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C. Biache, Catherine Lorgeoux, Stéfán Colombano, A. Saada, P. Faure. Multistep thermodesorption coupled with molecular analyses as a quick, easy and environmentally friendly way to measure PAH availability in contaminated soils. *Talanta*, 2021, 228, pp.122235. 10.1016/j.talanta.2021.122235 . hal-03187704

HAL Id: hal-03187704

<https://hal.univ-lorraine.fr/hal-03187704>

Submitted on 5 Oct 2021

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Multistep thermodesorption coupled with molecular analyses as a quick, easy and environmentally friendly way to measure PAH availability in contaminated soils

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Keywords: Gas chromatography; Mass spectrometry; Flame ionization detection; Polycyclic aromatic hydrocarbon; Potassium permanganate oxidation; Microbial incubation

Abstract

Whether it is for risk assessment or for remediation purpose, contaminant availability in polluted soils is a key parameter to determine. Two methods were recently standardized for the estimation of the environmental available fraction of non-polar organics but, in some cases, their application on real historically contaminated soils does not provide satisfactory results. The present study aimed at proposing an alternative method for the estimation of PAH availability in soils, based on analytical thermal desorption and molecular analyses with the hypothesis that the binding strength between PAH and the solid matrix is linked to the desorption temperature. This hypothesis was validated by comparing the thermodesorption molecular distribution of different contaminated soils and of their respective extractable organic matter. Then, comparing the thermodesorption profiles of each studied PAH to the efficiency of biological and chemical remediation treatments through principal component analysis allowed obtaining the desorption temperature corresponding to PAH fractions available towards both treatments. This method was proven to effectively estimate the PAH fraction available towards biological (microbial incubation) and chemical (KMnO₄ oxidation) treatments and present multiple advantages such as being fast, easy to execute and solvent free.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants presenting toxic, mutagenic and carcinogenic properties. They were introduced in the priority pollutant list in the 70s [1] and are, since then, monitored in food and different environmental compartments. The monitoring of PAHs in contaminated matrixes is either for risk assessment purpose or to anticipate the efficiency of remediation treatments. However, in aged materials such as historically contaminated soils, the total (16 listed) PAH concentration is not necessarily representative of the risk inherent to these materials or of the degradable fraction. Indeed, the contaminants can be strongly bound to the matrix through different physicochemical processes resulting in the contamination ageing [2,3]. The fraction to consider in these cases is the available fraction as it represents the part of the contamination that is readily accessible for uptake or degradation by living organisms or causing adverse effects to biota [4,5]. The available fraction can also readily react with soil constituents or chemicals. Currently there is no guarantee that these two fractions are identical or if it would be relevant to make the distinction between the bioavailable and the chemoavailable fractions. Indeed, various methods exist to evaluate the bioavailable fraction but, to our knowledge, there is no study dealing with the chemoavailable fraction.

This chemoavailable fraction is particularly relevant for remediation purpose. Indeed, chemical treatments, such as *in-situ* chemical oxidation, are often used to remediate contaminated soils [6]. It is known that the efficiency of such treatments depends on contaminant (chemo) availability and this availability differs according to the oxidant [7,8]. The determination of the fraction of the contamination chemoavailable towards a specific oxidant, in a specific matrix, would then allow assessing the remediation potential of this treatment. This would be particularly pertinent for aged contaminated soils where the amount of quantified contaminants may strongly differ from the available contaminants [9].

The methods existing to predict the bioavailable fraction are classically biological methods [10] such as biosensor, biodegradation tests and worm accumulation, however they present some limitations. The restriction of biosensors is linked to the hydrophobicity/low water solubility of PAHs as the tests are performed in liquid (aqueous) media. Organic solvent can be used to enhance the PAH dissolution but (i) the dissolved PAHs would not necessarily reflect the available fraction and (ii) organic solvents usually have negative effect on the biosensor [11]. An alternative is to use microbial incubation which is a good option to assess the bioremediation potential [12,13]. For risk assessment purpose, the earthworm uptake tests are generally preferred [14]. However these biological methods are costly, time-consuming and can be difficult to set up as the survival of the organisms will depend on several parameters, including the level of exposure which, if too high, can induce a toxicity and have lethal effect on the tested organisms [15]. To overcome these limitations, chemical methods have been proposed. Among them are mild-solvent extractions using solvent and solvent mixture such as butanol, methanol or water/methanol [16,17]. Passive samplers are also used as bioavailability measurement [18,19] as they are supposed to mimic the biomembrane and measure the freely dissolved compounds without significantly affecting the soil-water or sediment-water equilibrium [20]. Other methods are based on the partitioning between sediments or soils and water with an extractant acting as infinite sink such as cyclodextrin and Tenax [21]. Cyclodextrin has hydrophilic surface and an internal hydrophobic cavity that makes the molecule fairly stable in water and able to entrap hydrophobic molecules in the cavity. Tenax is a resin that adsorbs the PAHs from the water phase. Both cyclodextrin and Tenax based methods were standardized for the determination of the environmental availability for non-polar organics [22]. Some studies comparing bioavailability measurements reported consistent results obtained with both chemical and biological methods [11,17,23] but they were performed on spiked soils or sediments and considered only a limited number of PAHs. Consequently, they were not representative of real contaminated soils and sediments that (i) are often contaminated by a wide range of PAHs and (ii) underwent ageing process that influences the behavior of PAHs during these bioavailability tests.

Other studies aiming at comparing several bioavailability determination methods on real contaminated soils and sediments showed that results were not consistent [20,24–26]. It is not surprising as the available fraction differs according to the targeted organisms or even species as they are exposed from different routes [27], so one chemical method cannot measure several bioavailabilities. However, the discrepancies in the results presented in these

studies, especially the ones confronting biological and chemical methods, underline the necessity to propose a standardized way to measure availability.

