



**Estimation of hydrocarbon sequestration in soils: Influence of the chemical characteristics of humic substances**

**Estimación de secuestro de hidrocarburos en suelos: Influencia de las características químicas de las sustancias húmicas**

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**Abstract**

Soil organic matter (SOM) is a key factor controlling the fate of hydrocarbons in soils; however, there are no standardized methods to accurately estimate their sequestration. This study proposes an accessible analytical approach to estimate the capacity of soils to sequester hydrocarbons using the chemical characteristics of humic acids in SOM, saving time/costs by avoiding the extraction and analysis of hydrocarbons. A natural organic soil was modified by thermal treatments, obtaining five different soils. Their SOM was fractionated into humic (HA) and fulvic (FA) acids, which were characterized by UV-Vis and FTIR. The soils were contaminated with hydrocarbons (hexadecane [HXD], phenanthrene [PHE], and pyrene [PYR]), and the sequestered concentration of each hydrocarbon was quantified. The soil treated at 350 °C presented the highest capacity to sequester PHE and PYR (>100 mg·[kg soil]<sup>-1</sup>). The characteristics that significantly improved the sequestration of PHE and PYR were: i) FA of low molecular weight and degree of condensation, and ii) HA with high molecular weight, aromaticity, aliphaticity, polarity, and degree of condensation. Based the HA analysis by UV-Vis and FTIR, two equations are proposed to estimate - with > 80% accuracy - the sequestration of PHE and PYR in soils with different characteristics in their SOM.

*Keywords:* hydrocarbon sequestration, soil organic matter, humic acids, spectrophotometry.

**Resumen**

La materia orgánica (SOM) define el destino de los hidrocarburos en suelos; sin embargo, no hay métodos estandarizados para estimar su secuestro. Este estudio propone una herramienta analítica accesible para estimar la capacidad de un suelo para secuestrar hidrocarburos, con base en las características químicas de los ácidos húmicos de la SOM, reduciendo tiempo/costos al evitar la extracción y análisis de hidrocarburos. Un suelo orgánico natural se modificó térmicamente para obtener cinco suelos. La SOM de cada uno se fraccionó en ácidos húmicos (HA) y fúlvicos (FA) y cada fracción se caracterizó por UV-Vis y FTIR. Los suelos se contaminaron con hidrocarburos (hexadecano, fenantreno y pireno) y se cuantificó el secuestro de cada hidrocarburo. El suelo tratado a 350 °C presentó la mayor capacidad para secuestrar PHE y PYR (>100 mg·[kg suelo]<sup>-1</sup>). Las características que aumentaron el secuestro fueron: i) FA con bajo peso molecular y grado de condensación y ii) HA con alto peso molecular, aromaticidad, alifaticidad, polaridad y grado de condensación. Con base en el análisis de HA por UV-Vis y FTIR, se proponen dos ecuaciones para estimar (>80% de precisión) el secuestro de PHE y PYR en suelos con diferentes características en la SOM.

*Palabras clave:* secuestro de hidrocarburos, materia orgánica del suelo, ácidos húmicos, espectrofotometría.

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## 1 Introduction

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Soils are considered a sink for hydrophobic organic pollutants, such as hydrocarbons, whose fate in the environment depends mainly on the type of pollutant and the components and characteristics of the soil. High levels of hydrocarbons have been found in organic and humified soils compared to those levels found in mineral soils (Obrist *et al.*, 2015). Most organic soils are formed in peatlands (Mokma, 2005), improving water quality, carbon sequestration, and the regulation of biogeochemical cycles (Kingsford *et al.*, 2016). The main characteristic of this type of soils is their high content of organic matter, reaching more than 20% (FAO, 2014). Soil organic matter (SOM) includes all soil organic materials, and is a determining factor in the mobility, availability, and persistence of hydrocarbons (Pignatello and Xing, 1996; Ukalska-Jaruga and Smreczak, 2020). SOM is a heterogeneous mixture of compounds of plant and animal origin at various stages of decomposition, and of humic substances (HS) with specific properties and unknown structural formula (Tan, 2011; Ukalska-Jaruga and Smreczak, 2020). Humic substances are considered to be supramolecules, which are associations of heterogeneous molecules held together by weak linkages or hydrophobic interactions (Nebbioso and Piccolo, 2011). The structure of HS is based on aliphatic chains and aromatic rings containing various functional groups responsible for their chemical reactivity. The molecular size, chemical structure, redox-active functional groups, aromatic domains, and hydrophobicity of each fraction of HS together determine their affinity for trapping contaminants (Ukalska-Jaruga and Smreczak, 2020). They are classified into three main fractions, which are characterized by their different physicochemical properties, structures, and sorption affinities: i) humins (HU), ii) humic acids (HA), and iii) fulvic acids (FA) (Aiken *et al.*, 1985). Humic substances differ in their molecular weights, with HU being the heaviest, and FA the lightest. Likewise, FA and HA are relatively soluble in aqueous solutions while HU are insoluble in water (Tan, 2011).

In addition to SOM, soils contain inorganic (clays and quartz) and biological components as well as pores filled with air and water, making it difficult to elucidate the interactions between their components and exogenous compounds such as hydrocarbons. When hydrocarbons are in contact with the soil matrix,

they can either: i) be removed by physical, chemical, or biological processes; or ii) be irreversibly sorbed into SOM, mineral surfaces, or the complexes between them (Li *et al.*, 2008; Luthy *et al.*, 1997; Okere and Semple, 2011). Thus, the removal of hydrocarbons from soil depends on the predominantly occurring interactions, which according to their extractability are classified as: i) dissolved, ii) weakly adsorbed, iii) slowly desorbed, and iv) strongly bound (Umeh *et al.*, 2017). Hydrocarbons strongly bound to the soil matrix, known as residual, non-extractable or sequestered, are under limited processes of diffusion, adsorption, and partition (Luthy *et al.*, 1997). Sequestered hydrocarbons cannot be fully extracted or quantified (Kuppusamy *et al.*, 2017; Maletić *et al.*, 2019) even using exhaustive extraction techniques (Umeh *et al.*, 2018a). Sequestered contaminants in soil can be quantified only when using isotope labeling compounds (Loeffler *et al.*, 2020). Therefore, the traditional risk assessment does not consider sequestered hydrocarbons; instead, it focuses on total extractable concentrations (Umeh *et al.*, 2018b), thus underestimating the actual concentration in the soil. Sequestered hydrocarbons modify the physicochemical characteristics of soils—such as pH, texture, field capacity (FC), cation exchange capacity (CEC), and porosity—affecting their fertility and the occurrence of several processes such as those involved in biogeochemical cycles (Martínez-Prado *et al.*, 2014).

