

1 **Comparative effects of several cyclodextrins on the extraction of PAHs**
2 **from an aged contaminated soil**
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Preprint submitted to Pergamon Press

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 β -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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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^{-1} , 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 (β -cyclodextrin; β -CD) and three chemically modified CDs (2-
92 hydroxypropyl- β -cyclodextrin (HP- β -CD), randomly methylated- β -cyclodextrin
93 (RAMEB) and hydroxypropyl- γ -cyclodextrin (HP- γ -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⁻¹ solution in methanol. The

108 surrogate standard was a mixture containing naphthalene-d₈, acenaphthene-d₁₀,
109 phenanthrene-d₁₀, chrysene-d₁₂, and perylene-d₁₂ supplied by Supelco as a mix at 200
110 mg L⁻¹ solution in methanol. Anthracene-d₁₀, 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⁻¹ 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-d₁₀ was
148 added as an internal standard at a final concentration of 1 µg mL⁻¹. 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(NO₃)₂ as background electrolyte
157 and 200 mg L⁻¹ HgCl₂ 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(NO₃)₂ and 200 mgL⁻¹ HgCl₂. 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-d₁₀ was added to each vial before analysis
165 to a final concentration of 1 µg mL⁻¹. 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-d₁₀ 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⁻¹. 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⁻¹, and good linearity was observed using internal standard with both the surrogate
201 standards (for exhaustive extraction) and the internal standard anthracene-d₁₀. 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 ($\sum 16\text{PAHs}$) 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⁻¹, and $\sum 16$ PAHs was
213 1068±101 mg kg⁻¹. 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⁻¹ found by Morillo et al.
230 (2008) for $\sum 15$ 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⁻¹). In Spain a "risk assessment value" of 50 mg
235 kg⁻¹ 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- β -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 $\text{Ca}(\text{NO}_3)_2$, β -CD, HP-
279 β -CD, RAMEB and HP- γ -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 β -CDs (HP- β -CD and RAMEB) resulted in the highest extraction percentages.
283 With the $\text{Ca}(\text{NO}_3)_2$, β -CD and HP- γ -CD solutions, only 2.4, 6.5 and 6.1 %, respectively,
284 of the total PAH content was removed. However, the HP- β -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 $\text{Ca}(\text{NO}_3)_2$, β -CD,
290 HP- β -CD, RAMEB and HP- γ -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- β -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- γ -CD solutions. In general, for the 3-ring PAHs tested
296 (acenaphthene, fluorene, phenanthrene and anthracene), the extraction efficiencies of β -
297 CD and its derivatives were ranked in the following order: RAMEB \geq HP- β -CD \gg β -
298 CD.

299

300 Table 2 shows the percentages of PAHs (by number of rings) extracted from the soil
301 using the $\text{Ca}(\text{NO}_3)_2$, β -CD, HP- β -CD, RAMEB and HP- γ -CD solutions. When the β -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- β -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- γ -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- β -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- γ -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 β -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 β -CD cavity, leaving a large portion of their volume
335 in contact with the surrounding water. Therefore, lower solubilisation was achieved
336 with β -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 β -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 β -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 β -CD as an extracting agent.

353

354 When HP- γ -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- γ -CD, which allows their
357 complete inclusion in the cavity, but the distance between these molecules and the HP-
358 γ -CD cavity is not as small as for β -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- γ -CD, because these PAHs can be completely included in
361 the CD hydrophobic cavity and they have more contact points with the HP- γ -CD core
362 (as demonstrated by Morillo et al. (2012) for pyrene).

