



Review

The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges



A. Cachada^{a,*}, R. Pereira^{b,c}, E. Ferreira da Silva^d, A.C. Duarte^a

^a University of Aveiro, Department of Chemistry & CESAM, Campus de Santiago, 3810-193 Aveiro, Portugal

^b Department of Biology, Faculty of Sciences, University of Oporto, Rua do Campo Alegre, 4169-007 Porto, Portugal

^c Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal

^d University of Aveiro, Department of Geosciences, GeoBioTec–GeoBioSciences, Geotechnologies and Geoenvironment Research Center, Campus de Santiago, 3810-193 Aveiro, Portugal

HIGHLIGHTS

- Chemical availability should be included in risk assessment since it's more realistic.
- The ability of chemical methods to predict PAHs bioavailability is not yet evident.
- Bioaccessibility has potential to predict microbial mineralization.
- For higher organisms, it is crucial to improve biomimetic methods and existing models.
- Chemical availability is important to understand soil's bioavailability processes.

ARTICLE INFO

Article history:

Received 1 October 2013

Received in revised form 6 November 2013

Accepted 7 November 2013

Available online 1 December 2013

Keywords:

Bioavailability

Chemical availability

PAHs

Soils

ABSTRACT

The evaluation of the available fraction of hydrophobic organic contaminants (HOCs) is extremely important for assessing their risk to the environment and human health. This available fraction, which can be solubilized and/or easily extracted, is believed to be the most accessible for bioaccumulation, biosorption and/or transformation by organisms. Based on this, two main types of chemical methods have been developed, closely related to the concepts of bioaccessibility and freely available concentrations: non-exhaustive extractions and biomimetic methods. Since bioavailability is species and compound specific, this work focused only in one of the most widespread group of HOCs in soils: polycyclic aromatic hydrocarbons (PAHs). This study aims at producing a state of the art knowledge base on bioavailability and chemical availability of PAHs in soils, clarifying which chemical methods can provide a better prediction of an organism exposure, and which are the most promising ones. Therefore, a review of the processes involved on PAHs availability to microorganisms, earthworms and plants was performed and the outputs given by the different chemical methods were evaluated. The suitability of chemical methods to predict bioavailability of the 16 US EPA PAHs in dissimilar naturally contaminated soils was not yet demonstrated, being especially difficult for high molecular weight compounds. Even though the potential to predict microbial mineralization using non-exhaustive extractions is promising, it will be very difficult to achieve for earthworms and plants, due to the complexity of accumulation mechanisms which are not taken into account by chemical methods. Yet, the existing models could be improved by determining compound, species and site specific parameters. Moreover, chemical availability can be very useful to understand the bioavailability processes and the behavior of PAHs in soils. The inclusion of chemical methods on risk assessment has been suggested and it is promising, despite some methods overpredict risks.

© 2013 Elsevier B.V. All rights reserved.

Abbreviations: ACE, acenaphthene; ANT, anthracene; BaA, benzo(a)anthracene; BaP, benzo(a)pyrene; BC, black carbon; BCF, bioconcentration factor; BghiP, benzo(ghi)perylene; BSAF, biota-to-soil accumulation factor; BuOH, n-butanol; C_{free} , dissolved concentration in pore water; C_{org} , concentrations in an organism; C_{ps} , equilibrium concentration in the sampler; CRY, chrysene; C_{soil} , concentration in soil; EqPT, equilibrium partition theory; EtOH, ethanol; FLA, fluoranthene; Frap, rapid desorbing fraction; HMW, high molecular weight; HOCs, hydrophobic organic contaminants; K_{BC} , black carbon normalized partition coefficient; K_{disk} , partition coefficient between disks and soils; K_{OC} , organic carbon–water partition coefficient; K_{OW} , octanol–water partition coefficient; Lip, lipids; LMW, low molecular weight; MeOH, methanol; MGP, manufactured gas plant; NP, naphthalene; OC, organic carbon; PHE, phenanthrene; POM-SPE, polyoxymethylene solid phase extraction; PrOH, propanol; PYR, pyrene; SFE, supercritical fluid extraction; SOM, soil organic matter; SWE, subcritical water extraction; TECAMS, triolein embedded cellulose acetate membranes.

* Corresponding author at: Laboratórios Tecnológicos, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234401527; fax: +351 234370084.

E-mail address: acachada@ua.pt (A. Cachada).

¹ Tel.: +351 234401527; fax: +351 234370084.

Contents

1.	Introduction	464
2.	Review of existing chemical methods used to predict bioavailability of PAHs in soils	465
2.1.	Non-exhaustive extractions	466
2.1.1.	Mild solvent extraction	467
2.1.2.	Subcritical water extraction (SWE)	467
2.1.3.	Supercritical fluid extraction (SFE)	467
2.1.4.	Solid phase extraction (SPE) from soil water	468
2.1.5.	Solubilising agents	468
2.1.6.	Persulfate oxidation	469
2.2.	Biomimetic methods	469
2.2.1.	Solid phase microextraction (SPME)	469
2.2.2.	Polyoxymethylene solid phase extraction (POM-SPE)	470
2.2.3.	Extraction disks	470
2.2.4.	Composite membranes	470
3.	State of the art of the prediction of PAH availability by chemical methods	471
3.1.	Microbial degradation	471
3.1.1.	Chemical methods for assessing microbial availability	472
3.2.	Bioaccumulation in earthworms	474
3.2.1.	Chemical methods for assessing bioavailability to earthworms	475
3.3.	Bioaccumulation assays using plants	476
3.3.1.	Chemical methods for assessing phytoavailability	477
3.4.	Ecotoxicological assays/molecular biology methods	477
4.	Problems and challenges faced by chemical methods for assessing availability	478
5.	Conclusions	478
	Acknowledgments	479
	Appendix A. Supplementary data	479
	References	479

1. Introduction

The total amount of hydrophobic organic contaminants (HOCs) in soil, given by traditional chemical methods, may not relate directly to environmental or human health risk, since normally only a fraction of contaminants can be leached to groundwater or be uptaken by organisms (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2003). The fraction of contaminants accessible for bioaccumulation (through pore water, soil and food) or transformation by organisms, is normally called available or bioavailable, and is environmentally the most significant (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2003). Therefore, the chemical prediction of bioavailability established by assessing the most soluble or easily extracted fraction has been a major issue in the last years on environmental sciences (Cui et al., 2013; Ehlers and Loibner, 2006).

The major difficulty when dealing with the concept of bioavailability is that there are many definitions and several methods to measure and to calculate it, depending on the specific scientific discipline (Ehlers and Loibner, 2006; Reichenberg and Mayer, 2006; Semple et al., 2003, 2004). Indeed, bioavailability depends on the physical, chemical and biological properties of contaminants, soil and receptors and it is governed by three way interactions between contaminants, matrix and organism (Ehlers and Luthy, 2003; Sijm et al., 2000). Therefore, three distinct processes are involved: physicochemical, physiological uptake and toxicological. The physicochemical processes, which have been extensively discussed in recent years (Ehlers and Loibner, 2006; Reid et al., 2000a; Semple et al., 2003), include sorption, diffusion and partitioning and are controlled by soil and compound properties such as soil organic matter (SOM) content and quality, soil inorganic constituents and lipophilicity of compounds. The physiological uptake processes depends on receptor type and specific parameters such as anatomy, feeding strategy or lipid content of organism, whereas toxicological processes are controlled by metabolism, detoxification or accumulation capacity (Ehlers and Luthy, 2003).