In this study we propose a new method based on thermodesorption (Td) coupled to molecular analyses to determine PAH availability in soils. Few studies explored the potential of thermal methods to detect PAHs in environmental matrices [28–30] but these methods did not enable PAH quantification. More recently, a study proposed a new method for PAH quantification using pyrolysis-gas chromatography-mass spectrometry/flame ionization detection (Py-GC-MS/FID) coupling [31]. This method consists in direct thermodesorption at 450 °C, which is a good compromise between a temperature high enough to desorb heavier PAHs or PAHs associated with matrices with high retention properties and a temperature low enough to prevent thermal cracking of the soil organic matter. In this study, a link between the aging of the contaminated soil and the ability to thermodesorb PAHs is suspected. Indeed, it can be admitted that there is a link between the availability of a compound, the strength to which this compound is retained to the matrix and the energy required to break this bond [32]. The increase in the temperature would bring higher energy capable of desorbing molecules bound with increasing strength. Therefore, it could be possible to define for a given molecule the desorption temperature corresponding to different levels of availabilities (e.g. bioavailability and chemo availability). The aim of this study was then to determine the PAH thermodesorption profiles of several contaminated soil using the Td-GC-MS/FID coupling with several desorption steps and to compare them to the efficiency of several remediation treatments (microbial incubations, chemical oxidations) in order to define the temperatures corresponding to the PAH available fractions. This work will allow (i) determining if there are several availabilities according to the treatment (biological or chemical) and (ii) proposing a method able to predict the efficiency of different treatments in one set of analysis.

Table 1: List of the analyzed PAHs with their abbreviations and the minimal and maximal thermodesorption temperatures corresponding to the low and high available fractions towards microbial incubation and permanganate oxidation treatments.

PAHs	Abbreviations	Biodegradation		KMnO ₄	
		Low	High	Low	High
Acenaphthylene	Acy	250	300	250	300
Acenaphthene	Ace	250	300	200	250
Fluorene	Flu	400	450	300	350
Phenanthrene	Phe	400	450	200	250
Anthracene	Ant	350	400	300	350
Fluoranthene	Fluo	400	450	250	300
Pyrene	Py	400	450	350	400
Benz[<i>a</i>]anthracene	B(a)A	400	450	400	450
Chrysene	Chry	350	400	300	350
Benzo[<i>b</i>]fluoranthene	B(b)F	300	350	300	350
Benzo[<i>k</i>]fluoranthene	B(k)F	350	400	350	400
Benzo[<i>a</i>]pyrene	B(a)P	350	400	400	450
Indeno[1,2,3- <i>cd</i>]pyrene	IP	200	250	300	350
Dibenzo[<i>a,h</i>]anthracene	DB(ah)A	400	450	400	450
Benzo[<i>ghi</i>]perylene	B(ghi)P	100	150	400	450

2. Materials and methods

2.1. Soil samples

Soils were collected in (i) three former coking plants, Cok.P-1 (Neuves-Maisons, France), Cok.P-2 and Cok.P-4 (Homécourt, France) and Cok.P-3 in confidential site (Lorraine Region – France), (ii) one former gas plant: GasP (Rennes – France) and (iii) one active wood- treating facility: WoodTF (Midi-Pyrénées Region – France). After sampling, soils were stored at – 18 °C before being freeze-dried. Samples were then sieved (2 mm) and the undersize was crushed (<500 µm). After homogenization, a sub-fraction of the crushed samples was grinded more finely for Td

analyses (<100 µm). Indeed, as shown in our previous study [31], grinding the sample to such particle size allow to greatly decrease the variability in the measure.

2.2. Thermodesorption (Td) analyses

Thermodesorption were carried out on a Frontier Lab Multi-shot pyrolyzer EGA/PY-3030D and a Micro Jet Cryo-Trap MJT-1030Ex installed on an Agilent GC-FID 7890B coupled with a 5977A MS detector. The GC was equipped with an Agilent DB-5MS column (20 m × 0.18 mm i.d. × 0.18 µm film thickness). The carrier gas was helium at a constant flow of 1.6 mL/min. Deactivated fused silica columns connected both detectors to a dean switch allowing the column flow split between the detectors with a FID/MS flow separation of 1/3. The pulsed split mode was used with pulsed pressure of 50 psi until 0.5 min and a split ratio of 20:1. The inlet and transfer line temperatures were set at 320 and 340 °C, respectively and the MS quadrupole and ion source were set at 150 and 230 °C, respectively. For all analyses the MS was used in the combined selected ion monitoring (SIM)/full scan mode (Table S1). The FID temperature was set at 320 °C and the air, hydrogen and makeup (He) flows were 400 mL/min, 30 mL/min and 25 mL/min, respectively. The FID was calibrated for the quantification of 15 PAHs (Table 1) according to Biache et al. [31]. Briefly, solvent-washed pieces of glass fiber filter were placed into stainless steel cups and impregnated with 1 µL of calibration solutions at nine concentrations ranging from 0.5 to 100 µg/mL. The cups were then inserted in the Td oven set at 450 °C. The temperature of the pyrolyzer interface was maintained at 320 °C. Compounds were concentrated at the head of the column thanks to a cryo trap held at - 180 °C during 5 min before the beginning of the GC analysis according to the following oven temperature program: 1 min at 40 °C, from 40 to 195 °C at 25 °C/min, a hold-time of 2 min at 195 °C, from 195 to 315 °C at 20 °C/min, from 315 to 320 °C at 5 °C/min followed by a hold-time of 3 min at 320 °C.

For the soil Td analyses, samples (0.2–5 mg according to the PAH content) were placed into stainless steel cups which were inserted into the Td oven. To obtain a reference thermodesorption profile corresponding to the maximum available fraction, the extractable organic matter (EOM) of the untreated soil were analyzed as well by impregnating glass fiber filters placed in stainless steel cups with 1–4 µL of EOM. Samples were then heated according to seven successive 50°C-Td steps from 100 to 450 °C (i.e. from 100 to 150 °C, then from 150 to 200 °C, then from 200 to 250 °C, and so on) at 10 °C/min.

As discussed in a previous study [31], the selection of the maximum temperature was done in order to allow the vaporization of higher boiling point compounds but to avoid thermal cracking and aromatization reactions. Maximal temperature of 450 °C happened to be a good compromise. Even if 450 °C is consistent with pyrolysis, the aim of using such temperature was not to induce thermal cracking but the desorption of HMW PAH, that is why the term for the used technique is referred as “thermodesorption” throughout this paper.