363

364 However, the extraction of 4-ring PAHs with HP- γ -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_{ow} 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- γ -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- γ -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- β -CD or HP- γ -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- β -CD, RAMEB and HP- γ -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 (Kc) 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- β -CD, RAMEB and HP- γ -CD). Phenanthrene,
407 anthracene and fluoranthene were the PAHs that showed the best Se and the highest Kc
408 values in the presence of HP- β -CD and RAMEB. In contrast, pyrene had the highest Se
409 and Kc values when HP- γ -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- β -CD and RAMEB and that pyrene was the most

412 appropriate to form inclusion complexes with HP- γ -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- β -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- β -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- γ -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- β -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- β -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- β -CD and RAMEB extracted

480 a greater proportion of the PAH contaminants than did HP- γ -CD or the natural β -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.

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496 **Acknowledgement**

497 The authors thank to Dr. J.R. Gallego from the Universidad de Oviedo for providing the
498 contaminated soil sample. Support from projects CTM2006-04626 and CTM2009-07335, Spanish
499 Ministry of Science and Innovation (co-funded by Fondo Europeo de Desarrollo Regional,
500 FEDER), are greatly appreciated. M.A. Sánchez-Trujillo acknowledges a research contract from
501 CSIC (JAEPRe 0800763) cofinanced by Fondo Social Europeo (FSE).

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506 **References**

507

508 Aichner, B., Glaser, B., & Zech, W., 2007. Polycyclic aromatic hydrocarbons and
509 polychlorinated biphenyls in urban soils from Kathmandu, Nepal. *Organic*
510 *Geochemistry* 38, 700-715.

511

512 Ahn, C.K., Kim, Y.M., Woo, S.H., Park, J.M., 2008. Soil washing using various
513 nonionic surfactants and their recovery by selective adsorption with activated carbon.
514 *Journal of Hazardous Materials* 154, 153-160.

515

516 Badr, T., Hanna, C., de Braue, C., 2004. Enhanced solubilization and removal of
517 naphthalene and phenanthrene by cyclodextrins from two contaminated soils. *Journal of*
518 *Hazardous Materials* B112, 215-223.

519

520 Bakker, M.I., Casado, B., Koerselman, J. W., Tolls, J., Kolloffel, C., 2000. Polycyclic
521 aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery.
522 *Science of the Total Environment* 263, 91-100.

523

524 Balogh, K., Szaniszlo, N., Otta, K.H., Fenyvesi, E., 2007. Can cyclodextrins really
525 improve the selectivity of extraction of BTEX compounds? *Journal of Inclusion*
526 *Phenomena and Macrocyclic Chemistry* 57, 457-462.

527

528 Bardi, L., Martini, C., Opsi, F., Bertolone, E., Belviso, S., Masoero, G., Marzona, M.,
529 Ajmone-Marsan, F., 2007. Cyclodextrin-enhanced in situ bioremediation of
530 polyaromatic hydrocarbons-contaminated soils and plant uptake. *Journal of Inclusion*
531 *Phenomena and Macrocyclic Chemistry* 57, 439-444.

532

533 Cao, J., Zhao, C., Huang, L., Ding, Y., Wang, L., Han, S., 2000. Solubilization of
534 substituted indole compounds by β -cyclodextrin in water. *Chemosphere* 40, 1411-1416.

535

536 Dodziuk, H., 2006. *Cyclodextrins and their Complexes: Chemistry, Analytical Methods*
537 *and Applications*. Wiley-VCH GmbH & Co. KGaA, Weinheim.