Due to the need of incorporate bioavailability in risk assessment, efforts have been made to clarify and standardize the concept and develop methods to measure it (Brand et al., 2013; Ehlers and Luthy,

2003; Reichenberg and Mayer, 2006; Semple et al., 2004). Semple et al. (2004) suggested distinguishing the concepts of bioavailability and bioaccessibility according to their working definitions. The term bioavailability is related to the fraction actually or freely available for the organism at a given time, whereas bioaccessibility is the fraction potentially available over time (it encompasses what is actually bioavailable and what is potentially bioavailable). Further, Reichenberg and Mayer (2006) defined two major processes that rule the physicochemical component of bioavailability: chemical activity and bioaccessibility. Chemical activity quantifies the potential of contaminants for spontaneous processes such as partition, sorption or diffusion, being related to fugacity and freely dissolved concentrations. Bioaccessibility is directly comparable with the definition provided by Semple et al. (2004) and refers to the fraction of HOCs that is weakly or reversibly sorbed and can undergo rapid desorption from the solid phase to the aqueous phase. Both processes can be included in a generic concept of chemical availability. In practice, the existing analytical methods to measure chemical availability can be divided in two groups according to the definitions given by Reichenberg and Mayer (2006). Bioaccessibility is given by depletive non-exhaustive extractions (such as mild solvent or solid phase extraction) and the chemical activity, or freely dissolved concentrations, by non-depletive biomimetic methods (passive sample techniques such as solid phase microextraction or semipermeable membrane devices).

Estimation of bioavailability is traditionally performed by using direct (accumulation in organism's tissues or biodegradation) or indirect (related with effects of contaminants – ecotoxicological tests) bioassays. In fact, bioassays are considered the most accurate approach to assess the available fraction, since they integrate the three way interactions between contaminants, matrix and organism. However, there are some constraints regarding the use of bioassays. For example, regarding field bioaccumulation studies, it is not always possible to measure the contents of contaminants in organism's tissues for all the species (ethical issues, presence of organisms), whereas in bioaccumulation tests the culturing of organisms may be difficult and it can be time consuming since more than one species should be tested. Regarding ecotoxicological tests, the main problem is that generally they are not compound specific. There is a need to develop rapid, accurate, compound specific, cheap,

ethical, user- and more environmental-friendly methods, and it has been suggested that non-traditional chemical tests could meet these requirements. Nevertheless, it is necessary to understand and validate the results of chemical methods in order to perform a routine application and its incorporation in risk assessment. Indeed, this is the biggest challenge since bioavailability is species, soil or matrix and compound specific, as is also chemical availability (Kelsey et al., 1997; Reid et al., 2000a; Ten Hulscher et al., 2003). For example, it was already demonstrated that one single chemical method could not predict availability for different organisms and the results depend on the compound as well (Kelsey et al., 1997; Ten Hulscher et al., 2003). In addition to organisms and compound specificity, availability is also matrix specific (e.g.: sorption differences between soils and sediments). For these reasons this review will focus on the existing methods to study chemical availability of one group of HOCs, polycyclic aromatic hydrocarbons (PAHs) in soils, and the relationship with bioavailability in terrestrial organisms.

Due to the existence of a vast number of diffuse and point sources, their persistence and tendency to accumulate in soils, PAHs can be major contaminants in urban or industrial areas, where levels are often above the recommended guideline values (Cachada et al., 2012; Cai et al., 2008). Moreover, the importance and interest of studying this group of contaminants is related to their environmental significance (carcinogenic, mutagenic or endocrine disrupting properties), and due to the wide range of properties of the 16 priority PAHs defined by the US EPA.

Several chemical methods have been used to predict PAHs bioavailability to bacteria, earthworms and plants (Tables 1–4). Some authors found that chemical methods tested were able to predict bioavailability for a given organism, whereas others stated that it will not be possible (e.g.: Bogan and Sullivan, 2003; Liste and Alexander, 2002). Literature data is scattered and contradictory, and leaves the question on whether it is possible or not to predict PAH bioavailability using chemical

methods unanswered. This study aims at producing a state of the art of what is known about bioavailability and chemical availability of PAHs in soils, clarifying which chemical methods can provide a better prediction of an organism exposure, and which are the most promising ones. The importance of incorporating chemical availability in risk assessment is also discussed.

2. Review of existing chemical methods used to predict bioavailability of PAHs in soils

Chemical methods typically include vigorous extractions, normally called total or exhaustive, performed by hot solvent (Soxhlet), ultrasonic or accelerated solvent extraction. However, these procedures are not related with the available fraction, since it has been observed that they over predict availability to organisms by a factor that can reach 10–10,000 times and the correlations with bioassay data is normally poor (Gomez-Eyles et al., 2010, 2012; Kelsey et al., 1997). Therefore, other analytical methods that are environmentally more relevant have been developed. Despite measuring different components of the matrix, the non-exhaustive extractions and biomimetic methods, which are the two main approaches used, are based in the principle that the exposure of contaminants to soils organisms occurs mainly through the aqueous phase. Cui et al. (2013) recently published a review of the principles, advantages and limitations of the chemical methods most commonly used to predict the bioavailability of HOCs in soils and sediments. The paper also proposes operational protocols for the methods and guidelines to interpret data obtained, i.e. how to compare chemical availability with bioassay results. Focusing on methods that predict the bioavailability of PAHs in soils, compiling major findings and reviewing the knowledge about chemical availability of these compounds are the specific goals of this paper.

Table 1
Compilation of studies comparing biodegradation and chemical methods.

Chemical method	Compound	Contamination source	Species	Reference
BuOH	NP	Spiked	<i>Pseudomonas putida G7</i>	Kelsey and Alexander (1997)
BuOH	PHE; PYR	Spiked	<i>M. austroafricanum</i>	Bogan and Sullivan (2003)
BuOH	PHE, PYR	Spiked	<i>Pseudomonas putida G7</i>	Liste and Alexander (2002)
BuOH; 50% MeOH or EtOH	PHE	Spiked	<i>Pseudomonas R</i>	Kelsey et al. (1997)
Sequential extraction	PYR	Spiked	PYR-degrading (PAS1)	Macleod and Semple (2003)
SWE	PHE	Spiked	<i>Pseudomonas sp.</i>	Latawiec et al. (2008)
SFE	13 PAHs	Gas, wood and tar works	Indigenous (composting)	Cajthaml and Šašek (2005)
Sequential SFE	8 PAHs	MPC/railroad, steel and coal tar	PAH-degrading	Szolar et al. (2004)
Tenax	PHE	Spiked	<i>Pseudomonas R</i>	Braida et al. (2004)
Tenax	15 PAHs	MPG	PAH-degrading	Li et al. (2005)
HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Hickman and Reid (2005)
HPCD	PHE	spiked	Indigenous	Papadopoulos et al. (2007b)
HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Rhodes et al. (2008a) and Rhodes et al. (2008b)
HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Rhodes et al. (2010)
HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Allan et al. (2006)
HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Doick et al. (2006)
HPCD	4 PAHs	Roadside	Indigenous	Johnsen et al. (2006)
HPCD	9 PAHs	Creosote	Indigenous	Sabaté et al. (2006)
HPCD	14 PAHs	MPG	Indigenous	Papadopoulos et al. (2007a)
HPCD	16 PAHs	MGP/diesel, lubricating oil	PAH-degrading	Hickman et al. (2008)
HPCD	16/20 PAHs	Spiked/coke works	<i>Pseudomonas sp.</i>	Stokes et al. (2005)
HPCD	24 PAHs	Spiked/coke plant	Indigenous/PAH-degrading	Doick et al. (2005)
Persulfate	16 PAHs	MPG/wood works/railroad	PAH-degrading	Cuyppers et al. (2000)
BuOH; 50% or 100% PrOH; HPCD; persulfate	12 PAHs	Creosote	Indigenous	Juhasz et al. (2005)
BuOH; HPCD	PHE	Spiked	<i>Pseudomonas sp.</i>	Reid et al. (2000b)
BuOH; SWE; HPCD; surfactants	10 PAHs	Spiked/tar works	<i>Pseudomonas sp.</i>	Latawiec and Reid (2009)
BuOH; MeOH; 50% EtOH; surfactants	15 PAHs	Gasworks/coking plant	Indigenous	Thiele-Bruhn and Brümmer (2004)
SFE; XAD2	20 PAHs	MPG	Indigenous	Hawthorne and Grabanski (2000) and Hawthorne et al. (2001)
XAD4; HPCD	NP	Spiked	Indigenous	Patterson et al. (2004)
Tenax; HPCD	12 PAHs	Historical sites	Indigenous	Bernhardt et al. (2013)
SPME	PHE	Spiked	<i>Mycobacterium vanbaalenii</i> (PYR-1)	Yang et al. (2009)

