

1 **Comparative effects of several cyclodextrins on the extraction of PAHs**  
2 **from an aged contaminated soil**  
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16 **Abstract**

17

18 The objective of the present study was to characterize the polycyclic aromatic  
19 hydrocarbons (PAHs) content of an aged contaminated soil and to propose remediation  
20 techniques using cyclodextrins (CD). Four CDs solutions were tested as soil  
21 decontamination tool and proved more efficient in extracting PAHs than when an  
22 aqueous solution was used; especially two chemically modified CDs resulted in higher  
23 extraction percentages than natural  $\beta$ -CD. The highest extraction percentages were  
24 obtained for 3-ring PAHs, because of the appropriate size and shape of these  
25 compounds relative to those of the hydrophobic cavities of the CDs studied. A detailed  
26 mechanistic interpretation of the chemical modification of CDs on the extraction of the  
27 different PAHs has been performed, and connected with the role that the different  
28 hydrophobicities of the PAHs play in the extraction behaviour observed for the 16  
29 PAHs, limiting their accessibility and the remaining risk of those PAHs not extractable  
30 by CDs.

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32

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34 *Keywords: contaminated soil; cyclodextrins; PAHs; soil remediation; contaminant*  
35 *bioavailability.*

36

37 **Capsule:** Cyclodextrin solutions are useful and interesting tools for the decontamination  
38 of soils polluted by PAHs.

39

## 40 **1. Introduction**

41

42 Polycyclic aromatic hydrocarbons (PAHs) are persistent organic compounds that can  
43 accumulate in soils. Soils from coal storage areas, coke oven plants, manufactured gas  
44 plants and areas of coal tar spillage have high levels of contamination with PAHs,  
45 although their concentrations vary by several orders of magnitude. (Khodadoust et al.,  
46 2000; Paria and Juet, 2006; Vulava et al., 2007; Wu et al., 2010). Owing to the toxicity,  
47 mutagenicity and carcinogenicity of PAHs, the remediation of PAH-contaminated soil  
48 is needed (Ahn et al., 2008). In 2005, the Spanish government published new  
49 regulations (Ministerio de la Presidencia, 2005) that established the criteria and  
50 standards for declaring a soil as contaminated. These regulations consider soil to be a  
51 sensitive matrix for performing risk assessment if the soil has a total hydrocarbon  
52 content higher than 50 mg kg<sup>-1</sup>, and states that necessary actions have to be performed  
53 to promote environmental recovery.

54

55 Physical, chemical, biological, and combination technologies have been used to  
56 remediate soils contaminated with organic compounds. The *in situ* microbial  
57 degradation of PAHs is limited by the low bioavailability of these compounds (Badr et  
58 al., 2004). Increasing both the mass transfer from the soil particles to the aqueous phase  
59 and the solubility of the organic contaminant, bioavailability will be improved, and  
60 hence, the potential remediation of contaminated soils and groundwater. To enhance the  
61 desorption rate of organic pollutants various extraction agents have been used, such as  
62 solvent mixtures (e.g., ethanol/water and methanol/water) and various types of  
63 surfactants. The health and environmental hazards associated with the use of surfactants  
64 have been described in detail in the literature (Ying, 2006).

65

66 Recently, cyclodextrins (CDs) have been proposed as alternative agents to enhance the  
67 water solubility of hydrophobic compounds. CDs have a low-polarity cavity within  
68 which organic compounds of the appropriate shape and size can form inclusion  
69 complexes (Dodziuk, 2006) (CDs molecular structure in Figure S1 of the  
70 Supplementary Data). This property provides CDs a capacity to increase the apparent  
71 solubility of several hydrophobic pollutants such as PAHs, pesticides, and nitroaromatic  
72 compounds thus increasing the availability of these pollutants for biodegradation (Fava  
73 et al., 2002; Villaverde et al., 2004, 2005, 2006). CDs have several advantages over

74 organic solvents and non-ionic surfactants, including their non-toxicity to  
75 microorganisms and their higher biodegradability and negligible sorption to solids. For  
76 these reasons, CDs have emerged as useful tools for the removal of PAHs from soil.

77

78 Previous studies have shown that CDs improve the solubility of PAHs in contaminated  
79 soils, but, in general, no more than two or three PAHs are studied, and the comparison  
80 to the other 16 priority PAHs is lack (Badr et al., 2004; Viglianti et al., 2006a, b; Bardi  
81 et al., 2007; Petitgirard et al., 2009). Moreover, as far as we know, in the great majority  
82 of studies about PAH extraction from soils by CDs, the soils are artificially  
83 contaminated (Gao et al., 2012), and it is usually not representative of their desorption  
84 from weathered and aged contaminated soils (Wu et al., 2010; Reddy et al., 2011). Even  
85 in the most recent reviews about technologies for PAH remediation using CDs no  
86 references to studies on the extraction of the 16 EPA-PAHs from real aged  
87 contaminated soils are included (Gan et al., 2009; Mahanty et al., 2011; Landy et al.,  
88 2012). For these reasons, we considered very interesting and a novelty such research.  
89 Therefore, the objectives of the present study were (i) to thoroughly analyze a real  
90 contaminated soil to determine its PAH content, and (ii) to evaluate the abilities of a  
91 natural CD ( $\beta$ -cyclodextrin;  $\beta$ -CD) and three chemically modified CDs (2-  
92 hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD), randomly methylated- $\beta$ -cyclodextrin  
93 (RAMEB) and hydroxypropyl- $\gamma$ -cyclodextrin (HP- $\gamma$ -CD)), to extract the 16 PAHs  
94 considered priority pollutants by the United States Environmental Protection Agency  
95 (US-EPA) (Environmental Protection Agency, 2011). The paper discusses the potential  
96 of CDs to be used for the decontamination of PAHs from real aged soils.