538 Environmental Protection Agency, 2011. Contaminant Candidate List 3,
539 <http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm>.
540
541 Eom, I.C., Rast, C., Veber, A.M., Vasseur, P., 2007. Ecotoxicity of a polycyclic
542 aromatic hydrocarbon (PAH)-contaminated soil. *Ecotoxicology and Environmental*
543 *Safety* 67, 190-205.
544
545 Fava, F., Bertin, L., Fedi, S., Zannoni, D., 2002. Methyl- β -cyclodextrin enhanced
546 solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-
547 contaminated soils. *Biotechnology and Bioengineering* 81, 1-10.
548
549 Gan, S., Lau, E.V., Ng, H.K., 2009. Remediation of soils contaminated with polycyclic
550 aromatic hydrocarbons (PAHs). *Journal of Hazardous Materials* 172, 532-549.
551
552 Gao, Y., Zeng, Y., Shen, Q., Ling, W., Han, J., 2009. Fractionation of polycyclic
553 aromatic hydrocarbon residues in soils. *Journal of Hazardous Materials* 172, 897-903.
554
555 Gao, Y., Ren, L., Ling, W., Gong, S., Sun, B., Zhang, Y., 2010. Desorption of
556 phenanthrene and pyrene in soils by root exudates. *Bioresource Technology* 101, 1159-
557 1165.
558
559 Gao, H., Miles, M.S., Meyer, B.M., Wong, R.L., Overton, E.B., 2012. Assessment of
560 cyclodextrin-enhanced extraction of crude oil from contaminated porous media. *Journal*
561 *of Environmental Monitoring* 14, 2164-2169.
562
563 Hanna, K., de Brauer, C., Germain, P., 2003. Solubilization of the neutral and charged
564 forms of 2, 4, 6 trichlorophenol by β -cyclodextrin, methyl- β -cyclodextrin and
565 hydroxypropyl- β -cyclodextrin in water. *Journal of Hazardous Materials B100*, 109–116.
566
567 Hildebrandt, A., Lacorte, S., Barceló, D., 2009. Occurrence and Fate of
568 Organochlorinated Pesticides and PAH in Agricultural Soils from the Ebro River Basin.
569 *Archives of Environmental Contamination and Toxicology* 57, 247-255.
570

571 Jiao, W., Lu, Y., Li, J., Han, J., Wang, T., Luo, W., Shi, Y., Wang, G., 2009.
572 Identification of sources of elevated concentrations of polycyclic aromatic hydrocarbons
573 in an industrial area in Tianjin, China. *Environmental Monitoring and Assessment* 158,
574 581-592.
575

576 Ko, S.O., Schlautman, M.A., Carraway, E.R., 1999. Partitioning of hydrophobic organic
577 compounds to hydroxypropyl- β -cyclodextrin: experimental studies and model
578 predictions for surfactant-enhanced remediation applications. *Environmental Science &*
579 *Technology* 33, 2765-2770.
580

581 Khodadoust, A.P., Bagchi, R., Suidan, M.T., Brenner, R.C., Sellers, N.G., 2000.
582 Removal of PAHs from highly contaminated soils found at prior manufactured gas
583 operations. *Journal of Hazardous Materials B* 80, 159–174.
584

585 Landy, D., Mallard, I., Ponchet, A., Monflier, E., Fourmentin, S., 2012. Remediation
586 technologies using cyclodextrins: an overview. *Environmental Chemistry Letters* 10,
587 225-237.
588

589 Ling, W., Zeng, Y., Gao, Y., Dang, H., Zhu, X., 2010. Availability of polycyclic
590 aromatic hydrocarbons in aging soils. *Journal of Soils and Sediments* 10, 799-801.
591

592 Lorenzi, D., Cave, M., Dean, J.R., 2010. An investigation into the occurrence and
593 distribution of polycyclic aromatic hydrocarbons in two soil size fractions at a former
594 industrial site in NE England, UK using in situ PFE–GC–MS. *Environmental*
595 *Geochemistry and Health* 32, 553-565.
596

597 Mahanty, B., Pakshirajan, K., Venkata Dasu, V., 2011. Understanding the complexity
598 and strategic evolution in PAH remediation research. *Critical Reviews in Environmental*
599 *Science and Technology* 41, 1697-1746.
600

601 Maliszewka-Kordybach, B., 1996. Polycyclic aromatic hydrocarbons in agricultural
602 soils in Poland: preliminary proposals for criteria to evaluate the level of soil
603 contamination. *Applied Geochemistry* 11, 121–127.
604

605 Martínez, E., Gros, M., Lacorte, S., Barceló, D., 2004. Simplified procedures for the
606 analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels. *Journal*
607 *of Chromatography A* 1047, 181-188.
608