Table 2
Compilation of studies comparing earthworm accumulation and chemical methods.

Procedures/extraction	Compound	Contamination source	Species	Reference
BuOH	PYR; BAA	Spiked	<i>Aporrectodea longa</i>	Johnson et al. (2002)
BuOH	PYR, CRY	Spiked	<i>Eisenia fetida</i>	Liste and Alexander (2002)
BuOH; 50% MeOH; 35% EtOH	PHE	Spiked	<i>Eisenia fetida</i>	Kelsey et al. (1997)
BuOH; MeOH; PrOH	ANT, FLA, PYR	Spiked	<i>Eisenia fetida</i>	Tang and Alexander (1999)
BuOH; SFE	PYR	Spiked	<i>Eisenia fetida</i>	Sun and Li (2005)
SFE	PHE; PYR	Spiked	<i>Eisenia fetida</i>	Bielská et al. (2013)
SFE	16 PAHs	MGP	<i>Aporrectodea caliginosa</i>	Kreitinger et al. (2007)
XAD4	12 PAHs	MGP	<i>Eisenia fetida</i>	Bogan et al. (2005)
Tenax	PYR	Spiked	<i>Eisenia fetida</i>	Li et al. (2007)
Tenax	12 PAHs (+ chlorobenzenes)	Field contaminated soil/sediment	<i>Lumbricus rubellus</i>	Ten Hulscher et al. (2003)
HPCD	PHE	Spiked	<i>Lumbricus rubellus</i>	Hickman and Reid (2005)
BuOH; HPCD	PYR	Spiked	<i>Eisenia fetida</i>	Khan et al. (2011)
BuOH; HPCD; Tenax	5 PAHs	Spiked	<i>Eisenia fetida</i>	Gomez-Eyles et al. (2010)
95% EtOH; C18	ANT, PYR, CRY, BaP	Spiked	<i>Eisenia fetida</i>	Tang et al. (2002)
SPME	13 PAHs	MPG	<i>Eisenia fetida</i>	Jonker et al. (2007)
C18	15 PAHs	Urban	<i>Lumbricus terrestris</i>	Krauss and Wilcke (2001)
TECAM	NP, PHE, PYR, BaP	Spiked	<i>Eisenia andrei</i>	Tao et al. (2008b)
TECAM	NP, PHE, PYR, BaP	Field contaminated	<i>Eisenia andrei</i>	Tao et al. (2009)
BuOH; HPCD; SPME; POM-SPE	12 PAHs	Industrial	<i>Eisenia fetida</i>	Gomez-Eyles et al. (2012)
50% MeOH; 1% MeOH or BuOH; sequential leaching; surfactant; HPCD; SPME; SPMDs	15 PAHs	Gas plant	<i>Eisenia fetida</i>	Bergknut et al. (2007)

2.1. Non-exhaustive extractions

Non-exhaustive methods consist in simple shaking extractions to measure the fraction known as bioaccessible. They give the mass quantity of contaminants, which are or can become available under given conditions and a within time period (Reichenberg and Mayer, 2006; Semple et al., 2004). Therefore, measuring the desorption kinetics of contaminants can estimate bioaccessibility. It is believed that non-exhaustive methods may predict the bioavailable fraction because as uptake by organisms occurs, there is a depletion of the freely dissolved concentrations which may be replenished by the weakly or reversibly sorbed fraction.

Because HOCs are not uniformly distributed in particles, desorption kinetics is triphasic, due to the depletion of the contaminant pools from different compartments at rates inversely related to contaminant-particle sorption strength (Rhodes et al., 2010; You et al., 2011). The data is normally fitted to a three-compartment desorption model (Eq. (1)), assuming first order kinetics for each of the three compartments (Cui et al., 2013; You et al., 2011):

$$S_t/S_0 = F_{rap}(e^{-k_{rap}t}) + F_s(e^{-k_s t}) + F_{vs}(e^{-k_{vs}t}) \quad (1)$$

where S_t and S_0 represent the concentration in soil at time t and 0, respectively; F_{rap} , F_s and F_{vs} represent the rapid, slow and very slow desorbing fractions at time zero, and k_{rap} , k_s and k_{vs} are the respective desorption rate constants. The first phase, F_{rap} , includes the sorbed contaminants which can desorb towards the pore water within a short time, while the remaining phases, F_s and F_{vs} , are considered kinetically rate-limited. The F_{rap} is assumed to be the representative of release conditions and, based on this, the assessment of this fraction has been

suggested as a rapid approach to measure bioaccessibility, since the study of desorption kinetics including the F_s and F_{vs} may take several weeks or months. Still, this approach is used as a proxy since the F_{rap} may under- or overestimated. While some methods (solid phase extraction or solubilizing agents) have shown to be able to remove only the F_{rap} , under a defined time frame, others, such as mild solvent extraction, may also remove the F_s (Kelsey et al., 1997). Indeed, one of the difficulties in interpreting and comparing results is the fact that bioaccessibility is operationally defined, and different experimental conditions will give different results (e.g.: type of agitation, soil: solution ratio, desorption solution used or desorption time).

Bioaccessibility tends to decrease with increasing matrix contact time (sequestration), i.e., chemical available fractions (or extractability) declines with residence time of compounds in soil in opposition to fractions obtained by vigorous extractions. This was demonstrated for the methods discussed: mild solvent (Johnson et al., 2002; Kelsey et al., 1997); supercritical fluid extraction (Bogolte et al., 2007; Sun and Li, 2005) solid phase extraction (Li et al., 2007); solubilizing agents (Khan et al., 2011; Rhodes et al., 2008b; Swindell and Reid, 2006). Similarly, extractabilities of PAHs are expected to decrease with the increase of SOM content, while the total content of PAHs usually increases with the SOM content (Bogan and Sullivan, 2003; Cachada et al., 2012). Still, this behavior was not always verified, suggesting that other factors may also have influence on sequestration and desorption behavior of PAHs (Hawthorne et al., 2002). It is also expected that non-exhaustive extractions reflect individual PAH properties: in spite of being normally more abundant in naturally contaminated soils, high molecular weight (HMW) PAHs tend to have lower extractabilities due to their recalcitrant nature and higher tendency to associate with SOM (Bogan et al., 2005; Hawthorne and Grabanski, 2000).

Table 3
Compilation of studies comparing plant accumulation and chemical methods.

