Assisted attenuation of a soil contaminated by diuron using hydroxypropyl-β-cyclodextrin and organic amendments

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Highlights

- *HPBCD acts as an enhancer for diuron bioavailability*

- *Organic amendments (OAs) increase the rate of diuron mineralization*

- *OAs supplied specific diuron degrading microorganisms*

- *DOM from OAs acts as natural surfactant for diuron extraction*

- *HPBCD plus OAs application causes a positive synergy on diuron rate mineralisation*

30 **ABSTRACT**

31

32 Diuron desorption and mineralisation were studied on an amended and artificially

33 contaminated soil. The amendments used comprised two different composted organic

34 residues (i.e., sewage sludge mixed with pruning **wastes** (SS), and urban solid residues

35 (USR), and two different solutions (with inorganic salts as the micronutrients) and

36 hydroxypropyl-β-cyclodextrin (HPBCD). After applying micronutrients to activate the

37 soil flora, 15.5% mineralisation could be reached after 150 days, indicating that the soil

38 has a potential capacity to mineralise the herbicide through biostimulation-assisted

39 attenuation. Diuron mineralisation was also improved when HPBCD solutions were

40 applied. Indeed, the extent of herbicide mineralisation reached 29.7% with this

41 application. Moreover, both the lag phase and the half-life time (DT_{50}) were reduced to

42 33 and 1778 days, respectively, relative to the application of just microutrients (i.e., 39

43 and 6297 days, respectively). Organic amendments were also applied (i.e., USR and SS)

44 on the contaminated soil: it was found that the diuron mineralisation rate was improved

45 as the amendment concentration increased. The joint application of all treatments

46 investigated at the best conditions tested was conducted to obtain the best diuron

47 mineralisation results. The micronutrient amendment plus 4% USR or SS amendment

48 plus HPBCD solution (10-fold diuron initially spiked) caused an extent of diuron

49 mineralisation 33.2 or 46.5%, respectively.

50

51 *Keywords: contaminated soil; mineralisation; diuron; hydroxypropyl-β-cyclodextrin;*

52 *bioremediation; compost*

53

54   1. **Introduction**

55

56   **Phenylurea herbicides** are widely used for the general control of non-crop areas and

57   for selective pre- and post-emergence weed control on crops such as asparagus, cotton,

58   maize or wheat; consequently, these herbicides constitute important environmental

59   pollutants. **Diuron is one such herbicides and it is the one that is most frequently**

60   **detected in groundwater (Di Bernardo Dantas et al., 2011; Giaccomazzi and**

61   **Cochet, 2004). Moreover, this herbicide is included in the list of priority hazardous**

62   **substances of the European Union (Malato et al., 2003), and is subjected to**

63   **emission controls and quality standards to achieve "progressive reduction of**

64   **discharges, emissions and losses".**

65

66   **Diuron attenuation in the environment is primarily achieved through microbial**

67   **degradation processes (Villaverde et al., 2012). This mechanism yields the**

68   **corresponding aniline derivatives, for example 3,4-dichloroaniline (DCA), which**

69   **are considered to be more harmful to non-target organisms compared with the**

70   **parent herbicide. Although there are numerous studies on diuron dissipation in**

71   **soil-water systems, the complete diuron mineralisation has been described**

72   **relatively rarely in the literature (Sorensen et al., 2013; Villaverde et al., 2013a;**

73   **Villaverde et al., 2012; Bazot and Lebeau, 2009; Sorensen et al., 2008).**

74   **Mineralisation studies using** ^{14}**C-ring-labelled diuron have demonstrated slow**

75   **mineralisation rates, with half-life values up to 4000 days in different soils**

76   **(Muhamad et al., 2013). At this time it is appropriate to provide the definition of**

77   **bioavailability and an interesting definition is that supplied by Katayama et al.**

78   **(2010): "The amount of chemical available to be taken up or utilised by an**

organism/organisms in a defined time and environment". The most significant interaction between soils and xenobiotics that affects bioavailability is sorption, followed by aging and bound residue formation. The rate of uptake and subsequent degradation of chemicals by organisms is generally determined by the concentration of the chemicals in soil solution. It has been observed that the rate of desorption is proportional to the rate of the mineralisation of the organic chemical (Villaverde et al., 2012; Villaverde et al., 2013b; Zhu and Aitken, 2010; Dou et al., 2011; Sun et al., 2012). For these reasons, the present investigations are focused on the development of different strategies for increasing the diuron bioavailability and therefore, mineralisation rate in soil-water systems. One of the strategies is the use of cyclodextrins (CDs), which are cyclic organic compounds that are obtained through the enzymatic transformation of starch. These molecules posses a hydrophobic cavity and an exterior that is strongly hydrophilic. This peculiar structure allows organic molecules to be included in the cavity via non-covalent bonds to form inclusion complexes (Morillo et al., 2012). In previous studies, complexes between diuron and different CDs were successfully obtained in solution, where the most successful complexation parameters and the highest solubility increment were obtained for HPBCD (Villaverde et al., 2012). Equally, previous works have been published in this area by the authors, who used a HPBCD solution at a very low concentration (i.e., only 10 times the diuron equimolar concentration in soil) as a bioavailability enhancer. This approach has the effect of accelerating the passage of the diuron-desorbing fraction from the soil particle surface to the soil solution, thereby improving the accessibility of the endogenous microorganisms to the herbicide (Villaverde et al., 2012; Villaverde et al., 2013). Similarly, Villaverde et al. showed for the first time in 2012 that a

