

1 **Effect of contact time and the use of hydroxypropyl- β -cyclodextrin in**
2 **the removal of fluorene and fluoranthene from contaminated soils**

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13
14 **Abstract**

15 Sorption-desorption experiments of fluorene (FLU) and fluoranthene (FLT) in soils
16 were carried out and correlated to their removal from aged contaminated soils using
17 aqueous solutions in the absence and in the presence of hydroxypropyl- β -cyclodextrin
18 (HPBCD) as the extraction agent. FLU became more resistant to extraction in aged
19 contaminated soils due to its initial adsorption onto the mineral and amorphous soil
20 organic matter (SOM) domains, sites of lower binding energy from which, due to its
21 small size, it could spread towards the condensed SOM as the contact time increased.
22 Therefore, FLU will not be easily desorbed from aged contaminated soils due to
23 physical entrapment mechanisms, even when using HPBCD as extractant, presenting
24 FLU low risks to the environment. On the contrary, FLT was extracted from aged soils
25 in the presence of HPBCD in solutions to a much greater extent than in its absence. Due
26 to its more hydrophobic character FLT sorption in soils was relatively quicker,
27 remaining more or less fixed on hydrophobic sites of the organic matter (OM) with
28 different energies, and therefore the amount of FLT extracted was almost constant for
29 different ageing times. During extraction experiments, the influence of the OM quality
30 of the soils was also highlighted because an inverse proportionality between OM
31 content of soil and extractability of sorbed FLT was observed. It was concluded that

1 soils with lower OM content that had more diagenetically processed OM could block
2 the extraction of FLT more effectively than soils with higher OM content that are less
3 humified. This indicates the need to not only use adsorption-desorption data in
4 contaminant fate and transport models, but also extraction studies in aged contaminated
5 soils and other complementary analytical approaches when assessing soil
6 contamination-related risks.

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9 **Keywords:**

10 fluorene, fluoranthene, PAHs, cyclodextrins, sorption-desorption, extraction, aged
11 contaminated soil, organic matter quality.

12

1 **1. Introduction**

2 Polycyclic aromatic hydrocarbons (PAHs) are pollutants of great environmental concern
3 because of their toxic, mutagenic, and carcinogenic properties. Because of their low
4 solubility in water, the fate of hydrophobic organic compounds such as PAHs in
5 sediments and soils depends largely on their sorption to solid phases (Sullivan et al.,
6 2011), where they become resistant to environmental degradation. Microbial
7 degradation is the major process involved in effective bioremediation of contaminated
8 soils and sediments; however, unfortunately, PAHs removal during natural
9 bioremediation is incomplete, and residual concentrations are often too high to satisfy
10 the standards for clean soil.

11 High concentrations of residual PAHs are caused by their limited bioavailability,
12 controlled by physico-chemical processes such as sorption/desorption and diffusion into
13 the soil matrix, which cause the slow mass transfer of the hydrophobic contaminant to
14 the biodegrading microorganisms (Cuypers et al., 2002; Barnier et al., 2014). Moreover,
15 organic compounds that persist as adsorbed contaminants in the soil exhibit declining
16 extractability and bioavailability to microorganisms with increasing contact time or
17 “ageing”. Significant amounts of organic compounds are retained in soil as nonavailable
18 and nonextractable sequestered residues, whose proportion increases with increasing
19 soil contact time or ageing (Alexander, 2000; Ma et al., 2012). Ageing is associated
20 with a continuous diffusion of organic molecules into remote and inaccessible regions
21 within the soil matrix, thereby occluding the compounds and preventing them from
22 abiotic and biotic loss processes.

23 The toxicity of individual PAHs to soil organisms and other species, including humans,
24 is directly related to their availability, which is also related to their lipophilicity (log
25 K_{ow}) and soil sorption properties (i.e., log K_{oc} combined with information on the
26 organic carbon content of the soil) (Sverdrup et al., 2002). The absence of toxicity
27 could, in most cases, be explained by limited water solubility, due to a higher
28 lipophilicity and a tendency to remain adsorbed on the OM of the soil. Therefore, to
29 characterize PAHs availability, data on soil sorption (log K_{oc}) are needed. Sorption and
30 sequestration are usually the most important factors limiting clean-up of sites
31 contaminated by such organic compounds. This has resulted in extensive studies on the
32 remediation of PAHs-contaminated soils using different technologies, including: solvent
33 extraction, bioremediation, phytoremediation, chemical oxidation, photocatalytic
34 degradation, electrokinetic remediation, thermal treatment and integrated remediation

1 technologies (Gan et al., 2009). According to these authors, no single remediation
2 technology can be the solution for all PAH-contaminated soils, and remediation
3 technologies that combine the separation and degradation of PAHs seems to be one way
4 to increase removal efficiencies.

5 To improve desorption efficiency of PAHs from soils as well as the mobility and
6 bioavailability of PAHs in the aqueous phase, various solubility enhancement agents
7 have been used, such as co-solvents and surfactants (Saichek and Reddy, 2004; Gómez
8 et al., 2010), but themselves can present a health or environmental hazard. Organic co-
9 solvents can harm resident microbial population; surfactants can form high-viscosity
10 emulsions, which are difficult to remove, and can be toxic to humans and microbial
11 populations (Ying, 2006). Moreover, Zhou and Zhu (2007) reported that surfactant
12 micelles can be adsorbed by the soil matrix leading to PAH partitioning into immobile
13 adsorbed surfactants, increasing the adsorption of PAHs onto soils.

14 Cyclodextrins (CDs) have been proposed as a non-toxic and biodegradable alternative
15 to organic solvents and surfactants for the removal of PAHs and other hydrophobic
16 compounds from contaminated soils (Gomes et al., 2013). It is well known that CDs are
17 capable of forming inclusion complexes both in solution and in a solid state with a
18 variety of guest molecules, which are placed in their hydrophobic interior cavity (Pérez-
19 Martínez et al., 1999; Villaverde et al., 2007; Yáñez et al., 2012; Sánchez-Trujillo et al.,
20 2014). CDs are widely used in pharmaceutical science (Uekama et al., 2006), but, in the
21 last decade, CDs have gained considerable attention in many other fields, such as,
22 nanocomposite technologies, chromatography, biotechnology, and agriculture (Morillo,
23 2006), due to the low production cost of some compounds within the CD family.
24 Recently, CDs have been used in environmental applications to improve the
25 remediation efficiency of contaminated soil, because they have the ability to increase
26 the apparent water solubility of low-polarity organic compounds, reducing their sorption
27 and facilitating their transport through soil (Badr et al., 2004; Villaverde et al., 2006).
28 This transfer of the contaminants from the soil-solid phase into the soil solution is an
29 important way of increasing the biodegradable fraction of PAHs in soil (Stroud et al.,
30 2009). The use of CDs as extractants has the advantage of minimising environmental
31 impact because they have been approved as non-toxic compounds (for these reasons
32 they are commonly used in drug formulations), they do not harm resident microbial
33 populations, and are considered biodegradable due to their glucose-based composition
34 (Verstichel et al., 2004). However, hydroxypropyl- β -cyclodextrin (HPBCD) has been

1 shown to be resistant to biodegradation for time periods of at least a few months
2 (Fenyvesi et al., 2005).

3
4 In the present work, batch extraction experiments were conducted to find procedures,
5 using biodegradable molecules such as CDs, which might result in an increase in the
6 extraction of FLU and FLT from spiked soils through solubility enhancement, to
7 increase their availability to be degraded by microorganisms. In a previous paper,
8 Morillo et al. (2012) obtained FLU and FLT solubility approximately 200/300-fold
9 higher than their aqueous solubility when using HPBCD solutions. Because the cost of
10 HPBCD is within the range of common surfactants, and it has been demonstrated to be
11 toxicologically safe (Gould and Scott, 2005), it was selected as a good candidate to
12 study its ability to enhance FLU and FLT extraction after different ageing periods from
13 soils of different characteristics which were artificially contaminated. The influence of
14 their physico-chemical properties on extraction in comparison to extraction with
15 aqueous solution in the absence of CD has also been assessed. The two PAHs were
16 selected from amongst 16 proposed by the US-EPA as the most frequently occurring
17 and/or dangerous to the environment, but with reported log Kow values ≤ 5.2 (i.e.,
18 NAP, ACE, ACT, ANT, PHE, FLU, FLT and PYR), which are those PAHs that could
19 present a higher availability and, consequently, toxicity, making their effective
20 extraction from contaminated soils more necessary. FLU and FLT were also selected
21 due to their differences in aqueous solubility and hydrophobicity, and also due to the
22 scarcity of studies carried out with these two PAHs in relation to the use of CDs for soil
23 decontamination. Understanding that PAH sorption behaviour is important for
24 modelling their distribution between water and soil fractions and for assessing their
25 environmental risk, sorption-desorption experiments of FLU and FLT were also carried
26 out to observe the effects of their different chemical properties and to relate them with
27 their extraction from unaged and aged soils using HPBCD. These studies will contribute
28 to a deeper knowledge of PAH sorption-desorption behaviour and to the assessment of
29 their environmental risk and the potential for bioremediation of contaminated soils.