The cryo trap was maintained at - 180 °C during the Td steps, which were each followed by GC analysis runs according to the parameters described above. Finally, seven chromatograms were generated for each sample, each chromatogram corresponding to a 50 °C-temperature range. Td were done in triplicate for each soil sample.

Compound identification was confirmed by the mass spectra obtained with the MS detector in comparison with the NIST08 and Wiley database spectra, and calibrated PAHs were quantified on the FID response.

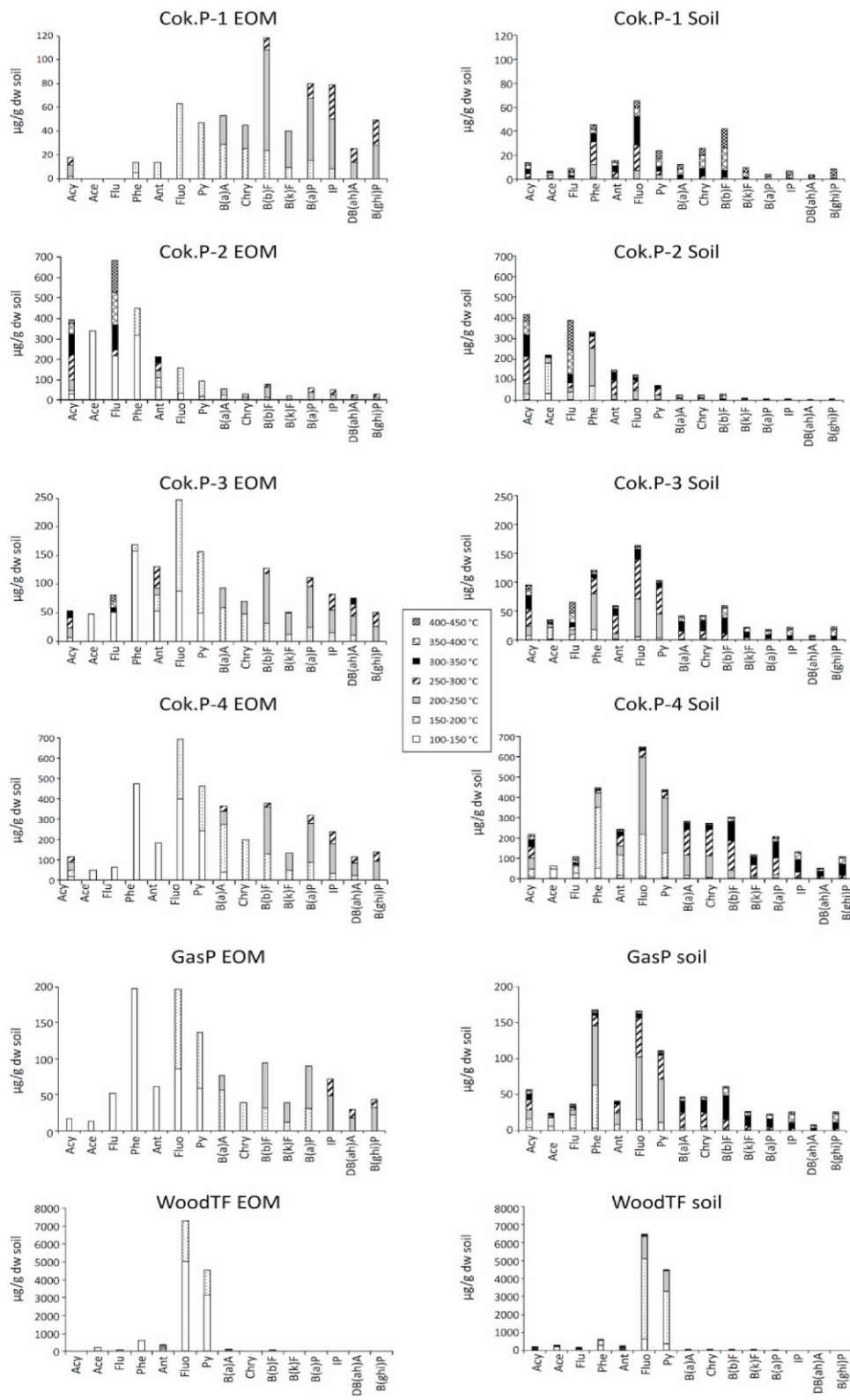


Fig. 1: Thermodesorption profiles (i.e. cumulated PAHs quantified during the seven 50°C-thermodesorption steps from 100 °C to 450 °C) for the six studied soil samples and their extractable organic matter (EOM). See Table 1 for PAH abbreviation meanings.

2.3. $KMnO_4$ oxidation

Procedures for chemical oxidation were detailed in previous studies [7,33]. Briefly, $KMnO_4$ treatments were applied on sieved and crushed (<500 μm) samples in order to reach at least twice the stoichiometric oxidant demand (SOD) based on the extractable organic matter (EOM) content according to Ranc et al. [34]. The following amounts of $KMnO_4$ were used for 1 g of soil: 0.3340 g for Cok-P-1, 0.4451 for Cok.P-3, 3.112 for Cok-P4, 0.3340 g for Gas-P and 7.4300 g for WoodTF. Samples were agitated (200 rpm) in the dark for one week.

2.4. Bioremediation experiment

Bioslurry experiments were described in detail in previous studies [35,36]. Briefly, inocula obtained from the "fresh" WoodTF, Cok.P-1, Cok.P-2 and GasP soils were added to their respective freeze-dried and crushed (<500 μm) soils with a nutritive solution (Bushnell Hass). The flasks containing the slurry were placed in an incubation chamber at 28 °C in the dark under continuous stirring (120 rpm). Samples were collected after 5 months (Cok.P-1 soil, GasP and WoodTF soils) and 9 months of experiment (Cok.P-2 soil) and were freeze-dried before analysis.

2.5. Extraction of OM and PAH quantification

In order to determine the efficiency of the oxidations and bioremediation experiments, PAHs were quantified before and after treatments.

First, EOM was obtained by extracting 1.5–2 g of dw soil using an accelerated solvent extractor (ASE 350, Dionex) with dichloromethane (130 °C, 100 bars, 10 min). Molecular sulfur and residual water were removed by adding to the extraction cells activated copper powder (2 g) and sodium sulfate (2 g), respectively. The extract volume was adjusted to 20 mL in volumetric flask after solvent evaporation under a gentle nitrogen stream. An aliquot (3 mL) was transferred into pre-weighed vials. The EOM content was determined by weighing the vial after solvent evaporation to dryness.