97

## 98 **2. Materials and Methods**

99

### 100 **2.1. Chemicals and reagents**

101

102 Sixteen US-EPA PAHs, were analysed: naphthalene, acenaphthylene, acenaphthene,  
103 fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene,  
104 chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno [1,2,3-  
105 cd] pyrene, dibenzo [a,h] anthracene and benzo [ghi] perylene (PAH molecular  
106 structures in Figure S2 of the Supplementary Data). The standard PAHs mix was  
107 supplied by Supelco (Barcelona, Spain) at 200 mg L<sup>-1</sup> solution in methanol. The

108 surrogate standard was a mixture containing naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>,  
109 phenanthrene-d<sub>10</sub>, chrysene-d<sub>12</sub>, and perylene-d<sub>12</sub> supplied by Supelco as a mix at 200  
110 mg L<sup>-1</sup> solution in methanol. Anthracene-d<sub>10</sub>, also from Supelco, was used as an internal  
111 standard. β-CD, HP-β-CD, RAMEB and HP-γ-CD (purity 97%) were supplied by  
112 Cyclolab (Budapest, Hungary), the solvents were from Merck (Darmstadt, Germany),  
113 the 5 g alumina SPE cartridges were supplied by IST (International Sorbent  
114 Technology, UK) and 99.995% pure nitrogen for drying was purchased from Air Liquid  
115 (Spain).

116

## 117 **2.2. Collection and preparation of contaminated soil samples**

118

119 The investigated soil was collected from the area surrounding a deserted chemical plant  
120 in Asturias (northern Spain) whose primary activity was the distillation of organic  
121 compounds such as naphthalene and phenol derivatives. The boiler system run on fuel  
122 oil, and important oil spills had occurred in the past.

123 All of the samples were collected from the A (0-20 cm) horizon. They were placed in  
124 amber glass containers and transported to the laboratory. The soil samples were air-  
125 dried, crushed and passed through a 2 mm sieve to remove the coarse materials. They  
126 were placed in amber glass pots and stored at -20°C. The soil pH was basic (9.2), with a  
127 calcium carbonate content of 22.6%, an organic matter content of 2.62 %, and a particle  
128 size distribution that was predominantly sandy (average sand content approximately  
129 89%).

130

## 131 **2.3. Exhaustive PAHs soil extraction and clean-up**

132

133 The soil was first characterized for PAHs composition. Soil samples were spiked with  
134 the surrogate standards at a concentration of 5 mg kg<sup>-1</sup> and extracted by sonication. A  
135 0.2 g portion of soil was placed in a glass tube containing 10 mL of a  
136 hexane/dichloromethane mixture (1:1) and this tube was placed in an ultrasonic bath for  
137 10 minutes. Afterward sonication the solution was centrifuged for 10 minutes at 3000  
138 rpm. This procedure was repeated two more times, using fresh solvent each time. The  
139 sonicated extracts were evaporated in a Turbo Vap LV from Caliper Life Sciences to  
140 near dryness (approximately 1 mL) prior to further clean up. The extracts were purified  
141 using 5 g neutral alumina solid phase extraction (SPE) cartridges. The alumina was

142 solvated and conditioned prior to sample loading with 40 mL of hexane-  
143 dichloromethane (1:1). After addition of the extracts, the analytes were eluted with 40  
144 mL of hexane-dichloromethane (1:1). The eluents were concentrated to a volume of less  
145 than 1 mL using a Turbo Vap LV evaporator and transferred to amber glass  
146 chromatographic vials. Then, the extracts were evaporated to near dryness and  
147 reconstituted with hexane to a final volume of 1 mL. At this stage, anthracene-d<sub>10</sub> was  
148 added as an internal standard at a final concentration of 1 µg mL<sup>-1</sup>. The extraction  
149 experiments were performed in triplicate.

150

#### 151 **2.4. Non-exhaustive extraction of PAHs from soil using an electrolyte solution and** 152 **aqueous β-CD, HP-β-CD, RAMEB and HP-γ-CD solutions**

153

154 Three sequential batch extractions were performed using the electrolyte and aqueous  
155 solutions of four different CDs. Soil samples (0.2 g) were placed in glass tubes  
156 containing 10 mL of an aqueous solution (0.01M Ca(NO<sub>3</sub>)<sub>2</sub> as background electrolyte  
157 and 200 mg L<sup>-1</sup> HgCl<sub>2</sub> to prevent bacterial growth). Other batch extraction experiments  
158 were performed using 0.01 M β-CD, HP-β-CD, RAMEB or HP-γ-CD solutions that also  
159 contained 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub> and 200 mgL<sup>-1</sup> HgCl<sub>2</sub>. Extraction experiments were  
160 performed in triplicate. The glass tubes were shaken on a horizontal plate shaker at 200  
161 r.p.m for 20 h at a constant temperature of 20°C. After centrifugation at 3000 rpm for 10  
162 min, the target compounds were liquid-liquid extracted (LLE) from a 3 mL aliquot of  
163 the supernatant with 3 mL of hexane, and 1 mL of the organic phase was transferred to  
164 an amber glass vial. At this stage, anthracene-d<sub>10</sub> was added to each vial before analysis  
165 to a final concentration of 1 µg mL<sup>-1</sup>. The remainder of the supernatant was decanted,  
166 and then, the same amount of a fresh electrolyte solution or CDs solutions was added.  
167 The extractions were carried out three times in total, and the extracts were analysed  
168 individually.

169

#### 170 **2.5. Instrumental analysis**

171 The samples were analysed using a GC system (Agilent GC 6890 N) coupled with a  
172 quadrupole mass spectrometer (MS, Agilent MD 5975B). This system was operated in  
173 electron ionization mode (EI 70 eV). Separation was achieved with a 30 m x 0.25 mm  
174 I.D. DB-5 MS column (J&W Scientific, Agilent Technologies) coated with 5% phenyl-  
175 methylpolysiloxane (film thickness 0.25 µm). The oven temperature was programmed

176 to increase from 80°C (holding time 1 min) to 175°C at 6°C/min (holding time 4 min), to  
177 235°C at 3°C/min and finally to 320°C at 8°C/min. The final temperature was held for 5  
178 min. Two microlitres were injected using the splitless mode. Helium was used as carrier  
179 gas (50 cm/s). The injector, transfer line and ion source temperatures were set at 280°C,  
180 250°C and 200°C, respectively. For increased sensitivity and specificity, quantification  
181 was performed with time scheduled Selected Ion Monitoring (SIM) using three ions for  
182 each compound. For exhaustive extraction of soil, internal standard quantification was  
183 performed using the surrogate deuterated PAHs present in each elution window. For  
184 non exhaustive extraction, quantification was performed using the internal standard  
185 anthracene-d<sub>10</sub> added to the 1 mL of the final extract after LLE. Peak detection and  
186 integration were carried out using Agilent ChemStation software. The details of the  
187 analytical procedure are presented in Martinez et al. (2004).