31 **2. Materials and methods**

32 *2.1. Chemicals and soils*

33 FLU (C₁₃H₁₀) and FLT (C₁₆H₁₀) stock solutions in methanol (5000 mg l⁻¹, purity >99%)
34 were purchased from Sigma-Aldrich (Madrid, Spain). Their molecular weights are

1 166.2 and 202.3 g mol⁻¹ and their water solubility about 1.90 and 0.26 mg l⁻¹ at 25°C,
2 respectively. HPBCD (2-hydroxypropyl-β-cyclodextrin) (purity > 97%) was supplied
3 by Cyclolab (Budapest, Hungary). HPLC-solvents used were purchased from Merck
4 (Darmstadt, Germany). All other reagents were of analytical grade and used as received.
5 The three soils used were from Seville province (south-western Spain). The climate of
6 the area is Mediterranean, with an average annual temperature of about 18 °C and a
7 mean annual rainfall of 500 mm. Soil 1 presented a loamy sand texture and was located
8 at the CSIC experimental farm ‘La Hampa’ in Coria (37°16′59″ N, 06°04′03″ W);
9 loam soil 2 was located in the Guadalquivir River Valley at the CIFA Las Torres
10 experimental farm in Alcalá del Río (37°8′33″N, 5°16′4″W); soil 3 presented a clay
11 texture and was located at the CIFA Tomejil experimental farm in Carmona (37°24′07″
12 N, 05°35′10″ W). All soils were sampled from the top 0-20 cm layer, air dried and
13 crushed to pass through a 2-mm aperture sieve before their use. They were analyzed for
14 pH in saturated paste, total carbonate content, particle size distribution, cationic
15 exchange capacity, OM content, and percentages of C and N. The above-indicated
16 properties of the soils used are given in Table 1. Organic carbon content (OC) was
17 determined as follows: OC (%) = 0.58 x OM (%). Preliminary tests showed that
18 negligible contents of native FLU and FLT were present in the soils.

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20 *2.2. Sorption-desorption experiments in soils.*

21 FLT and FLU solutions were prepared by diluting the corresponding stock solution
22 (5000 mg l⁻¹ in methanol) with 0.01 M Ca(NO₃)₂ aqueous solution as background
23 electrolyte, containing 200 mg L⁻¹ of HgCl₂ as biocide to minimize microbial activity
24 throughout the experiments. The concentration of methanol in the final solutions was
25 always kept ≤ 0.02% (v/v), since methanol concentrations up to 0.2% (v/v) do not
26 modify the PAHs sorption properties on soils and their colloidal components, rendering
27 its co-solvent effect negligible (Weber and Huang, 1996; Hundal et al., 2001).
28 Adsorption experiments were performed in triplicate by mixing 0.2 g of the soils with
29 10 mL of FLT or FLU aqueous solutions of initial concentrations in the range 0.5-5.0
30 μM in 25 ml glass centrifuge bottles (Corex) sealed with Teflon-lined screw cap
31 silicone septa. Controls without soil were also prepared in the same way to account for
32 possible loss of FLT or FLU other than sorption to soils, such as losses due to
33 evaporation or to adsorption on the walls of the centrifuge bottles. The samples and
34 controls were shaken in an orbital shaker at 120 rpm in the dark for 3 days for FLT and

1 7 days for FLU at 20 ± 1 °C (the choice for equilibration time was based on previous
2 kinetic studies, data not shown). After equilibration, the suspensions were centrifuged
3 and the aqueous supernatant separated. Analysis of 1:1 (v/v) hexane extract of the
4 aqueous supernatant solutions was performed (PAHs were completely recovered in the
5 separated hexane phase due to their much higher solubility in hexane than in CD
6 solutions). Calibration curves were also prepared in hexane. The concentration of FLT
7 and FLU was determined by HPLC coupled to a fluorescence detector (Shimadzu RF-
8 10APXL) according to the conditions described by Morillo et al. (2008). The difference
9 in concentrations between the initial and final equilibrium solutions was assumed to be
10 due to sorption, and the amount retained by the adsorbent was calculated. Non
11 significant losses were observed in the controls without soil, and no correction was
12 required.

13 The adsorption data of both PAHs were fitted to the linearized form of the Freundlich
14 equation ($\log C_s = \log K_f + n \log C_e$), where C_s ($\mu\text{mol kg}^{-1}$) is the amount of PAH
15 adsorbed at the equilibrium concentration in solution C_e ($\mu\text{mol l}^{-1}$), and K_f and n are
16 constants that characterize the sorption capacity and sorption intensity, respectively. K_f
17 corresponds to PAH adsorption for an equilibrium concentration $C_e = 1 \mu\text{mol l}^{-1}$. The
18 influence of soil OM on the sorption behaviour of the PAHs was calculated using the
19 normalized distribution coefficient (K_{foc}) of organic carbon (OC) calculated from K_f
20 values ($K_{foc} = K_f / \text{OC} \times 100$). The coefficient K_{foc} is often used to characterize the
21 sorption of nonpolar hydrophobic compounds; the concept is based on organic carbon
22 exhibiting the same affinity for a nonpolar compound, independent of the source of OC
23 (Morillo et al., 2004).

24 Desorption experiments were performed only in soil samples treated with 0.5 and 5.0
25 μM after adsorption equilibrium was reached by removing 5 ml of the supernatant after
26 centrifugation and replacing it by 5 ml of 0.01 M $\text{Ca}(\text{NO}_3)_2 + 200 \text{ mg l}^{-1} \text{HgCl}_2$ solution.
27 Equilibration was allowed for an additional 24-h period, operating after that as in the
28 adsorption experiment. This process was repeated twice more.

29

30 *2.3. Spiking and ageing of soils with fluorene and fluoranthene.*

31 Previous to the spiking, soils were sterilized using an autoclave Auster-G, P-Selecta
32 with three cycles at 121 °C, inlet pressure of 103 kPa, during 20 min. After that, 10 g of
33 each soil were weighed in 25 mL glass centrifuge bottles (Corex) in quadruplicate and
34 combined with 10 ml of each of the following four solutions in methanol: FLT or FLU

1 of 5 and 50 mg l⁻¹, to reach 5 and 50 mg kg⁻¹ in the soil. The mixtures were then
2 vigorously shaken for 30 s by using a Vortex mixer, to allow the PAHs to mix with the
3 soil, and were maintained at room temperature under the fume hood during the time
4 necessary to evaporate any traces of methanol (measured by weight loss until constant
5 values). The soil water contents were adjusted to 60% of the water holding capacity of
6 each soil. After spiking and moistening the soils, 0.2 g (dry matter basis) were weighed
7 into Corex centrifuge bottles which were incubated for 1, 35, 78, 135, and 195 days at
8 20°C in the darkness in triplicate for each ageing period. Closed/sealed bottles were
9 stored vertically in order to be aged.

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11 2.4. Extraction of fluorene and fluoranthene from spiked and aged soils.

12 Extraction experiments were performed in triplicate adding 10 ml of 0.01 M Ca(NO₃)₂
13 + 200 mg l⁻¹ HgCl₂ solution (from now on named aqueous solution) or 10 ml of this
14 solution plus 0.01M HPBCD (named HPBCD solution) to a weight of 0.2 g of dry soil.
15 HgCl₂ was added to prevent microbial degradation of both, CD and PAHs. Bottles were
16 closed with screw caps Teflon-lined silicone septa and the mixtures were shaken
17 horizontally on an orbital shaker at 200 rpm in the dark for 20 h at 20 ± 1 °C. Blanks
18 were prepared at the same time under the same conditions. The samples were
19 centrifuged at 3,000 rpm for 10 min, the soil and liquid phases separated, and the
20 supernatant analysed to determine the quantities of FLT and FLU desorbed from the
21 solid matrix. The rest of the supernatant was decanted and then 10 ml of fresh aqueous
22 or HPBCD solution were added. The extractions were carried out three times in total,
23 and each extract was analysed individually.