609 Ministerio de la Presidencia. Real Decreto 9/2005 de 14 de Enero. Actividades
610 potencialmente contaminantes del suelo y los criterios y estándares para la declaración
611 de suelos contaminados. *Boletín Oficial del Estado* 2005 (BOE 18/1/2005); No
612 15:1833–1843.
613

614 Moret, S., Purcaro, G., Conte, L.S., 2007. Polycyclic aromatic hydrocarbon (PAH)
615 content of soil and olives collected in areas contaminated with creosote released from
616 old railway ties. *Science of the Total Environment* 386, 1-8.
617

618 Morillo, E., Romero, A.S., Maqueda, C., Madrid, L., Ajmone-Marsan, F., Greman, H.,
619 Davidson, C.M., Hursthouse, A.S., Villaverde, J., 2007. Soil pollution by PAHs in
620 urban soils: a comparison of three European cities. *Journal of Environmental*
621 *Monitoring* 9, 1001-1008.
622

623 Morillo, E., Romero, A.S., Madrid, L., Villaverde, J., Maqueda, C., 2008.
624 Characterization and Sources of PAHs and Potentially Toxic Metals in Urban
625 Environments of Sevilla (Southern Spain). *Water Air Soil Pollution* 187, 41-51.
626

627 Morillo, E., Sánchez-Trujillo, M.A., Moyano, J.R., Villaverde, J., Gómez-Pantoja,
628 M.A., Pérez-Martínez, J.I., 2012. Enhanced solubilisation of six PAHs by three
629 synthetic cyclodextrins for remediation applications. *Molecular modelling of the*
630 *inclusion complexes*. *PloS ONE*. DOI: 10.1371/journal.pone.0044137.
631

632 Navarro, R.R., Ichikawa, H., Iimura, Y., Tatsumi, K., 2007. Removal of Polycyclic
633 Aromatic Hydrocarbons from Contaminated Soil by Aqueous DNA Solution.
634 *Environmental Science and Technology* 41, 4240-4245.
635

636 Paria, S., Yuet, P.K., 2006. Solubilization of naphthalene by pure and mixed surfactants.
637 *Industrial & Engineering Chemistry Research* 45, 3552-3558.
638

639 Petitgirard, A., Djehiche, M., Persello, J., Fievet, P., Fatin-Rouge, N., 2009. PAH
640 contaminated soil remediation by reusing an aqueous solution of cyclodextrins.
641 *Chemosphere* 75, 714-718.
642

643 Reddy. K., Al-Hamdan, A., Ala, P., 2011. Enhanced soil flushing for simultaneous
644 removal of PAHs and heavy metals from industrial contaminated soil. *Journal of*
645 *Hazardous, Toxic, and Radioactive Waste* 15, 166-174.
646

647 Reichemberg, F., Karlson, U.G., Gustafsson, O., Long, S.M., Pritchard, P.H., Mayer, P.,
648 2010. Low accessibility and chemical activity of PAHs restrict bioremediation and risk
649 of exposure in manufactured gas plant soil. *Environmental Pollution* 158, 1214-1220.
650

651 Reid, B.J., Stokes, J.D., Jones, K.C., Semple, K.T., 2000. Nonexhaustive Cyclodextrin-
652 Based Extraction Technique for the Evaluation of PAH Bioavailability. *Environmental*
653 *Science and Technology* 34, 3174-3179.
654

655 Rekharsky, M.V., Inoue, Y., 1998. Complexation Thermodynamics of Cyclodextrins.
656 *Chemical Reviews* 98, 1875-1917.
657

658 Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C., Semple, K.T., 2005. Prediction of
659 polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous
660 hydroxypropyl-beta-cyclodextrin extraction technique. *Environmental Toxicology and*
661 *Chemistry* 24, 1325-1330.
662