104   **cyclodextrin-based bioremediation technology coupled with the application of a**

105   **specific bacterial diuron degrader consortium (bioaugmentation) was able to**

106   **achieve almost a complete mineralisation of diuron in a soil-water system.**

107

108   **The other strategy is the use of compost obtained from different residues for**

109   **diuron soil remediation. Composts are rich sources of xenobiotics-degrading**

110   **microorganisms, which can degrade pollutants to innocuous compounds through**

111   **the process of mineralisation. However, the application of composts can also**

112   **decrease the bio-accessibility of chemicals, sequestering pollutants within the**

113   **organic matrix of the soil. Although recycling different organic wastes provides**

114   **benefits from an environmental point of view, to date, the use of composts has not**

115   **been widely applied as a method for bioremediation (Semple and Reid, 2001). The**

116   **success or failure of a compost remediation strategy depends on a number of**

117   **factors, the most important of which are the bioavailability and biodegradability of**

118   **the pollutant. The use of HPBCD has been demonstrated, as noted above, to**

119   **accelerate the mineralisation rate of persistent contaminants in water-soil systems,**

120   **which has enhanced their bioavailability. Therefore, the main objective of the**

121   **present work will be to test if the joint application of both strategies can improve**

122   **the mineralisation of diuron. These results will contribute to the discussion of**

123   **establishing a bioremediation technique as a practical choice based on chemical**

124   **bioavailability.**

125

126   **2. Materials and Methods**

127

128   *2.1. Materials*

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2.1.1. Chemicals

131

Technical grade (98%) *diuron* [N-(3,4-dichlorophenyl)-N,Ndimethylurea] was provided by PRESMAR S.L. (Seville, Spain). Radiolabelled [ring-U-^{14}C]-diuron was purchased from the Institute of Isotopes (Budapest, Hungary) with specific activity of 36 mCi mmol^{-1}, chemical purity of 99.9% and radiochemical purity of 100%. The CD employed was hydroxypropyl-β-CD (HPBCD) from Cyclolab (Budapest, Hungary) and had a chemical purity of 97 %.

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2.1.2. Soil

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A loamy sandy agricultural *soil* from southwestern Spain with a pH of 8.7, 0.66% of organic matter (OM), 0.03 g kg^{-1} of dissolved organic matter (DOM) and a particle size distribution of 74% sand, 16% silt, and 10% clay was selected for this study. The sample was taken from the horizon (0−20 cm), air-dried for 24 h, and sieved through 2 mm to remove stones and plant materials. The soil was frozen until its use. The soil was analysed for particle size distribution (as measured using a Bouyoucos densimeter), organic matter (as measured by $K_2Cr_2O_7$ oxidation), pH (determined in the 1:2.5 soil/water extract), and total carbonate content (as measured using the manometric method).

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2.1.3. Amendments

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The micronutrients (trace elements, TEs) $CaSO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $Al_2(SO_4)_3 \cdot 16H_2O$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 2H_2O$, KBr, KCl, $MnCl_2 \cdot 4H_2O$, $SnCl_2 \cdot 2H_2O$ and $FeSO_4 \cdot 7H_2O$ were used as the inorganic amendment (Fenlon et al., 2011) in the mineralisation experiments. Additionally, two composts were used as the organic amendments. The first, USR compost, was obtained from an experimental reactor that uses food residues at the University of Huelva (López et al., 2011). The second, a composted Biosolid (SS), was obtained from sewage sludge from a Wastewater Treatment Plant in Sevilla. The SS compost was mixed with pruning residues (15%) to optimise the composting process and the final product. Both amendments are described in Table 1. OM was determined by dry combustion at 450°C (CEN, 1999), and the nitrogen (N) content was determined by distillation after Kjeldahl digestion. The total content of mineral nutrients and trace elements was determined after aqua regia digestion (CEN, 2002). The pH and the electric conductivity were determined in 1:5 (weight) compost/water extracts (USDA, 2001). Composts have a distinct nutrient content, which can help improving the microbial activity within the soils. The main difference between the residues is the organic matter content (i.e., it is much higher in the USR compost than in the SS compost because of the former´s origin). The potentially toxic elements (Cd, Cu, Pb, Zn) of SS do not cause negative effects following its application to soil, as demonstrated by Madrid and Florido (2010), who did not observe increases in soil metal solubility using the same SS amendment, even at higher rates than used in this assay.

2.2. Methods

178   *2.2.1. Mineralisation assays*

179

180   Assays to study the mineralisation of ^{14}C-labelled diuron were performed in

181   **respirometers** to evaluate the potential soil attenuation and assisted-biodegradation

182   using different amendments, as described by **Villaverde et al. (2013b).** Briefly, the soil

183   (10 g) in each respirometer was spiked with diuron (50 mg kg^{-1}) and a radioactivity of

184   approximately 450 Bq in a MMS medium (50 mL). A volume of 1 mL of

185   micronutrients (TEs) solution was added to evaluate the effect of an inorganic

186   amendment application on soil diuron mineralisation and the necessity of biostimulation

187   of the endogenous microflora. **To determine** the effect of CD application on soil diuron

188   mineralisation, a HPBCD solution, with a concentration 10 times the millimoles of

189   diuron concentration, was used as in **Villaverde et al., 2012**. To evaluate the effect of

190   the compost on the soil diuron mineralisation, the organic amendments were added to

191   the mineralization flasks at different concentrations: 0.5, 2 and 4% (with respect to soil-

192   dry weight).