24 The plots of compound extractability versus aging exhibit an exponential decrease. The
25 extraction curves were fitted to the following kinetic models: a simple first-order model
26 (SFO) and a first-order two-compartment model (2CFO) (Cornelissen et al., 2000), :

$$27 \quad [PAH]_t/[PAH]_0 = e^{-kt} \quad (\text{SFO})$$

$$28 \quad [PAH]_t/[PAH]_0 = F_1 e^{-k_1 t} + F_2 e^{-k_2 t} \quad (\text{2CFO})$$

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30 where $[PAH]_t$ and $[PAH]_0$ are the concentrations of PAH extractable at time t and just
31 after spiking the soil, respectively (mg kg⁻¹), k is the rate constant of extraction (day⁻¹).

32 In the 2CFO model, k_1 and k_2 are the rate constants of extraction for the fast and the
33 slow fractions, respectively, and F_1 and F_2 are the rapidly and slowly extractable
34 fractions of the total amount present in the soils.

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2.5. Infrared spectroscopy

The investigation of the quality of SOM was carried out by FTIR spectroscopy. Due to the low organic carbon content of the three soils they were treated with 10% (w/w) hydrofluoric acid (HF) to remove mineral matter and concentrate the amount of SOM. The soils were subjected to four successive treatments as in Gonçalves et al. (2003) who observed that, although losses of C occurred, no consistent alterations in the distribution of carbon functional groups take place. FTIR spectra of the treated samples prepared in KBr pellets (2% w:w) were recorded using a JASCO FT-IR 6300 spectrometer with a DTGS detector, in the range of 4000-400 cm^{-1} . The resolution was set to 2 cm^{-1} . Because of the typical broadband IR spectra obtained from SOM, it is often useful to apply resolution enhancement techniques. In this case an algorithm was applied that was based on subtracting the original spectrum (previously sized to 2000 data points) after baseline correction from a positive multiple (300) of its second derivative (50-point moving averages smoothing) (Fernández-Getino et al., 2013).

3. Results and discussion

3.1. Fluorene and fluoranthene sorption experiments.

Adsorption-desorption experiments of FLT and FLU were carried out using three soils, each with different soil characteristics (Table 1). Figure 1 shows the sorption isotherms of both PAHs on the soils studied. Isotherms fit the Freundlich equation, with r^2 values > 0.99 , and the estimated K_f and n parameters are shown in Table 2. Isotherms are nonlinear and n values are lower than unity in agreement with the L shape of the isotherms according to the classification of Giles et al. (1960). The non-linearity of PAHs sorption suggests the saturation of sorption sites, and could be a consequence of the low OM content of these soils, and its heterogeneity in relation to the varying degrees of hydrophobicity of the adsorption sites. This nonlinear sorption is consistent with many earlier observations for hydrophobic organic compounds in the literature (Xing and Pignatello, 1997; Undabeytia et al., 2004; Rivas et al., 2008), and, according to Oren and Chefetz (2005), we also conclude that the more hydrophobic fraction of the OM is responsible for the rapid and linear adsorption, and the less hydrophobic fractions are those which contribute to the non-linearity of the sorption isotherm.

The sorption capacity of the soils for FLU and FLT were compared using the K_f parameter. K_f varied between 30.4 and 60.5 for FLU, and between 193 and 308 for

1 FLT, indicating the strong influence of soil characteristics and also the influence of
2 PAH type on its adsorption. This fact is clearly observed in Table 3, where the amount
3 (in $\mu\text{mol kg}^{-1}$) and percentage of both PAHs adsorbed for the lowest and highest
4 concentrations used (0.5 and 5.0 μM) are shown.

5 For both PAHs, the adsorption on the soils follows the sequence soil 1 < soil 2 < soil 3,
6 which corresponds to their OM content, in spite of the narrow range of differences
7 among them (0.79-1.76%). From Figure 1 and data on Tables 2 and 3, it is obvious that
8 FLT was adsorbed by the three soils to a greater extent than FLU. This can be attributed
9 to its higher hydrophobicity and, therefore, its higher tendency to be adsorbed on the
10 soil OM. In fact, FLT presents n-octanol-water partition coefficients ($\log K_{ow}$) of 5.20
11 in comparison to that of FLU, 4.23 (Sverdrup et al., 2002). One of the most important
12 factors in the adsorption of hydrophobic compounds in soils is their percentage of OM
13 because the more hydrophobic the molecule is, the higher is the probability to be
14 transported from the aqueous phase to the hydrophobic surfaces of the soils.

15 The role that the formation of hydrophobic bonds with OM plays in the adsorption of
16 organic compounds in soils can be compared using K_{foc} , i.e., the distribution coefficient
17 K_f , but referred to the percentage of OC. When hydrophobic bonds are responsible for
18 the adsorption of a molecule, K_{foc} values should be relatively constant among different
19 soils (Morillo et al., 2002). However, in many studies that does not happen, due to
20 adsorption in different multi-domains (Pan et al., 2007), especially due to the OM
21 heterogeneity (Zhang and He, 2009), although the contribution of inorganic constituents
22 to the sorption process has to also be taken into account. For each PAH, K_{foc} values
23 present a slight variability among the three soils (Table 2), indicating that the amount of
24 organic C alone cannot account for the different sorptive properties of the soil.

25 However, when they are expressed as $\log K_{foc}$, the values obtained are in agreement
26 with those presented by other authors (Maagd et al., 1998; Burgess et al., 2004). In the
27 present research, these values are in the range 3.77-3.82 for FLU and 4.48-4.62 for FLT.
28 K_{foc} values are higher for soil 1 than for soils 2 and 3, which are more similar to each
29 other. Most likely the higher carbonate contents of soils 2 and 3 (>20%) could reduce
30 the sorptivity of both PAHs. El Arfaoui et al. (2012) observed that soils with more than
31 15% calcite exhibited relatively low sorption capacities for the non-ionic pesticide
32 isoproturon due to calcite coating of OM, making sorption sites less accessible to non-
33 polar pollutants.

34

3.2. Fluorene and fluoranthene desorption experiments with water

Table 3 also contains the amount and percentages of FLU and FLT desorbed from the soils after three cycles of desorption. The desorption of FLT follows the logic sequence: soil 1 > soil 2 > soil 3, contrary to the adsorption, indicating that desorption percentages are inversely related to the affinity of FLT molecules for soil adsorption, expressed as K_f values. The values obtained show that the desorption of FLT is quite irreversible because only 15.7, 12.2 and 7.90% is desorbed from soils 1, 2, and 3, respectively, when the lowest concentration was used (0.5 μM). Using the highest concentration (5.0 μM), the maximum desorption of FLT was only 23.6% of that previously adsorbed in soil 1. In comparison, the percentages of desorption for FLU are higher, indicating that weaker interaction mechanisms predominate between FLU and the soils. The adsorption of FLT is more irreversible when the concentration used is the lowest, that is, when FLT surface covering is low because FLT molecules are occupying the highest energy adsorption points available on the soil, from which desorption will be more difficult or even irreversible. An increase of concentration led to an increase in the percentages of this PAH desorbed, due to their adsorption on sites of lower affinity when those of higher affinity are occupied (Morillo et al., 2002; Wu and Sun, 2010). Therefore, increasing concentrations may have different effects on desorption and persistence of this contaminant in soils.

However, this behaviour is not observed for FLU because the percentages desorbed were very similar for both concentrations and for the three soils used (approximately 30% desorbed), in spite of their different OM content, as occurred for FLT.

Although Müller et al. (2007) propose that mineral surfaces would only influence the retention of hydrophobic organic contaminants under OC threshold values < 0.1%, Weber and Huang (1996) postulate that mineral surfaces are important domains in the sorption of hydrophobic organic contaminants. According to these authors, three types of domain exist associated with a soil particle: an exposed mineral domain, an amorphous soil OM domain, and a condensed soil OM domain. The sorption of hydrophobic compounds by condensed OM appears to be energetically more favourable due to its less polar character, but it is very slow due to slow intradomain diffusion. On the contrary, sorptions associated with mineral surfaces and amorphous soil OM are relatively fast processes. In regard to this point, we have to consider also the different hydrophobicity of FLU and FLT. FLT presents high hydrophobicity and, therefore, a high tendency to be adsorbed in the less polar soil OM. The less hydrophobic FLU

1 perhaps has a greater tendency to be adsorbed primarily in the mineral and amorphous
2 soil OM domains and to diffuse slowly into the condensed soil OM. This is most likely
3 the reason why FLU desorption is higher for both concentrations in the three soils.
4 Wang et al. (2008) observed that carbonate provided sorption sites for PAH sorption
5 which mainly affected sorption and desorption processes of lower ring PAHs, such as
6 FLU. Their results indicated that FLU are closely fixed onto carbonate during the
7 adsorption procedure, but very little FLT was fixed. Additionally, Becker et al. (1997)
8 demonstrated that calcium carbonate can selectively sequester PAHs from an aqueous
9 solution. Due to the presence of carbonate in the three soils under study, especially in
10 soils 2 and 3, adsorption of FLU is likely to take place primarily on these amorphous
11 surfaces, and it could be the reason for similar desorption from the three soils.