Multiple attempts have been made to relate the sequestration of hydrocarbons in different soils with the content of SOM (Nam *et al.*, 1998), the qualitative characteristics of the HS contained in the SOM (Sun *et al.*, 2010), and the type and quantity of clays (Spasojević *et al.*, 2018). In particular, the relationship between SOM content and the ability of soil to sequester hydrocarbons varies substantially depending on the study. It has been recently reported that the persistence and availability of these pollutants in soil depend more on the composition of the SOM fractions than on their total content (Ukalska-Jaruga and Smreczak, 2020). Therefore, studies focused on hydrocarbon sequestration and its relationship with the chemical characteristics of HS in the SOM are key to determining the persistence and the right selection of a suitable remediation technique (Jonsson *et al.*, 2007; Rivas, 2006; Schäffer *et al.*, 2018). However, there are no standardized methods to quantify the concentration of hydrocarbons sequestered in soil up to this moment. To overcome this, we hypothesized that the capacity of a soil to sequester hydrocarbons can be

estimated through the chemical characteristics of HS, which could be estimated using spectroscopic and spectrophotometric techniques. Thus, this study aimed to propose an accessible analytical approach that allows estimating the capacity of a soil to sequester hydrocarbons based on the chemical characteristics of HS in the SOM. For such a purpose, the main methods and criteria used to obtain soil samples with different proportions of SOM are described, as well as the methods to quantify and characterize their humic fractions and relate them with hydrocarbon sequestration. Correlation analyses were used as a criterion to propose two equations to estimate the sequestration capacity of HXD, PYR and PHE by the studied soil as a function of the chemical characteristics of HA. The accuracy and applicability of the proposed equations to estimate the sequestration of polycyclic aromatic hydrocarbons (PAHs) other than PYR and PHE, and in soils with different characteristics are discussed.

## 2 Methods and materials

### 2.1 Soil sampling and characterization

The sampling site was chosen within the wetland of Pantanos de Centla Biosphere Reserve (PCBR) in Tabasco, Mexico (N 18° 29' 30.9", W 92° 38' 43.8"). This site was chosen due to the high content of SOM in the soil (> 20%) thus allowing to generate samples with decreasing SOM content. With the purpose to obtain a representative sample of the soil in the site, according to the Mexican Standards (NOM-021-SEMARNAT 2000) and considering the sampled area, a composite sample (30 kg) was obtained by mixing the five subsamples (blocks of 30 x 30 x 30 cm<sup>3</sup>) collected from horizon A (topsoil: first 30 cm). The sample was air dried and roots and large particles were removed; then, it was sieved (2 mm) and analyzed. This soil (Soil 1) was used to obtain four modified soils and was analyzed physicochemically by quantifying the SOM content, pH, soil texture, and CEC.

The SOM content in Soil 1 was determined by loss on ignition (LOI), which is appropriate for soils with a high content of organic matter (Agus *et al.*, 2011). For this, 25 g (CV 0.2%) of the sample was oven dried (Binder, 105 °C, 12 h), and 5 g (CV 0.02%) of the dry sample (1.4% moisture) was treated at 550 °C for 3 h (Felisa, FE341). The SOM content was quantified by

the difference between initial and final weights.

Soil pH was measured by potentiometry in a suspension (soil/water ratio 1:2.5 (w/v)) prepared with distilled water and stirred for 30 min. Particle size was analyzed using an ASTM 152H hydrometer (Bouyoucos, 1962) to determine soil texture according to the USDA soil textural triangle (García-Gaines and Frankenstein, 2015). The CEC was measured by the EDTA titration method, in which the sample is saturated with 1N CaCl<sub>2</sub> (pH 7), the excess solution was removed with ethanol (96%), and the adsorbed calcium was displaced with 1N NaCl (pH 7). The calcium-containing solution was buffered to pH 10 and titrated with a 0.02 N EDTA standard solution (Sigma Aldrich).

### 2.2 Experimental setup

To evaluate the sequestration of hydrocarbons, soil samples with different content and chemical characteristics of SOM, but with the same mineral composition, were generated. To avoid variations between the inorganic components that have soils from different origins, the composite soil sample (Soil 1) was thermally treated to modify both the SOM content and the HS characteristics.

Soil 1 was oven-dried (Lab-line Duo-Vac Oven, 3629) at 105 °C for 12 h and stored in a desiccator. Then, samples of 5 g (CV 0.02%) ( $\leq 2 \text{ mm}^3$ ) were heated (Felisa, FE-360) at a constant temperature for 3 h following the LOI protocol. The temperatures of the thermal treatments were selected based on the inflection points of the maximum mass losses related to the SOM content of the Soil 1 sample, which were obtained by thermogravimetric analysis (TGA). The thus determined temperatures were 250, 350, 450, and 550 °C. The thermal treatments resulted in four modified soils-named as Soils 2, 3, 4 and 5, respectively-, each one containing different amounts and chemical characteristics of SOM but retaining the original mineral composition. Consequently, we analyzed five soils: the natural sampled soil (Soil 1) and the four modified soils (Soils 2, 3, 4, and 5).

The SOM and the HS were analyzed in each soil. To evaluate the chemical characteristics of the SOM, the HS from each soil were extracted and quantified, and the HA and FA fractions were characterized by UV-Vis spectrophotometry and FTIR spectroscopy. The HU fraction in the remnant of each sample was gravimetrically quantified. Then, samples of all five soils were spiked with a hydrocarbon mixture.

Table 1. Description of UV-Vis and FTIR ratios used to characterize fractions of SOM in soils.

Technique	Ratio	Meaning
UV-Vis	A <sub>250</sub> /A <sub>365</sub> : 250/365 (nm)	Molecular weight and aromaticity <sup>a</sup>
	A <sub>240</sub> /A <sub>420</sub> : 240/420 (nm)	Degree of condensation <sup>b</sup>
FTIR	2925/1000 (cm <sup>-1</sup> )	Aliphatic groups <sup>c,d</sup>
	1620/1000 (cm <sup>-1</sup> )	Aromatic groups <sup>c,d</sup>
	3200/1000 (cm <sup>-1</sup> )	Hydroxyl groups <sup>c,d</sup>
	1710/1000 (cm <sup>-1</sup> )	Carboxyl groups <sup>c,d</sup>

<sup>a</sup>Peuravuori and Pihlaja (1997); <sup>b</sup>Wang *et al.* (2020); <sup>c</sup>Kovács *et al.* (2012); <sup>d</sup>Tatzber *et al.* (2007)

### 2.3 Thermogravimetric analysis (TGA)

TGA was used to determine temperatures for further thermal treatments of Soil 1, based on mass losses. A sample of about 15 mg (CV 0.08%) of dry soil was analyzed in a thermogravimetric analyzer (TA Instrument, New Castle, USA). The analysis was carried out under an inert atmosphere (N<sub>2</sub>) with a flow rate of 60 cm<sup>3</sup> min<sup>-1</sup>, in a temperature range of 25 to 600 °C at a heating rate of 10 °C·min<sup>-1</sup>.