193

194   *2.2.2. Diuron extraction experiments*

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196   Diuron extraction experiments were conducted to test the effect of micronutrients,

197   HPBCD and/or the composts **studied on herbicide availability**. For this purpose,

198   propylene centrifuge tubes containing 1 g of soil (previously spiked with 50 mg kg^{-1} of

199   diuron) and 5 mL of the medium were spiked with micronutrients, HPBCD and/or

200   organic amendment at different concentrations while maintaining the same soil:solution

201   ratio as in the mineralisation assays). **The tubes were agitated on an orbital shaker**

202   **for 72 h at 20±1 °C. Subsequently, the supernatant was removed following**

203 **centrifugation (10 min, 7000 rpm) and filtered through a 0.22 μm Millipore glass**

204 **fibre membrane. The diuron concentration in the supernatant was determined in**

205 **the filtrate with a Shimadzu HPLC** equipped with a UV detector (wavelength 230

206 nm) using the following conditions: mobile phase, acetonitrile/water (60:40); flow rate,

207 0.6 mL/min; temperature, 30 °C; and chromatographic column, Kromasil C18 reverse

208 phase.

209

210 *2.2.3 Bacterial enumeration*

211

212 Enumeration of viable bacteria (potential diuron specific degraders) was achieved by

213 counting the colony forming unit counts/gram of soil or compost (CFUs g^{-1}). Bacterial

214 enumeration was carried out using 1 g of soil or compost. The solids were whirl-mixed

215 for 30 s with 10 ml of Ringer's solution, and then sonicated for 1 min and allowed to

216 stand for 2 min. Aliquots (100 μl) of these dilutions were applied on agar-agar plates

217 prepared from a R2a diluted medium (1:40), which is the herbicide content (40 mg l^{-1})

218 that limits the carbon source for the soil endogenous flora and permits the selection of

219 diuron specific degraders. CFUs were counted after 48 h.

220

221 *2.2.4. Model of mineralisation kinetics*

222

223 All the diuron mineralisation curves obtained were fit to the best kinetic model

224 employing an excel file provided by the FOCUS (2006) workgroup on degradation

225 kinetics to facilitate kinetic analysis for parent compounds curves and the solver tool

226 (Microsoft statistical package).

227

228   The mineralisation curves were fitted to two kinetic models: a simple first-order model

229   (SFO) and a first-order sequential model (Hockey-Stick, HS). Parameters were

230   optimized adapting the recommendations by FOCUS (2006) to our mineralisation

231   processes, using the least-squares method with Microsoft Excel Solver and the

232   following equations:

233

234    $[C]_t = [C]_0\ e^{-kt}$ (SFO)

235    $[C]_t = [C]_0\ e^{-k_1 t_b}\ e^{-k_2(t-t_b)}$ (HS)

236

237   where $[C]_t$ and $[C]_0$ are the concentrations of mineralised diuron at time t and just after

238   spiking the soil, respectively (mg kg^{-1}), k is the rate constant of mineralisation (day^{-1}).

239   In the HS model, k_1 and k_2 are the rate constants of mineralisation for the fast and the

240   slow fractions, respectively, and tb is the time at which rate constant changes. **These**

241   **models have been selected for consideration based on their relative simplicity and**

242   **their potential to better fit the measured dissipation kinetics datasets for diuron**

243   **that appear to be monophasic or biphasic (Sharma and Rhoan, 2012; Beulke et al.,**

244   **2005).**

245

246   **3. Results and Discussion**

247

248   *3.1. Effect of composts on soil diuron mineralisation.*

249

250   The diuron soil mineralisation curves in the presence of the different amendments

251   applied at different concentrations are shown in Figure 1. All mineralisation curves fit

252   to a single first-order kinetic model (SFO). Diuron mineralisation without

253   micronutrients amendment was negligible even in the presence of both composts (data

254   not shown). **The ability of soil to recover from chemical contamination is primarily**

255   **dependent on the presence of a microbial community with the ability to remove it**

256   **(Caracciolo et al., 2013).** The degradation of a chemical depends on abiotic and biotic

257   processes, but only the latter are responsible for its complete degradation

258   (mineralisation) and removal from the environment **(Alexander, 1999)**. Although the

259   selected soil was managed over several decades with different herbicides, and its

260   endogenous flora would potentially be able to biodegrade these type of molecules, the

261   experimental results showed that these microorganisms require different essential

262   micronutrients. However, the addition of inorganic micronutrients (TEs) was not

263   sufficient to cause a significant increase in the mineralisation rate of the herbicide, and

264   only a 15.5% extent of mineralisation could be reached (DT_{50}: 6297 days, Table 2).

265

266   **Diuron mineralisation curves after** compost application are also shown in Figure 1.