188

## 189 **2.6. Quality Control/Quality assurance**

190 Quality parameters are given in Table S1 of Supplementary Data. Recovery studies  
191 were performed using an agricultural soil, previously washed with acetone, spiked with  
192 target PAHs at 50 mg kg<sup>-1</sup>. The extraction proved to be efficient, with recoveries from  
193 46±3 to 110±14. The recovery of the surrogate standards were from 77 to 101%. For  
194 non-exhaustive extraction, recoveries were not calculated since it represents the  
195 decontamination procedure where each CD provides unique performance in terms of  
196 efficiency. Laboratory blanks were performed with empty vial (no soil) and with the  
197 same clean agricultural soil, spiked with the surrogate standards and extracted as  
198 depicted before for exhaustive extraction. No background contamination was observed.  
199 Finally, the calibration curve was performed over a concentration range of 0.01 to 1 µg  
200 mL<sup>-1</sup>, and good linearity was observed using internal standard with both the surrogate  
201 standards (for exhaustive extraction) and the internal standard anthracene-d<sub>10</sub>. The  
202 precision of the method was excellent, with standard deviations from 3 to 23%, with  
203 worse precision for the more volatile and the last eluting compounds.

204

205

## 206 **3. Results and Discussion**

207

### 208 **3.1. PAHs content in the contaminated soil**

209

210 The concentrations of individual PAHs and the total concentration of all 16 PAHs  
211 ( $\Sigma$ 16PAHs) in the contaminated soil are given in Table 1. The concentrations obtained  
212 for individual PAHs were within the range of 0.77-270 mg kg<sup>-1</sup>, and  $\Sigma$ 16 PAHs was  
213 1068±101 mg kg<sup>-1</sup>. This high PAH level in the soil is due to the heavy industrial use of  
214 this area. From the beginning of the 1970's until the end of the 1980's and from 1991 to  
215 1995 a chemical plant operated in this area and there were numerous fuel oil spills  
216 during this period of time. This region of Spain is characterised by a low mean  
217 temperature (12 °C mean annual temperature), very humid conditions (coastal region  
218 with a mean annual humidity of 77% and a mean annual rainfall from 1000 to 1200  
219 mm) and a significant mean level of organic carbon in soil. All of these factors play  
220 important roles in the accumulation of PAH in the soil and they contribute to the very  
221 high PAH levels detected (Jiao et al., 2009; Moret et al., 2007) even though the  
222 chemical plant has been closed for 16 years. Morillo et al. (2007) analysed the PAH  
223 content of urban soils from three European cities and concluded that the humidity, mean  
224 temperature, industrial activity and organic matter content of the soil were the primary  
225 factors that influenced the PAHs content of the soil.

226 The total PAH soil content was similar to or higher than that at other industrial sites  
227 (Bakker et al., 2000; Eom et al., 2007; Lorenzi et al., 2010), in contrast with the very  
228 low PAH levels recorded by Hildebrandt et al. (2009) in agricultural soils that have not  
229 been impacted by industry, or the levels from 0.09 to 4 mg kg<sup>-1</sup> found by Morillo et al.  
230 (2008) for  $\Sigma$ 15 PAH in forty-one urban topsoil samples.

231

232 According to the classification of soil contamination by PAHs developed by  
233 Maliszewska-Kordibach (1996), the concentrations in the soil greatly exceed those for  
234 heavily contaminated soils (>10 mg kg<sup>-1</sup>). In Spain a "risk assessment value" of 50 mg  
235 kg<sup>-1</sup> has been set for total petroleum hydrocarbons in soils (Ministerio de la Presidencia,  
236 2005). According to this regulation and considering only the total 16 PAH content, the  
237 analysed soil exceeds the proposed limit by 20-fold. Hence, it can be concluded that the  
238 investigated soil site is highly contaminated, and therefore, further remediation should  
239 be performed because it represents an environmental and human health risk.

240

241 Among the 16 PAHs examined, phenanthrene and anthracene were found at the highest  
242 concentrations followed by naphthalene and fluoranthene (Table 1). The percentages of



243 these four PAHs with respect to the  $\Sigma 16$  PAH content in the soil were 25, 25, 17 and 12  
244 %, respectively (79 % of the  $\Sigma 16$  PAH).

245

246 With respect to the PAH profile by ring size, in general, low-molecular-weight (LMW)  
247 PAHs were more abundant than high-molecular-weight (HMW) PAHs. PAHs with 2- or  
248 3-rings accounted for more than 70% of the  $\Sigma 16$  PAH content, whereas the percentage  
249 of 4- to 6-ring PAHs was approximately 27% of the  $\Sigma 16$  PAH content in the soil. In  
250 fresh liquid fuels (petrogenic sources) LMW PAHs are typically more abundant,  
251 whereas in combustion residues (pyrogenic sources), HMW PAHs are predominant  
252 (Aichner et al., 2007). According to this pattern, it is evident that the PAHs in our soil  
253 were mainly derived from past spills of the fuel oil used when the plant was operating.

254

255 Most studies of PAH contaminated soils have not provided information on the detection  
256 or quantification of naphthalene because of its high volatility (Moret et al., 2007). In our  
257 study, the naphthalene content in the soil was one of the highest found among the  
258 studied PAHs. Although naphthalene can be easily volatilised, the distillation of  
259 naphthalenes performed in this plant contributed to the continuous input of this  
260 compound into the soil.