12

13 *3.3. Extraction of fluorene and fluoranthene from spiked soils with water and with* 14 *HPBCD.*

15 Figures 2 and 3 show the percentages of FLU and FLT, respectively, extracted by water
16 and HPBCD after the first extraction step and the cumulated quantities after three
17 extraction processes, from the soils spiked with 5 and 50 mg kg⁻¹ of FLU or FLT and
18 after 1, 35, 78, 135, and 195 days of spiking.

19 In general, the extraction of FLU and FLT from the soils is always more effective using
20 HPBCD, as previously observed for other organic contaminants (Pérez-Martínez et al.,
21 2000; Villaverde et al., 2005, 2013; Sánchez-Trujillo et al., 2013), due to the formation
22 of inclusion complexes in solution between the hydrophobic molecules of FLU and FLT
23 and HPBCD. However, in the case of FLU (Fig. 2), these differences become less
24 apparent as time from spiking increases. Moreover, after three successive extractions
25 these differences diminished greatly, especially in the cases of soils 1 and 3. It is
26 interesting to observe that in both extractions (with the aqueous and HPBCD solutions)
27 the amount of FLU extracted in the second and third steps dropped drastically. This is
28 most likely mainly caused by the relatively large concentration extracted in the first
29 step, resulting in a depletion of the more easily extractable fraction of FLU.

30 The similarity in the results obtained using HPBCD or water in FLU extraction is
31 related to the relatively high aqueous solubility of FLU (1.90 mg l⁻¹). For the spiking
32 concentrations used (5 and 50 mg kg⁻¹), even if 100% of FLU on the soil was extracted

1 in the first step, the concentration in the extractant solution would be 0.1 and 1 mg l⁻¹,
2 respectively, lower than FLU aqueous solubility.

3 However, the extraction of FLU decreased drastically as the time after spiking
4 increased, with only approximately 20% being extracted from any soil after 195 days of
5 ageing for both FLU concentrations (5 and 50 mg kg⁻¹) and for both extractants
6 (HPBCD or water) after three successive extractions. This seems to indicate that the
7 long contact of FLU with soils increases its retention, most likely by diffusion into the
8 pores of the sorbent, moving the molecules of the adsorbate towards the interior of the
9 soil particles (Rudzinski and Plazinski, 2007). From mineral domains, FLU diffuses
10 relatively quickly towards amorphous soil OM domains, and then slowly to more
11 hydrophobic soil OM. For this reason, the desorption of FLU is more difficult as contact
12 time increases because some portion of it is sorbed by inner and more hydrophobic
13 sites, becoming practically irreversible some days after spiking.

14 To confirm this assumption, an experiment of adsorption-desorption of FLU was
15 conducted for the three soils using the highest concentration (5.0 µM), but the
16 adsorption was carried out for only 3 days, instead of the 7 days necessary to reach FLU
17 adsorption pseudo-equilibrium. As expected, the percentage of FLU adsorbed was
18 lower (16.3, 26.7, and 34.2% instead of 34.5, 48.5, and 48.9% for soils 1, 2, and 3,
19 respectively), but the percentages desorbed were quite higher (98.9, 73.0, and 71.8%
20 instead of 34.4, 25.1, and 32.6% for soils 1, 2, and 3, respectively). This suggests that
21 FLU is adsorbed first onto surfaces from which desorption is quite easy, but the process
22 of diffusion towards more hydrophobic domains (such as amorphous OM) is very fast,
23 as the percentage of desorption decreased quickly if the sorption has taken place in 7
24 days in comparison to 3 days.

25 The plots of FLU extractability vs. ageing exhibit an exponential decrease that could be
26 fitted to a first-order two-compartment model (2CFO), and model parameters are
27 summarized in Table SD1. A fast initial decrease of the percentage of PAH extracted is
28 often followed by a slower decline, indicating that the adsorption of FLU in the three
29 soils is more irreversible as the time after spiking increases. It must be taken into
30 account that loss due to FLU biodegradation in the soil is not possible because all the
31 experiments were carried out in the presence of an HgCl₂ solution to prevent microbial
32 degradation of the PAH.

33 These results indicate that there are few advantages in using HPBCD as the extractant
34 solution in comparison with extraction with water for soils contaminated with FLU after

1 an ageing period subsequent to contamination, which is what usually occurs in real
2 contaminated soils. However, the advantage of the observed behaviour of FLU in aged
3 soils is that it will present very few problems for subsequent desorption, such as
4 leaching, runoff or damage for the microbial population.

5 On the contrary, FLT presented different extraction behaviour from that of FLU (Fig.
6 3). When FLT was extracted with HPBCD solutions the amount extracted after all
7 ageing times was much higher than in the absence of the CD. In addition, the
8 percentages of FLT extracted with $\text{Ca}(\text{NO}_3)_2$ or HPBCD solutions are similar for all
9 ageing times greater than 35 days, and in the case of the lowest concentration of
10 contamination, even from the first day after spiking. This behaviour suggests that
11 sorption pseudo-equilibrium was reached within the first few days after spiking for the
12 low level of contamination because FLT molecules have reached adsorption points of
13 high hydrophobicity immediately after spiking.

14 However, in the case of soils spiked with 50 mg kg^{-1} , the amount extracted 1 day after
15 spiking was, in general, higher than for the rest of ageing times, for which the amount
16 extracted remained constant (opposite to the observed behaviour of FLU). This
17 indicates that a diffusion process of some portion of FLT adsorbed towards the interior
18 part of the soil particles is occurring. For longer ageing periods sorbed molecules reside
19 on adsorption sites with different energies: readily extractable sites for water extraction,
20 readily extractable sites for HPBCD extraction, and extraction resistant sites (Yang et
21 al., 2012). This behaviour suggests that FLT sorption in soils is rather faster than that of
22 FLU, due to its higher hydrophobic character and also to the high affinity and energy of
23 the interaction formed, which do not vary with time, so that the percentages of
24 extraction were constant. This is the reason for the low values obtained for k_2 , the rate
25 constant corresponding to the slower extracting fraction in first-order two-compartment
26 model (2CFO), which is three or even four orders-of-magnitude lower than those for k_1
27 (Table SD1).

28 The heterogeneity of the sorption sites on the OM of each of the soils under study can
29 be easily observed in Table 4, where the extraction enhancement of FLT obtained using
30 HPBCD relative to the extraction using the $\text{Ca}(\text{NO}_3)_2$ solution is shown. The percentage
31 of extraction is multiplied by a greater factor when the soil was spiked with the higher
32 concentration of FLT for the three soils.

33 It is important to emphasise, in the case of FLT, that the difference between $\text{Ca}(\text{NO}_3)_2$
34 and HPBCD extractions is greater than that observed in the case of FLU, what is due to

1 the much lower aqueous solubility of FLT (0.26 mg L^{-1}). Unlike FLU, the amount of
2 FLT in the soils far exceeds its aqueous solubility in the case of the soil spiked with 50
3 mg kg^{-1} (1.011 mg L^{-1} if all FLT were solubilized in only one extraction).
4 That is the reason of the higher differences between the percentages of FLT extracted
5 with one or three extractions (Fig. 3) in comparison to this difference in the case of FLU
6 (Fig.2). It means that the more hydrophobic compounds, such as FLT, will exhibit a
7 greater improvement in contaminant mass removal with HPBCD (compared with water)
8 than that exhibited for the more hydrophilic one (McCray et al., 2000; Balogh et al.,
9 2007). This is also observed in Table 4 where the factors of extraction enhancement are
10 much greater for FLT than for FLU. Morillo et al. (2012) previously observed that the
11 solubility of the least soluble compounds, when using CDs, was improved to a greater
12 extent than that of the more soluble compounds (Sánchez-Trujillo et al., 2013).
13 The differences among the three soils studied related to FLT extraction demonstrates the
14 influence not only of their OM content but also of their quality and/or other soil
15 properties. In the case of soil 3, although it presented a greater tendency than soils 1 and
16 2 to adsorb FLT due to its higher OM content (Figure 1), the percentage of FLT
17 extracted was similar to or even higher than those found for soils 1 and 2 (Fig. 3). This
18 was opposed to the inverse proportionality, reported in the majority of studies, between
19 the OC content of a soil and the extractability of the sorbed contaminants. This
20 phenomenon could be due to a higher microporosity of soils with low OC content
21 (Ncibi et al. 2007; Luo et al. 2012), and/or to differences in the quality of their OM.
22 The different microporosity of the three soils studied is not likely the cause of such
23 behaviour because, as observed in Table SD2, microporosity follows the sequence: soil
24 3 > soil 2 > soil 1. In relation to the quality of the OM, FTIR studies have been carried
25 out on these soils previously treated with hydrofluoric acid (HF) to remove mineral
26 matter and concentrate the amount of SOM. Figure 4 shows the IR spectra after
27 resolution enhancement techniques described in section 2.5 (restricted ranges of
28 wavelength between $1500\text{-}1800 \text{ cm}^{-1}$ and $1200\text{-}1500 \text{ cm}^{-1}$ have been included as Figure
29 SD1 for more details of the bands).
30 The degree of oxidation of humic substances in soils is reflected by the intensity of the
31 band at about 1720 cm^{-1} (C=O groups band) (Aranda et al., 2011). Soil 1 presented only
32 a shoulder at 1727 cm^{-1} as a result of overlapping of different individual bands,
33 indicating weak oxidation of the OM and suggesting that it has probably suffered a high
34 diagenetic transformation. This is followed by soil 2, which presents a small band at

