



On the sequestration of polycyclic aromatic hydrocarbons in aged artificial soil

Sobre el secuestro de hidrocarburos aromáticos policíclicos en suelos artificiales añejados

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Abstract

The fate of polycyclic aromatic hydrocarbons is a major environmental concern that has attracted much attention in the current literature. The physicochemical properties of contaminants, along with soil characteristics and aging, translate into unusual sorption that leads to high sequestration and low bioavailability even in artificial systems. In the present study, fresh (non-sterile) and aged (sterile) artificial soils (sand-perlite 1:1 v/v) were characterized and spiked (hexadecane, phenanthrene and pyrene 2:1:0.5 w/w) to evaluate extractability. Hydrocarbons extraction differed for sand and perlite in fresh samples, on the contrary, the artificial aging process affected equally the extraction in the artificial soil components. In the artificial soil, the extractability of hexadecane and phenanthrene were 80 and 60%, respectively, in fresh samples, but for both hydrocarbons, it was only 20% in aged samples. Pyrene extractability was not affected by the components of the artificial soil or by aging, and this may be related to an in-situ crystallization process. The characteristics of the soil and its contaminants, coupled with long-term interactions, led to the formation of non-extractable residues, also known as sequestered contaminants. This study expands the current knowledge about the use of artificial materials for contaminants' environmental fate evaluation and should serve as motivation for further experimental and theoretical research on this subject.

Keywords: polycyclic aromatic hydrocarbons, extractability, artificial soil, sequestration, aging.

Resumen

El destino de hidrocarburos aromáticos policíclicos es una gran preocupación medioambiental que ha atraído la atención en literatura reciente. Las propiedades fisicoquímicas de los contaminantes, junto con las características y el añejamiento de suelos se traducen en un comportamiento inusual de sorción, alto secuestro y baja biodisponibilidad incluso en suelos artificiales. En el presente estudio, muestras frescas añejadas (estériles) y frescas (sin esterilizar) de suelo sintético (arena-agrolita 1:1 v/v) fueron caracterizadas y contaminadas (hexadecano, fenantreno y pireno 2:1:0.5 p/p) para evaluar extractabilidad. En muestras frescas se encontraron diferencias en la extracción de hidrocarburos para arena y agrolita de manera individual, sin embargo, estas diferencias se eliminaron por el proceso de añejamiento. En muestras de suelo frescas, la extractabilidad de hexadecano y fenantreno fue de 80 y 60% respectivamente. Mientras que, para muestras añejadas, se extrajo solamente el 20% para ambos hidrocarburos. La extractabilidad de pireno no se afectó por los componentes del suelo o por el añejamiento, y esto puede estar relacionado con un proceso de cristalización *in situ*. Las características del suelo y los contaminantes, acoplada con interacciones a largo plazo, favorecen la formación de residuos no extraíbles, conocidos también como contaminantes secuestrados. Este estudio contribuye con el conocimiento actual sobre el uso de materiales sintéticos en evaluaciones de destino ambiental de contaminantes, y debe servir como motivación para futura investigación teórica y experimental en la materia.

Palabras clave: hidrocarburos aromáticos policíclicos, extractabilidad, suelo artificial, secuestro, añejamiento.

1 Introduction

The environmental presence of hydrophobic organic contaminants (HOCs), is a major concern in scientific, industrial, economic and political areas, and, in recent

years, contamination of soils by polycyclic aromatic hydrocarbons (PAHs) has gained attention (Martínez-Prado *et al.*, 2014; Trellu *et al.*, 2016). It is well known that soil components in nature—such as pore geometry and soil organic matter—play an important role in the fate of HOCs (Nam & Alexander, 1998; Chung & Alexander, 1998). The environmental persistence

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of these contaminants in soils is important because of their toxicity and potential bioavailability. In this sense, the physicochemical characteristics of these contaminants, combined with soil properties, may affect the long-term interactions formed between the soil and the chemical species and thus lead to trapping phenomena that interfere with bioavailability (Lamichhane *et al.*, 2016, Martínez-Prado & Soto-Álvarez, 2017). The mechanisms on the release of trapped PAHs are highly related to the chemical associations formed with soil constituents, therefore they require a better understanding because the release rates of HOCs control intrinsic bioavailability (Luthy *et al.*, 1997).