261

### 262 **3.2. PAH extraction by $\text{Ca}(\text{NO}_3)_2$ and four different cyclodextrin solutions**

263

264 Batch extraction experiments were performed to evaluate the ability of four CDs to  
265 remove PAHs from the contaminated soil relative to the extraction with  $\text{Ca}(\text{NO}_3)_2$   
266 solution. 0.01M  $\text{Ca}(\text{NO}_3)_2$  was used as a background electrolyte to mimic closely as  
267 possible the characteristics of soil-water solutions. According to previous studies, little  
268 increase is expected in the apparent solubility of PAHs in the presence of the  
269 electrolyte, presumably because of the non-polar characteristics of pollutants  
270 (Whitehouse, 1984; Wang and Brusseau, 1995; Badr et al., 2004) and to the fact that  
271 inorganic ions do not interact significantly with the low-polarity cavity of CDs  
272 (Rekharsky and Inoue, 1998). Although “salting out” effects can lead to increases in the  
273 partition coefficients of hydrophobic organic compounds, Ko et al. (1999) calculated  
274 that the small increase in phenanthrene-HP- $\beta$ -CD complexation with increasing ionic  
275 strength appears to be within statistical limits, with a 6% estimated increase.

276

277 Figure 1 shows the percentages of PAHs extracted from the contaminated soil after the  
278 first extraction and after three sequential extractions with 0.01 M  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD, HP-  
279  $\beta$ -CD, RAMEB and HP- $\gamma$ -CD solutions. The differences between the total amount of  
280 PAHs removed by 0.01 M  $\text{Ca}(\text{NO}_3)_2$  and by the different CD solutions were relevant,  
281 being higher using CDs than using only the electrolyte solution. The two chemically  
282 modified  $\beta$ -CDs (HP- $\beta$ -CD and RAMEB) resulted in the highest extraction percentages.  
283 With the  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD and HP- $\gamma$ -CD solutions, only 2.4, 6.5 and 6.1 %, respectively,  
284 of the total PAH content was removed. However, the HP- $\beta$ -CD and RAMEB solutions  
285 increased the extraction percentages to 19 and 18%, respectively. In all cases, one  
286 extraction was sufficient to remove more than half of the total extractable PAHs from  
287 the soil.

288

289 Figure 2 shows the percentage of individual PAHs extracted by the  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD,  
290 HP- $\beta$ -CD, RAMEB and HP- $\gamma$ -CD solutions from the contaminated soil. Phenanthrene,  
291 anthracene and fluoranthene were the PAHs that exhibited the greatest differences in the  
292 extraction percentages obtained using  $\text{Ca}(\text{NO}_3)_2$  (4 %, 2 % and 0.5 %, respectively) and  
293 using HP- $\beta$ -CD (27 %, 26 % and 6 %, respectively) or RAMEB (26 %, 27 % and 8 %,  
294 respectively) solutions. In the case of pyrene, the greatest difference (from 0.25 to 7.2  
295 %) was obtained using HP- $\gamma$ -CD solutions. In general, for the 3-ring PAHs tested  
296 (acenaphthene, fluorene, phenanthrene and anthracene), the extraction efficiencies of  $\beta$ -  
297 CD and its derivatives were ranked in the following order: RAMEB  $\geq$  HP- $\beta$ -CD  $\gg$   $\beta$ -  
298 CD.

299

300 Table 2 shows the percentages of PAHs (by number of rings) extracted from the soil  
301 using the  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD, HP- $\beta$ -CD, RAMEB and HP- $\gamma$ -CD solutions. When the  $\beta$ -CD  
302 solution was used, the extraction percentage of 3-ring PAHs increased (from 4.71 to  
303 9.02%), but 4-ring PAHs had extraction percentages lower than 1%, very similar to the  
304 result obtained when using the  $\text{Ca}(\text{NO}_3)_2$  solution. With the HP- $\beta$ -CD and RAMEB  
305 solutions, the highest extraction percentages were obtained for 3-ring PAHs (26.57 and  
306 26.38 %, respectively), and the extraction percentages were much lower for 4-ring  
307 PAHs (4.85 and 6.36 %, respectively). With the HP- $\gamma$ -CD solution, the extraction  
308 percentages for 3-ring and 4-ring PAHs were similar (6.94 and 5.38%, respectively). In  
309 general, when HP- $\beta$ -CD and RAMEB solutions were used, the PAH extraction  
310 percentages were ranked in the following order: 3-ring  $\gg$  2-ring  $>$  4-ring  $>$  5-ring. In

311 the case of HP- $\gamma$ -CD, the PAH extraction percentages were in the order of 3-ring > 4-  
312 ring  $\geq$  2-ring > 5-ring.

313

314 The differences observed in the PAH extraction percentages when using CDs were most  
315 likely due to the combination of several factors. First, 3-ring PAHs were the most  
316 abundant in the polluted soil (approximately 57% of the total PAH content). Wu et al.  
317 (2010) found that the most polluted soil sample had the highest PAH extraction  
318 percentage (60% of the PAH content), whereas in samples with lower PAH  
319 concentrations, the percentages extracted were much lower (2-8 %). Therefore, the total  
320 PAH concentration is an important factor affecting the removal of PAHs from  
321 contaminated soils.

322

323 Second, the molecular sizes of some PAHs are more favourable than others for the  
324 formation of inclusion complexes with fixed CDs (Ko et al., 1999; Reid et al., 2000;  
325 Stokes et al., 2005; Navarro et al., 2007).

326

327 Third, the major driving forces for the formation of CD inclusion complexes are  
328 hydrophobic and van der Waals interactions between the inner surface of the CD ring  
329 and the hydrophobic sites on the guest molecule (Wenz et al., 2006). Thus, the better the  
330 guest molecule fills the CD cavity, the stronger the hydrophobic and van der Waals  
331 interactions are. Morillo et al. (2012) demonstrated that 3-ring PAHs have the most  
332 appropriate structure and molecular size to fully fill the cavities of  $\beta$ -CD and its  
333 derivatives (5.8-6.5 Å internal diameters and a minimum depth of 7.9 Å). Four-ring  
334 PAHs can only partially enter the  $\beta$ -CD cavity, leaving a large portion of their volume  
335 in contact with the surrounding water. Therefore, lower solubilisation was achieved  
336 with  $\beta$ -CD derivatives because the inclusion complexes formed had large hydrophobic  
337 parts, and consequently lower extraction percentages from the contaminated soil are  
338 expected.