### 2.4 Extraction and characterization of humic substances (HS)

The HS of each soil sample were extracted and fractionated into HA and FA using the Schnitzer method (Pansu and Gautheyrou, 2006). The extraction of HS was done with 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>·NaOH in a N<sub>2</sub> atmosphere at 200 rpm for 24 h. The suspension was centrifuged (Beckman J2-HS) at 8,000 x g for 15 min, and the so obtained solid pellet was oven-dried and used for HU determination, following the steps described in the LOI protocol used for SOM determination. The alkaline extract was acidified with 2 M HCl until pH ≤ 2 and left in static conditions for 24 h. The HA was separated from the supernatant (FA extract) by centrifugation (8,000 x g during 15 min) and washed with Milli-Q deionized and degassed water until a negative Cl<sup>-</sup> test was obtained using AgNO<sub>3</sub>. The HS were lyophilized, and the recovered fractions were gravimetrically quantified. HA and FA were used to estimate the HA/FA ratio for each soil sample.

HA and FA were characterized (in triplicate) by UV-Vis spectrophotometry and HA by FTIR spectrometry. The UV-Vis analysis (Perkin Elmer, Lambda 25) was done in the range from 1000 to 200 nm using quartz cells. For this purpose, 1-2 mg of lyophilized HS was dissolved in 5 mL of 0.05 M NaHCO<sub>3</sub> (pH = 8.3); this solution was also used as a blank. Absorption ratios A<sub>250</sub>/A<sub>365</sub> and A<sub>240</sub>/A<sub>420</sub>

were estimated by relating the absorbance values obtained at wavelength (nm) ratios of 250/365 and 240/420 respectively (Peuravuori and Pihlaja, 1997; Wang *et al.*, 2020) (Table 1).

The FTIR spectra of HA extracts were obtained in an infrared spectrometer (Frontier, Perkin Elmer) coupled to attenuated total reflectance (FTIR-ATR). A background spectrum was taken every three measurements. The spectra were obtained using Perkin Elmer software (Spectrum 10). Each spectrum was measured in the 4000-400 cm<sup>-1</sup> range using 20 scans and processed (OriginPro 8.5). Processing included baseline correction and range definition for each band. For each spectrum, the normalized area under each band was calculated using the total area of the spectrum. Normalized areas were used to estimate ratios using the bands obtained at 3200, 2925, 1710 and 1620 cm<sup>-1</sup> related to the band obtained at 1000 cm<sup>-1</sup> as a reference (Table 1).

### 2.5 Hydrocarbon sequestration assay

Soil samples were contaminated with a hydrocarbon mixture (30,000 mg·[kg soil]<sup>-1</sup>) containing HXD, PHE, and PYR in a 100:1:1 (w/w) proportion, using a mixture of hexane (HXN) and acetone (ACE) (1:1 v/v) as the carrier. This hydrocarbon mixture was chosen since HXD is a major component in diesel (Jung *et al.*, 2015) whilst PHE and PYR are commonly considered as model hydrocarbons of three- and four-ringed PAHs (Ding *et al.*, 2020; Spasojević *et al.*, 2018). The proportion of hydrocarbons in the mixture is similar to that of aliphatic-PAHs in a Premium Diesel Fuel (Lin *et al.* 2006); being diesel one of the most common substances involved in soil spills in Mexico, reaching almost 20% of all accidents in the country (Palacios *et al.* 2020). The spiking was done by adding 1 mL of the hydrocarbon mixture to 1 g of each soil sample, completely covering the soil mass, simulating a superficial oil spill. The contaminated soil samples were left inside a fume hood until the solvent

mixture evaporated (3 h) and reached the equilibrium (12 h,  $25 \pm 0.5^\circ\text{C}$ ). Afterward, 5 mL of distilled water was added to tubes containing the contaminated soil samples; the content in each tube was gently mixed with a glass rod for 3 min, in order to displace not sorbed hydrocarbons to the aqueous phase. The aqueous solutions containing not sorbed hydrocarbons were separated from the soils by decantation. The hydrocarbons dissolved in the aqueous solutions were recovered by liquid-liquid extraction and quantified by gas chromatography (GC); those that remained in the soil matrices were subjected to solid-liquid extraction.

The sequestered quantity of each hydrocarbon constituting the mixture was quantified and related to the SOM content-particularly HA and HF-and their chemical characteristics.

### 2.5.1 Liquid-liquid extraction

The aqueous phase obtained from each contaminated soil sample was treated with 5 mL of the solvent mixture (HXN:ACE, 1:1) in an extraction funnel. Both liquids were mechanically stirred for 1 min and left to stand for 30 min, allowing phase separation. The aqueous phase was discarded, and the organic phase was left overnight in a fume hood to evaporate the solvent. Then, the recovered hydrocarbons were suspended in hexane and filtered (nylon membrane,  $0.45 \mu\text{m}$ , Whatman). Finally, the hydrocarbons were quantified by GC.

### 2.5.2 Solid-liquid extraction

The soil matrix with hydrocarbons was oven-dried ( $60^\circ\text{C}$ , 48h) and subjected to microwave-assisted extraction (CEM MarsXpress) with a mixture of solvents (HXN:ACE) at 175 psi. The extraction procedure consisted of three steps: i) heating at a constant ramp up to  $150^\circ\text{C}$  (5 min), ii) standing at  $150^\circ\text{C}$  (30 min); iii) cooling (5 min). Then, the so obtained extracts were concentrated in a rotary evaporator (Büchi R-295), suspended in HXN, and filtered (nylon membrane,  $0.45 \mu\text{m}$ , Whatman). Finally, the hydrocarbons were quantified by GC.

## 2.6 Hydrocarbon quantification

Hydrocarbons were quantified by GC (Shimadzu GC-2010, coupled to a Shimadzu AOC-20i auto-injector) using an Agilent DB-1HT column (15m x  $0.250\text{mm}$  x  $0.10\mu\text{m}$ ) and a flame ionization detector (FID). External standards of HXD (JT Baker), PHE (Sigma-Aldrich), and PYR (Aldrich) were used. Since

sequestered hydrocarbons cannot be extracted from the soil, they were estimated indirectly through a mass balance, according to the following equation (Gómez-Flores *et al.*, 2021):

$$Seq_{HC} = HC_{ini} - (HC_{aq} + HC_{So} + HC_{lost}) \quad (1)$$

where  $Seq_{HC}$  is the concentration ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) of sequestered hydrocarbons;  $HC_{ini}$ , the initial concentration of each hydrocarbon in the mixture;  $HC_{aq}$ , the mixture of hydrocarbons in the aqueous phase;  $HC_{So}$ , the hydrocarbons extracted from the soil matrix; and  $HC_{lost}$ , corresponds to the loss of hydrocarbons during the experimental procedures, mainly during L-L and S-L extractions and filtration ( $HC_{lost}$  6-8%).

Glass beads (0.5 mm) were used to quantify the hydrocarbon mass losses during the extraction process. The beads (1 g) were artificially contaminated and subjected to the same extraction procedure used for the soil. Total losses were quantified by subtracting the recovered hydrocarbons from the glass beads after their extraction from the hydrocarbons initially added to the mixture.