Sequestration and bioavailability are highly intricate phenomena that have been studied in natural and artificial controlled environments. Artificial soils have been used as model systems where natural soil properties are mimicked. Complex artificial systems (such as those proposed by OECD) have been used to test bioavailability and toxicity of contaminants (Šmídová *et al.*, 2012; Šmídová, *et al.*, 2017), while more simple systems have allowed a better understanding in enzymatic biological responses to HOCs (Cruz-Hernández *et al.*, 2013; Mendarte-Alquisira *et al.*, 2017). Nevertheless, these artificial systems have been found highly variable, and the implications of this variability need to be understood and identified before any conclusions are made (Hofman *et al.*, 2014).

Exposure time is also considered an important factor that induces variability and favors soil-contaminants interactions causing PAHs recalcitrance, giving rise to the so-called "aged soils" (Ma *et al.*, 2012). Aging is a natural, complex, time-dependent process, and a variety of biological, chemical, and physical methods have been used to artificially mimic this phenomenon (for example, heating or freezing cycles, γ -irradiation and exposure to microorganisms) (Hale *et al.*, 2011). Another method used is sterilization by autoclave, which has shown to drastically affect soil chemical and physical properties (e.g. CEC, porosity, dissolved organic matter), and it has been hypothesized that may induce changes in hydrophobicity of organic matter in the soil, modifying its adsorptive capabilities (Slizovskiy *et al.*, 2010). During soil aging, HOC sorption and desorption mechanisms take place, these mass transport processes substantially diminish the extractability of contaminants and modify their fate, leading to sequestration (Medina-Moreno *et al.*, 2009; Luo *et al.*, 2012; Khan *et al.*, 2018).

Through aging, strong bonding between contaminants and some components of the soil (for example, organic matter complexes and minerals) take place and favor the formation of non-extractable residues (NERs), which can also be regarded as sequestered pollutants (Roberts, 1984; Kästner *et al.*, 2014). Two processes are governing the interactions that favor sequestration (ECETOC, 2013): (i) covalent associations related with the most strongly bonded contaminants, which are ligand or ionic exchange; and (ii) weaker interactions such as van der Waals forces, hydrophobic partitioning or charge transfer complexes and hydrogen bonds. Both interactions may directly affect the bioavailability of contaminants in soils.

Sequestered contaminants and aging are highly related to bioavailability. Hence, when artificial soils are used, it is necessary to clearly define contaminants extractability extents and the measurable variables that represent them, along with the suitable quantitative techniques that allow inferring about the environmental fate of contaminants in soils. This work aims to correlate PAHs extractability with artificial soil properties (porosity and organic matter content) in freshly and aged samples. Hypothetically, a decrease in extractability is directly proportional to an increase in sequestration, which would allow making inferences about the bioavailability of contaminants when model systems are used. The paper is organized as follows: Firstly, the necessary experimental materials and methods to synthesize the artificial soil and the aging process are described. Secondly, the main results of this research are presented and carefully described. A highlight of this section is the proposition of a sequestration mechanism. Finally, the corresponding conclusions and perspectives are presented.

2 Materials and methods

2.1 Artificial soil and porosity

The artificial soil used in this study simulates the mechanical characteristics of agricultural soil, therefore, its water retention capacity allows the proper development of plants (Mendarte-Alquisira *et al.*, 2017). The artificial soil consisted of a perlite and sand mixture, and the pore size ranged between 0.01-100 and 10-100 μm , respectively (Minagawa *et al.*, 2008b; Grosse, 2007). Perlite (Dicalite, Mexico) was sieved (0.40-0.28 mm) and washed with hot tap water

and then air-dried. Sand (Sand Silica, Juanita, Mexico) was sieved separately (0.42-2.00 mm) and left to stand in contact with a hydrochloric acid (HCl) solution (10%, v/v) for 24 h, washed 10 times with tap water and washed 10 times with distilled water to remove residual HCl and to reach neutral pH. Both perlite and sand were separately dried in an oven at 60 °C and stored at room temperature. Before use, perlite and sand were mixed in a 1:1 proportion (v/v) to assemble the artificial soil.