Procedures/extraction	Compound	Contamination source	Species	Ref
BuOH; MeOH; PrOH	ANT	Spiked	Wheat; barley	Tang and Alexander (1999)
Tenax	16 PAHs	MGP	<i>Festuca arundinacea</i> ; <i>Panicum virgatum</i>	Cofield et al. (2008)
Butanol; HPCD; Tenax	5 PAHs	Spiked	<i>Lolium multiflorum</i>	Gomez-Eyles et al. (2010)
BuOH; 50% MeOH; TECAM	NP, PHE, PYR, BaP	Field contaminated	<i>Triticum aestivum</i> L.	Tao et al. (2008a)
BuOH; HPCD; SPME; POM-SPE	12 PAHs	Industrial	<i>Lolium multiflorum</i>	Gomez-Eyles et al. (2012)

Table 4
Compilation of studies comparing ecotoxicological tests and chemical methods.

Procedures/extraction	Compound	Matrix	Test	Ref
BuOH	BaP	Spiked	Bacterial genotoxicity	Alexander and Alexander (2000)
SFE	16 PAHs	MGP	Earthworm toxicity	Kreitinger et al. (2007)
SFE	13 PAHs	Wood/gas works	Earthworm and plant growth inhibition and mortality; bioluminescent inhibition	Čvančarová et al. (2013)
Tenax	16 PAHs	Gas plant	Nematode and earthworm survival, lettuce emergence, microbial respiration	Cofield et al. (2008)
HPCD	PHE	Spiked	¹ H NMR metabolomics	Brown et al. (2010)
SPME	PYR	Spiked	Springtail toxicity	Styrishave et al. (2008)
SPME	13 PAHs	MGP	Earthworm toxicity	Jonker et al. (2007)

2.1.1. Mild solvent extraction

This is a very simple approach that involves the agitation of soil with polar solvents (typically primary alcohols), or a mixture of solvent with water, for a determined period of time, and finally the analysis of PAHs in the extraction solvent (Cui et al., 2013). The solvent most commonly used is n-butanol (BuOH), but others such as methanol (MeOH), ethanol (EtOH) or propanol (PrOH) have been also tested (Tang and Alexander, 1999; Tang et al., 2002). In addition to the different solvents used, different operational conditions (Table S1) turned the comparison of results very difficult to perform. Moreover, very few studies tested the 16 PAHs, being the majority focused on 3- and 4-ring compounds.

Some authors used sequential leaching, i.e., solvents of decreasing polarity, to assess the relative sorption strength of various PAHs pools. For example, Bergknut et al. (2007) used a sequence of MeOH, BuOH, acetone, n-hexane and toluene to extract PAHs from a gaswork's site. However, these authors found that MeOH already extracted 83% of the Σ 15 PAHs. Also, Macleod and Semple (2003) used a sequential extraction with 50% MeOH, BuOH and dichloromethane to extract pyrene (PYR) from spiked soils and, for 24 weeks aging, the percentages extracted ranged between 0.8–2.2% for the first step, 32–46% for the second and 22.5–24.7% for the final step. These results suggest that extractable percentages could be close to exhaustive extractions, when using pure MeOH or BuOH. Indeed, other studies using single BuOH extractions reported extractabilities of individual compounds in naturally contaminated samples ranging between 41 and 72%, either using a 48 h or a 120 s vortex extraction (Latawiec and Reid, 2009; Tao et al., 2008a). Moreover, no differences were observed when extracting naphthalene (NP) in freshly spiked and in aged soils (99.9 and 93.3%, respectively) (Kelsey and Alexander, 1997).

In addition, the pattern of individual compounds extracted by MeOH was found to be similar to total extractions (Bergknut et al., 2007). Likewise, BuOH extracted a similar amount of 2 to 5-ring PAHs in 15 naturally contaminated soils: 55.1 ± 3.73 for NP, 44.3 ± 3 for PHE, 53.8 ± 4.28 for PYR and $52.2 \pm 4.73\%$ for benzo(a)pyrene (BaP) (Tao et al., 2008a). In another study it was observed that BuOH extractabilities of individual compounds, in a naturally contaminated soil, increased with the octanol–water partition coefficient (K_{OW}): 41% for NP to 72% for benzo(a)anthracene (BaA) (Latawiec and Reid, 2009). Again, it's noteworthy that different BuOH contact times seem to have a little effect on PAH extraction pattern (Gomez-Eyles et al., 2010). On the other hand, using a mixture with 50% MeOH, it was possible to relate the fraction of individual PAHs extracted with their properties. In urban soils, for instance, the extractability ranged between 68% for acenaphthene (ACE) and 5.9% for benzo(ghi)perylene (BghiP), decreasing linearly with increasing log K_{OW} (Krauss et al., 2000). Tao et al. (2008a) found that in 15 field contaminated soils this mixture extracted $8.8 \pm 0.67\%$ for NP, $5.16 \pm 0.43\%$ for PHE, $3.66 \pm 0.27\%$ for PYR and $1.17 \pm 0.07\%$ for BaP. Moreover, decreasing the amount of solvent drastically reduces the percentage of PAHs extracted: 83% using pure MeOH, 5% using 50% MeOH and 0.16 or 0.13% using either 1% MeOH or BuOH for the Σ 15 PAHs (Bergknut et al., 2007). Still, the pure BuOH extractability has been related to soil properties: in 13 coal tar soils aged for 120 days, the fraction of PAHs (3 to 6 ring) extracted with this solvent

was found to be inversely correlated to the TOC content (Bogan and Sullivan, 2003).

2.1.2. Subcritical water extraction (SWE)

Latawiec et al. (2008) suggested SWE as a promising method to predict long-term release rates of PAHs from soils. The principle is to lower the polarity of water by increasing its temperature (subcritical water when $T < 374$ °C) while maintaining the liquid state by controlling the pressure. This method allows changing the water properties to obtain an aqueous solvent with similar properties to organic solvents. These authors performed an extraction similar to the conventional pressurized liquid extraction and tested several conditions, reaching the conclusion that 200 °C during 10 min was the best option (Latawiec and Reid, 2009). However, only these two studies were undertaken, and only in the latest several compounds were analyzed (10 PAHs with 2-, 3- and 4-ring) in a field contaminated soil. A decrease in extractability according to the ring size, ranging from 17% for NP to 2% for anthracene (ANT) and BaA was observed. The authors advise that there is a need to understand the influence of factors such as soil ratio, dispersing agent or water volume.

2.1.3. Supercritical fluid extraction (SFE)

SFE with pure CO₂ has been suggested as a rapid method to study the available fraction of PAHs in soils. Pure CO₂ has been chosen since it has a polarity similar to biological lipids, and, when using appropriate conditions of temperature and pressure, the solubility of PAHs is similar to the ones of water (Hawthorne et al., 2001). Yet, supercritical CO₂ is considered relatively lipophilic, transferring HOCs from nonpolar matrices more efficiently than water. It easily allows a characterization of the desorption kinetics by performing several extraction phases (sequential leaching) with successively harsher conditions (increase fluid temperature and density) that mobilize compounds from different soil particle sites (Hawthorne and Grabanski, 2000; Hawthorne et al., 2001; Szolar et al., 2004). One of the major advantages is that this method does not alter the soil organic matrix (Hawthorne and Grabanski, 2000; Kreitinger et al., 2007). Hawthorne and Grabanski (2000) concluded that the best conditions to extract and calculate the F_{rap} of 2- to 5-ring PAHs was 200 bar at 50 °C, but other authors used different conditions (Bielská et al., 2013; Cajthaml and Šašek, 2005; Sun and Li, 2005).