## 2.7 Statistical analysis

Experiments were independent and performed in triplicate. The statistical analysis done on the obtained data was performed by means of the IBM SPSS Statistics 25 software. All the results are presented as mean values ( $n = 3$ ), with their corresponding standard deviation (SD). The normality of data was evaluated by the Shapiro-Wilk test and the variance equality by the Levene test. One-way ANOVA tested significant differences between soils, and the comparison of means was made by a Duncan test ( $\alpha = 0.05$ ). A correlation analysis between variables was done using the Pearson correlation with a bilateral significance test ( $\alpha = 0.05$  and  $\alpha = 0.01$ ).

Data on the quantity and chemical characteristics of HA and FA in the SOM were used to search for a correlation with the sequestration of hydrocarbons. Variables with a correlation greater than 0.7, according to the Pearson's coefficient, were used to propose linear adjustments directly or indirectly. In the latter case, the data were treated by a double reciprocal plot, according to the Lineweaver-Burk equation of enzyme kinetics (Lineweaver and Burk, 1934). With these results, from the analysis of HA and FA by UV-Vis spectrophotometry and FTIR spectroscopy, two equations were proposed to estimate the sequestration of PHE and PYR (Table 4).

### 3 Results

#### 3.1 Physicochemical and thermogravimetric characterization of soil

Soil 1, which was the natural soil used to generate the rest of the samples, was characterized through physicochemical and thermogravimetric analysis, TGA. This soil presented a high content of SOM ( $26.58 \pm 0.25\%$ ), and its mineral fraction was found to be composed of 62% sand, 19% silt, and 19% clay. The soil had a slightly acidic pH value ( $6.28 \pm 0.02$ ) and a high CEC ( $42.04 \pm 3.86 \text{ cmol}_c \cdot \text{kg soil}^{-1}$ ).

The TGA analysis revealed four weight losses in the Soil 1: i) ~2% between 25 and 100 °C; ii) ~1% in the range of 100 to 200 °C; iii) 20% from 200 to 550 °C; and iv) 5% at temperatures above 550 °C. These changes are observed in the derivative obtained from weight loss data vs. temperature, which indicates the inflection points (i.e., the temperatures at which the loss of mass is maximal). The inflection points were found around 250, 350, 450, and 550 °C, which is why these temperatures were selected for the thermal treatments to obtain the 4 different modified soils.

#### 3.2 Characterization of SOM and HS in the modified soils

The thermal treatments of Soil 1 led to losses of SOM content in the generated soils. Both the SOM content and the CEC values decreased linearly as temperature increased (Table 2), finding almost

complete elimination of SOM in Soil 5. Thermal treatments modified not only the SOM content of the soil, but also the quantity (Fig. 1) and chemical characteristics of the HS (Tables 2 and 3). In general, while the HU fraction remained almost constant ( $57\% \pm 5$ ) in Soils 1 to 4, the proportion of HA decreased and that of FA increased with increasing treatment temperature (Fig. 1), consequently decreasing the HA/FA with increasing treatment temperature (Table 2). The greatest change in this ratio was found at 350 and 450 °C (Soils 3 and 4, respectively), which showed a decrease greater than 5 times related to the untreated soil (Soil 1) and at 250 °C (Soil 2). At 450 °C (Soil 4), the ratio decreased to a value close to zero due to the low HA content. Since the SOM content of Soil 5 was close to zero, no results of this ratio are presented.

#### 3.3 Spectrophotometric and spectroscopic characterization of humic substances (HS)

From the UV-Vis characterization of the HA, the lowest  $A_{250}/A_{365}$  and  $A_{240}/A_{420}$  ratios were found in Soils 2 and 3 (Table 3). Concerning the FA, except in Soil 4, the value of the  $A_{250}/A_{365}$  ratio increased as the SOM content decreased, finding the highest value in Soil 3. As for the  $A_{240}/A_{420}$  ratio, the highest values were found for Soils 2 and 3.

According to the FTIR analysis (Table 3), Soils 2 and 3 have a higher proportion (2-4 times) of aromatic, aliphatic and hydroxyl groups compared to Soils 1 and 4. Likewise, the HA in Soil 2 contains two times more carboxyl groups than Soils 1 and 3, and six times more than in Soil 4.

Table 2. Characterization of the five soils in terms of organic matter (SOM) content, cation exchange capacity (CEC), and the humic to fulvic acids mass ratio (HA/FA) obtained in the SOM of each soil\*.

Soil	Treatment temperature (°C)	CEC ( $\text{cmol}_c \cdot \text{kg soil}^{-1}$ )	SOM (%)	HA/FA
1	25	$42.04 \pm 3.86^a$	$26.58 \pm 0.25^a$	$2.80 \pm 0.17^a$
2	250	$37.28 \pm 0.32^a$	$15.70 \pm 0.40^b$	$2.59 \pm 0.31^a$
3	350	$25.13 \pm 0.92^a$	$10.31 \pm 0.89^c$	$0.45 \pm 0.12^b$
4	450	$17.08 \pm 0.93^c$	$6.14 \pm 1.40^d$	$0.03 \pm 0.01^c$
5	550	$14.98 \pm 0.15^c$	$0.33 \pm 0.26^f$	n.d.

\*Values are the average of triplicates  $\pm$  their correspondent SD. Different letters by column indicate significant differences ( $p < 0.01$ ). n.d. not determined.

Table 3. Ratios obtained from UV-Vis, and FTIR analyses of extracted humic substances (HA and FA).

	Ratio		Soil 1	Soil 2	Soil 3	Soil 4
UV-Vis	HA	A <sub>250</sub> /A <sub>365</sub>	1.60 ± 0.12 <sup>b</sup>	1.13 ± 0.02 <sup>c</sup>	1.15 ± 0.03 <sup>c</sup>	3.09 ± 0.05 <sup>a</sup>
		A <sub>240</sub> /A <sub>420</sub>	2.67 ± 0.22 <sup>b</sup>	1.37 ± 0.17 <sup>c</sup>	1.49 ± 0.29 <sup>c</sup>	5.24 ± 0.07 <sup>a</sup>
	FA	A <sub>250</sub> /A <sub>365</sub>	4.50 ± 0.47 <sup>d</sup>	6.47 ± 0.37 <sup>b</sup>	7.33 ± 0.42 <sup>a</sup>	5.22 ± 0.02 <sup>c</sup>
		A <sub>240</sub> /A <sub>420</sub>	9.85 ± 1.24 <sup>b</sup>	16.25 ± 1.15 <sup>a</sup>	18.36 ± 2.38 <sup>a</sup>	10.82 ± 0.43 <sup>b</sup>
FTIR	HA	Aromatic	0.16 ± 0.02 <sup>b</sup>	0.31 ± 0.01 <sup>a</sup>	0.27 ± 0.04 <sup>a</sup>	0.06 ± 0.01 <sup>c</sup>
		Aliphatic	0.23 ± 0.07 <sup>b</sup>	0.50 ± 0.04 <sup>a</sup>	0.50 ± 0.04 <sup>a</sup>	0.10 ± 0.01 <sup>c</sup>
		Hydroxyl	0.26 ± 0.04 <sup>b</sup>	0.50 ± 0.05 <sup>a</sup>	0.57 ± 0.26 <sup>a</sup>	0.13 ± 0.02 <sup>b</sup>
		Carboxyl	0.06 ± 0.01 <sup>b</sup>	0.13 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	0.02 ± 0.00 <sup>c</sup>