Artificial soil porosity was determined by loading a graduated glass cylinder with soil. Next, distilled water was added until the solid volume was covered by the liquid (Pinder and Gray, 2008). The porosity value was calculated as the ratio of void space (water) and solid occupied space (artificial soil).

2.2 Organic matter in artificial soil

Organic matter was quantified by the Walkley-Black method (Walkley, 1947), in which total organic carbon is determined by oxidation with potassium dichromate in a strong acidic medium. After a stage of oxidation and mineralization, the excess of non-reduced dichromate was back titrated by ferrous iron.

Organic matter identification was performed by gas chromatography-mass spectrometry (GC/MS) (Agilent 5973), with an HP-5MS capillary column (30 m × 0.32 mm × 0.25 μm) and helium as a carrier (2 mL/min, 10 μL samples in split mode 1:10). The injector and interphase temperatures were 300 °C. The initial oven temperature was 150 °C, then increased up to 250 °C in intervals of 25 °C/min. The Wiley7n.L mass spectral library was used.

Organic matter was removed from the artificial soil by three different digestion methods: (i) room temperature digestion, (ii) single-heated digestion, and (iii) double-heated digestion. For each digestion, 1 g of dried artificial soil was mixed with 10 mL of 1 N potassium dichromate and 20 mL of concentrated sulfuric acid. The mixture was gently stirred and left to stand for 30 min. Then, 100 mL of distilled water and 10 mL of phosphoric acid were carefully added. For heated digestions, the initial acidic mixture was kept in contact with the artificial soil at 97 °C for 2 h in the presence of boiling beads.

2.3 Spiking and aging

Dry soil samples (1 g) were artificially contaminated with a concentration of 800 mg/kg of the

contaminants. The spiking solution used consisted of a blend of three model hydrocarbons: hexadecane (HXD), phenanthrene (PHE), and pyrene (PYR) (2:1:0.5 w/w) (Sigma-Aldrich, > 98%) dissolved in acetone (Mallinckrodt Baker). After contamination, excess acetone was evaporated. HXD is an aliphatic hydrocarbon present in complex mixtures of fuels, like Diesel; whilst PHE and PYR are PAHs listed as priority pollutants by the US Environmental Protection Agency (US EPA). These hydrocarbons are commonly used as standard molecules in sequestration and bioavailability studies.

Autoclave sterilization was proposed as a simple and practical artificial aging strategy since the phenomena triggered by this procedure have proved to affect the sequestration of HOCs and their bioavailability (Slizovski, 2010). Contaminated artificial soil samples were sterilized in an autoclave (121 °C for 15 min) and defined as aged, while contaminated non-sterilized artificial soil samples were classified as fresh.

2.4 Hydrocarbons extraction and quantification

Residual hydrocarbons were recovered from the artificial soil samples by microwave-assisted extraction (MAE) (Mars Express, CEM), as proposed by US EPA in Method 3546, with the following modifications: 1 g of contaminated dried soil was mixed with 30 mL of dichloromethane-acetone (1:1 v/v) in extraction vessels. Extraction was conducted for 30 minutes at 175 psig and 150 °C. The liquid phase was recovered for later concentration and quantification.

Extracted hydrocarbons present in the liquid phase were concentrated in a vacuum rotavapor (R-295, Büchi) (3 min, 48 °C, 450 psi). Finally, the extract was quantified by gas chromatography using a flame ionization detector (FID) (Shimadzu GC-2010 Plus with Shimadzu AOC-20i auto-injector), with a DB-H1T column (15 m × 0.25 mm × 0.10 μm) and helium as a carrier (2 mL min⁻¹, 2 μL samples in splitless mode). The injector and detector temperature were 300 °C; the column temperature was 100 °C initially, then increased up to 200 °C in intervals of 20 °C/min. External standards were used for the quantification of each hydrocarbon.

The extractability value was defined as the ratio of hydrocarbons extracted by MAE and the initial hydrocarbons added to the artificial soil in aged or fresh samples.

2.5 Statistical analysis

All statistical analyses were carried out in SigmaPlot 12.5 (Systat Software Inc, 2011) with a 95% confidence interval. All results are presented as average values from triplicates and their associated standard deviations. The normality of data was tested using the Shapiro-Wilk test. An analysis of variance (ANOVA) test and the Tukey post hoc tests were performed to identify differences in organic matter content. t-tests were used to compare means for fresh and aged samples.