The extraction behavior of individual compounds has been related with their molecular weight (Čvančarová et al., 2013; Szolar et al., 2004). In addition, Hawthorne and Grabanski (2000) observed that the two and three-ring PAHs were associated with F_{rap} , while 5- and 6-ring were mostly in the slower fractions. Regarding the effect of soil properties, Szolar et al. (2004) concluded that SOM controls the sorption–desorption behavior for only some of the field contaminated soils studied, and that no relationship was observed for particle size. Similarly, Hawthorne et al. (2002) did not observe significant correlations between release rates and soil properties (elemental analysis, organic carbon and thermal gravimetric analysis) for the 6 PAHs extracted from manufactured gas plant (MGP) soils, suggesting that other parameters may have influence. Also Bielská et al. (2013) suggest that the

properties of SOM rather than the OC content have influence on the extractability of PHE and PYR using SFE. On the other hand, the influence of SOM and particle size was observed for low molecular weight (LMW) PAHs in spiked samples (Bogolte et al., 2007; Sun and Li, 2005).

2.1.4. Solid phase extraction (SPE) from soil water

In SPE methods, water is used as solvent to extract soil-associate compounds, combined with a compound scavenging resin. The matrix is mixed with an adsorbent and water in batch mode and after physical separation, the resin or the soil are analyzed in order to determine the amount that was transferred to the aqueous phase. The principle is keeping the aqueous concentrations close to zero using the resin as an “infinite” sink of desorbed compounds, maximizing the transfer to soil-water interface due to the diffusion gradient created.

One type of resin used is the XAD (styrene divinylbenzene copolymer), which is non-ionic, non-polar and hydrophobic adsorbent, but few studies have used it to predict PAH bioavailability (Tables 1–4). In fact, the resin most commonly used is Tenax, which is a solid porous polymer based on 2,6-diphenyl- ρ -phenylene oxide and it was originally used as column packing. SPE methods have been widely used to determine the desorption kinetics of HOCs from soils, due to the high sorptive rate and capacity of the resins to retain these contaminants. A typical operational protocol for calculating the desorption kinetics using Tenax is presented by Cui et al. (2013), and a compilation of SPE extraction procedures used in literature is given in Table S2. A simplified Tenax extraction of 6 or 24 h has also been used as a simple method to measure the F_{rap} . One of the advantages of using these methods lies on the fact that resin is easily separated from soil solution, since it floats on the top of aqueous phase, and PAHs can be then extracted using an organic solvent and the resin re-used. Moreover, the resin does not interact with the matrix, it can be used for different types of soil and it's easy to implement a protocol and QA/QC. The main disadvantage of this method is that it is time and labor consuming.

Still, some problems may arise for HMW PAHs, due to their low desorption rates and low solubility. For example, Hawthorne et al. (2001) did not calculate the F_{rap} of HMW PAHs in MGP soils using XAD-2 during 120 days, because the release was too slow to observe a two phase behavior. In fact, it was observed in other studies using MGP contaminated soils that Tenax or XAD-4 desorption percentages (at 120 days or 15 days, respectively) decrease with ring size, being negligible for HMW PAHs (Bogan et al., 2005; Li et al., 2005). For desorbed compounds, the fraction also depended on the ring size, being observed values of 58, 3.3 and 1.1% for NP, PHE and fluoranthene (FLA), respectively (Bogan et al., 2005). Very low desorption percentages (6%) for \sum 16 PAHs (profiles dominated by HMW PAHs) were also observed in coke oven soils after 210 days (Ahn et al., 2005). Little is known regarding the influence of soil properties. Unlike what would be expected, on MGP soils a poor positive correlation for the \sum 15 PAHs and organic carbon (OC) content was observed (Bogan et al., 2005). The rate constant of rapid desorption of PYR has been shown to be inversely dependent of SOM content in spiked soils aged for 120 days, but no relationship was observed for the desorption percentage (Li et al., 2007). Braida et al. (2004) did not observed a relationship between OC content and the PHE fraction resistant to desorption in 15 spiked soils.

2.1.5. Solubilising agents

Some authors stated that for HOCs, simply measure the fraction extracted with water, may not be the most appropriate, since they have low aqueous solubility's and tend to associate with SOM and mineral fractions (Hickman and Reid, 2005; Reid et al., 2000b). Therefore, the use of solubilizing agents as infinite sink of HOCs desorbed from soils has been suggested as an alternative approach. These agents interact with the matrix by providing a phase into which labile compounds can partition and thus enhancing their extractability.

One of the most frequently used is hydroxypropyl- β -cyclodextrin (HPCD), which was first proposed by Reid et al. (2000b). This is a ring-shaped oligosaccharide comprised of seven α -1,4-linked glucose units, having a high aqueous solubility due to the hydrophilic shell (hydroxyl functional groups). It also has a toroidal shape hydrophobic cavity, with approximately 6.5 Å in diameter, which enables the formation of 1:1 inclusion complexes with hydrophobic organic moiety, increasing the organic compound's aqueous solubility (Latawiec and Reid, 2009; Reid et al., 2000b). One assumption of this method is that the size and shape of the organic molecules and HPCD are complementary, which is the case of LMW PAHs, but it is suspected that HMW PAHs may be too large be included in the HPCD cavity, resulting in low extraction efficiency. However, the formation of 2:1 complexes has been suggested for HMW compounds (Latawiec and Reid, 2009).

Extraction with HPCD has been used to calculate desorption kinetics (Rhodes et al., 2010; Sabaté et al., 2006), though the typical operational protocol consists in single extractions. Most studies performed a 20 h extraction (Table S3), which is considered the weakly bound fraction, corresponding to F_{rap} . Yet, other extraction parameters varied between studies (e.g. concentration of HPCD, ratio soil: solution). Besides, after extraction, the solution is separated from soil (filtration or centrifugation) and some authors analyze the soil pellet whereas others analyze the HPCD solution, which has been suggested as being one of the causes of the different results obtained (Gomez-Eyles et al., 2012; Sabaté et al., 2006; Stokes et al., 2005). Latawiec and Reid (2009) concluded that in spite of being a good predictor, it can be more expensive than other methods such as Tenax. Still, using solubilizing agents may be fast and of easy operation.

Due to the working principle, it is expected to have higher extractabilities than those obtained with SPE methods, being closer to mild solvent extraction. However, the extraction of \sum 15 PAHs from a gasworks' site was 0.99% using HPCD, which was higher than using 1% MeOH or BuOH (0.16 and 0.13 respectively), but much lower than using 50% MeOH (5%) (Bergknut et al., 2007). On the other hand, higher extracted percentages were observed in MGP soil (ranging between 20 and 29% for the \sum 15 PAHs), and the extraction decreased with increasing ring number (77% for NP and 10% for BghiP), being the relationship with $\log K_{OW}$ statistically significant (Papadopoulos et al., 2007a). Also, in a creosote contaminated soil, the extractability was higher than 90% for 3-ring PAHs and FLA, between 80 and 90% for PYR, BaA and CRY, but 5- and 6-ring PAHs were not recovered (Sabaté et al., 2006). The influence of soil properties in PAHs extractability using HPCD has been widely studied, but results are not unequivocal. Several studies (Hickman and Reid, 2005; Papadopoulos et al., 2007a; Rhodes et al., 2008a, 2008b; Swindell and Reid, 2006) found lower extractabilities in soils with high SOM or black carbon (BC) contents. Moreover, the PAHs extracted after 155 h with HPCD in roadside soils ranged between 1 and 5%, in which the low values were attributed to the presence of soot (Johnsen et al., 2006). On the other hand, Patterson et al. (2004) concluded that NP extractability was related to dissolved OC rather than to texture or SOM content in spiked soils. Rhodes et al. (2010) did not observe any trend between desorption rate constants and soil type, for PHE spiked soils.