339

340 In contrast, in the case of the less voluminous PAHs such as naphthalene and  
341 acenaphthylene, the molecular volume is too small relative to the volume of the cavities  
342 of all the CDs tested in this study. In these cases, although 2-ring PAH molecules can  
343 fully enter the CDs' cavities, the interactions will be extremely weak. Therefore, the  
344 extraction of these PAHs from the soil by the four CDs solutions was less efficient than

345 the extraction of 3-ring PAHs. In these cases, the use of CDs presents no advantage  
346 relative to the use of the electrolyte solution.

347

348 It is important to emphasise the large difference between the extraction efficiency when  
349 using  $\beta$ -CD and those obtained when using its derivatives. One of the reasons could be  
350 the formation of water-insoluble complexes between some of the PAHs and  $\beta$ -CD due  
351 to its low solubility (Cao et al., 2000; Hanna et al., 2003; Badr et al., 2004). These facts  
352 tend to limit the use of  $\beta$ -CD as an extracting agent.

353

354 When HP- $\gamma$ -CD was used, the difference between the extraction percentages for 3-ring  
355 and 4-ring PAHs was much lower (6.94 % and 5.38 %, respectively) for several  
356 reasons. Three-ring PAHs have smaller widths than HP- $\gamma$ -CD, which allows their  
357 complete inclusion in the cavity, but the distance between these molecules and the HP-  
358  $\gamma$ -CD cavity is not as small as for  $\beta$ -CD derivatives, and the interaction is weaker. Four-  
359 ring PAHs have molecular characteristics that are more favourable for the formation of  
360 inclusion complexes with HP- $\gamma$ -CD, because these PAHs can be completely included in  
361 the CD hydrophobic cavity and they have more contact points with the HP- $\gamma$ -CD core  
362 (as demonstrated by Morillo et al. (2012) for pyrene).

363

364 However, the extraction of 4-ring PAHs with HP- $\gamma$ -CD from this real contaminated soil  
365 was less favoured than the extraction of 3-ring PAHs. This behavior is the result of the  
366 increase in the hydrophobicity of PAHs as their molecular weight increases (4-ring  
367 PAHs have octanol/water partition coefficients ( $\log K_{ow}$ ) in the range of 5.20 to 5.80,  
368 in comparison to 3.94-4.60 for 3-ring PAHs (Sverdrup et al., 2002)). This increased  
369 hydrophobicity indicates a greater tendency to remain adsorbed to organic matter in the  
370 soils. For this reason the system is very unlikely to be in equilibrium. Although the  
371 incorporation of the contaminant molecules into the CD cavities is rather fast, the  
372 desorption of the compounds from the soil particles is rather slow, especially for those  
373 PAHs with higher molecular weights and hydrophobicity. In addition, intra-particle  
374 diffusion needs to be considered because of the ageing of the pollutants in the soil.  
375 These factors indicate that only a minor fraction of the 3- or 4-ring PAHs will desorb  
376 from the aged contaminated soil within 20 hrs, the extraction time used in this study.

377

378 Extraction will be much more difficult in the case of 5- and 6-ring PAHs, with higher  
379 molecular weights and hydrophobicity (log K<sub>ow</sub> from 6.20 to 6.70), and with a much  
380 greater tendency to remain adsorbed to the hydrophobic surfaces of the soil. In addition,  
381 for 5- and 6-ring PAHs, the formation of inclusion complexes (even with HP- $\gamma$ -CD, the  
382 most voluminous CD used in this study) is extremely difficult owing to steric hindrance,  
383 which limits the penetration of the guest molecules into the CD cavity. However, 5-ring  
384 PAHs studied (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo  
385 [a,h] anthracene) could to be extracted by some of the CDs studied but in quite small  
386 percentages (<3%). It is most likely possible because in all them one of the ends is  
387 narrower than the rest of the molecule (see PAH molecular structures in Figure S2 of  
388 the Supplementary Data). The interaction with the CDs could occur through this  
389 narrower region, which could be partially inserted in the hydrophobic CD cavity. The  
390 fact that RAMEB is the CD that was able to extract the four 5-ring PAHs (Figure 2)  
391 despite having a narrower cavity than HP- $\gamma$ -CD indicates that the methyl groups and  
392 their arrangement permit a closer interaction with these PAHs, and this interaction is not  
393 favoured by the hydroxypropyl groups of HP- $\beta$ -CD or HP- $\gamma$ -CD. Six-ring PAHs are too  
394 voluminous to interact with the hydrophobic cavities of the CDs, and none of them  
395 could be extracted. The data presented in Table 2 and Figure 2 show that no 6-ring  
396 PAHs and only about 0.15 %, 1.07 % and a 0.62 % of 5-ring PAHs were removed when  
397 using HP- $\beta$ -CD, RAMEB and HP- $\gamma$ -CD solutions as extracting agents, respectively.  
398 These results are related not only to the possibility of complex formation with CDs, but  
399 also to the hydrophobicity of these compounds: PAHs with a greater number of  
400 aromatic rings in their structures are more hydrophobic and are more strongly adsorbed  
401 by soil, and they are less likely to transfer to the aqueous phase (Gao et al., 2009).

402

403 The results presented in Table 2 are in agreement with the solubility efficiency (Se) and  
404 apparent solubility constants of the inclusion complexes (K<sub>c</sub>) obtained by Morillo et al.  
405 (2012). These researchers performed a comparative study of the solubilisation of six  
406 PAHs by three synthetic CDs (HP- $\beta$ -CD, RAMEB and HP- $\gamma$ -CD). Phenanthrene,  
407 anthracene and fluoranthene were the PAHs that showed the best Se and the highest K<sub>c</sub>  
408 values in the presence of HP- $\beta$ -CD and RAMEB. In contrast, pyrene had the highest Se  
409 and K<sub>c</sub> values when HP- $\gamma$ -CD was used, concluding that phenanthrene, anthracene and  
410 fluoranthene were the PAHs whose shapes and sizes were more appropriate to form  
411 inclusion complexes with HP- $\beta$ -CD and RAMEB and that pyrene was the most

412 appropriate to form inclusion complexes with HP- $\gamma$ -CD. Similar results were obtained  
413 by Gan et al. (2009) and Viglianti et al. (2006a), who obtained the same order of CD  
414 efficiency after extracting three PAHs from an industrially aged-contaminated soil.  
415 Additionally, Petitgirard et al. (2009) compared the abilities of HP- $\beta$ -CD and RAMEB  
416 to remove phenanthrene and pyrene from soil, observing that less pyrene was removed  
417 than phenanthrene.