3 Results and discussion

3.1 Porosity and aging

In this section, the components of artificial soil were evaluated in both aged and fresh samples. The artificial aging method is hypothesized to potentially modify the extent of contaminant extractability favoring sorption reactions between the artificial soil and the hydrocarbon blend.

Because artificial soil components differ, sand and perlite (porosity equal to 0.60 and 0.41, respectively) were individually tested in fresh and aged samples. The measurement of porosity is relevant since it is

related to the intrinsic properties of the porous medium such as permeability or even effective diffusivity (Whitaker, 1999). Figure 1 shows the extractability extents of both individual artificial soil components.

Significant differences were found between the two artificial soil components in fresh samples for HXD, PHE, and PYR ($P < 0.05$). Extractability for sand reached 86, 62, and 100% for HXD, PHE, and PYR respectively. Extractability from perlite reached 53, 21, and 100% for HXD, PHE, and PYR respectively. These results suggest that in the porosity and pore size ranges tested, extractability behavior differed due to each artificial soil component nature in fresh samples. On the contrary, aging strongly reduced extractability to lower than 5% for HXD and PHE, but not PYR, for both artificial soil components ($P < 0.05$). According to our results, the application of an aging method did modify the extractability for HXD and PHE, but not PYR, which showed an unusual behavior, remaining completely extractable in all cases. These results differ from those reported by Hale *et al.* (2011), who used temperature as an aging factor. After submitting natural soil and biochar to temperatures as high as 110 °C in airtight closed vessels, a slight increase in PYR sorption strength was observed when compared to predicted values by the Freundlich model, reflected in an increase of the Freundlich K_{Fr} constant. Sterilization conditions support the establishment of wet-dry cycles under temperatures as high as 120 °C. Temperature variations and moisture content during the aging process have been found to promote PAH sequestration, reducing its bioavailability and extractability (Yu *et al.*, 2018).

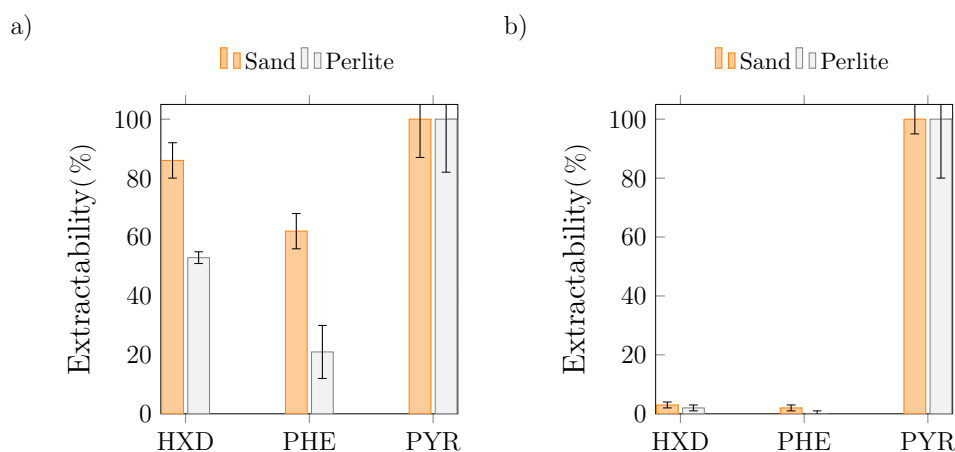


Figure 1. Extractability extents of hexadecane (HXD), phenanthrene (PHE) and pyrene (PYR) from sand and perlite in a) fresh and b) aged samples (porosity equal to 0.60 and 0.41, respectively).

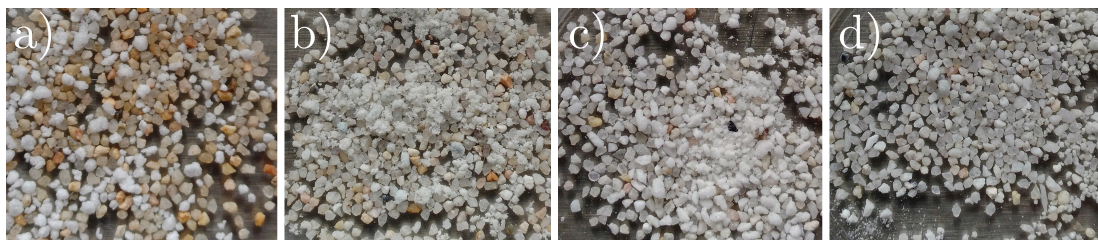


Figure 2. Artificial soil coloring after each digestion method: a) no digestion, b) room-temperature digestion, c) heated-digestion and d) double-heated digestion.