Data with different letter by row indicate significant differences ( $p < 0.01$ ;  $n = 3$ ). Soil 5 was excluded from the analysis as extractable humic substances were not enough for analysis

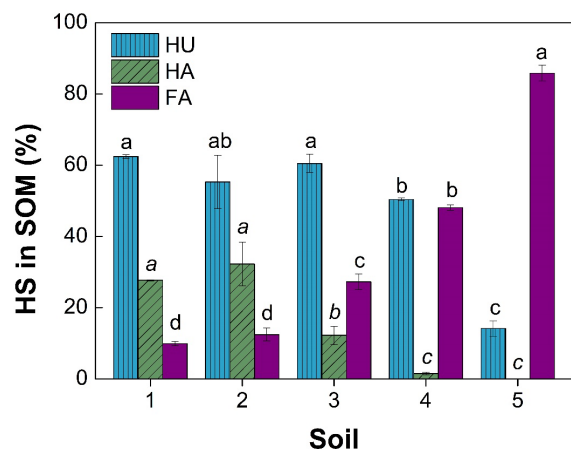


Figure 1. Proportion of humic substances (HS) contained in the organic matter of each studied soil: humins (HU), humic acids (HA) and fulvic acids (FA). Error bars show the standard deviation of each mean value ( $n=3$ ). Different letters indicate significant differences ( $p < 0.01$ ) among each HS fraction in the five soils.

### 3.4 Hydrocarbon sequestration

For all the soil samples, the concentration of HXD sequestered was below  $100 \text{ mg} \cdot [\text{kg soil}]^{-1}$ , that is, less than 1% of its initial concentration (Fig. 2). In contrast, all the soils showed the capacity to sequester PHE and PYR, referred in this study as PAHs (Fig. 2). Soil 1 was the only one that showed more affinity to sequester PYR than PHE. In contrast, Soil 3 sequesters more PHE than PYR, and the rest of the modified soils showed a similar affinity for both PAHs. Soils 2 and 3 showed between two- and six-times greater

sequestration capacity for both PHE and PYR ( $> 40\%$ , or  $\geq 100 \text{ mg} \cdot [\text{kg soil}]^{-1}$ , compared to the rest of the soils (10-20%).

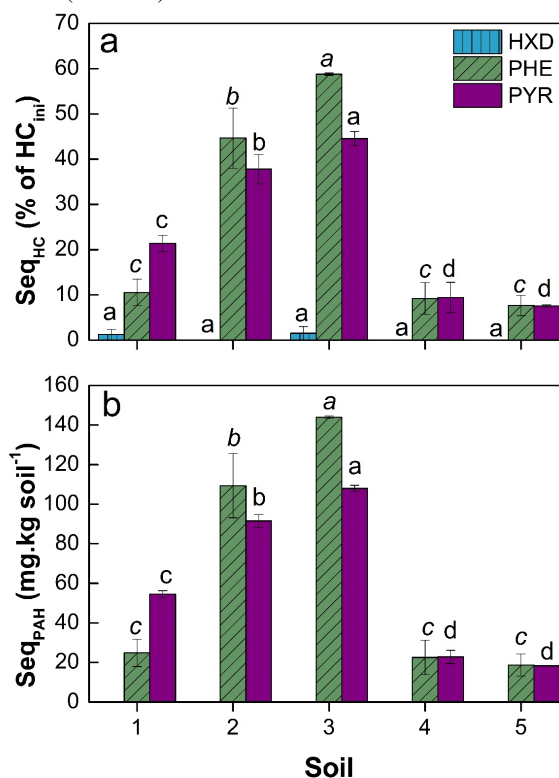


Figure 2. Proportion (%) of HXD, PYR, and PHE based on the initial concentration of each (HXD 25,426.0; PHE 244.8, and PYR 242.3  $\text{mg} \cdot [\text{kg soil}]^{-1}$ ) (a), and concentration of sequestered PHE and PYR in thermally-treated soils (b). Different letters indicate significant differences ( $p < 0.01$ ) between the mean value of each hydrocarbon in each soil.

Table 4. Parameters obtained from the correlation analysis between PHE or PYR sequestration ( $\text{Seq}_{\text{PAHs}}$ ) and different ratios obtained from UV-Vis ( $\text{UV-Vis}_{\text{ratio}}$ :  $A_{250}/A_{365}$  and  $A_{240}/A_{420}$ ) and FTIR ( $\text{FTIR}_{\text{ratio}}$ : aromatic and aliphatic) analysis of humic acids (HA) extracted from the soils under study.

Ratio	Plot	Constant <sup>a</sup>	Seq PHE				Seq PYR			
			P <sup>b</sup>	k <sub>1</sub>	k <sub>2</sub>	r <sup>2c</sup>	P	k <sub>1</sub>	k <sub>2</sub>	r <sup>2</sup>
$A_{250}/A_{365}$	1/Seq <sub>PAHs</sub> vs.	$k_1 = 1/m$	-0.98	-11.12	-0.97	0.95	-0.98	-22.08	-1.08	0.95
$A_{240}/A_{420}$	1/UV-Vis <sub>ratio</sub>	$k_2 = b/m$	-0.92	-12.77	-0.84	0.83	-0.86	-27.17	-0.99	0.71
Aromatic	Seq <sub>PAHs</sub> vs.	$k_1 = m$	0.91	441.58	-13.84	0.81	0.92	317.66	5.61	0.84
Aliphatic	FTIR <sub>ratio</sub>	$k_2 = b$	0.93	281.99	-18.69	0.85	0.97	184.07	7.95	0.94

<sup>a</sup>Constants obtained from the slope (m) and the intercept (b) of the linear plot; <sup>b</sup>Pearson coefficients; <sup>c</sup>determination coefficient obtained from the linear fit between the Seq<sub>PAH</sub> and the different UV-Vis and FTIR ratios of HA.