663 Sverdrup, L., Nielsen, T., Henningkrogh, P., 2002. Soil ecotoxicity of polycyclic
664 aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility.
665 *Environmental Science and Technology* 36, 2429-2435.
666

667 Viglianti, C., Hanna, K., Brauer, C., Germain, P., 2006a. Removal of polycyclic
668 aromatic hydrocarbons from aged-contaminated soil using cyclodextrins: Experimental
669 study. *Environmental Pollution* 140, 427-435.
670

671 Viglianti, C., Hanna, K., Brauer, C., Germain, P., 2006b. Use of cyclodextrins as an
672 environmentally friendly extracting agent in organic aged-contaminated soil
673 remediation. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 56, 275-280.
674

675 Villaverde, J., Morillo, E., Pérez-Martínez, J.I., Ginés, J.M., Maqueda, C., 2004.
676 Preparation and Characterization of Inclusion Complex of Norflurazon and β -
677 Cyclodextrin to Improve Herbicide Formulations. *Journal of Agricultural and Food*
678 *Chemistry* 52, 864-869.
679

680 Villaverde, J., Maqueda, C., Morillo, E., 2005. Improvement of the desorption of the
681 herbicide norflurazon from soils via complexation with β -cyclodextrin. *Journal of*
682 *Agricultural and Food Chemistry* 53, 5366-5372.
683

684 Villaverde, J., Maqueda, C., Morillo, E., 2006. Effect of the simultaneous addition of β -
685 cyclodextrin and the herbicide norflurazon on its adsorption and movements in soils.
686 *Journal of Agricultural and Food Chemistry* 54, 4766-4772.
687

688 Villaverde, J., 2007. Time-dependent sorption of norflurazon in four different soils: Use
689 of β -cyclodextrin solutions for remediation of pesticide-contaminated soils. *Journal of*
690 *Hazardous Materials* 142, 184-190.
691

692 Vulava, V.M., McKay, L.D., Driese, S.G., Menn, F.-M., Sayler, G.S., 2007.
693 Distribution and transport of coal tar-derived PAHs in fine-grained residuum.
694 *Chemosphere* 68, 554-563.
695

696 Wang, X., Brusseau, M.X., 1995. Simultaneous complexation of organic compounds
697 and heavy metals by a modified cyclodextrin. *Environmental Science and Technology*
698 29, 2632-2635.
699

700 Wenz, G., Han, B.H., Müller, A., 2006. Cyclodextrin rotaxanes and polyrotaxanes.
701 *Chemical Reviews* 106, 782–817.
702

703 Whitehouse, B.G., 1984. The effects of temperature and salinity on the aqueous
704 solubility of polynuclear aromatic hydrocarbons. *Marine Chemistry* 14, 319–332.

705

706 Wu, J., Gong, Z., Zheng, L., Yi, Y., Jin, J., Li, X., Li, P., 2010. Removal of high
707 concentrations of polycyclic aromatic hydrocarbons from contaminated soil by
708 biodiesel. *Frontiers of Environmental Science & Engineering in China* 4, 387-394.

709

710 Ying, G.G., 2006. Fate behaviour and effects of surfactants and their degradation
711 products in the environment. *Environment International* 32, 417-431.

712

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714

715 **Figure captions**

716

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

719

720 **Figure 2.** Percentage of individual PAHs extracted from the contaminated soil by
721 $\text{Ca}(\text{NO}_3)_2$, β -CD, HP- β -CD, RAMEB or HP- γ -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 (mg kg^{-1}) and percentage of individual PAHs related to $\Sigma 16$ PAHs (determined after exhaustive extraction).

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

Table 2. Percentage of PAHs (by number of rings) extracted from the contaminated soil using Ca(NO₃)₂, 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).