Table 1. Organic matter content in artificial soil samples before and after digestion methods.

Sample	Organic matter content (%)
No digestion	1.34 ± 0.12
Room-temperature digestion	0.41 ± 0.08
Single-heated digestion	0.14 ± 0.06
Double-heated digestion	0.14 ± 0.03

3.2 Organic matter and aging

In the present study, organic matter content was evaluated as an important factor related to aging and sequestration in artificial soils. To obtain samples of the same artificial soil with different organic matter content, samples were digested by the three different methods, described in the materials and methods section. As shown in Table 1, the organic matter content in samples diminished up to ten-fold for the single and double-heated digestion methods.

Changes in organic matter content were also visible in artificial soil samples. For example, Figure

2 demonstrates how a loss of color was consistent with a loss in organic matter content, reaching almost transparent sand grains with a more aggressive digestion method. A change in color was not observable in perlite, but there was a perceptible change in consistency and particle size.

The fresh and aged artificial soil samples with different organic matter content were tested for extractability. Figure 3 shows extractability profiles for HXD, PHE, and PYR, as a function of organic matter content for (a) fresh and (b) aged artificial soil.

In fresh artificial soil samples, HXD and PHE extractability were significantly diminished as organic matter content increased, decreasing from 96 to 65% for HXD and from 99 to 80% for PHE ($P < 0.05$). Ti *et al.* (2018) observed similar behavior for polychlorinated biphenyls, where an increase in soil organic matter and a decrease in soil particle size favored and strengthen adsorption, restricting mass transfer rates of these organic pollutants. In contrast, no significant differences were found in the extractability of the different organic matter content in aged samples for either HXD or PHE ($P < 0.05$).

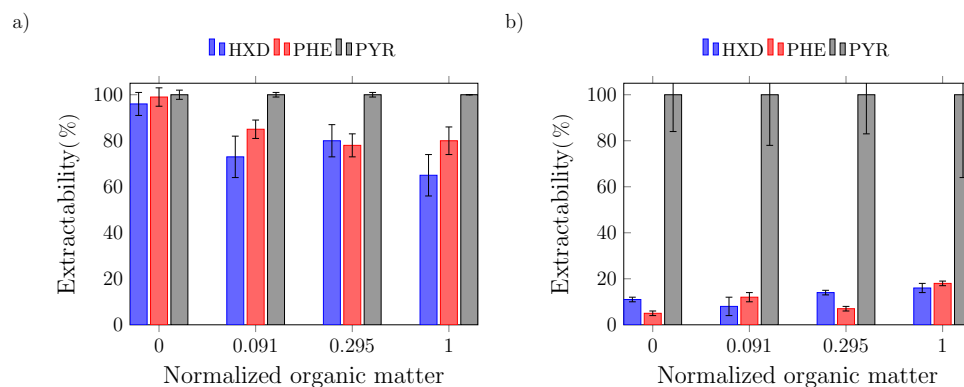


Figure 3. Extractability extents of hexadecane (HXD), phenanthrene (PHE) and pyrene (PYR) as a function of organic matter content in a) fresh and b) aged synthetic soil samples. Organic matter data was normalized with the highest content of organic matter.