1 1724 cm^{-1} , and lastly by soil 3, with a sharp feature located at 1722 cm^{-1} , which
2 indicates a progressive oxidation of soil C forms, and suggests oxidative breakdown of
3 humic substances as a major factor controlling SOM concentration in soil 3.
4 The broad band in the 1650-1600 cm^{-1} region can be attributed to the superimposition of
5 bands in the 1600-1620 cm^{-1} region (planar vibrations of aromatic C=C and
6 antisymmetric stretching of carboxylate ions, COO^-) with bands in the 1630-1655 cm^{-1}
7 region (carbonyl groups linked to hydrogen bonds, quinones and aminoacids) (Aranda
8 et al., 2011). The contribution of amide I band of aminoacids appears at the highest
9 wavenumbers (1640-1655 cm^{-1}) and OM with this broad band is characterised by higher
10 protein content, indicating weakly evolved forms of nitrogen. In contrast, if the
11 maximum is located at 1610 cm^{-1} , it reflects a higher content of aromatics. In soils 2 and
12 3 this broad band appears at 1645 cm^{-1} , but in soil 1 it appears at 1636 cm^{-1} , with a
13 shoulder at 1618 cm^{-1} , indicating that the latter soil contains a higher proportion of
14 aromatics, and confirming a higher humification stage. In the fingerprint region below
15 1500 cm^{-1} , the typical lignin pattern appeared with characteristic bands near 1460, 1420,
16 and 1380 cm^{-1} . In general, spectra with comparatively broad bands and smoothed lignin
17 peaks indicate chaotic macromolecular complexity typical of mature OM. On the
18 contrary, spectra with a clearly marked lignin band signature are interpreted as the
19 existence of macromolecular domains to some extent based on repeating structures. The
20 band at 1460 cm^{-1} (associated with aliphatic C-H groups and related to the band at
21 approximately 2920 cm^{-1}) and the band at 1420 cm^{-1} (associated with accumulation of
22 amide forms and related to the bands at 1540 and 1640 cm^{-1}) suggest the accumulation
23 of OM of aliphatic character, indicative of early stages of humification. In soil 1, only
24 smoothed lignin bands can be observed, whereas they are clearly marked in soil 3, and
25 less marked in soil 2, indicating that soil 3 is the least humified of the three soils, which
26 could be the reason of the extraction behaviour observed. That is, in spite of the fact
27 that soil 3 has a higher number of hydrophobic sites due to its higher OM content, these
28 sites most likely have a lower hydrophobic character than those of soils 1 and 2
29 (Kukkonen et al. 2003). Older and more diagenetically processed OM is known to bind
30 certain organic contaminants strongly, thereby decreasing their extraction rate (Huang
31 and Weber, 1997).

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34 **4. Conclusions**

1 There are great differences between FLU and FLT in relation to their extraction from
2 aged contaminated soils. FLU becomes more resistant to extraction as the contact time
3 with soil increases. Due to the more hydrophilic character of FLU, it seems to have a
4 greater tendency to be adsorbed first of all on the mineral and amorphous soil OM
5 domains (Wang et al., 2008), sites of low binding energy from which it diffuses slowly
6 to the condensed soil OM inside the soil structure as the time since spiking increases,
7 due to its small size. It is concluded that FLU will not be easily desorbed from aged
8 contaminated soils, and, therefore, it will not present problems due to leaching or
9 runoff. The similarity in the results obtained using HPBCD or water as extractants for
10 FLU is related to this fact and also to its higher aqueous solubility, and there could be
11 few advantages in the use of HPBCD as extractant of FLU from aged contaminated
12 soils.

13 On the contrary, when FLT was extracted from soils with HPBCD solutions the amount
14 extracted after all ageing times was much higher than in its absence, revealing the
15 heterogeneity of the sorption sites with different energies on the OM of the soils: readily
16 extractable sites for water extraction, readily extractable sites for HPBCD extraction,
17 and extraction resistant sites. Therefore, increasing concentrations may have different
18 effects on desorption and persistence of FLT in soils. The percentages of FLT extracted,
19 both with water or HPBCD solutions, are constant for the different ageing times,
20 indicating that FLT sorption in soils occurs rather more quickly than that of FLU, due to
21 its higher hydrophobic character, and the energy of the interaction do not vary
22 throughout the contact time.

23 The influence of the OM quality of the soils was also highlighted during extraction
24 experiments. The percentage of FLT extracted from the aged contaminated soil with the
25 highest OM content was similar or higher than from the others (opposed to the inverse
26 proportionality between OM content of soil and extractability of sorbed contaminants),
27 and FTIR measurements revealed that its OM was the least humified of the three soils,
28 while the soil with the lowest OM content presented the most diagenetically processed
29 OM. This demonstrates the need for not only using the conventional adsorption-
30 desorption isotherms incorporated into contaminant fate and transport models but also
31 studies of resistant extractions in aged contaminated soils and other complementary
32 analytical approaches for risk assessment of soil organic contaminants.

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Supplementary data

Supplementary data associated with this article can be found in the online version. These data include two tables, one of them with the Specific Surface Area (SSA) and pore and micropore values of the soils studied, and the other with the kinetic parameters of the model relative to the extraction of both PAHs from aged soils. Additionally, a figure with FTIR spectra in the restricted ranges of wavelength between 1500-1800 cm^{-1} and 1200-1500 cm^{-1} has been also included.

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1 **Figure captions**

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3 Figure 1. Adsorption isotherms of FLU and FLT in the soils studied.

4

5 Figure 2. Percentages of FLU extracted with $\text{Ca}(\text{NO}_3)_2$ 0.01M (water) and HPBCD
6 0.01M (HP) solutions after one and three consecutive cycles of extraction, from the
7 soils spiked with 5 and 50 mg kg⁻¹ at 1, 35, 78, 135, and 195 days after spiking.

8

9 Figure 3. Percentages of FLT extracted with $\text{Ca}(\text{NO}_3)_2$ 0.01M (water) and HPBCD
10 0.01M (HP) solutions after one and three consecutive cycles of extraction, from the
11 soils spiked with 5 and 50 mg kg⁻¹ at 1, 35, 78, 135, and 195 days after spiking.

12

13 Figure 4. Infrared spectra of the OM of the soils under study after removing mineral
14 matter and applying resolution enhancement techniques.

15

Table 1. Some characteristics of the soils used.

Soil	pH	CaCO₃ (%)	OM (%)	CEC (cmol_c kg⁻¹)	C (%)	N (%)	Sand (%)	Silt (%)	Clay (%)
1	8.0	6.9	0.79	4.8	1.63	0.092	87.6	4.0	8.4
2	8.2	21.8	1.31	14.9	3.62	0.145	28.5	45.8	25.7
3	8.0	24.1	1.76	39.0	4.13	0.207	2.7	31.5	65.9

Table 2. Freundlich adsorption isotherm parameters (K_f and n values), coefficients of determination (r^2) and organic carbon normalized distribution coefficients (K_{foc}) of FLU and FLT sorption on the soils used.

PAH	Soil	K_f	n	r^2	K_{foc}	$\log K_{foc}$
FLU	1	30.4	0.89	0.99	6641	3.82
	2	45.3	0.83	0.99	5967	3.78
	3	60.5	0.78	0.99	5932	3.77
FLT	1	193	0.77	0.99	42100	4.62
	2	257	0.84	0.99	33812	4.52
	3	308	0.75	0.99	30153	4.48

Table 3. FLT and FLU adsorbed on the three soils under study for the lowest and the highest concentrations used (expressed as $\mu\text{mol kg}^{-1}$, and as % of the concentrations applied) and desorbed after three desorption cycles ($\mu\text{mol kg}^{-1}$ and % of the concentration previously adsorbed).