418

419 It is important to emphasise that after three sequential extractions using HP- $\beta$ -CD and  
420 RAMEB solutions, the total PAH content in the soil was approximately 83% and 70%  
421 of the initial concentration, respectively. However, when an aqueous salt solution was  
422 used as the extracting agent, 98% of PAHs remained in the soil. The soil used in this  
423 study had an organic matter content of 2.62 %, and therefore, it has a certain sorption  
424 capacity for hydrophobic compounds. It could explain why the PAHs were not all  
425 released from the soil when these two CD solutions were used as extracting agents,  
426 because there were competing hydrophobic interactions of contaminants between the  
427 hydrophobic sites on the soil organic matter and those of the CDs cavities (Badr et al.,  
428 2004., Ling et al., 2010). Additionally, the desorption of PAHs from soil diminishes  
429 with time because of the ageing process, in which the sequestration of PAH molecules  
430 makes them less available over time for further extraction and degradation (Villaverde,  
431 2007; Gao et al., 2010). The studied soil had been contaminated for more than 16 years,  
432 and during this period of time, the PAH molecules could enter soil micropores, partition  
433 into the organic matter in the soil and/or undergo strong surface adsorption. As a  
434 consequence, these compounds would become more persistent and more difficult to  
435 remove or mobilize (Reichemberg et al., 2010).

436 In order to emphasise the importance of the hydrophobic interactions of PAHs with the  
437 organic matter of soils, Figure 3 shows the extraction enhancement obtained for each  
438 PAH, calculated from the ratio of the percentage extracted when using CD solutions to  
439 that obtained when using  $\text{Ca}(\text{NO}_3)_2$  solution. Only 8 PAHs are included because the rest  
440 of them were not extracted with  $\text{Ca}(\text{NO}_3)_2$  solution and the enhancement cannot be  
441 calculated due to the zero-denominator. The extraction enhancement was higher as  
442 higher was the hydrophobicity of the PAH, being the extraction of pyrene about 28-fold  
443 higher in HP- $\gamma$ -CD solutions than in  $\text{Ca}(\text{NO}_3)_2$  solution. Morillo et al. (2012) had  
444 observed previously that the solubility of the least soluble compounds was improved to  
445 a higher extent than that of the more soluble compounds, and a similar trend was also

446 observed by Balogh et al. (2007). Gao et al. (2012) explained this behaviour by the  
447 greater affinity of PAHs for the low polarity of the CD cavity as hydrophobicity of  
448 PAHs increased, but, according to the results of apparent stability constants obtained for  
449 the formation of the different PAH-CD complexes by Morillo et al. (2012), this is not  
450 totally true. The reason for this behaviour is related to the higher difficulty to extract  
451 these compounds from the hydrophobic surfaces of the soil as the hydrophobicity of the  
452 PAHs increases, and Figure 3 demonstrates that this is the determining factor in the  
453 extraction of PAHs from soil. Afterwards, the appropriate size and shape of these  
454 compounds relative to those of the cavities of the CDs plays also an important role, as it  
455 can be also visualized in Figure 3.

456

457 This behaviour is closely related to the toxicities of the different EPA-PAHs in the  
458 environment. The bioavailable fraction of contaminants in soils (and not their total  
459 content) is the primary factor determining their influence on living organisms. Sverdrup  
460 et al. (2002) deduced that limitations in water solubility were likely the reason why the  
461 highly lipophilic PAHs ( $\log K_{ow} > 5.6$ ) adsorbed in soils were not toxic to springtails.  
462 Eom et al. (2007) also observed that the toxicity of a soil polluted with 16 EPA-PAHs  
463 could be explained by only 4 PAH congeners (fluorene, phenanthrene, pyrene and  
464 fluoranthene). Reid et al. (2000) observed that the extraction of PAHs from soils using  
465 an aqueous solution of HP- $\beta$ -CD closely mimics the mass transfer mechanisms that  
466 govern the bioavailability of PAHs. Taking into account that in the present study the  
467 PAHs extraction percentages when using aqueous solutions of HP- $\beta$ -CD and RAMEB  
468 were similar and the most efficient, it seems to indicate that this extraction was more  
469 than sufficient to extract the bioavailable PAHs, and those that were not extracted are  
470 not potentially toxic to the environment (Reichemberg et al., 2010). Therefore, the  
471 PAHs extracted from the contaminated soil when using these CDs are only those that  
472 can have an adverse effect on the environment and on living organisms.

473

474

#### 475 **4. Conclusions**

476

477 The results presented in this study show that, an useful and interesting tool for the  
478 decontamination of aged soils polluted by PAHs could be the treatment with CD  
479 solutions. Overall, the two chemically modified CDs, HP- $\beta$ -CD and RAMEB extracted

480 a greater proportion of the PAH contaminants than did HP- $\gamma$ -CD or the natural  $\beta$ -CD.  
481 Among the sixteen selected PAHs, the highest extraction percentages were obtained for  
482 the 3-ring PAHs, due to the more appropriate size and shape of these compounds with  
483 respect to the cavity dimensions of the CDs studied. Taking into account that 3-ring  
484 PAHs were the most abundant in the contaminated soil studied, the use of these CD  
485 derivatives in soil remediation would allow the extraction of higher percentages of these  
486 more abundant PAHs. The extractable fraction of PAH was inversely correlated to the  
487 number of benzene rings and the octanol–water partition coefficient, but the PAHs that  
488 were not extracted by the more effective CDs almost certainly are not bioavailable and,  
489 therefore, they are not potentially toxic to the environment. The enhanced solubility  
490 and, therefore, enhanced extraction of PAHs when using some of the studied CDs  
491 makes PAHs more available for many remediation technologies, including flushing and  
492 bioremediation, but also electrokinetic and chemical oxidation/reduction technologies.