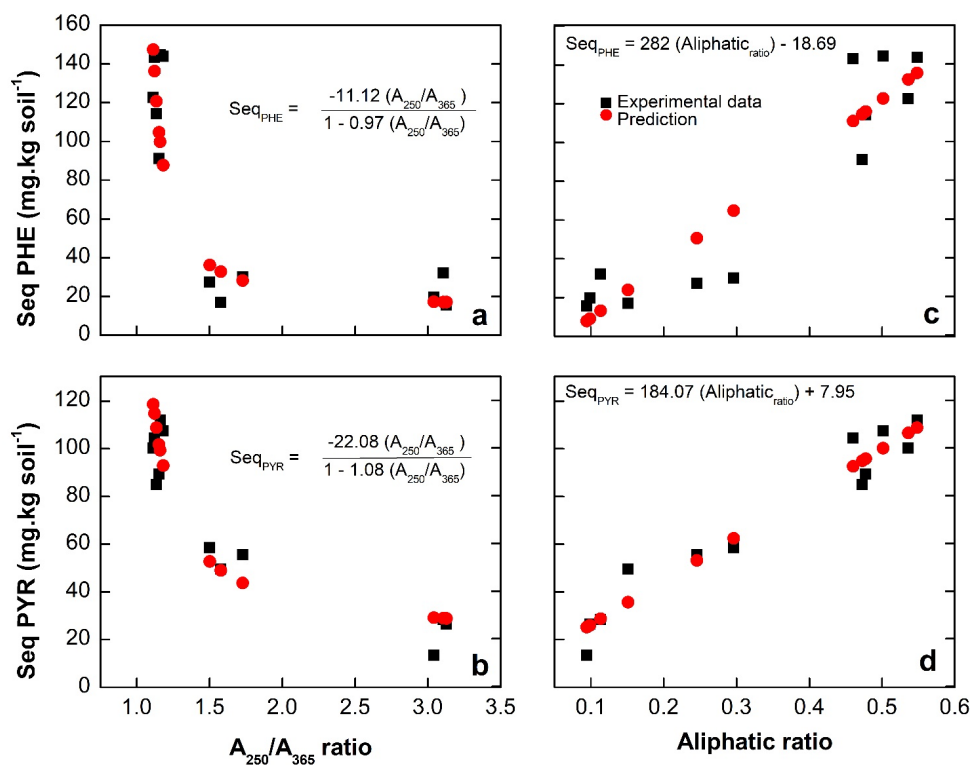


Figure 3. Sequestration of PHE (a, and c) and PYR (b, and d) vs UV ratio ( $A_{250}/A_{365}$ ) and FTIR ratio (Aliphatic) of HA. Experimental data (black squares) and estimation of PHE and PYR sequestration (red circles) by the equations 2 and 3, using the calculated constants ( $k_1$  and  $k_2$ ).

### 3.5 Estimation of hydrocarbon sequestration based on the analysis of humic substances

Although no significant correlation was found between the sequestration of PYR or PHE and the amount of SOM or its fractions, sequestration was positively and significantly ( $p < 0.01$ ) correlated with some of the HA ratios estimated from the FTIR and UV-Vis analyzes (Table 4). The best fit between UV-

Vis ratios and sequestration data for both PAHs was obtained with the  $A_{250}/A_{365}$  ratio using a double reciprocal plot-also known as the Lineweaver-Burk plot (Lineweaver and Burk, 1934). Regarding the FTIR ratios of the HA, the best fit for the sequestration data resulted in a linear equation, this model represents better the sequestration data for PYR than that of PHE. The best estimator for PHE and PYR sequestration based on the linear fit was the aliphatic-FTIR ratio of HA. Based on the previous results, two equations are



proposed to estimate the capacity of a soil to sequester hydrocarbons:

$$Seq_{PAHs} = \frac{k_1 UV_{ratioHA}}{1 + k_2 UV_{ratioHA}} \quad (2)$$

$$Seq_{PAHs} = k_1 \cdot FTIR_{ratioHA} + k_2 \quad (3)$$

The constants  $k_1$  and  $k_2$  were calculated through linear adjustments and used to estimate the PHE and PYR sequestration capacity of the soil based on the characteristics of the HA (Table 4);  $k_1$  and  $k_2$  are valid for Eq. 2 and Eq. 3. Equation 2, based on the  $A_{250}/A_{365}$  ratio of HA, can estimate the sequestration of PHE and PYR (95% accuracy) in soils containing SOM of different composition (Figs. 3a, 3b). For its part, Eq. 3 provides information (>85% accuracy) about the affinity of the soil for PHE and PYR as a function of the aliphatic groups quantified by the FTIR ratio of HA (Figs. 3c, 3d).

## 4 Discussion

Soil 1 presented high content of SOM ( $26.58 \pm 0.25\%$ ), characteristic of organic soils named histosols or peatlands (FAO, 2014). According to Mexican standards (NOM-021-SEMARNAT-2000, 2002), this soil can be classified as slightly acidic ( $pH = 6.28 \pm 0.02$ ) with a high CEC ( $42.04 \pm 3.86 \text{ cmol}_c \cdot \text{kg soil}^{-1}$ ). Together, these values suggest a histosol associated with gleysol, a mineral soil with  $pH_{H_2O}$  values around 7.4, and a CEC of  $42.1 \text{ cmol}_c \cdot \text{kg soil}^{-1}$ . This histosol-gleysol association was previously reported for the sampling site (Palma-López *et al.*, 2007).

According to the TGA analysis of Soil 1, the first weight loss (2%, between 0-100 °C) corresponds to the evaporation of the incorporated or adsorbed water (Giannetta *et al.*, 2018). In the range of 100 to 200 °C, the recorded weight loss (~1%) may be related to the loss of water strongly linked to the soil components. The weight loss (20%) from 200 to 550 °C is associated with the degradation of different fractions of the SOM, while above 550 °C it is attributed to the degradation of organo-mineral structures (Kučerík *et al.*, 2018). Both the SOM content and the CEC decreased in Soil 1 as the treatment temperature increased (Table 2), finding a negative linear relationship. This confirms that thermal treatments can be used as a controlled method to modify the SOM content in the studied soil.

The reduction in CEC can be attributed to the fact that it is directly related to the content of SOM and

the content and type of clay (Parfitt *et al.*, 1995). In fact, the CEC value in Soil 5 is utterly related to the content and type of clay, since that soil barely contains any SOM. The thermal stability of the soil mineral components, including clays, depends on their type (non-expanding or expanding) and composition (Heller-Kallai, 2006). Previous results suggest that the type of clay in the soil under study is kaolinite, since it has been reported that it is associated with gleysols in Tabasco (Palma-López *et al.*, 2007). In fact, the CEC value in the Soil 5 is in the range reported for kaolinite, i.e.,  $3\text{-}15 \text{ cmol}_c \cdot \text{kg soil}^{-1}$  (NOM-021-SEMARNAT-2000, 2002). The chemical structure of this type of clays (kaolinite or metakaolinite) is stable at temperatures up to 900 °C. This suggests that the clay in the studied soil, even during the treatment at 550 °C, could have been dehydrated but not partially decomposed (Yanti and Pratiwi, 2018). Since the soils under study contained the same type and quantity of clay, the differences between them are explained by the content and nature of the SOM, and so is the fate of the hydrophobic compounds in these soils.

The fate of hydrophobic compounds in soils is commonly related to the SOM and organic carbon content (SOC). Most studies support a direct correlation between PAHs sorption and SOM content, which means that the higher the SOM content, the greater the PAHs retention capacity (Oleszczuk, 2007; Pignatello and Xing, 1996; Yang *et al.*, 2010). However, in the soils here studied, the sequestration of PHE and PYR did not correlate neither with the content of SOM nor with its HS. In fact, Soils 2 and 3, with intermediate values of SOM and CEC, had the highest capacity to sequester PHE and PYR (Fig. 2). This implies that the sequestered fraction behaves differently from the extractable fraction.