	SOIL 1		SOIL 2		SOIL 3	
	0.5 (μM)	5.0 (μM)	0.5 (μM)	5.0 (μM)	0.5 (μM)	5.0 (μM)
FLUORENE						
Adsorption ($\mu\text{mol kg}^{-1}$)	10.9 (± 1.6)	84.9 (± 4.9)	13.6 (± 0.2)	119 (± 7)	16.1 (± 0.7)	119 (± 4)
Adsorption (%)	43.6	33.9	54.4	47.6	64.4	47.6
Desorption ($\mu\text{mol kg}^{-1}$)	3.77 (± 1.24)	29.2 (± 6.7)	4.08 (± 1.18)	29.9 (± 5.36)	4.00 (± 1.41)	38.8 (± 6.19)
Desorption (%)	34.6	34.4	30.0	25.1	24.6	32.6
FLUORANTHENE						
Adsorption ($\mu\text{mol kg}^{-1}$)	21.8 (± 0.2)	196 (± 2)	22.3 (± 0.3)	210 (± 2)	23.3 (± 0.5)	219 (± 3)
Adsorption (%)	87.1	78.3	89.1	84.2	93.2	87.5
Desorption ($\mu\text{mol kg}^{-1}$)	3.42 (± 0.50)	45.8 (± 3.5)	2.73 (± 1.60)	37.0 (± 4.6)	1.84 (± 0.55)	27.8 (± 3.3)
Desorption (%)	15.7	23.6	12.2	17.7	7.90	12.8

Table 4. Increment factors for the extraction of FLU and FLT from soils when using HPBCD with respect to their extraction with Ca(NO₃)₂ aqueous solution.

		SOIL 1		SOIL 2		SOIL 3	
		5 mg kg ⁻¹	50 mg kg ⁻¹	5 mg kg ⁻¹	50 mg kg ⁻¹	5 mg kg ⁻¹	50 mg kg ⁻¹
FLUORENE							
<u>1 extraction</u>	1 day	1.39	1.36	1.77	1.56	1.90	1.34
	35 days	1.21	1.21	1.76	1.63	1.62	1.33
	78 days	1.25	1.19	1.78	1.51	1.49	1.38
	135 days	1.24	1.18	1.61	1.60	1.55	1.42
	195 days	1.25	1.18	1.45	1.57	1.76	1.62
<u>3 extractions</u>	1 day	1.09	1.10	1.34	1.17	1.30	1.05
	35 days	1.04	1.03	1.31	1.25	1.11	1.02
	78 days	1.20	1.02	1.23	1.19	1.09	1.02
	135 days	1.07	1.01	1.22	1.24	1.13	1.09
	195 days	1.03	1.02	1.19	1.27	1.26	1.22
FLUORANTHENE							
<u>1 extraction</u>	1 day	2.65	3.22	2.35	3.86	2.54	4.55
	35 days	2.26	3.45	2.35	3.65	2.10	3.53
	78 days	2.16	3.40	2.27	3.72	1.87	3.19
	135 days	2.12	3.47	2.31	3.74	1.93	3.08
	195 days	2.13	3.53	2.09	3.79	1.70	3.01
<u>3 extractions</u>	1 day	1.69	2.27	1.86	2.63	1.66	2.78
	35 days	1.47	1.81	1.87	2.47	1.65	2.63
	78 days	1.47	1.81	1.81	2.49	1.65	2.47
	135 days	1.42	1.89	1.72	2.50	1.61	2.36
	195 days	1.46	1.95	1.73	2.50	1.53	2.29

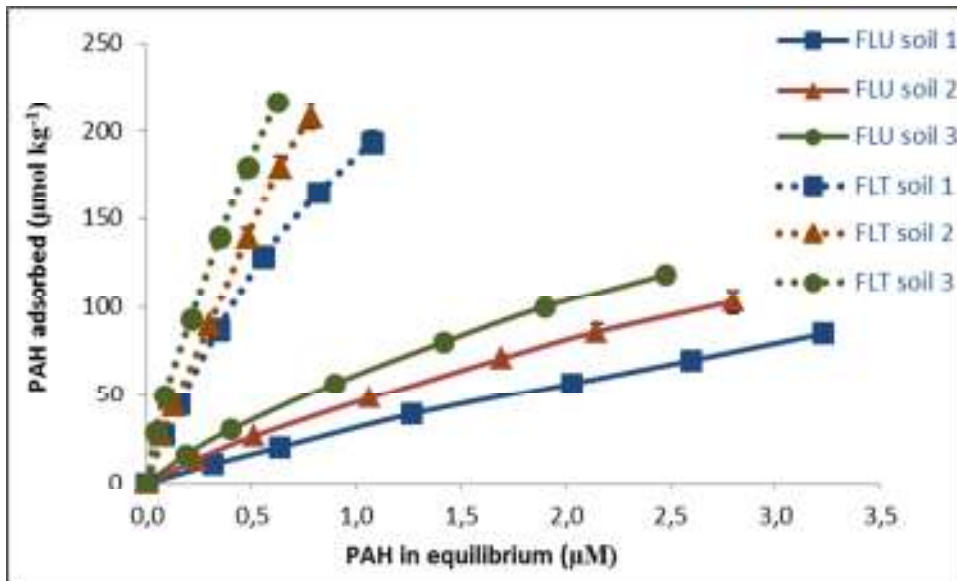
Table SD 1. First order kinetic parameters (Single First-Order, SFO, or first-order two-compartment model (2CFO)) for extraction from the soils (after 3 successive cycles) of FLU and FLT (5 and 50 mg kg⁻¹) at different ageing times using aqueous solution (W) or cyclodextrin solution (HP).

Soil	Treatment	Kinetic model	k_1	k_2	R ²
FLUORENE					
1	5W	2CFO	3.6x10 ⁻²	3.8x10 ⁻³	0.99
	5HP	2CFO	5.7x10 ⁻²	5.9x10 ⁻³	0.99
	50W	2CFO	1.3 x10 ⁻²	1.4x10 ⁻³	0.93
	50HP	2CFO	1.6 x10 ⁻²	8.4x10 ⁻³	0.95
2	5W	2CFO	5.9 x10 ⁻¹	3.2x10 ⁻³	0.99
	5HP	2CFO	4.6 x10 ⁻²	3.8x10 ⁻³	0.99
	50W	2CFO	1.3 x10 ⁻²	6.9x10 ⁻³	0.98
	50HP	2CFO	1.2 x10 ⁻²	1.2x10 ⁻³	0.94
3	5W	2CFO	9.5 x10 ⁻²	5.4x10 ⁻³	0.99
	5HP	2CFO	6.0 x10 ⁻²	3.5x10 ⁻³	0.99
	50W	2CFO	5.6 x10 ⁻¹	7.2x10 ⁻³	0.99
	50HP	2CFO	7.4 x10 ⁻²	5.9x10 ⁻³	0.99
FLUORANTHENE					
1	5W	2CFO	5.9x10 ⁻¹	4.8x10 ⁻⁴	0.99
	5HP	2CFO	6.1x10 ⁻¹	4.4x10 ⁻⁴	0.99
	50W	2CFO	5.1x10 ⁻¹	6.0x10 ⁻⁴	0.97
	50HP	2CFO	1.3x10 ⁻¹	1.1x10 ⁻⁴	0.99
2	5W	2CFO	5.3x10 ⁻¹	2.4x10 ⁻⁴	0.97
	5HP	SFO	7.3x10 ⁻⁴		0.99
	50W	2CFO	1.2 x10 ⁻¹	3.6x10 ⁻⁵	0.99
	50HP	2CFO	5.6 x10 ⁻¹	7.3x10 ⁻⁵	0.99
3	5W	SFO	4.7x10 ⁻⁴		0.93
	5HP	SFO	1.1x10 ⁻³		0.96
	50W	2CFO	4.2 x10 ⁻²	4.6x10 ⁻⁴	0.99
	50HP	2CFO	2.6 x10 ⁻²	8.3x10 ⁻⁴	0.99

Table SD 2. Specific Surface Area (SSA) and pore and micropore values of the soils studied. Errors are less than 2%.