493

494

495

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712

713

714

715 **Figure captions**

716

717 **Figure 1.**  $\Sigma$ 16PAHs extracted (%) from the soil after one and three sequential  
718 extractions using  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD, HP- $\beta$ -CD, RAMEB or HP- $\gamma$ -CD aqueous solutions.

719

720 **Figure 2.** Percentage of individual PAHs extracted from the contaminated soil by  
721  $\text{Ca}(\text{NO}_3)_2$ ,  $\beta$ -CD, HP- $\beta$ -CD, RAMEB or HP- $\gamma$ -CD aqueous solutions after three  
722 sequential extractions (relative to their total content determined by exhaustive  
723 extraction).

724

725 **Figure 3:** Extraction enhancement effect of the different cyclodextrins studied on some  
726 PAHs with respect to the extraction obtained with  $\text{Ca}(\text{NO}_3)_2$  aqueous solution.

727

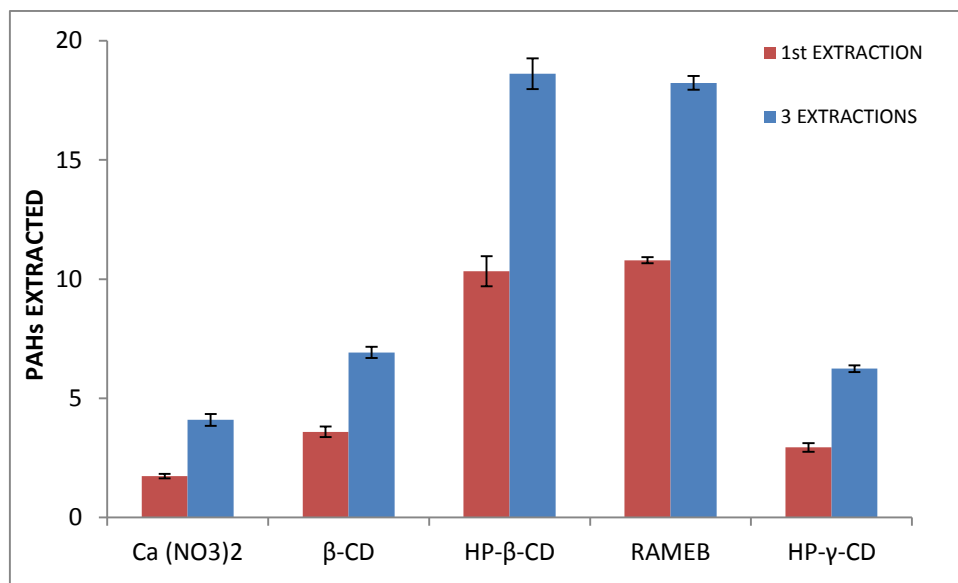


**Table 1. Content of PAHs in the contaminated soil ( $\text{mg kg}^{-1}$ ) and percentage of individual PAHs related to  $\Sigma 16$  PAHs (determined after exhaustive extraction).**

| PAHs                   | $\text{mg kg}^{-1}$ | %                   |
|------------------------|---------------------|---------------------|
| Naphtalene             | 183 ( $\pm 18$ )    | 17.18 ( $\pm 1.7$ ) |
| Acenaphtylene          | 8.96 ( $\pm 1.37$ ) | 0.84 ( $\pm 1.13$ ) |
| Acenaphtene            | 22.9 ( $\pm 4.2$ )  | 2.14 ( $\pm 0.39$ ) |
| Fluorene               | 37.4 ( $\pm 8.0$ )  | 3.49 ( $\pm 0.75$ ) |
| Phenanthrene           | 271 ( $\pm 30$ )    | 25.3 ( $\pm 2.8$ )  |
| Anthracene             | 264 ( $\pm 36$ )    | 24.7 ( $\pm 3.4$ )  |
| Fluoranthene           | 133 ( $\pm 12$ )    | 12.5 ( $\pm 1.1$ )  |
| Pyrene                 | 78.1 ( $\pm 7.2$ )  | 7.30 ( $\pm 0.68$ ) |
| Benzo(a)anthracene     | 23.5 ( $\pm 1.8$ )  | 2.20 ( $\pm 0.17$ ) |
| Chrysene               | 17.4 ( $\pm 1.3$ )  | 1.63 ( $\pm 0.12$ ) |
| Benzo(b)fluoranthene   | 12.2 ( $\pm 0.2$ )  | 1.14 ( $\pm 0.02$ ) |
| Benzo(k)fluoranthene   | 3.38 ( $\pm 0.06$ ) | 0.32 ( $\pm 0.01$ ) |
| Benzo(a)pyrene         | 7.31 ( $\pm 0.45$ ) | 0.68 ( $\pm 0.04$ ) |
| Indeno(1,2,3-cd)pyrene | 3.72 ( $\pm 0.30$ ) | 0.35 ( $\pm 0.03$ ) |
| Dibenzo(a,h)anthracene | 0.77 ( $\pm 0.05$ ) | 0.07 ( $\pm 0.01$ ) |
| Benzo(g,h,i)perylene   | 3.05 ( $\pm 0.25$ ) | 0.29 ( $\pm 0.02$ ) |
| $\Sigma 16$ PAHs       | 1068 ( $\pm 101$ )  | 100 ( $\pm 9.4$ )   |

**Table 2. Percentage of PAHs (by number of rings) extracted from the contaminated soil using Ca(NO<sub>3</sub>)<sub>2</sub>, β-CD, HP-β-CD, RAMEB or HP-γ-CD aqueous solutions after three sequential extractions (relative to their total content determined by exhaustive extraction).**

| <b>PAHs</b>    | <b>Ca(NO<sub>3</sub>)<sub>2</sub></b> | <b>β-CD</b>  | <b>HP-β-CD</b> | <b>RAMEB</b> | <b>HP-γ-CD</b> |
|----------------|---------------------------------------|--------------|----------------|--------------|----------------|
| <b>2-rings</b> | 7.84 (±0.91)                          | 8.08 (±0.93) | 9.63 (±0.54)   | 9.33 (±0.32) | 5.05 (±0.29)   |
| <b>3-rings</b> | 4.71 (±0.17)                          | 9.02 (±0.68) | 26.6 (±1.5)    | 26.4 (±0.6)  | 6.94 (±0.13)   |
| <b>4-rings</b> | 0.40 (±0.02)                          | 0.69 (±0.04) | 4.85 (±0.13)   | 6.36 (±0.21) | 5.38 (±0.01)   |
| <b>5-rings</b> | 0.00 (±0.00)                          | 0.00 (±0.00) | 0.15 (±0.01)   | 1.07 (±0.05) | 0.62 (±0.01)   |
| <b>6-rings</b> | 0.00 (±0.00)                          | 0.00 (±0.00) | 0.00 (±0.00)   | 0.00 (±0.00) | 0.00 (±0.00)   |