267   DT_{50} was drastically reduced as the percentage of compost application was increased

268   (Table 2), reaching a value of approximately 240 days, which is 26 times lower than

269   that obtained with only TEs when 4% SS compost was added. Moreover, the extent of

270   mineralisation reached approximately 33% for both composts with doses of 4%.

271   Ellegaard-Jensen et al. (2013) showed that degradation of diuron was faster in carbon-

272   and nitrogen-rich media, whereas suboptimal nutrient levels restricted degradation. In

273   this way, enhanced bioremediation may be achieved adjusting the nutrient content of

274   contaminated soil (Tejada et al., 2010; Teng et al., 2010).

275

276   Adding exogenous organic matter improves the biological, physical and chemical soil

277   properties and its fertility (Thevenot et al., 2008). These changes also influence

278   biodegradation, retention and the transport of pesticides in soil (López-Piñero et al.,

279   2013; Herrero-Hernández et al., 2011; Romero et al., 2010). The increase of organic

280   matter in soil generally results in an increase in the sorption of hydrophobic pesticides

281   (Wauchope et al., 2002). However, this is not the only soil property associated with

282   hydrophobic pesticide sorption (Morillo et al., 2004). Villaverde et al. (2013b) and

283   Undabeytia et al. (2011) showed that the diuron sorption mechanism is related to some

284   other soil properties in addition to the OC content, such as the content of clay minerals.

285

286   **Most published works have described** a retention-sequestration effect on organic

287   contaminants that **gives rise to** a decrease in their availability (Puglisi et al., 2007;

288   Tejada et al., 2011). In contrast, Barriuso et al. (1997) showed that although the addition

289   of compost generally favoured the stabilisation of herbicide residues, this effect depends

290   on the type of organic amendment. Exogenous and endogenous dissolved organic

291   matter (DOM) may influence the transport of pesticides, through the formation of

292   DOM-pesticide complexes and/or the competition between DOM and pesticides for the

293   adsorption sites in the soil (Williams et al., 2002). Thevenot et al. (2009) performed

294   diuron leaching experiments using columns to investigate DOM–diuron interactions and

295   the possible co-transport of diuron and DOM using four different organic amendments.

296   These interactions appear to be related to the aromatic and aliphatic content of the

297   DOM, which suggests the formation of hydrogen and other non-covalent bonds.

298   Wischmann and Steinhart (1997) showed that supplementation of contaminated soils

299   with compost materials could enhance PAHs biodegradation.

300

301   Diuron extraction experiments (Figure 2) were performed to determine the effect of

302   both composts on diuron availability. The soil:solution relation used was the same as

303   that used in the mineralisation assays. The DOM concentration of the two composts

304   were very different, i.e., 0.7 and 0.1 g kg^{-1} for USR and SS, respectively (Table 1).

305   However, this difference did not influence the amount of diuron extracted from soil

306   (i.e., 4.45% for USR and 4.47 % for SS, fig. 2) when using 4 % of each compost. These

307   amounts are 4.6 times higher than that obtained when only micronutrients were applied

308   in the soil (0.96 %, Figure 2), and this increase is because of the formation of DOM-

309   diuron complexes as observed previously (Thevenot et al., 2008; Cox et al., 2004;

310   Imache et al., 2012). Although SS and USR possess different DOM concentrations

311   (0.28 and 0.04 mg DOM/g of soil, respectively), a similar diuron extraction was

312   obtained, indicating that the quality of DOM supplied plays also an important role in

313   diuron availability.

314

315   The formation of DOM-diuron complexes in solution does not fully explain the

316   significant decrease of DT_{50} for diuron mineralisation, which was divided by 26 after

317   the application of 4% of both composts (Table 2). **In addition to DOM, another**

318   **explanation would be the nutrients supplied by the composts, particularly organic**

319   **labile nutrients from the USR and SS organic matter (Table 1), which would act as**

320   **a carbon source for the soil endogenous flora. However, the amounts of available**

321   **micronutrients such as K, Fe, Mn, Zn or Ni supplied as biostimulants by the**

322   **composts are likely to be in low concentrations, which would explain why**

323   **significant mineralisation was not observed when only SS and USR were supplied**

324   **to the soil in the absence of TEs.**

325

326

327   However, the application of compost can act as a soil ameliorant capable of improving

328   the contaminated soil environment by introducing microbial degradative activity

329   (Semple et al., 2001). Composts are capable of sustaining diverse populations of

330   microorganisms with the potential to degrade a variety of aromatic pollutants. In our

331   case, potential diuron specific degrading CFUs were determined, both in the selected

332   soil and in composts (Table 1). **We noted the presence of a high number of CFUs in**

333   **the composts used, which were multiplied by 28 and 82 in the soil in the presence**

334   **of USR and SS, respectively.**

335

336   *3.2. Effect of HPBCD on soil diuron mineralisation.*

337

338   The diuron soil mineralisation curves in the presence of HPBCD are shown in Figure 3a

339   and 3b. Unlike the previously discussed mineralisation curves (Table 2), degradation

340   could not always be described by single first-order kinetics, as recommended by

341   FOCUS (2006). **Many simulation models accept only first-order half-life as an**

342   **input degradation parameter; however, in recent years attempts have been made**