Quantification of 15 regulatory PAHs was reported in previous studies [7,35]. Briefly, it was carried out using internal calibration. An internal PAH standard mix of [2H_8]naphthalene, [$^2H_{10}$]acenaphthene, [$^2H_{10}$]phenanthrene, [$^2H_{12}$]chrysene, [$^2H_{12}$]perylene, (Cluzeau®, 20 μL at 16 $\mu g/mL$) was added to 80 μL of the EOM before being injected in a gas chromatograph coupled with a mass spectrometer (GC-MS). For each quantified compound, internal calibration curve was drawn with six concentrations (0.3, 0.9, 1.5, 3, 6 and 9 $\mu g/mL$). Two GC-MS instruments were used: Shimadzu system for samples from bioslurry experiment and Agilent system for samples from $KMnO_4$ oxidation. Untreated samples were analyzed with both systems. The gas chromatographs (GC) were a Shimadzu GC-2010 plus and an Agilent 6890, equipped with a capillary column in silica glass DB-5MS (60 m \times 0.25 mm i.d. \times 0.1 μm film thickness and 60 m \times 0.25 mm i. d. \times 0.25 μm film thickness, respectively) coupled to a QP2010-Ultra (Shimadzu) electronic ionization mass spectrometer (MS) and an Agilent 5973 Inert Mass Selective Detector, respectively, operating at 70 eV. The Shimadzu system operated in alternated fullscan/selected ion monitoring mode and the Agilent system operated in fullscan mode, and both had their transfer line heated at 300 °C. The oven temperature program was as follow: 70 °C for 2 min, from 70 to 130 °C at 15 °C/min, then from 130 to 315 °C at 3 °C/min and then a 15 min hold at 315 °C. The carrier gas was helium at 1.4 mL/min constant flow. Injections were done with one microliter in split mode with a split ratio of 1:5 for the Shimadzu and in splitless mode for the Agilent system.

2.6. Statistical analyses

The PAH concentrations obtained during the thermodesorption steps and degraded by the remediation treatments were subjected to principal component analyses (PCA) to investigate their relationships and similarities. PCA calculates new variables or principal components, which are linear combinations of the original variables accounting for as much of the variance of the original data as possible [37].

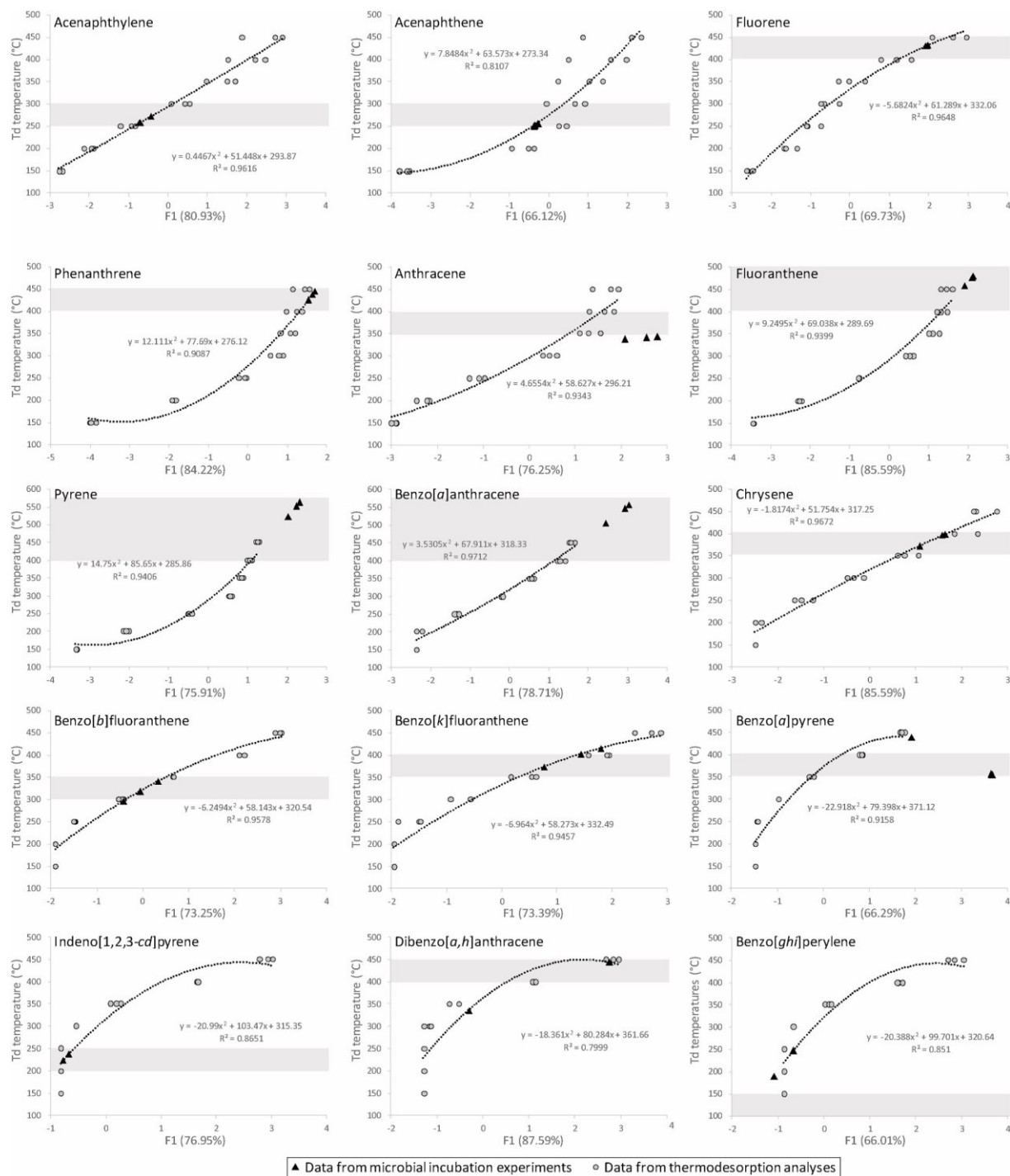


Fig. 2: Plot of the desorption temperature as a function of the F1 coordinate of the principal component analysis for the 15 PAHs degraded during microbial incubation. The grey areas have been added to facilitate the reading of the desorption temperature of the incubated samples.