**Figure 1**

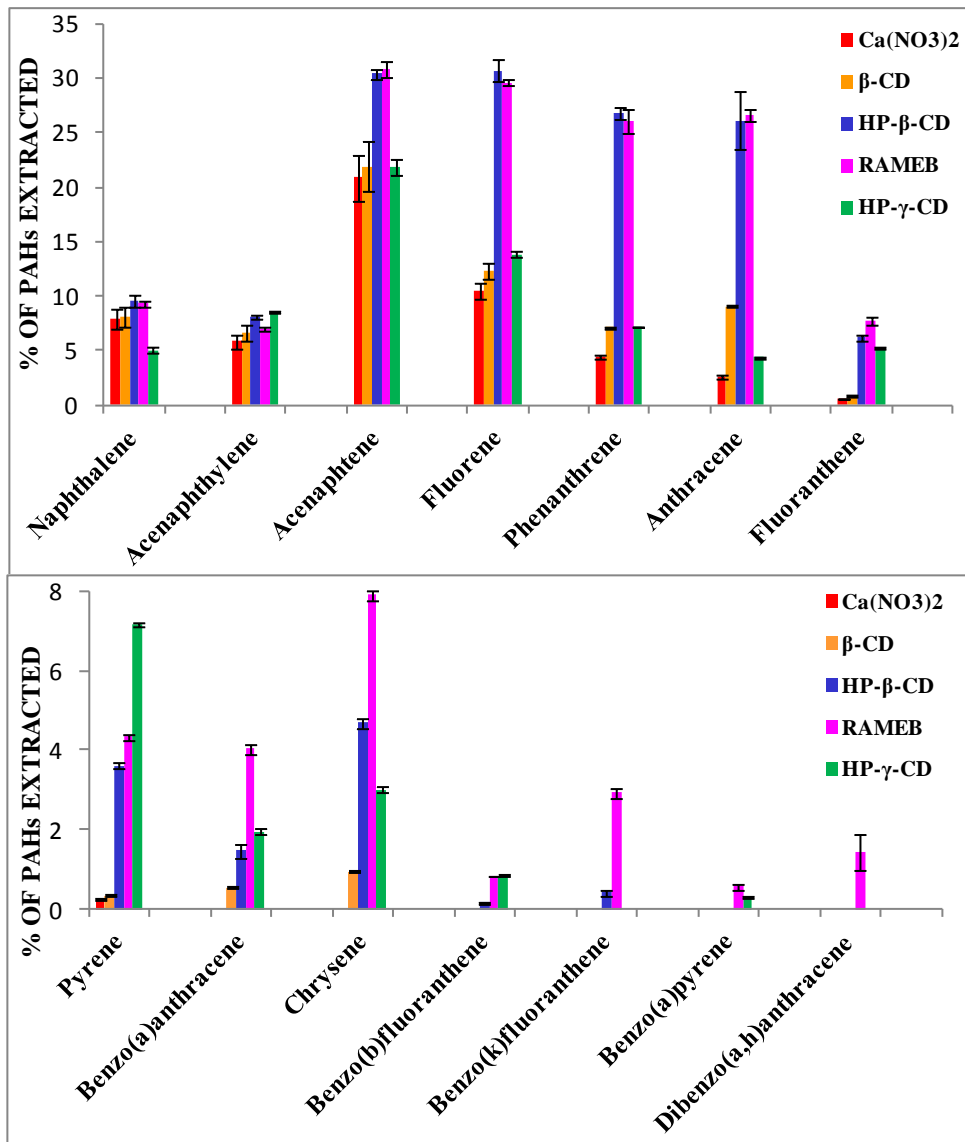


Figure 2

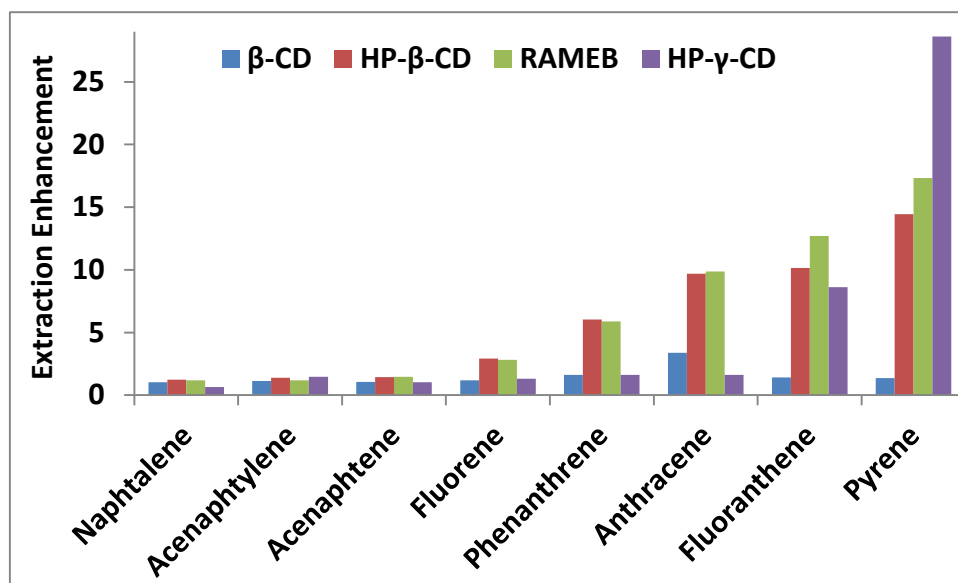


Figure 3