343   **to incorporate degradation parameters obtained through models other than first-**

344   **order kinetic (Sarmah et al., 2009). Using the SFO model for all types of decay**

345   **patterns could lead to the under- or overestimation of the dissipation endpoints**

346   **when the deviation from first-order is significant. Srinivasan et al., (2014)**

347   **concluded that the dissipation times for the antibiotic sulfamethoxazole in soil**

348   **demonstrated that the non-linear biphasic models improved the goodness-of-fit**

349   **parameters for all datasets of the SFO model. Further, Sarmah and Rohan (2011)**

350   **observed that the biexponential model could describe the dissipation kinetics of 4-**

351   **n-nonylphenol and bisphenol-A in groundwater-aquifer material slurry.**

352 The diuron mineralisation curves in the presence of a HPBCD solution were fit to a

353 hockey-stick first-order kinetic model (HS), which consists of two sequential first-order

354 curves, where a fast initial decrease in pesticide concentrations is followed by a slower

355 decline. This is usually referred to as a bi-phasic pattern of pesticide degradation. The

356 pesticide concentration initially declines according to first-order kinetics with a rate

357 constant k_1. However, at a certain point in time (i.e., the breakpoint, tb), the rate

358 constant changes to a different value k_2.

359

360 As observed in Table 2, the diuron mineralisation rate k_1 was multiplied by 5 relative to

361 the case when only **TEs were** applied to the soil. In a previous work, the ability of

362 HPBCD to extract the diuron soil bioaccessible fraction was confirmed **by** Villaverde et

363 al., (2013b). **HPBCD can extract the entire bioavailable diuron fraction, resulting**

364 **in its rapid mineralisation, as shown in the first part of the mineralisation curve**

365 **(Figure 3). Here, approximately 30% diuron was mineralised in the first 40 days**

366 **after HPBCD was applied.** The recalcitrant or strongly sorbed herbicide fraction is

367 considered unimportant from a toxicological and environmental point of view

368 (Mahmoudi et al., 2013). Semple et al. (2007) asserted that HPBCD extraction is able to

369 assess the bioaccessible fraction in a soil-water system of low molecular weight

370 polycyclic aromatic hydrocarbons (PAHs). Additionally, CD extractions were capable

371 of predicting the microbial degradation of organic contaminants using laboratory-spiked

372 soils.

373

374 The role of HPBCD as a diuron bioavailability enhancer and, as a consequence, a

375 mineralisation increaser could be due to two different effects: a) the formation of an

376 inclusion complex in solution that may increase diuron solubility and/or b) HPBCD

377 may be used as a carbon source by the indigenous microorganisms of the soil and

378 promote their growth. This latter effect has been demonstrated to be invalid, because

379 various tests (data not shown) have shown that the number of specific diuron degraders

380 CFUs did not increase in the presence of HPBCD, suggesting that the bacterial

381 consortium tested does not use HPBCD as a carbon source.

382

383 However, the first effect has been observed in previous work (Villaverde et al., 2012),

384 where a significant increase in diuron hydrosolubility in the presence of HPBCD was

385 noted. These diuron solubility studies in the aqueous phase in the presence of different

386 CDs showed that the best complexation parameters for the formation of CD-diuron

387 water-soluble inclusion complexes were obtained in the case of HPBCD. CDs are

388 considered novel hosts for the complexation of hydrophobic compounds in the

389 environment, including PAHs and pesticides and even some emerging contaminants

390 such as alkylphenols (Morillo et al., 2014; Villaverde et al., 2014; Sánchez-Trujillo et

391 al., 2013). Consequently, the use of CDs can address some of the largest problems

392 facing chemistry, such as the increase of pollution in the environment (Albelda et al.,

393 2012). CDs are considered non-toxic, biodegradable (Fenyvesi et al., 2005) and

394 environmentally friendly molecules (Badr et al., 2004; Gould and Scott, 2005). The role

395 of HPBCD to act as an enhancer of diuron bioavailability has been shown in previous

396 desorption studies of organic contaminants in different soils (Villaverde et al., 2013a;

397 Sánchez-Trujillo et al., 2013; **Villaverde et al., 2005; Pérez-Martínez et al., 2000**) and

398 soil colloidal components (Villaverde et al., 2013b). In the present paper it has also been

399 demonstrated through extraction experiments that the presence of HPBCD causes an

400 increase in the diuron desorbing fraction, reaching 14.7% of diuron desorbed relative to

401 the amount desorbed in the absence of HPBCD (0.96 %, Figure 2).

402   *3.3. Combined effect of compost and HPBCD on soil diuron mineralisation*

403

404   Based on the results obtained for accelerating diuron mineralisation following the

405   application of different composts on diuron contaminated soil, the joint application of

406   each amendment at the best ratio (i.e., 4% for SS and USR) and HPBCD was

407   conducted. The corresponding diuron mineralisation curves are shown in Figures 3a and

408   3b. In both cases, the curves best fit to a hockey-stick first-order kinetic model with a

409   drastic increase for samples including TEs, in the diuron mineralisation rate in the first

410   part of the curve (k_1= 2.0 x 10^{-2} and 2.5 x 10^{-2} d^{-1} for SS- and USR-HPBCD,

411   respectively).