On the other hand, the HA/FA ratio of HS fractions was obtained (Table 2) as a measure of soil humification (Putra *et al.*, 2016), which represents the formation of thermodynamically stable aromatic and polyaromatic structures that originate from more labile structures, such as carbohydrates and amino acids (Klavins *et al.*, 2008). The values of HA/FA in Soils 1 and 2 were very similar and were within the reported values for natural soils (Putra *et al.*, 2016). However, the affinity for PHE and PYR and the ability to sequester them were different in both soils, suggesting that they have different chemical structures in their HS fractions. Although the HA/FA provides an insight into the characteristics of SOM and is indicative of the conversion of HA to FA, it is not sufficient to explain the differences in PHE and PYR

sequestration. The chemical characteristics of HA may influence the PHE and PYR sequestration, since they bind more strongly to HA than to FA (Banach-Szott *et al.*, 2014). So, spectrophotometric, and spectroscopic analyzes are a more reliable measure to determine the chemical characteristics of FA and HA in SOM (Table 1). These analyses were performed by means of UV-Vis spectrophotometry and FTIR spectroscopy through various ratios (Table 3). Commonly, the  $A_{250}/A_{365}$  ratio estimated from UV spectra decreases as molecular size and aromaticity increase (Peuravuori and Pihlaja, 1997); that is, the larger the molecule, the greater the absorption at longer wavelengths (Rodríguez *et al.*, 2016). For its part, the value of the  $A_{240}/A_{420}$  ratio decreases as the condensation degree increases (Wang *et al.*, 2020). Absorption in the UV range is attributed to the  $\pi$  electron transition and involves activated aromatic groups ( $\lambda > 250$  nm) (Rodríguez *et al.*, 2016), carboxylic electron systems ( $\lambda < 250$  nm) (Uyguner and Bekbolet, 2005), and charge-transfer interactions ( $\lambda > 350$  nm) between hydroxy-aromatic donors and quinoid acceptors; the latter groups being typical components of partially oxidized lignin structures (Del Vecchio and Blough, 2004).

Each soil studied presented a different capacity for hydrocarbon sequestration (Fig. 2), which can be attributed to the differences in the chemical characteristics of SOM of the soils, particularly those of HA and FA. Each HS fraction showed a different affinity for PYR or PHE sequestration, which was demonstrated by significant ( $p < 0.01$ ) correlations and the spectroscopic and spectrophotometric ratios of HS (Table 4). In fact, not all types of interactions between the soil matrix and hydrocarbons lead to their sequestration. For this to occur, the interaction must be strong enough to prevent their release, such as the formation of charge-transfer complexes or donor-acceptor  $\pi$ - $\pi$  interactions (Chianese *et al.*, 2020). In this study, no soil sequestered HXD, since it being an aliphatic alkane it has neither free electrons nor functional groups to establish charge-transfer interactions with the HS. Although HXD interacts with different soil components, the results suggest that such interactions only involve surface sorption in the mineral fraction and the SOM (Stroud *et al.*, 2007), allowing its release from the soil matrix. In contrast, PHE and PYR are strong  $\pi$ -electron donors due to their stability through resonance, and both PHE and PYR were sequestered by the soils under study.

Soil 1 presented a greater capacity to sequester PYR than PHE, which can be attributed to the

chemical differences between both PAHs. The fact that PYR is more lipophilic than PHE, since its octanol/water partition coefficient is higher ( $\log K_{ow} = 5.32$ , and 4.46, respectively) (Housani *et al.*, 2019), makes it less extractable from the SOM when strong interactions are formed. Soil 1 has low sequestration capacity due to the presence of larger and more condensed FA molecules (high  $A_{250}/A_{365}$  and  $A_{240}/A_{420}$  FA ratios) compared to the FA in the modified soils; this limits the diffusion and access of PHE and PYR through the soil matrix, hindering their sequestration. This observation is supported by a positive correlation ( $> 0.80$ ) between sequestration and the  $A_{250}/A_{365}$  and  $A_{240}/A_{420}$  ratios of FA (Table 4). PAHs can be extracted when they bind to the most labile and soluble fraction of the SOM—that is, FA (Yang *et al.*, 2010b). Consequently, the fact that the magnitude of sequestration in Soil 1 is not the highest (Fig. 2) may be the result of the lack of contact between PHE and PYR and the core of the most recalcitrant fractions in the SOM (HA and HU).

The highest sequestration of PYR and PHE ( $\geq 100$  mg·[kg soil]<sup>-1</sup>) in Soils 2 and 3 can be attributed to the chemical nature of their HS fractions. According to the estimated ratios, and compared to Soils 1 and 4, HA in Soils 2 and 3 have higher molecular weight and degree of condensation (lowest  $A_{250}/A_{365}$  and  $A_{240}/A_{420}$ , respectively) and higher content of aromatic, aliphatic and hydroxyl groups (Table 3). This is supported by significant correlations ( $> 0.7$ ) between PHE and PYR sequestration and UV-Vis or FTIR ratios of HA (Table 4). An increase in the aromaticity of HA is related to the decarboxylation of surface carboxyl groups (Plante *et al.*, 2009), leaving the core of the HA available for interaction with other molecules, such as PHE and PYR. Likewise, the increase in aromatic, aliphatic and polar groups in HA together with the decrease in the molecular weight, condensation, and polarity of FA due to thermal treatments improves the mobility and diffusion of PHE and PYR, thus increasing their interaction with the most recalcitrant fraction in the SOM—where  $\pi$ - $\pi$  interactions can occur—and, consequently, enabling their sequestration (Bogan and Trbovic, 2003). The greater sequestration capacity in Soil 3 compared to Soil 2 can be attributed to the latter having a higher content of carboxyl groups in HA, thus increasing its polarity and solubility. This modifies the electronic density and the conformation of supramolecules, decreasing the capacity to sequester PHE and PYR in Soil 2. Soils 4 and 5 showed the lowest sequestration capacity for both PAHs (Fig. 2), which may be the

result of the chemical composition of their SOM, particularly the absence of HA and the presence of the highest proportion of FA (Fig. 1), which is the most labile and soluble (extractable) fraction. Since Soil 5 contains practically no SOM, its low capacity to sequester PHE and PYR can be entirely attributed to the clays present in the soil, particularly of the non-expandable type.