Principal component analysis was realized using the software XLStat (Addinsoft, 2018) with the PAH concentrations obtained during the different thermodesorption steps and the amount of PAHs degraded during the microbial incubation and chemical treatment. The PCA was performed using a covariance matrix. A PCA was obtained for each of the studied 15 PAHs, the different soil samples constituted the variables and the thermodesorbed concentrations and amount of PAHs degraded during the remediation treatments were the individuals used for the PCA.

3. Results and discussion

3.1. Are the thermodesorption profiles dependent on the solid matrix?

In order to confirm that the PAH desorption temperatures are related to the bond between the compound and the matrix, thermodesorption profiles of the studied soils were compared to their corresponding EOM from which PAHs are classically quantified (Fig. 1). For both types of sample, PAHs with higher boiling points (and molecular weights) are desorbed at higher temperatures (boiling points of the studied PAHs are reported in Table S1). For the EOM, thermodesorption profiles were similar for the studied samples. Most of the PAHs were desorbed at 150–200 °C and 250 °C for the PAHs with molecular weights up to 202 g/mol and higher than 228 g/mol, respectively. These desorption temperatures were comparable to those obtained with a standard PAH mixture (i.e. solution used for calibration; Figure S1). These observations seem to indicate that whatever the initial soil composition, the PAH desorption temperatures from the EOM are similar. However, Cok.P-2 and Cok.P-3 EOM thermodesorption profiles showed some particularities as low molecular weight (LMW) PAH kept being released at higher temperatures, especially for Cok.P-2. This behavior could indicate a cracking of heavy compounds/macromolecules. This hypothesis tends to be supported by the fact that Cok.P-2 EOM present a high proportion of asphaltenes (69%) compared to the other samples (from 25 to 43%) that could be responsible for the LMW PAH release at high temperature (Table S2) due to thermal cracking. This assumption is confirmed by the fact that the amounts of these LMW PAHs were higher for EOM Td analysis than for classical EOM GC-MS analysis (Table S3).

3.2. Are the PAH thermodesorption profiles indicators of the contaminant status?

Differences between soil behaviors were observed with contrasted profiles obtained with successive thermodesorptions (Fig. 1). In some cases (e.g. GasP, WoodTF, Cok.P-4), PAHs were mostly desorbed between 100 and 250 °C, and between 250 and 350 °C for LMW and high molecular weight (HMW) compounds, respectively. In other cases (e.g. Cok.P-1, Cok. P-2, Cok.P-3), LMW PAHs were desorbed mostly between 200 and 350 °C and HMW PAHs between 300 and 450 °C. These differences in thermodesorption temperatures can be linked to PAH status, i.e. their availability. Indeed, availability level can be associated with the level of energy necessary to break the bond between the PAH and the matrix and therefore to release it, and this energy can be brought by increasing temperature.

The studied soils are known to exhibit contrasted availability levels as they responded differently to various remediation treatments. The contrasted thermodesorption profile can then be linked to the contrasted response to treatments according to the soil sample. For instance, when applied to Cok.P-1, bioremediation treatment [35] and Fenton-like oxidation [7,8,38] were not effective to remove PAH contamination as they resulted in low PAH degradation in both cases (34% and 0% of PAH degradation, respectively). On the contrary, PAHs from GasP were easily degraded by microbial incubation (76% PAH degradation) and Fenton-like oxidation (78% PAH degradation). From these studies, it can be established that the response of the soil contamination towards the remediation treatment also depends on the type of treatment applied. Indeed, for the same soil, different treatments lead to different levels of contamination degradation. For instance, application of potassium permanganate to Cok.P-1 soil lead to a PAH degradation of 52% whereas no PAH decrease was observed with Fenton-like treatment [7]. Therefore, the fraction of the contaminants available for potassium permanganate oxidation is much more important than the fraction available for Fenton-like treatment. Consequently, there are as many availabilities as remediation treatments; in this example we can consider the fraction available for microbial degradation, the fraction available for Fenton-like oxidation and the fraction available for potassium permanganate. The soil showing lower PAH desorption temperature (i.e. gas plant soil) also shows higher contaminant availability towards the considered treatments.