412

413   The application of SS-HPBCD led to a DT_{50} of only 174 days, which is a 36-fold

414   reduction in time to mineralise 50% of the diuron initially applied versus the application

415   of just TEs. Additionally, the joint application of SS-HPBCD achieved an important

416   enhancement in the rate of mineralisation, reaching 46.5 % of the herbicide mineralised

417   (Table 2). From these latter results, it seems that the diuron bioavailable fraction was

418   increased, because a higher percentage of the herbicide was mineralised than when only

419   HPBCD was applied, which could be due to the combined effect of the natural

420   surfactant, DOM, the formation of an inclusion complex between HPBCD and diuron

421   and the supplied of the specific diuron degraders. Yu et al. (2011) observed a similar

422   combined effect of DOM and biosurfactants on the desorption of PAHs in a soil-water

423   system, concluding that the synergistic effect caused by increasing the potential

424   hydrophobic surface of the complex DOM-HPBCD was able to extract organic

425   compounds (Nkambule et al., 2009).

426

427 It is also notable that the SS-HPBCD treatment elicited a larger effect on diuron

428 mineralisation than did the USR-HPBCD treatment (i.e., DT_{50}: 174 versus 1606 days;

429 lag phase: 11 versus 16 days, for SS- and USR-HPBCD, respectively). In Figure 2

430 diuron extraction in the presence of HPBCD and SS compost was much higher (50.7%)

431 than that of the system with HPBCD or compost individually (14.7 and 4.47%,

432 respectively). In the case of the USR-HPBCD application, this effect was not as evident,

433 although the combined effect was also higher than the isolated applications (34%). The

434 fact that the USR compost possesses an OM content >3.5 times higher than that of the

435 SS could be the reason for the lower increase in both the extraction and the

436 mineralisation of diuron in the presence of HPBCD. For this reason, the USR compost

437 possesses a higher diuron adsorption than the SS compost, and hence, diuron extraction

438 this will be lower in the soil amended with the compost with the higher OM content,

439 which in this case is (USR).

440

441 **4. Conclusions**

442

443 **Diuron mineralisation was possible only after the application of the essential**

444 **nutrients for soil microorganisms (TEs). The improvement in the rate of diuron**

445 **mineralisation following the application of compost on soil was mainly due to: i)**

446 **the supply of specific diuron degraders present in the organic amendments; ii) the**

447 **presence of labile organic nutrients from compost OM used as a carbon source by**

448 **soil microorganisms; and iii) DOM acting as a natural extractant that increases the**

449 **concentration of diuron in the soil solution. The joint application of all treatments**

450 **investigated at the best conditions tested was conducted to obtain the best diuron**

451 **mineralisation results. The use of HPBCD has been demonstrated to accelerate the**

mineralisation rate because improvement of diuron bioavailability in water-soil systems. The joint application of all treatments investigated at the best conditions tested was conducted to obtain the best diuron mineralisation results. The micronutrient amendment plus 4% USR or SS amendment plus HPBCD solution (10-fold diuron initially spiked) caused an extent of diuron mineralisation 33.2 or 46.5%, respectively.

It is important to highlight that the soil bioremediation strategy proposed in this work presents a feasible remediation technology from both an environmental and economic point of view. Indeed, a significant increase in the diuron mineralisation rate was achieved, which makes this bioremediation technique a viable option.

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Figure Captions

Figure 1. Mineralisation of ^{14}C-labeled diuron in soil in the presence of micronutrients (●) and after the addition of a) SS compost and b) USR compost applied at different concentrations: measured residues 4% (▲), 2% (■), 0.5% (♦), with the fit for SFO or HS model (dash line).

Figure 2. Extracted diuron in the presence of micronutrients (TEs), HPBCD, USR and SS compost, 4% and compost + HPBCD.