Surfactants have also been used to increase the solubility of HOCs. Surfactant molecules aggregate forming micelles with a hydrophobic core, which incorporate the HOCs (Ehlers and Loibner, 2006; Guha et al., 1998). The surfactant molecules penetrate into pores and the solid phase causing intrasorbent swelling of the soil, therefore resulting in an increased concentration gradient at the soil-water interface and matrix diffusivity of PAHs (Yeom et al., 1996). The amount of PAHs solubilized depends on the properties of the compounds and the structure and concentration of surfactant (Guha et al., 1998; Thiele-Bruhn and Brümmer, 2004). The critical micelle formation concentration is particularly important, being the PAH solubilization proportional to the surfactant dose above this concentration (Thiele-Bruhn and Brümmer, 2004). The procedure is similar to the one used for HPCD, but different

conditions and several surfactants have been tested (Table S3): Genapol88, Synperonic LF/RA 30 (Thiele-Bruhn and Brümmer, 2004); Brij 700 (Latawiec and Reid, 2009); and Tween-80 (Bergknut et al., 2007). Extractability of PAHs with surfactants seems to be higher than with HPCD. For instance, Tween-80 extracted 6.3% of the total PAHs ($\Sigma 15$) from a gaswork site, whereas HPCD extracted only 0.99% (Bergknut et al., 2007). Moreover, Thiele-Bruhn and Brümmer (2004) found that the used surfactants dissolved up to 71% of each individual PAH (3- to 6-ring), in different industrial soils. Even so, these authors observed that the surfactant-extractable PAH concentration decreased with decreasing water solubility and increasing molecular weight of compounds.

2.1.6. Persulfate oxidation

Cuyper et al. (2000) proposed a rapid method to predict PAHs bioavailability based on persulfate ($S_2O_8^{2-}$) oxidation. The principle is that organic matter is oxidized by sulfate radicals formed during the heating of persulfate. The persulfate oxidation affects the expanded organic matter, which contains the readily available fraction (low sorption affinity with PAHs and lower resistance to microbial degradation), in opposition to condensed organic matter which contains the non-available fraction. Little is known about the behavior of PAHs in soils when using this method since few studies have been made (Tables 1–4). Even so, it was observed that the amount of HMW PAHs extracted by this method in creosote-contaminated soils, was insignificant in opposition to LMW PAHs (Juhász et al., 2005).

2.2. Biomimetic methods

Biomimetic (or passive sampling) methods give the concentrations actually or currently available and are related with the chemical activity of contaminants (Reichenberg and Mayer, 2006). The chemical activity of a compound is logarithmically related to its fugacity and linearly related to its freely dissolved concentration (Mayer et al., 2003). Hence, passive samplers include all the techniques that measure the free flow of contaminants from the matrix to the sampler in a non-depletive manner, since only a minor portion of the analyte is removed from the matrix (Brand et al., 2013; You et al., 2011). These methods allow for a selective partitioning and adsorption of analytes to a surrogate phase in order to simulate desorption from soil and, after achieving the equilibrium, to estimate the bioavailable concentrations by calculating the freely dissolved concentration in pore water (C_{free}). The exchange kinetics between sampler and pore water can be expressed by a first order one-compartment model: $C_{PS}(t) = C_{free} * (k_1/k_2 * (1 - e^{-k_2 * t}))$, where C_{PS} is the concentration in the passive sampler and k_1 and k_2 are the uptake and desorption rate coefficients, respectively.

It is assumed that, at equilibrium conditions, the concentrations in the different compartments (sampler, pore water and soil) are proportional to each other. This assumption is closely related to the basis of the Equilibrium Partition Theory (EqPT), which is the most widely accepted theory concerning chemical's uptake by organisms in sediments, and considers that bioavailability of HOCs is controlled by equilibrium partitioning between sediment OC, water and the lipids of organisms. This theory has been also applied to soils, even though some of the assumptions are normally not verified in this case. For example, calculations of C_{free} may not be appropriate to describe exposure in dry media such as soil, since they are usually not saturated and, therefore, contaminants in pore water are not in equilibrium (Reichenberg and Mayer, 2006).

In EqPT it is assumed that OC fraction (f_{OC}) and organic carbon-water partition coefficient (K_{OC}) are the most important factors that determines soil-water partition coefficient (K_d), which is given by $K_d = f_{OC} * K_{OC}$. According to this theory, it is possible to calculate the C_{free} once the concentration in soil (C_{soil}) is known: $C_{free} = C_{soil} / (f_{OC} * K_{OC})$. Concentrations in an organism

(C_{org}) can be further estimated by the relationship between pore water and the bioconcentration factor (BCF), using the following equation: $C_{org} = BCF * C_{free}$. BCF is, therefore, the partition of the compound between water and the lipid content of the organism and it is generically assumed to be equal to K_{OW} . Moreover, at steady state concentrations in an organism (C_{org}), lipid (lip) normalized, are related to the OC normalized C_{soil} through a constant factor, which is the biota-to-soil accumulation factor (BSAF): $BSAF = (C_{org} * f_{OC}) / (C_{soil} * lip)$. This constant factor is independent of the soil type, species or compound properties and it is expected to be independent of K_{OW} (Krauss et al., 2000; Ma et al., 1998; Sijm et al., 2000). Yet, EqPT is not always valid and deviations are likely to occur due to several factors such as sequestration of pollutants in the soil (Sijm et al., 2000). When comparing with other compounds, for instance, PAHs are normally more strongly adsorbed to soils and have a slower desorption rates (Jager et al., 2003; Krauss et al., 2000). In addition, ter Laak et al. (2006b) showed that EqPT models can predict sorption coefficients of spiked soils (fresh or aged) but not of field contaminated soils, where C_{free} are normally lower than predicted. Besides, these models normally use generic K_{OC} values and it has also been shown that they do not reflect the behavior of naturally contaminated samples, since factors such as the quality of SOM may have a strong influence (Jonker et al., 2007). For example, Gustafsson et al. (1997) demonstrated that BC can affect the sorption of PAHs in marine environment, but this can be also applicable to water saturated soils. The authors suggested to expand the hydrophobic partition model to include BC partitioning and predict PAH sorption by using K_d ($K_d = f_{OC} * K_{OC} + f_{BC} * K_{BC} * C_{free}^{-1}$), where f_{BC} is the BC fraction of the solid matrix and K_{BC} is the BC normalized partition coefficient. A direct method to measure the predicted bioavailable fraction is the determination of HOCs present in soil pore water, but the problem of this approach is that the levels are often below the detection limit and the presence of dissolved organic matter may affect the measured concentrations. Biomimetic methods are indeed the best approach to refine the estimations based on EqPT.