PAHs	Ca(NO₃)₂	β-CD	HP-β-CD	RAMEB	HP-γ-CD
2-rings	7.84 (±0.91)	8.08 (±0.93)	9.63 (±0.54)	9.33 (±0.32)	5.05 (±0.29)
3-rings	4.71 (±0.17)	9.02 (±0.68)	26.6 (±1.5)	26.4 (±0.6)	6.94 (±0.13)
4-rings	0.40 (±0.02)	0.69 (±0.04)	4.85 (±0.13)	6.36 (±0.21)	5.38 (±0.01)
5-rings	0.00 (±0.00)	0.00 (±0.00)	0.15 (±0.01)	1.07 (±0.05)	0.62 (±0.01)
6-rings	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

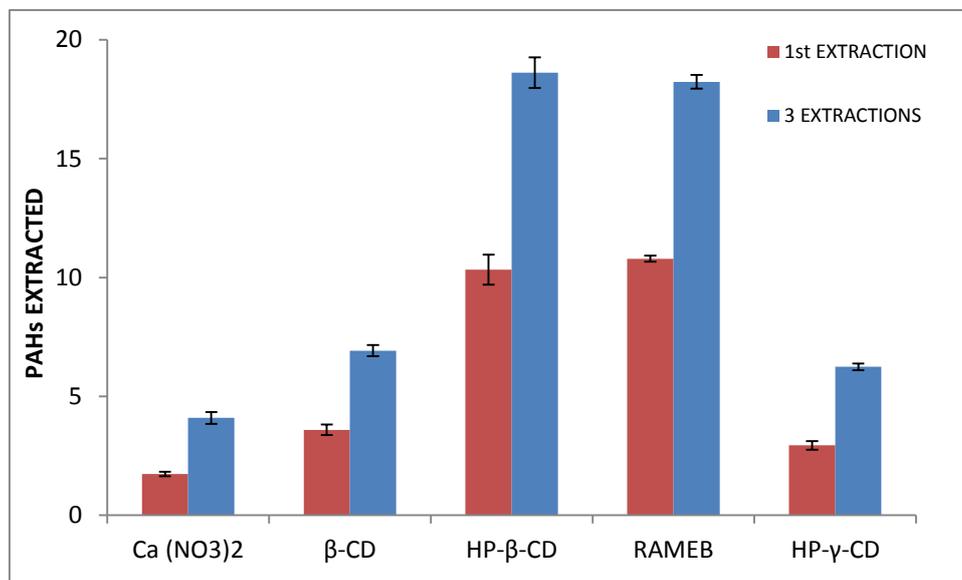


Figure 1

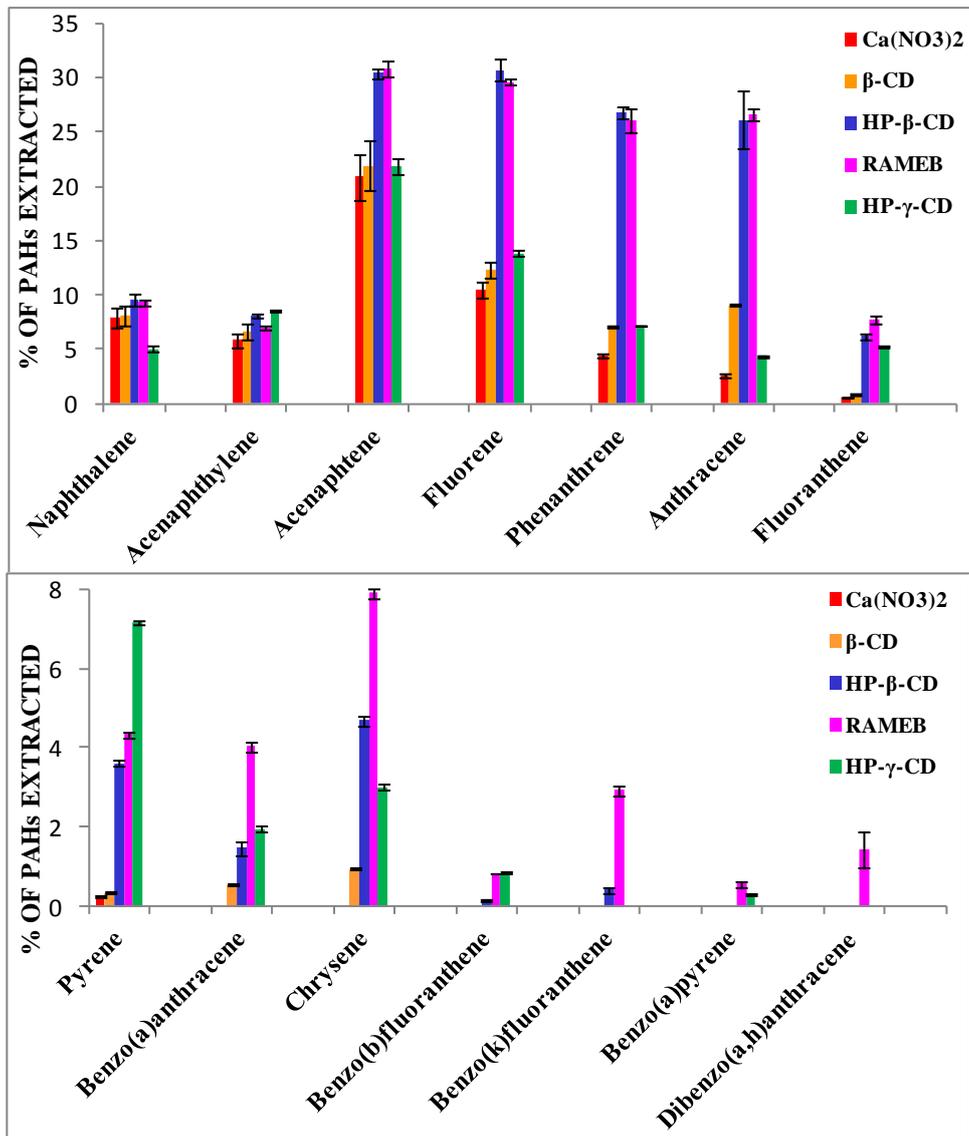


Figure 2

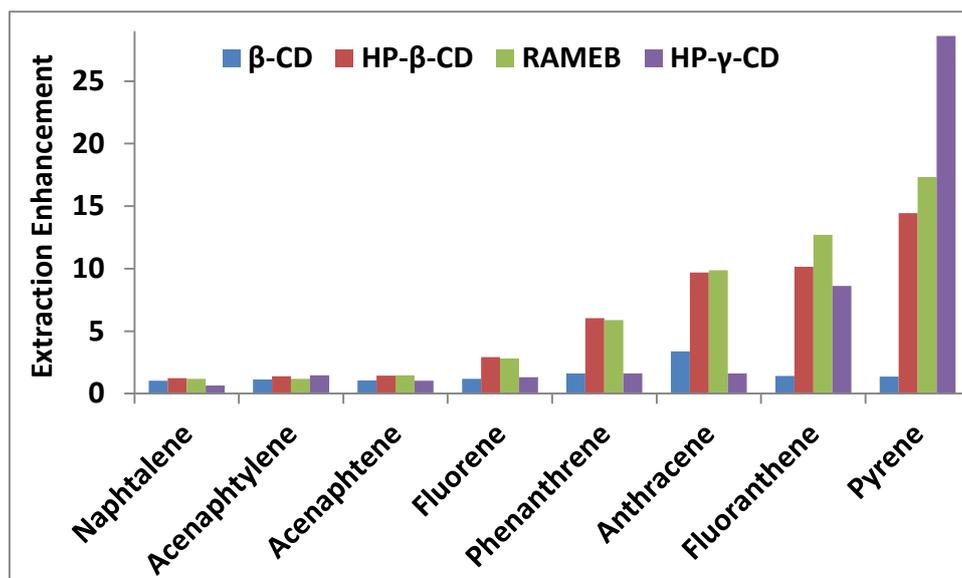


Figure 3