Figure 3. Mineralised diuron in soil in the presence of 4% SS compost (a) and USR compost (b). The different treatments are only compost (▲), only HPBCD (♦) and in combination: composts + HPBCD (■), with the fit for SFO or HS model (solid line).
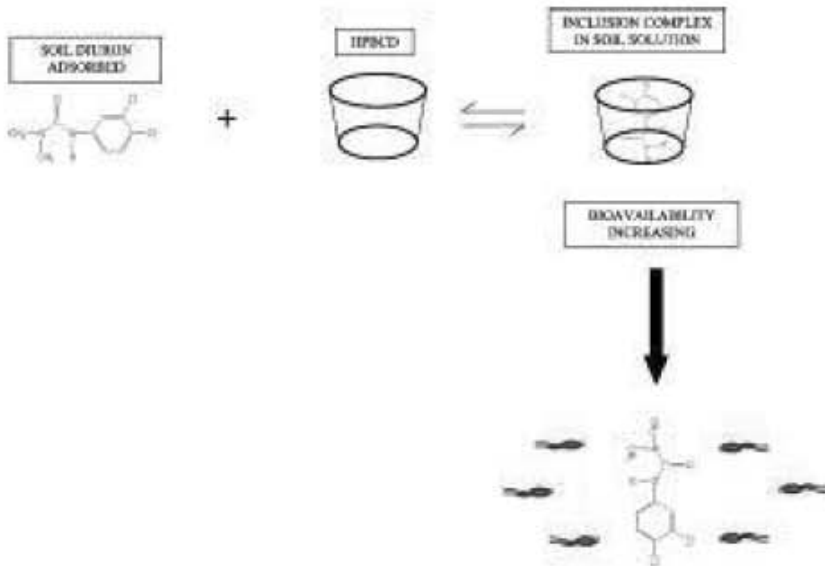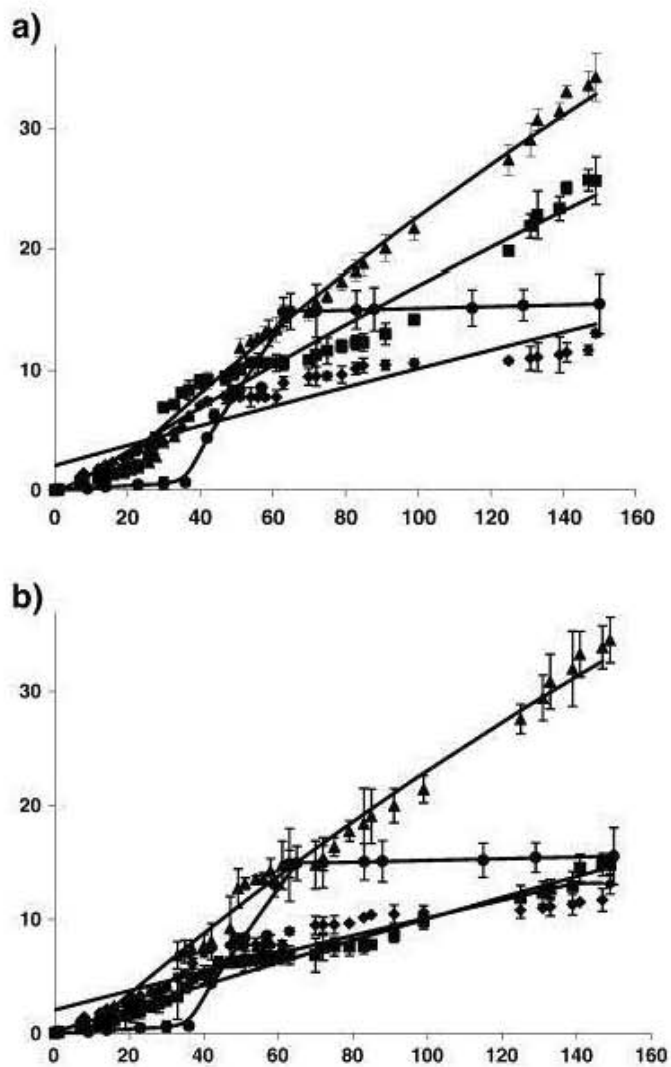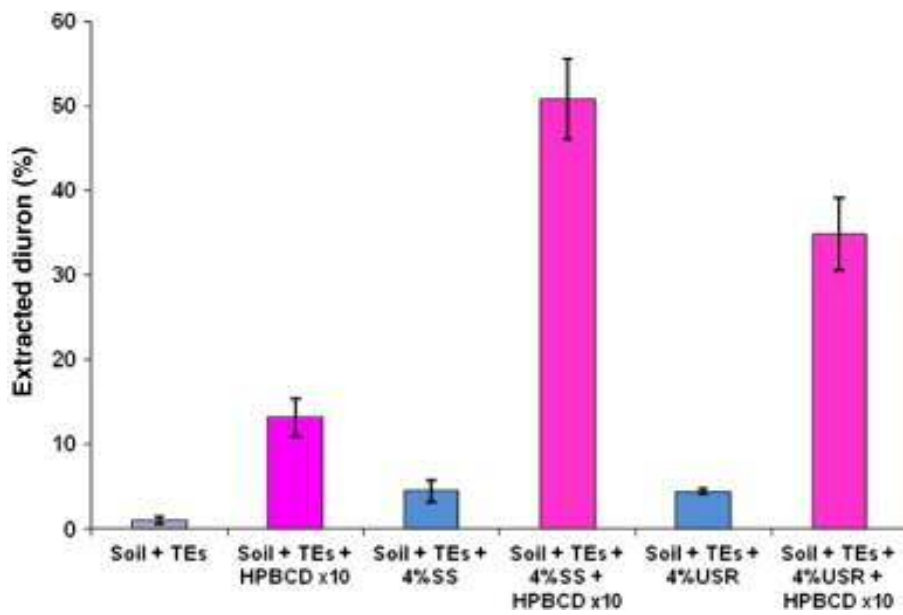
Figura 1.

Mineralisation of ^{14}C-labelled diuron in soil in the presence of micronutrients (●) and after the addition of a) SS compost and b) USR compost applied at different concentrations: measured residues 4% (▲), 2% (■), 0.5% (♦), with the fit for SFO or HS model (solid line).

Figura 2.

Extracted diuron in the presence of micronutrients (TEs), HPBCD, USR and SS compost (4%), and compost + HPBCD.

