity. In general, a positive correlation between SOM content and calculated Kᵢ was observed. However, the soil that showed the highest diuron adsorption capacity, soil 6 (Kᵢ, 14.3), did not have the highest SOM content (1.41%). Soil 1, with the highest SOM content (3.37%) had not the highest Kᵢ value (13.0), being very similar to those observed for soil 2 and soil 3 (13.8 and 13.2) with a SOM content of 2.13% and 1.76%, respectively. SOM is a very complex and heterogeneous mixture of humic acids, lignin, polysaccharides, proteins, organic acids, inter alia. (Sheng et al., 2001), and hence different types of interactions between sorbent and adsorbate can be observed depending on SOM composition (Morillo et al., 2014). Inoue et al. (2006) observed diuron Kᵢ values ranging from 5.99 to 9.33 for 6 soils with different properties, and sorption was correlated positively with the organic carbon and clay content of the soil 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.
To confirm the most relevant parameters to predict diuron adsorption, including amorphous oxides presented in Table 1, statistical approach studies were carried out. Initially, the selected 7 soils were included in the prediction model, and after that, only 5 soils were studied, excluding soils 2 and 6, that is, those soils with the highest amorphous oxides content.

Table 3 gives statistical correlations between $K_f$ and selected soil properties of the seven investigated soils (a) and with only five soils (b), excluding soils 2 and 6, to know which soil properties influenced diuron adsorption. When all soils are considered only amorphous $\text{Al}_2\text{O}_3$ content was observed to correlate significantly ($P < 0.05$) with $K_f$. This fact suggests that some of the soils alter the correlation between adsorption and OM content influenced by other soil properties. If soils 2 and 6, which have the highest total amorphous oxides content (TAO) are excluded, $K_f$ is positively correlated with SOM content. For these two soils, TAO promotes diuron adsorption, and hence, a higher adsorption capacity is observed than that expected according to their OM contents.

The automated linear regression equation obtained by applying the selection step forward method and using the criterion value input $P < 0.05$, including the analyzed properties of the 7 soils investigated for $K_f$, was as follows:

$$K_f = 0.654 + 0.880 \text{Al}_2\text{O}_3 \ (%) + 0.156 \text{clay fraction \ (\%)}$$

$K_f$ appears to be related not only with $\text{Al}_2\text{O}_3$ content, but also with clay fraction despite no significant relationship with $K_f$ was found (Table 3). Other authors confirm these results about the role of clay fraction in Diuron adsorption. Inoue et al. (2006) observed that clay content significantly affected $K_f$ values. Wang and Keller (2009) reviewed the strong adsorption of diuron on the clay fraction, concluding that in soils with clay minerals/OM contents ratios $> 60$, herbicide adsorption could be on both SOM and clay minerals surfaces. Villaverde et al. (2008) observed a similar result for 4 herbicides, 2,4-D, dicamba and two sulfonyluracils, on soils from southern Spain, with a content of clay minerals 30 times greater than SOM content. Smernik and Kookana (2015) investigated potential causes of variation in diuron $K_{oc}$ based on OM-mineral interactions attributing the $K_{oc}$ increase after treatment with hydrofluoric acid to the elimination of the mineral fraction, which was blocking the OM sorption sites. However, as far as we know, no references exist dealing with the adsorption of diuron specifically on amorphous oxides.
The linear regression equation was also obtained for only 5 soils using the same procedure as previously indicated:

\[ K_f = 0.373 + 4.032 \text{ OM(\%)} \]

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

Diuron desorption isotherms when using a Ca(NO\textsubscript{3})\textsubscript{2} 0.01 M solution as extractant are shown in Appendix (Fig. 1) and the results are summarised in Table 4. Diuron desorption results deviated markedly from those corresponding to the adsorption isotherms showing positive hysteresis in all cases and indicating that diuron soil adsorption was not completely reversible. Lewartz and Louchart (2007) also observed positive hysteresis for Diuron, but Liu et al. (2013) only observed it on 3 of the 6 soils studied. Total percentages of diuron desorbed (%D) for an initial concentration of 10 mg L\textsuperscript{-1} without HPBCD are shown in Table 4. The percentages of desorption observed for each soil are, in general, in agreement with the values obtained for sorption (Table 2), that is, the higher the \(K_f\), the lower the percentage of diuron removed from the soil. The only exception was soil 2 that had the highest TAO percentage and especially in large amounts of amorphous Al\textsubscript{2}O\textsubscript{3} (Table 1).

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

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

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

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

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

Table 5 also shows the distribution of diuron residues in the soil columns as a function 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
Soil desorption of the herbicide diuron has always been more effective in the presence of HPBCD because diuron tends to form an inclusion complex with this cyclodextrin, which acts as an extractant towards the diuron adsorbed in soils. HPBCD did not cause an increasing soil leaching of the herbicide, which is considered key for deciding on the use of HPBCD in soil bioremediation. Finally, its application led to an increase in the rate of mineralisation, thanks to an increase in the apparent solubility of the herbicide, and thus its greater bioavailability. These results showed that the application of HPBCD was used for decontamination of diuron-polluted soils with different properties.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found in the online version. These data include one table with the Solution nutrients (SNs). Additionally, two figures have been also included. One of them with diuron adsorption-desorption isotherms on soils, using Ca(NO₃)₂ or HPBCD as extractant solutions. And the other with Diuron break through curves in soils 7 and 1 in the presence or absence of HPBCD.

**Reference**