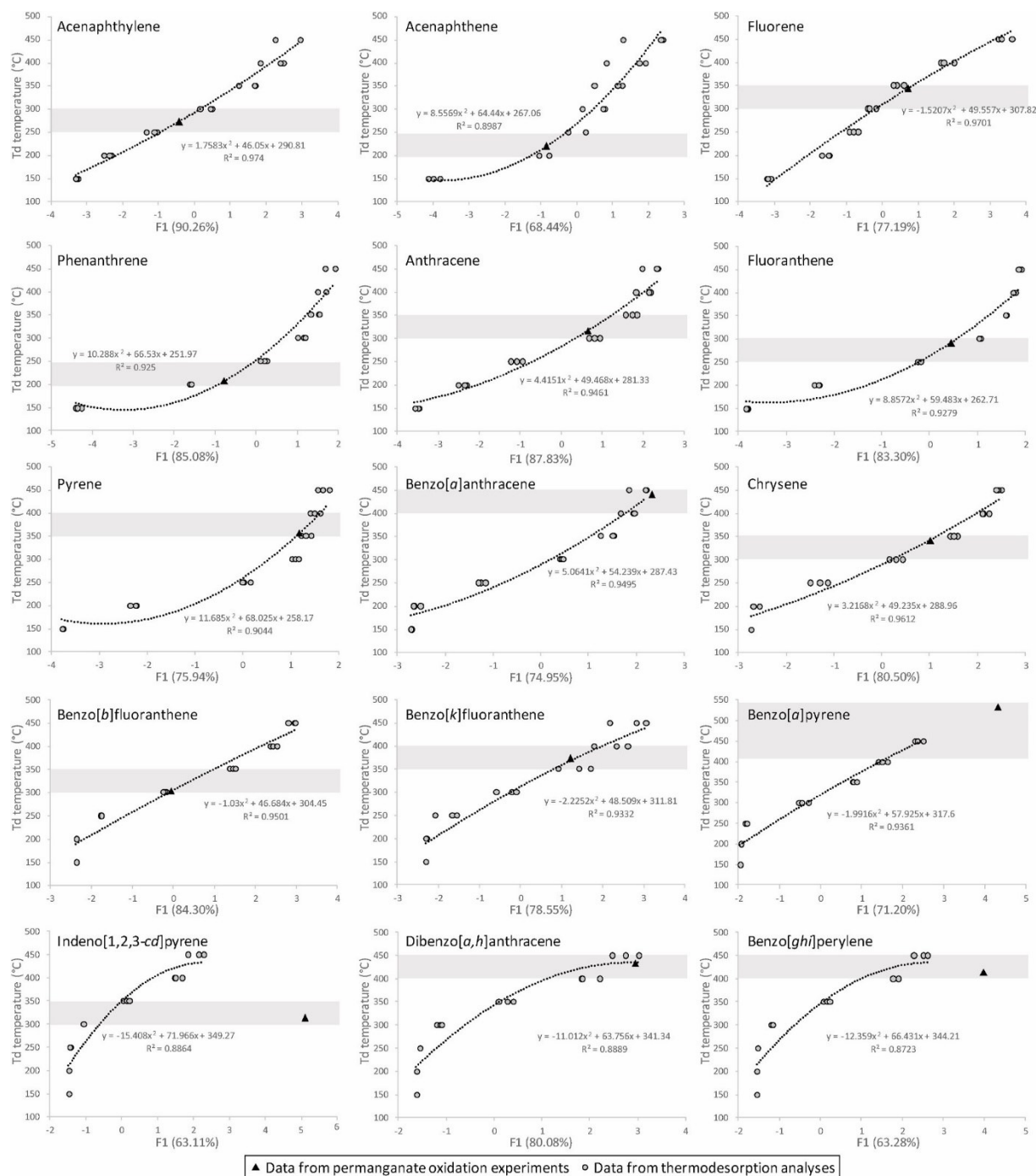


Fig. 3: Plot of the desorption temperature as a function of the F1 coordinate of the principal component analysis for the 15 PAHs degraded during $KMnO_4$ oxidation. The grey areas have been added to facilitate the reading of the desorption temperature of the oxidized samples.

3.3. Determination of the desorption temperatures corresponding to the available fractions

Principal component analyses were done for each compound and each treatment to compare the cumulated PAH concentrations obtained during the thermodesorption steps and the PAHs actually degraded during the treatments in order to determine the desorption temperature leading to the release of the bio- and chemoavailable PAHs. For all PCAs the first two components explained more than 90% of the total variance (Table S4), with the first component (F1) contributing for more than 77% on average. A regression analysis was performed after plotting the desorption temperatures as a function of F1 coordinates of the thermodesorption individuals (Figs. 2 and 3) and the curve

equation with the determination coefficient determined. The curve equation was then used to calculate the desorption temperature corresponding to the F1 coordinates of the individuals from the remediation experiments. In other words, for each PAH and each treatment, the x variable of the equation was replaced by the F1 coordinates of the remediation treatment in order to obtain the desorption temperature corresponding to the degraded fraction of the PAH during the remediation treatments (Table 1). As thermodesorptions were performed according to 50 °C-steps, desorption temperatures corresponding to the available fractions were given as 50 °C-windows which will be further referred as low and high availability estimations (Table 1).

Overall, the temperatures corresponding to the bioavailability estimation were higher than those of the chemoavailability estimation for LMW compounds, and lower for HMW compounds. It is consistent with the fact that (i) microorganisms degrade more easily LMW compounds than HMW compounds [39] and (ii) KMnO_4 degrade non-selectively LMW and HMW compounds [7].

3.4. Method application

The different availabilities were determined for the six studied soils according to the temperature ranges presented in Table 1 and compared to the PAH amounts actually degraded by biological (microbial incubation) (Fig. 4) and chemical (KMnO_4 oxidations) treatments (Fig. 5).