Figura 3.
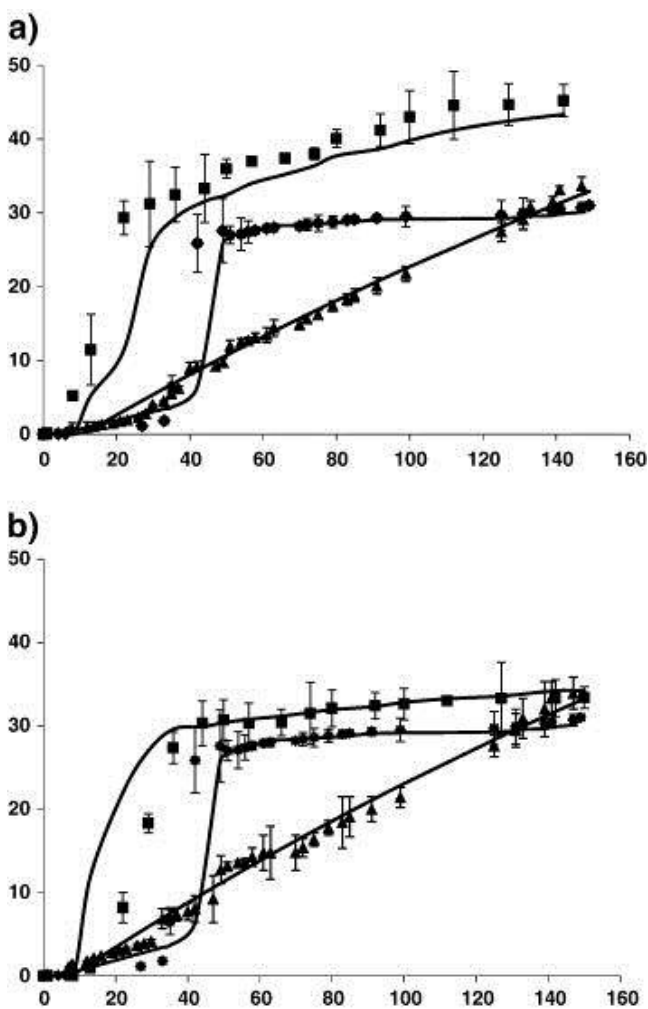
Mineralised diuron in soil in the presence of 4% SS compost (a) and USR compost (b). The different treatments are only compost (▲), only HPBCD (♦) and in combination: composts + HPBCD (■), with the fit for SFO or HS model (solid line).

Table 1. Main properties of the composts used.

Properties	USR	SS
Organic matter (%)	79	22
pH	6.7	6.4
Electrical conductivity (dS m^{-1})	8.7	8.0
N Kjeldahl (%)	1.8	1.7
P_2O_5 (%)	0.6	3.9
K_2O (%)	1.2	0.8
CaO (%)	5.4	9.5
MgO (%)	0.4	1.2
Na (%)	0.6	0.0
Fe (%)	0.5	1.9
Mn (mg kg^{-1})	86	252
Cu (mg kg^{-1})	57	325
Zn (mg kg^{-1})	49	563
Pb (mg kg^{-1})	60	184
Ni (mg kg^{-1})	16	59
Cd (mg kg^{-1})	< 1	2.0
C/N ratio	44	13
Dissolved organic matter (g kg^{-1})	0.7	0.1
Microbial content (CFU g^{-1}). Specific diuron degraders	23×10^5	68×10^5
Soluble phosphorous (mg L^{-1})	90	17
Soluble nitrogen (mg L^{-1})	670	60×10^3

Table 2. First order kinetic parameters (Single First Order, SFO, or Hockey–Stick, HS, models) for mineralisation curves of diuron obtained after micronutrients (TEs), cyclodextrin (HPBCD) and/or composts application (SS and USR) at different concentrations.

Treatments	Kinetic model	k_1 (day^{-1})	k_2 (day^{-1})	tb (day^{-1})	Lag phase (days)	DT$_{50}$ (days)	Extent of mineralisation (%)
Soil + TEs	HS	$5.1 \cdot 10^{-3}$	$0.9 \cdot 10^{-4}$	68.0	39	6297	15.5
Soil + TEs + 0.5% SS	SFO	$8.6 \cdot 10^{-4}$	–	–	60	804	13.9
Soil + TEs + 2% SS	SFO	$2.0 \cdot 10^{-3}$	–	–	27	360	24.5
Soil + TEs + 4% SS	SFO	$3.0 \cdot 10^{-3}$	–	–	18	240	32.9
Soil + TEs + 0.5% USR	SFO	$9.5 \cdot 10^{-4}$	–	–	54	734	13.4
Soil + TEs + 2% USR	SFO	$1.2 \cdot 10^{-3}$	–	–	49	660	14.6
Soil + TEs + 4% USR	SFO	$3.0 \cdot 10^{-3}$	–	–	18	244	33.1
Soil + TEs + HPBCD × 10	HS	$2.6 \cdot 10^{-2}$	$4.0 \cdot 10^{-4}$	42.2	33	1778	29.7
Soil + TEs + HPBCD × 10 + 4% SS	HS	$2.0 \cdot 10^{-2}$	$3.0 \cdot 10^{-3}$	21.4	11	174	46.5
Soil + TEs + HPBCD × 10 + 4% USR	HS	$2.5 \cdot 10^{-2}$	$3.6 \cdot 10^{-4}$	40.2	16	1606	33.2