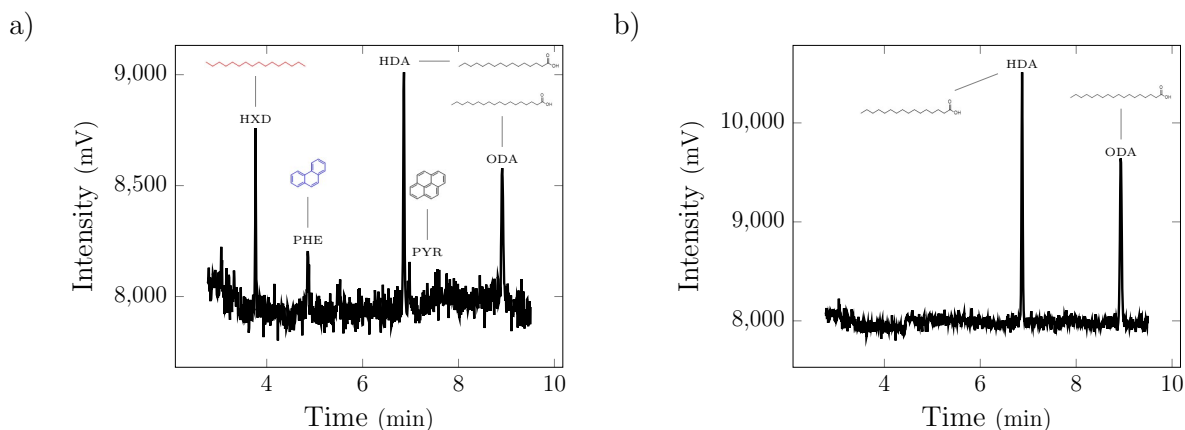


Figure 4. Gas chromatography-mass spectrometry chromatogram obtained from: a) contaminated and b) clean synthetic soil samples, after microwave assisted extraction. (HXD: hexadecane, PHE: phenanthrene, PYR: pyrene, HDA: hexadecanoic acid, ODA: octadecanoic acid).

A plausible explanation is that artificial aging (that is, sterilization) may favor irreversible sorption reactions, and it may favor a combination of soil organic matter with PAH that results in the formation of NERs (Katayama *et al.*, 2010; Kong *et al.*, 2013). In the same way, sterilization conditions may lead to the exchange of interlayer mineral water for organic compounds, sequestering hydrocarbons in inner soil layers (Chorover & Brusseau, 2008). This mineral interlayer sorption of PAHs was recently reported by Ghavami *et al.* (2017); they observed how organic molecules, such as surfactants and PAHs, change mineral microstructure surfaces, favoring sorption reactions for organic compounds. Masakorala *et al.* (2014) observed a correlation between total organic matter content as total organic carbon (TOC) and the presence of PAHs. Šmídová *et al.* (2012) investigated the effect of sterilization by radiation on the fate and behavior of ^{14}C -PYR in artificial and natural soils with different TOC concentrations. They observed a clear pattern of decline in the extractability efficiency for PYR during the incubation periods in both sterile natural and artificial soils, dropping from 7.4 to 5.4% and 69.1 to 41.2% respectively. This decrease was also influenced by a higher TOC concentration in soils.

Unlike HXD and PHE, PYR reached 100% extractability in all cases. This result differed from those previously reported for natural and artificial soils. PYR is considered one of the most recalcitrant PAHs; therefore, it has been used in many bioavailability and extractability studies where it seems to be easily incorporated into plant

biomass (Cruz-Hernández *et al.*, 2013) and native or added microorganisms (Wang *et al.*, 2012). Such a discrepancy might be explained by the nature of PYR crystallization, which occurs after solvent evaporation, during spiking and before it reaches and enters soil pores, leaving it incapable of creating interactions with organic matter in the artificial soil. This extraction behavior may have an extremely different outcome in a sample of natural soil since PYR would encounter with SOM with rich aromatic fractions, where PYR sorption would be likely to increase (Pan *et al.*, 2008).

3.3 Sequestration model

HXD and PHE sequestration may be more easily explained by the organic matter present in the artificial soil. GC/MS was performed to identify some of the components of the organic matter in the artificial soil samples, and the results are shown in Figure 4.