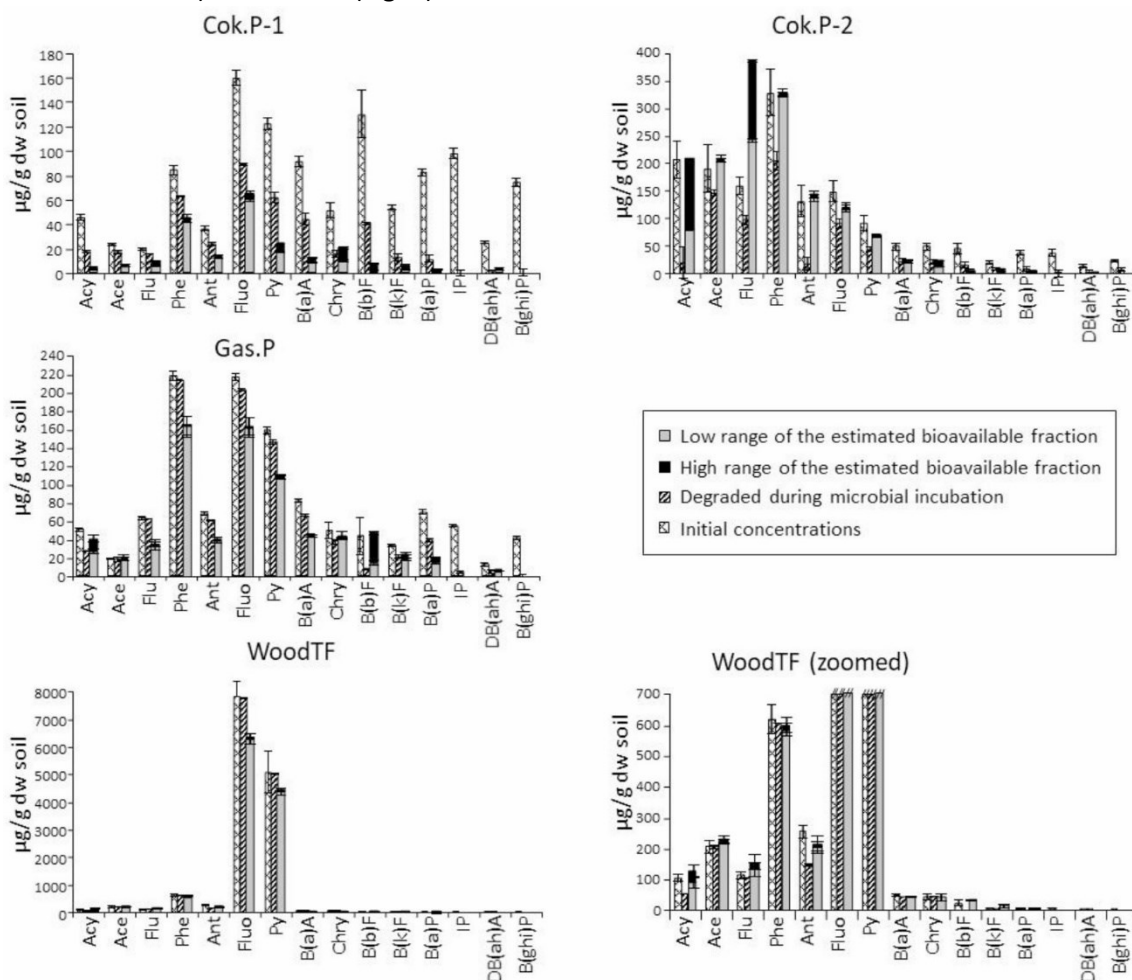


Fig. 4. Bioavailable PAHs estimated by thermodesorption compared to PAHs quantified in the initial samples and the actually degraded PAHs during microbial incubation for Cok.P-1, Cok.P-2, GasP and WoodTF soil samples. Available fractions are reported as ranges with low (in grey) and high (in black) estimations obtained with the thermodesorption temperatures reported in Table 1. See Table 1 for abbreviation meanings. The error bars represent the SD ($n = 3$).

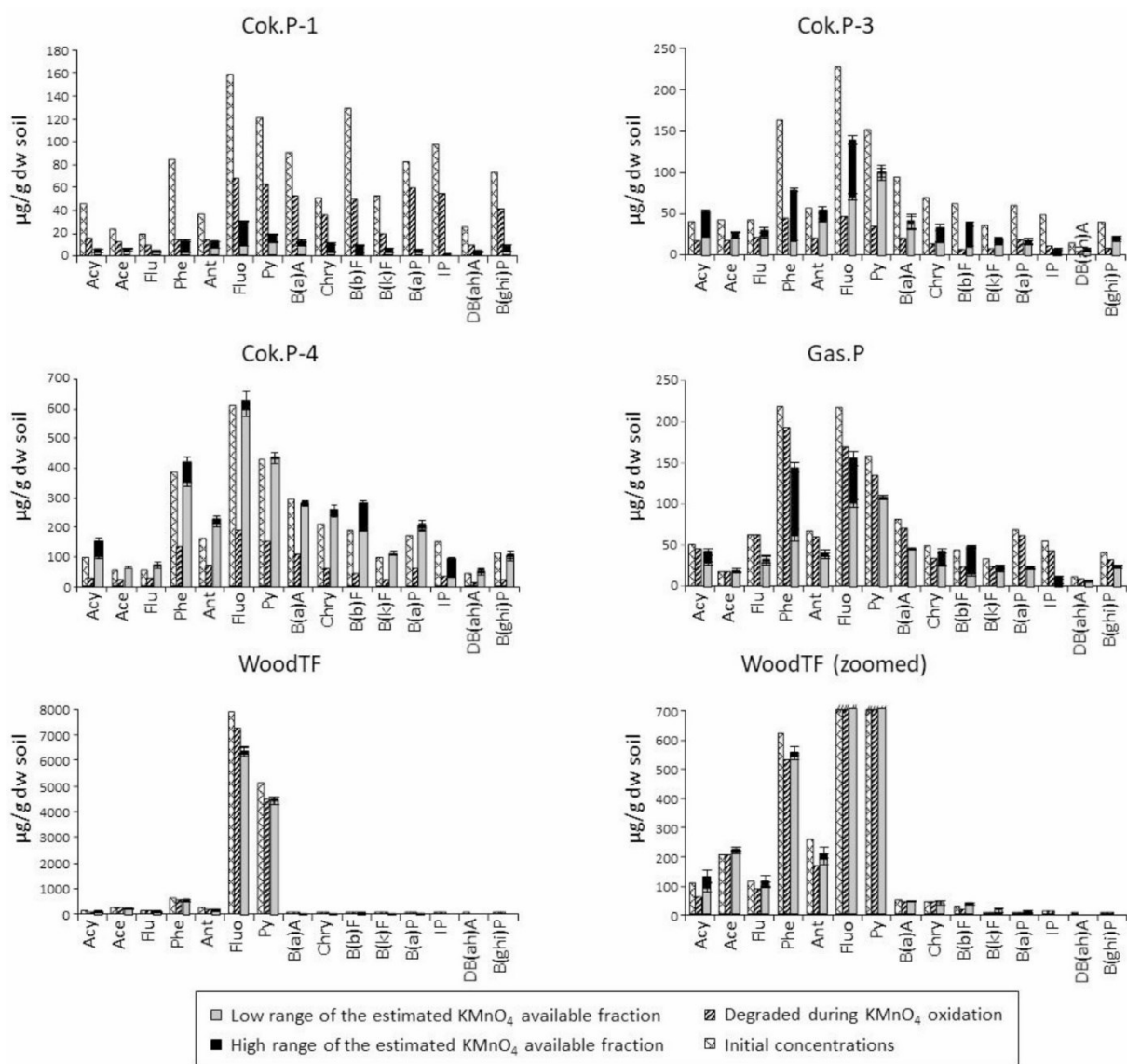


Fig. 5: Chemoavailable (KMnO_4 oxidation) PAHs estimated by thermodesorption compared to the initial samples and the actually degraded PAHs during microbial incubation for Cok.P-1, Cok.P-3, Cok.P-4, GasP and WoodTF soil samples. Available fractions are reported as ranges with low (in grey) and high (in black) estimations obtained with the thermodesorption temperatures reported in Table 1. See Table 1 for abbreviation meanings. The error bars represent the SD ($n = 3$).