The sequestration of PHE and PYR can be estimated based on the chemical characteristics of HA in the SOM through UV-Vis and FTIR analyses (Table 4 and Fig. 3), using Eq. 2 and 3, respectively. This result indicates that the HA of the SOM is the main responsible for PHE and PYR sequestration in the studied soils, and that both analytical techniques can be used independently. Eq. 2, using the  $A_{250}/A_{365}$  ratio, reliably estimates (95% confidence) the PYR and PHE sequestration (Fig. 3a, 3b), implying that the higher the aromaticity and molecular weight of the HA (low values of the  $A_{250}/A_{365}$  ratio), the greater the sequestration capacity. Therefore, the affinity of PHE for HA is greater than that of PYR when the HA are more aromatic, resulting in a greater capacity to sequester PHE than PYR (140 and 120 mg·[kg soil]<sup>-1</sup>, respectively). The higher the value of  $A_{250}/A_{365}$  in HA-i.e., HA with low aromaticity and molecular weight-the higher the affinity for PYR. These differences in affinity reflect the complexity of the interactions between PHE and PYR and HA, depending on features such as chemical composition, self-assembly properties, and particle size (Yu *et al.*, 2018). In contrast, Eq. 3-based on the FTIR ratio corresponding to the aliphatic groups in HA-estimates the PHE and PYR sequestration with higher accuracy ( $r^2 \geq 0.85$ ) (Fig. 3d) compared to the aromatic FTIR ratio ( $r^2 > 0.80$ ) (Fig. 3c). These results indicate that the content of aliphatic compounds in HA also play an essential role in the sequestration of PHE and PYR, particularly for PHE. This is consistent with the strong affinity previously found between PHE and aliphatic domains in the SOM (Salloum *et al.*, 2002). The data fitting (Table 4) shows that the UV-Vis ( $A_{250}/A_{365}$ ) and FTIR (aliphatic) ratios allowed the highest correlation with PYR and PHE sequestration. This information has not been previously published and is the basis that supports the proposal to estimate the sequestration capacity of a soil without the need to handling a hydrocarbon-polluted sample. For this purpose, Eqs. 2 or 3 can be used, once the data of a routine characterization of HA in a soil are available. This study demonstrates that only some chemical characteristics of HA are useful to estimate

PYR and PHE sequestration by using the obtained equations. This first approach shows the possibility of estimating the hydrocarbon sequestration in soil using the chemical characteristics of HS. Nonetheless, more studies are still needed to elucidate the interactions that explain the differences in the affinity of the different SOM domains for the different types of hydrocarbons.

The two equations proposed are useful to estimate the amount of PHE and PYR that can be sequestered in a soil, using the aromaticity and the degree of condensation (by UV-Vis analysis) as well as the proportion of aliphatic groups (by FTIR analysis) in HA. As far as we know, this is the first approach to estimate PHE and PYR sequestration in natural soil based on the chemical characteristics of the HA of the SOM using standardized and affordable techniques. Commonly, available techniques to quantify non-extractable compounds in soils are based on exhaustive extraction with solvents (Umeh *et al.*, 2018a), <sup>14</sup>C labeling complemented with cleavage methods of the SOM (silylation, KOH hydrolysis, methanolic saponification) or combustion followed by scintillation (Claßen *et al.*, 2019; Schäffer *et al.*, 2018; Umeh *et al.*, 2018b), being most of them quantified by chromatography. The cost of the latter techniques makes them not readily accessible (Umeh *et al.*, 2018a). From the equipment accessibility point of view and the accuracy of the results, we suggest the use of the Eq. 2 and the UV-Vis ratio  $A_{250}/A_{365}$  of HA to estimate the PHE and PYR sequestration in soils. Although PHE and PYR have been considered as model hydrocarbons for three- and four-ringed PAHs (Ding *et al.*, 2020; Rastegari *et al.* 2016; Spasojević *et al.* 2018), more studies are needed to determine the accuracy and applicability of the equations proposed to estimate the sequestration of other PAHs structurally different from PYR and PHE. Since interactions leading to PAHs sequestration include tridimensional molecular interactions with soil components, PHE and PYR cannot be considered synonyms with PAHs. Given that humic substances are supramolecular structures (small self-assembled molecules held together by weak interactions), they could adopt different tridimensional conformations (Nebbio and Piccolo, 2012) depending on the nearby molecules, thus modifying the sequestration capacity.

This set of results shows that the sequestration of PHE and PYR in this type of natural and modified soils depends substantially on the chemical characteristics of their SOM rather than on their

content. Consequently, sequestration can be estimated using the proposed equations (2, and 3), which are based on the chemical characteristics of HA. Although there is not a unique chemical structure of HA, their general chemical characteristics in all types of soils are the same (Chianese *et al.*, 2020), and they can be analyzed systematically by UV-Vis spectrophotometry and FTIR spectroscopy, which are techniques commonly used to characterize HS. Thus, Eqs. 2 and 3 could be useful to estimate PYR and PHE sequestration in organic soils, considering two restrictions: i) the values of  $A_{250}/A_{365}$  and aliphatic ratios in samples should be within the range of 1.0 - 3.5 and 0.1 - 0.6, respectively, and ii) the soil should contain only non-expansive clays, such as kaolinite. However, it is still necessary to corroborate this first approach through studies with other types of soils and consider that, in soils with a mineral fraction different from that of this study, it might not be consistent with the results here presented. Furthermore, the obtained results show that the concentrations of PHE and PYR sequestered in Soil 3 and PHE in Soil 2 are above the maximum permissible limits in soils according to Mexican standards (NOM-138-SEMARNAT/SSA1-2012, 2013). In fact, the concentration of sequestered pollutants in a soil can commonly exceed the maximum permissible limits established in the regulations (Ding *et al.*, 2020).

## Conclusions

The sequestration of PAHs by a natural soil is a complex process that involves, more than just the content of the SOM, the chemical characteristics of the fractions that constitute it. The molecular weight of the FA and HA in the SOM, their degree of condensation, and the content of aromatic and aliphatic groups are key factors that define the sequestration of PHE and PYR in the soils studied.

To our knowledge, this study proposes a first approach that allows estimating PHE and PYR sequestration based on the chemical characteristics of HS in the SOM using accessible spectrophotometric and spectroscopic techniques. Moreover, the proposed approach estimates PHE and PYR sequestration with an accuracy greater than 80%. Further research involving other PAHs, soil types, and SOM is essential to increase the understanding about the interactions between soil components and PAHs, and to validate the proposed approach. The use of easily

measurable variables as reliable estimators of the PHE and PYR sequestration capacity in soils could avoid underestimating the concentration of PHE and PYR that remains sequestered in a soil matrix. This approach could be useful for soil remediation and soil risk assessment, especially in contaminated sites with high ecological value.

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## Nomenclature

- $Seq_{HC}$  is the concentration ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) of sequestered hydrocarbons.  
 $HC_{ini}$ , the initial concentration ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) of each hydrocarbon in the mixture.  
 $HC_{aq}$ , the concentration ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) of hydrocarbons in the aqueous phase.  
 $HC_{So}$ , the concentration of hydrocarbons ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) extracted from the soil matrix.  
 $HC_{lost}$ , the concentration of hydrocarbons ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ) lost in the process.  
 Constants  $k_1$  and  $k_2$  ( $\text{mg}\cdot[\text{kg soil}]^{-1}$ ).

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