The sand used in this study was industrial grade, therefore this material is thought to have another component besides quartz, possibly organic traces whose origin could not be identified. Two organic acids were identified by GC/MS: hexadecanoic acid (HDA, retention time 6.9 min) and octadecanoic acid (ODA, retention time 8.9 min) (89% certainty for both compounds). Because these organic acids were found in soil samples submitted for the extraction process, their presence and origin can only be attributed to the organic matter in the original artificial soil (control samples were analyzed to discard organic acids coming from other sources,

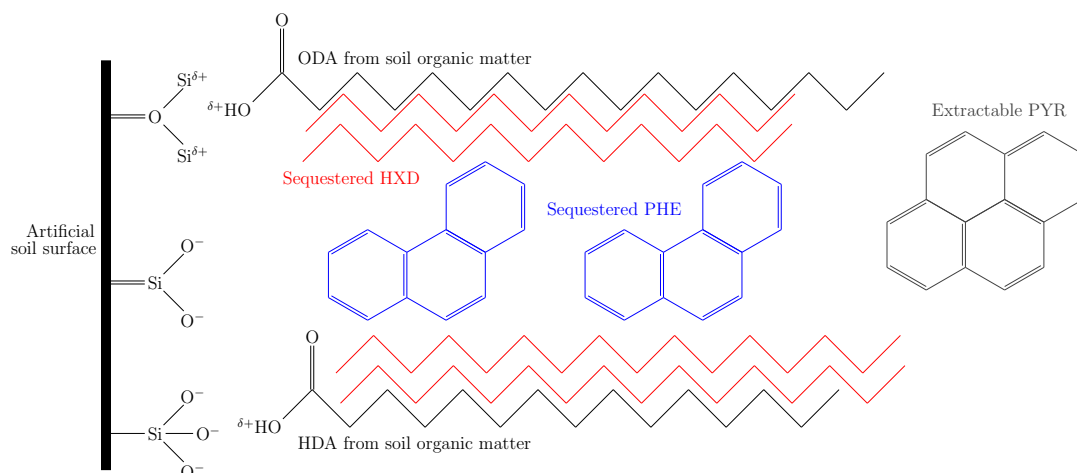


Figure 5. Proposed sequestration model for PAHs in the organic matter present in the aged artificial soil. (HXD: hexadecane, PHE: phenanthrene, PYR: pyrene, HDA: hexadecanoic acid, ODA: octadecanoic acid).

such as spiking solution). According to the aliphatic nature of organic matter, the sequestration of HXD was a natural outcome due to its structure, which favors sorption processes because of the hydrophobic interactions between organic matter and hydrocarbon chains (Salloum *et al.*, 2002). Once this interaction is formed, a hydrophobic environment is created and affinity for more hydrophobic compounds increases, resulting in the sequestration of PHE. Zhang, *et al.* (2016) have previously reported how PHE is a PAH with a high affinity for aliphatic organic matter fractions in soil. Based on our results, a sequestration model is proposed and presented in Figure 5.

Considering that artificial soil particles are silicate materials, which expose hydrophilic surface characteristics under moisturized conditions, the attachment of amphiphilic molecules, such as organic acids, becomes highly probable. This attaching creates a rich hydrophobic environment inside and outside soil pores where PHE and HXD become sequestered, and, apparently, non-extractable by aggressive physicochemical methods. In contrast, PYR does not seem to come in direct contact with organic matter in the soil, leaving it outside the tight hydrophobic places where sorption reactions take place making its extraction plausible; therefore, this hydrocarbon remains non-sequestered and increases the possibility of its bioavailability.

Conclusions

Understanding the environmental persistence of HOCs has been a concern of scientists for decades. The recalcitrance of these contaminants is directly related to soil characteristics, the interactions formed between them during their time of contact, and with the environmental factors they are submitted to. In this study, practical and simple methodologies (artificial soil and artificial aging) were proposed to shed light on the sequestration phenomena. The results demonstrate that porosity and pore size have greater effects on extractability extents than organic matter in the fresh artificial soil samples. These observations are related to the chemical characteristics of the organic matter identified in the artificial soil samples. However, artificial aging overcomes these two factors, leading to the formation of non-extractable residues and, consequently, sequestration. Besides, selective sequestration was observed, which also depended on the chemical characteristics of the organic matter. This work aims to serve as an approximation in the usage of artificial methodologies in sequestration and environmental fate studies, and the importance of evaluating the interactions formed between contaminants and the solid matrix. This study intends to make noticeable that extrapolating extractability results from artificial to natural soils would not always be direct, and much attention must

be paid to the synergistic effects of soil characteristics. The extractability hindrance is associated with important consequences, as total hydrocarbons are not properly quantified before and after treatments. These errors in quantification may lead to incorrect conclusions and overestimation on bioavailability, toxicity, and its consequences.

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