Overall the thermodesorption method gave satisfactory results for the estimation of PAH availability. Indeed, using the high availability range values, the Td methods gave a good estimation, i.e. 86% and 88%, of what was actually degraded during microbial incubation of the GasP and WoodTF soils, respectively. In the same way, Td method estimation for KMnO_4 treatment efficiency reach 80% and 95% for the same samples, with the high availability estimation.

The low availability estimation fitted better with the chemically degraded and biodegraded PAHs for Cok.P-3 (146% of the degraded fraction) and Cok.P-2 soils (179% of the degraded fraction), respectively. The overestimation of the Cok.P-2 bioavailability is mainly due to high values obtained for LMW compounds, probably due to a contribution of thermal degradation of asphaltenes and heavier fractions. Indeed, as previously mentioned, this sample exhibit a high content of asphaltenes (69% of the EOM). When considering PAHs with ring number of four or higher, the estimation satisfactorily reached 112% of the biodegraded fraction. For the Cok.P-4 sample, the fraction available for KMnO_4 oxidation was overestimated as less PAHs were degraded by KMnO_4 oxidation than those predicted by the Td method, even when considering the low availability estimation.

Using the Td method to predict the chemo- and bioavailable PAHs of Cok.P-1 led to an underestimation of this fraction in both cases. During the validation of a method for PAH quantification by flash-thermodesorption (450 °C) coupled with molecular analysis [31], issues were already encountered with this soil. Indeed, the PAHs quantified by flash-Td were lower than the PAHs quantified using traditional method (solvent extraction and GC-MS analyses). As already mentioned, Cok.P-1 soil is an aged contaminated soil and is known to present low PAH availability [7–9,35,38,40,41] probably because of strong PAH-matrix bonds resulting in limited PAH thermodesorption, even at 450 °C. Because of its persistence, KMnO_4 can penetrate deeper into the soil particles [42], access and degrade PAHs that cannot be released with Td, even at 450 °C. The high level of PAH sequestration can also affect the bioavailability estimation. However, in previous studies bioavailability measurements were carried out on Cok.P-1 using both standardized chemical methods (i.e. Tenax and cyclodextrin extractions) Cebon et al. [40] reported a value of 12 mg/kg for the sum of 15 PAHs (the listed PAHs without the naphthalene) obtained after Tenax extraction. In the same way, Ouvrard et al. [9] measured a PAH available fraction (sum of the 16 listed PAHs) of 16 mg/kg using also Tenax extraction. Finally, Bourceret et al. [43] estimated the PAH bioavailable fraction using cyclodextrin extraction and obtained a value of 40 mg/kg for the sum of 15 PAHs (listed PAHs without the naphthalene). With the Td method, the amount of bioavailable PAHs (16 listed PAHs without naphthalene) was estimated at 165 and 217 mg/kg, for low and high availability, respectively. If these values remained lower than the actually biodegraded PAHs (413 mg/kg), they are still more realistic than the one obtained with Tenax and cyclodextrin that represented only 3% and 10% of biodegraded PAHs, respectively.

4. Conclusion

A new method, based on Td coupled with molecular analyses, was proposed to estimate the availability of PAHs in several contaminated soils towards chemical (KMnO_4) and biological (microbial incubation) treatments. As mentioned and already demonstrated in previous studies (aforesaid references), the efficiency of chemical (KMnO_4 oxidation) and biological (microbial incubation) remediation treatment differs. Consequently, a distinction can be made between the bio – and the chemo-availabilities.

The comparison between the chemo-available fraction for permanganate potassium treatments determined by Td and the PAH fractions actually degraded during these treatments showed a good match except for two soils. In one case, Cok.P-4, the estimated available fractions were higher than the degraded fraction. For the other sample, Cok.P-1, the chemoavailability measurements underestimated the fraction really degraded, and it was also the case for the bioavailability estimation performed on this soil. PAHs in this soil are probably highly sequestered and the maximum desorption temperature was not able to release them from the matrix, leading to this underestimation. However, comparison with bioavailability measurements performed with other methods (cyclodextrin and Tenax extractions) showed that even if the Td method underestimated the available fraction, the obtained results remained much closer to reality than the one obtained using “traditional” methods. For the other tested soils, the estimation of bioavailable PAHs by thermodesorption matched the amount of biodegraded PAHs. From these results we can conclude that this method was proven satisfactory for the estimation of PAH bio- and chemo-availability. It could then be used (i) prior the application of remediation treatments in order to determine the best way to manage the contamination, (ii) during the application of remediation treatment to monitor the degradation of available PAHs, and (iii) after remediation to ensure that the degradation of the available fraction is complete and/or the applied dose of oxidant was sufficient to degrade it.

The main advantages of this method are its quickness as it takes less than 4 h to obtain a thermodesorption profile, and the fact that one set of analysis allows to estimate at the same time the chemo- and the bio- availabilities. Another benefit is the fact that this method is environment-friendly since it is solvent free.

In this study, even if the concentration range varied from 917 to 13000 $\mu\text{g/g}$ of 15 PAHs (16 listed PAHs without naphthalene – Table S3), these soils present relatively high contamination level. It would be now interesting to test our method on less impacted soils and to other contaminated matrixes such as sediments. It is also planned to broaden the comparison with other methods classically used to determine the available fraction on a wider range of

soils. As this method is adapted even to compounds with high boiling points such as HMW PAHs (e.g. dibenzo[*a,h*]anthracene boiling point = 797 °C [44]), we can also consider extending and adapting this method to other organic contaminants such as petroleum hydrocarbons.

Acknowledgements

This study was funded by the Lorraine Energy and Environment Carnot Institute (ICEEL) and the French Geological Survey (BRGM) (Grant numbers: 1.12Action54OTELoMACRODISP and CR213/233- BFC30033895, respectively). This work is included in the scientific program of the GISFI research consortium dedicated to the knowledge and the development on remediation technologies for degraded and polluted lands (Groupement d'Intérêt Scientifique sur les Friches Industrielles - <http://www.gisfi.univ-lorraine.fr>). We also thank Dr Marc Offroy for his expertise and valuable advises on multivariate analysis and Axel Bart from SRA for technical support.

Appendix A. Supplementary data

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