The use of passive samplers allow the determination of specific K_{OC} values, derived from C_{free} ($K_{OC} = C_{soil} / (C_{free} * f_{OC})$). The C_{free} is based on the measurement of the equilibrium concentration in the sampler (C_{PS}) and the sampler-to-water partition coefficient (K_{PS-W}): $C_{free} = (C_{PS}/K_{PS-W})$ (Mayer et al., 2003). Once the C_{free} is known, it's possible to estimate the C_{org} as described before, assuming that the BCF values are equal to K_{OW} (Jonker et al., 2007). Accurate measurements of C_{free} and K_{OC} with biomimetic methods require the sampler to be in equilibrium and soil concentration to be unaffected by the sampler (ter Laak et al., 2006b). Accurate K_{PS-W} calculations are also needed, including the use of the same experimental conditions of sampling. If sampling is not performed at equilibrium, kinetic data is needed to estimate C_{free} and rate constants, by using a one or two-phase uptake model (Mayer et al., 2003; ter Laak et al., 2006b). Another approach when equilibrium is not reached is to use deuterated PAHs that allow correcting and calculating the data (Ghosh and Hawthorne, 2010).

The biomimetic methods can also be used as a surrogate of the organisms, i.e. C_{PS} can be assumed to be an estimation of C_{org} , or EqPT can be applied by calculating the passive sampler-to-soil accumulation factor – PSSF = $(C_{PS} * f_{OC}) / (C_{soil} * lip)$ – which theoretically would be equivalent to BSAF.

2.2.1. Solid phase microextraction (SPME)

Several studies have established SPME as a reliable method to assess the C_{free} for several HOCs, including PAHs (ter Laak et al., 2006b). This approach uses a fiber with a silica core that contains a thin film of organic phase (normally polydimethylsiloxane – PDMS) to extract HOCs from the aqueous phase. Compounds are absorbed via diffusion into the organic phase, with no competition between compounds or saturation of the layer, until the thermodynamic equilibrium is established (Ehlers and Loibner, 2006; You et al., 2011).

Both injector type or disposable SPME may be used, though the present study will be focused on the latter since it's much more flexible, practical and cheap (fibers can be regenerated) (Jonker et al., 2007). This method is normally called matrix-SPME, because sampling is achieved by immersing the fiber in an aqueous suspension of soil with gentle shaking (Table S4). After exposure, the analytes are extracted from fibers using solvent extraction and concentrations in fibers are then transformed in C_{free} using predetermined fiber-to-water partition coefficients ($K_{\text{SPME-W}}$). Some of the disadvantages of this method (Cui et al., 2013; Jonker et al., 2007; You et al., 2011) are: fiber stability (both physical and chemical) and sensitivity; SPME may be influenced by several parameters (temperature, etc.); fiber surface fouling (may affect uptake kinetics or increase sorption capacity); matrix effects; too long sampling times (it can take weeks or months to reach equilibrium); and difficulty to define a universal equilibration time. However, the sensitivity and sampling times may be adjusted by adjusting the surface-volume ratio of sampler, mass of sample and size of passive sampler (ter Laak et al., 2006a).

Ter Laak et al. (2006b) observed that uptake kinetics of seven PAHs (3- to 6-ring) in spiked soils, aged for 3 weeks, was different from field contaminated soils, with equilibrium reached in 3 days in the former and several months in the latter. The reason given was that in spiked soil desorption from soils is not the rate-limiting step, whereas in field contaminated soils it is. A similar conclusion was obtained by Jonker et al. (2007): differences in uptake kinetics of fibers (from 1 to 10 weeks to reach the equilibrium for several MGP soils) were due to differences in PAH desorption. However, the authors argue that the different behaviors between samples are most likely to be related to sources rather than soil characteristics. On the other hand, other authors observed that OC/SOM inversely affected the C_{free} of both PHE and PYR in spiked soils (Styrishave et al., 2008; Yang et al., 2009). Variations due to aging (up to 553 days) were not very clear for 3- to 6-ring PAHs (ter Laak et al., 2006b; Yang et al., 2009), but in another study conducted with PYR, a decrease of C_{free} with soil contact, depending of the SOM content was observed (Styrishave et al., 2008).

2.2.2. Polyoxymethylene solid phase extraction (POM-SPE)

POM is a polymer that contains a repeated polar group and it has been used to determine the partition of HOCs in sediment systems. It has only recently been tested to predict bioavailability in soils and only one study used this polymer to predict PAHs bioavailability (Tables 1–4). Advantages of this method include their stability, resistance to organic solvents and low propensity to fouling, whereas the main disadvantages are the long sampling times and large volumes of sample needed (Cui et al., 2013). The operational procedure is similar to SPME, but the soil:water ratio is normally higher. For example Gomez-Eyles et al. (2012) used 3 g of soil to 110 ml of 0.01 M CaCl_2 (76 μm thick POM, exposure time of 28 days at 20 °C, in a shaker at 150 rpm).

2.2.3. Extraction disks

C18 membranes have been used as passive sampler, but few studies have used them to predict availability of PAHs (Tables 1–4). As for the other passive samples referred previously, the method consists in the suspension of a disk in soil slurry and a further extraction of contaminants from the sampler using a solvent. The main disadvantage is that due to the high volume of the hydrophobic C18, a volume of water higher than in other materials such as composite membranes or SPME is required.

Krauss and Wilcke (2001) used a soil:solution ratio of 10:50 ml and a 50 mg C18 disk with a gentle agitation performed every second day for 15 days. In the other study, it was used a soil:solution ratio of 1:30 ml and also one C18 disk, shaken in an end-over-end for 14 days (Tang et al., 2002). Albeit being performed in spiked soils, the last study did not prove that the equilibrium was reached. Krauss and Wilcke (2001) observed that after 15 days, at 20 °C, the calculated PAH concentrations in disks were $79 \pm 12\%$ of the theoretical steady

state but if disks were exposed at 40 °C this value rises to $92 \pm 6\%$. These calculations were performed for 20 PAHs in one urban soil and the authors assumed that, for the other 24 soils, the steady-state was also achieved using these conditions. The C18 disks only extracted 0.1% of individual PAHs and the partition coefficient between disks and soils (K_{disk}) varied widely, reaching 0.41 (for ACE and fluorene) and decreasing with K_{OW} , probably due to desorption rate limitation.

2.2.4. Composite membranes

Semi-permeable membrane devices (SPMDs) are made of low-density polyethylene tubes with octanol or triolein inside and they commonly used as surrogate to predict availability of HOCs to aquatic organisms. Recently, Tao et al. (2008a), have used triolein embedded cellulose acetate membranes (TECAMs) to predict the bioavailability of PAHs in soil samples. Later, Li et al. (2010) proposed a new type of composite membrane (cellulose acetate membranes embedded with petroselenic acid) to specifically predict the availability of HOCs to plants, since this compound is the major component of plant lipids. However, these membranes were not yet tested in soil samples and will not be discussed in the present paper.

In all cases, membranes mimic the passive transfer between the organism's fat tissues and dissolved HOCs (excluding contaminants that are attached to particles or associated with colloidal material) which are available to pass the membrane and accumulate in the inter-lipid (Cui et al., 2013; MacRae and Hall, 1998). In the case of TECAMs, since the triolein is uniformly mixed with the cellulose acetate, the resistance of mass transfer to the lipid is minimal, compared with SPMD.

The method consists in the exposure of membranes to soil (without agitation), and then their extraction with a solvent. The soils are normally kept at 60–70% of water holding capacity (similar conditions to organism's uptake), which is one of the advantages of composite membranes compared with the previous described methods. However, a depletion of PAHs nearby the sampler may occur due to the diffusive transport of small and more mobile PAHs. Other problems may be the slow sorption kinetics (it can take weeks of exposure), the high quantity of the sample (to obtain acceptable analytical sensitivity) and the problem of fouling of membranes. The use of TECAMs may reduce the high sampling times required by SPMDs, since the cellulose dialysis membranes have hydrophilic hydroxyl groups which can decrease the surface tension between membrane and soil solution and consequently have a fast exchange kinetics (Li et al., 2010; Tao et al., 2009). Other advantages of TECAMs include having a large contact area (1 ml triolein on approximately 15,000 cm^2 surface area of cellulose acetate), being easily established in laboratory and being inexpensive (Tao et al., 2008a, 2008b).