Sample	SSA m^2g^{-1}	Pore volume cm^3g^{-1}	Pore diameter nm	Pore width nm	Micropore Surface m^2g^{-1}	Micropore Volume cm^3g^{-1}
CR	6.8763	0.008856	8.2687	5.15184	1.2399	0.000504
LT	14.1059	0.021134	9.8758	5.99298	1.8114	0.000690
TM	49.5827	0.051107	7.1949	4.12299	10.2924	0.004240

Figure 1



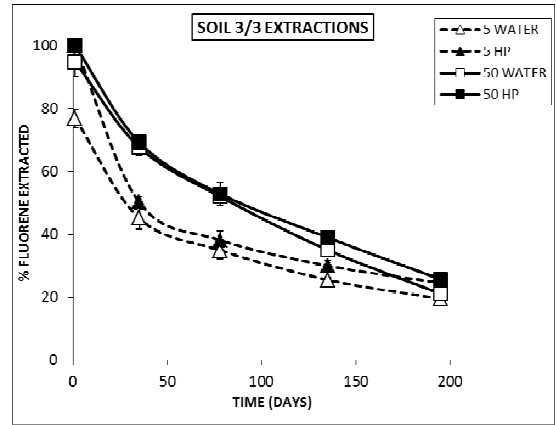
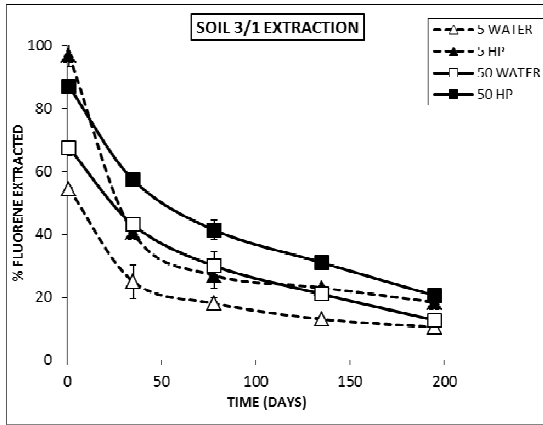
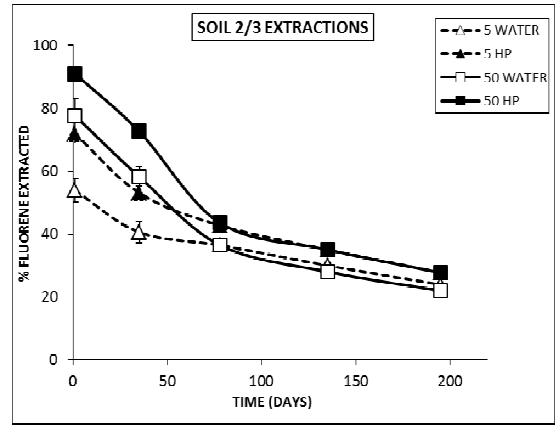
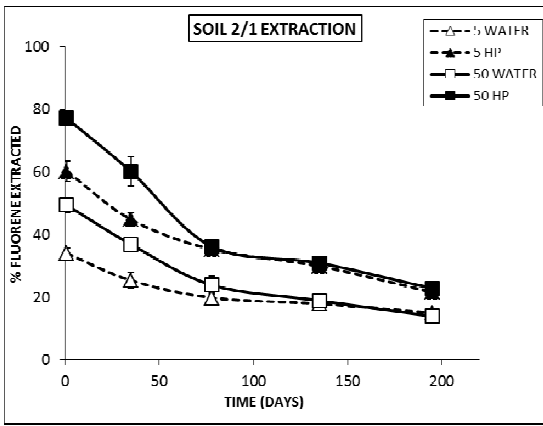
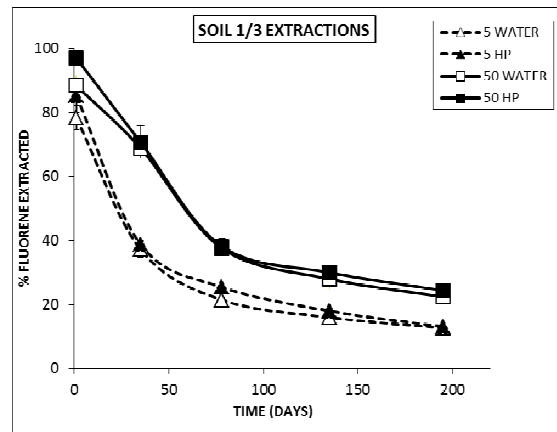
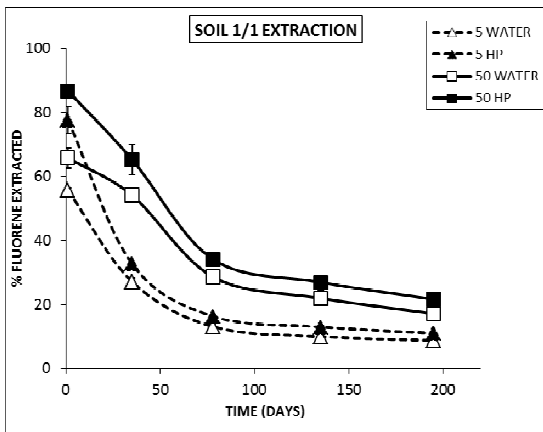


Figure 2

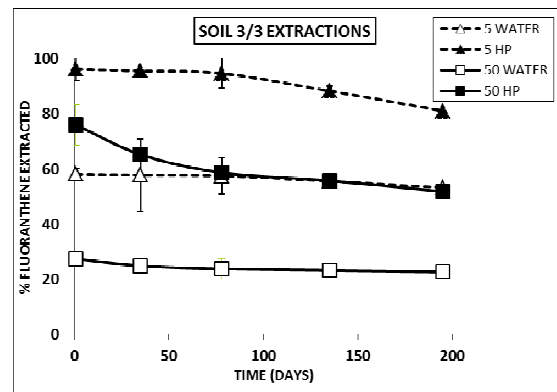
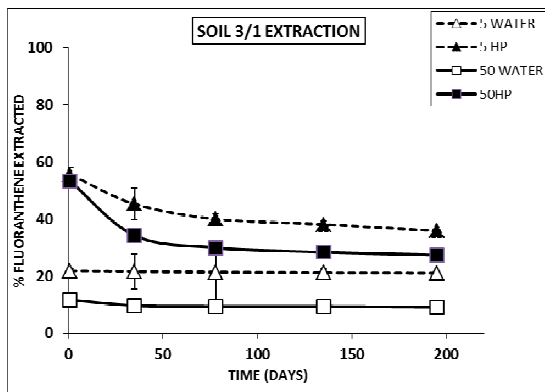
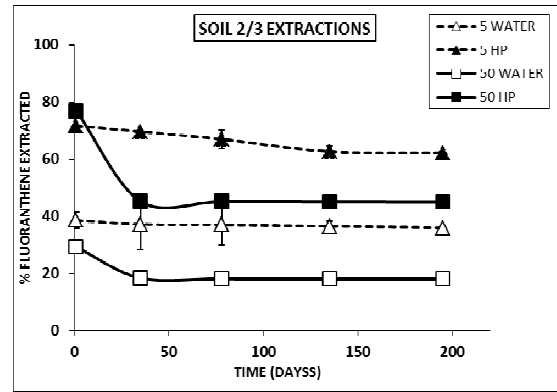
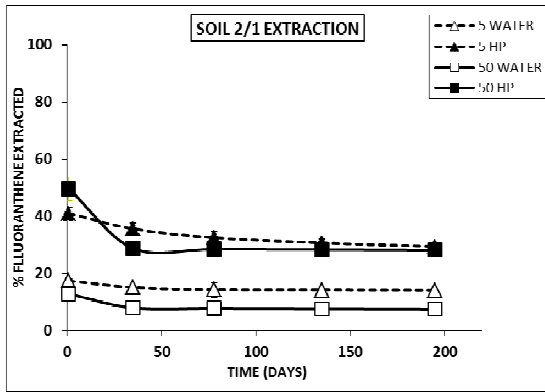
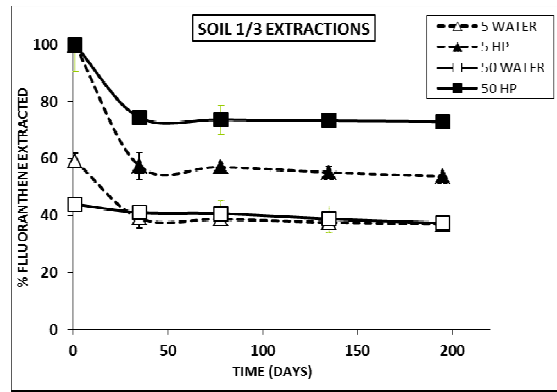
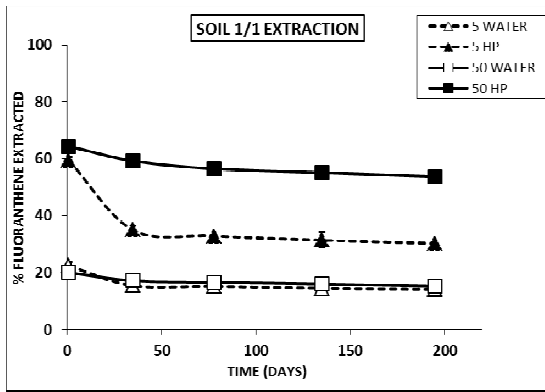


Figure 3

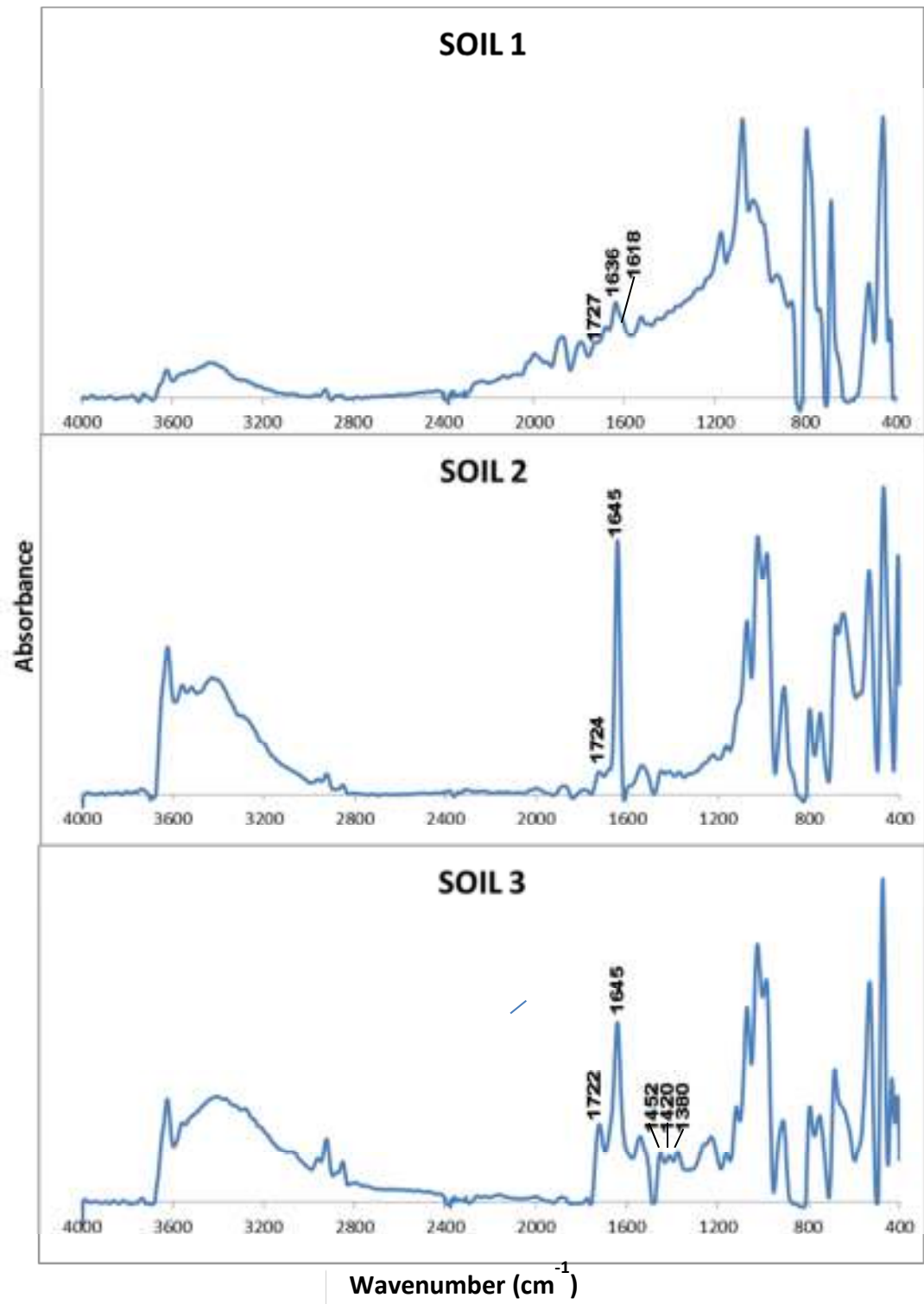


Figure 4

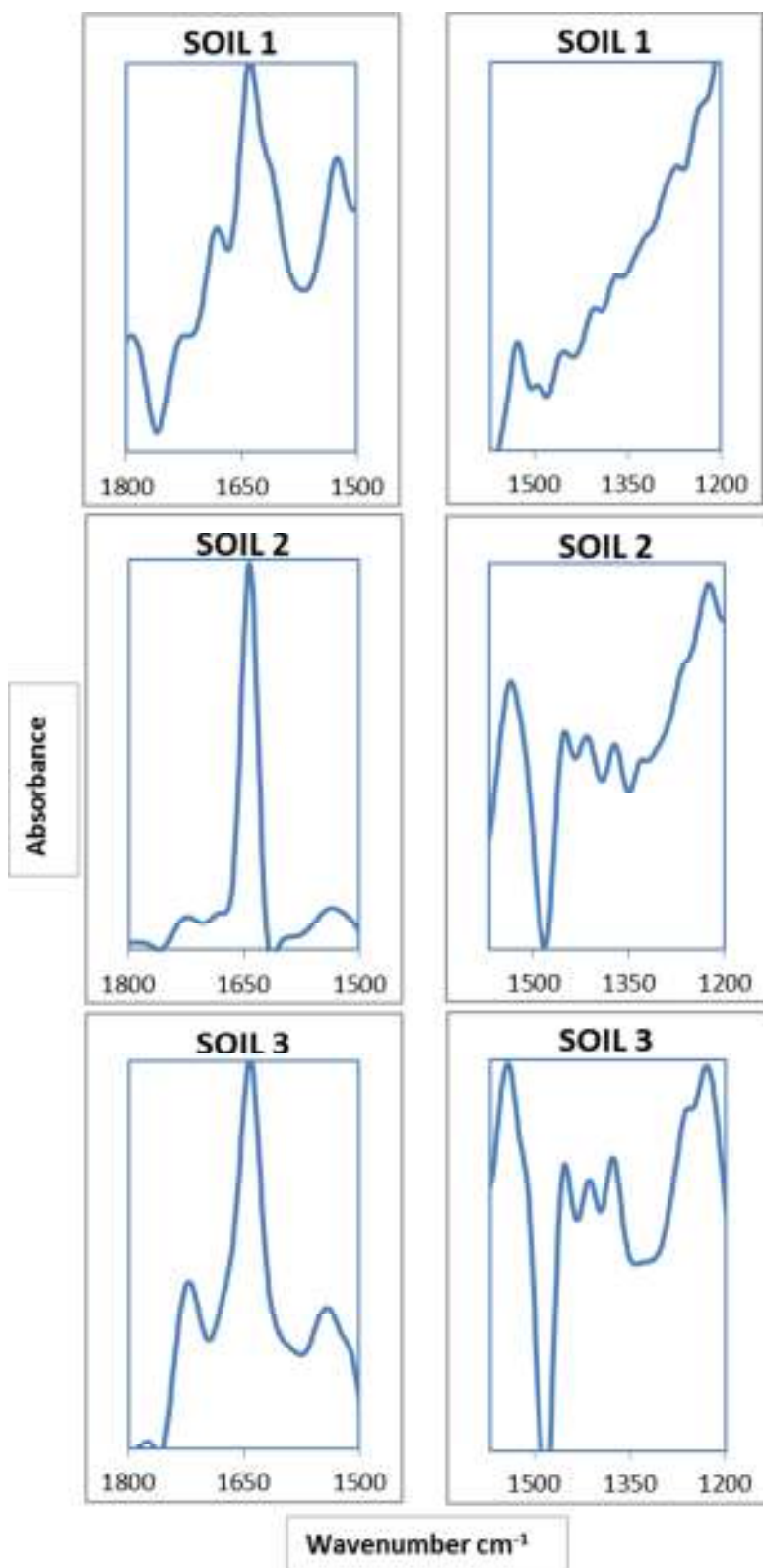


Figure SD 1. Infrared spectra of the OM of the soils under study after removing mineral matter and applying resolution enhancement techniques. Ranges 1200-1500 cm^{-1} and 1500-1800 cm^{-1} .