Bergknut et al. (2007) concluded that PAH profiles in SPMDs had relatively high proportions of small PAHs, being one possibility that steady state was reached only for the 3-ring PAHs during the 3-week exposure. Another possibility is the referred rapid depletion of the reservoirs of these LMW PAHs closer to membranes. Tao et al. (2009) observed that the sampling process of 4 PAHs (NP, PHE, PYR and BaP) by TECAMs in naturally contaminated soils corresponded with first-order kinetics model and steady state was reached after 7 day exposure (being the time for 95% equilibration of 9.3 h for NP to 18.5 h for BaP).

Tao et al. (2008a) found that the amount of the same 4 compounds that accumulate in TECAMs, in field contaminated samples, was not very different for each one (0.27 ± 0.03 , 0.16 ± 0.01 , 0.21 ± 0.03 , and $0.33 \pm 0.08\%$ of the total amount for NP, PHE, PYR and BaP, respectively). In another study (Tao et al., 2009) also using field contaminated soils, the relationships between concentrations of PAHs in TECAMs and soils (K_{TECAM}) varied for each compound according to their K_{OW} (53, 52, 41, and 8.1 for NP, PHE, PYR, and BaP, respectively), suggesting that the desorption limitation is the most probable reason. However, these factors were much lower in spiked soils: 0.47, 2.84, 1.24 and 0.33 for NP, PHE, PYR and BaP, respectively (Tao et al., 2008b). Nevertheless, no explanation is given for these contradictory results, since it was expected

to observe higher K_{TECAMs} in spiked soils than in field contaminated. Moreover, in this study no linear relationship between $\log K_{TECAM}$ and $\log K_{OW}$ was observed, but if excluding NP it seems that there was also a decrease with $\log K_{OW}$. Yet, a significant positive linear relationship ($R^2 = 0.988$, $p < 0.01$) was observed between $\log K_{TECAM-W}$ (partition coefficient between TECAM and water) and $\log K_{OW}$. In the same study it was also observed that the quantities of the 4 PAHs accumulated by TECAMs were negatively related to SOM and positively related to dissolved OC and an effect of aging was also observed (Tao et al., 2008b).

3. State of the art of the prediction of PAH availability by chemical methods

In order to understand the meaning of the chemical assays and in an attempt to use them as alternative to bioassays, several studies investigated their correlation with biological assays. The approach normally used is either the comparison of how they correlate with the amount of HOCs degraded by microbes, accumulated by biota (earthworms, plants) or the response of ecotoxicological tests. Bioavailability is organism's specific, due to differences in animal behavior or different metabolic fates in organisms, which result in dissimilar uptake and/or elimination rates (Ten Hulscher et al., 2003). For example, availability of HOCs to microorganism is unlikely to be related with its availability to higher organisms (Bogan and Sullivan, 2003). Moreover, although several studies have concluded that aging affects bioavailability, there are some differences between organisms. Yet, several studies conclude the feasibility of a chemical method by comparing it with other studies where different bioassays were used (e.g. Khan et al., 2011). Therefore, the further discussion will be separated between microbial degradation, earthworm and plant's accumulation and ecotoxicological tests. Some difficulties were faced, since similar methods may use different conditions, or even different target species within an organism group, which can give different results, making it difficult to evaluate them. The timescale considered will also affect the amounts of PAHs accumulated or degraded. Another problem when comparing different methods is the data presentation and different normalization approaches used.

In the case of non-exhaustive extractions, direct comparisons are typically made by performing a linear regression between the extracted percentage as a function of the biodegraded or accumulated percentage. However, some authors suggest that it's possible to predict concentrations in organisms by applying the EqPT calculations, i.e. based on C_{free} (Gomez-Eyles et al., 2012; van der Heijden and Jonker, 2009). In this case, C_{free} is determined by dividing the OC-normalized concentration extracted from soils (C_{ext}) by the generic organic carbon–water partition coefficient (K_{OC}) derived from K_{OW} : $C_{free} = C_{ext} / (K_{OC} * f_{OC})$. Other authors (Ten Hulscher et al., 2003) also suggest an approach based on EqPT models, but by calculating the BSAF based on desorbing fraction (C_{ext}) instead of total concentrations $BSAF = (C_{org} * f_{OC}) / (C_{ext} * lip)$.

Outputs of biomimetic methods can be used in several ways. If passive samplers are considered a surrogate of the organisms, a direct comparison of concentrations can be made ($C_{ps} = C_{org}$), while also performing a linear regression between the concentration in the sampler as a function of the concentration accumulated, with or without lipid normalization. A direct comparison of the passive sampler-to-soil accumulation factor with the BSAF can also be made. If using the C_{free} it's possible to estimate the C_{org} as previously described (Section 2.2) or to make a direct comparison of C_{free} with C_{org} .

A compilation of literature results of comparisons between chemical methods and bioassays is presented in Tables 5–7. In some cases, the relationship was performed by calculating the ratio between chemical method and bioassay, whereas in other cases it represents the slope of the line of the best fit. Therefore, when the relationship is presented, it either represents the ratio of chemical method vs. bioassay, the slope of the linear correlation of the extracted fraction by chemical method as a function of the fraction biodegraded or accumulated, or the residual fraction after bioassay, as a function of the residual fraction after

chemical extraction. Yet, results are always interpreted in the same way: a slope higher than one means that chemical methods overpredict biodegradation and if it is lower than one there is an underestimation.

3.1. Microbial degradation

Microbial degradation is one of the most important processes for PAHs declining in soils, due to their catabolic activity (Haritash and Kaushik, 2009; Ling et al., 2009). Degradation parameters of contaminants are used as indicators of bioavailability to assess the potential of bioremediation, for instance, the availability of the sorbed HOCs to microorganisms is still not well understood. Yet, it is known that two different processes are involved: the transfer of contaminant from the soil to the aqueous phase (physical or chemical) and the metabolism of the compound (biological) (Semple et al., 2003). Regarding the latter, most important aspects were reviewed in the study of Haritash and Kaushik (2009). However, it is important to highlight that it is the species and compound's specific and that soil properties such as pH, humidity and its nutrients' availability rule the microbial activity (Haritash and Kaushik, 2009; Li et al., 2005; Sabaté et al., 2006). Yet, the effect of mixtures on biodegradation is not well known, since some studies refer that the presence of some compounds may inhibit the degradation of others, whereas other studies refer the stimulation of degradation of PAHs present in mixtures (Haritash and Kaushik, 2009).

Microbial degradation has the similar biphasic profile of desorption: an initial fast biodegradation phase, where the rate of PAHs removal is suggested to be primarily limited by microbial degradation kinetics, and a second slow phase, in which biodegradation should be limited by desorption rates (Allan et al., 2006; Doick et al., 2005; Haritash and Kaushik, 2009; Rhodes et al., 2008b). However, it is suspected that microbes not only utilize contaminants present in the liquid phase (the ones that are rapidly desorbed from particles) or in submicrometric particles dispersed in the aqueous phase, but they are also able to degrade the sorbed contaminants, suggesting that other factors may affect the relationship between biodegradation and desorption rates (Brida et al., 2004; Haritash and Kaushik, 2009; Semple et al., 2003; Yang et al., 2009). Moreover, it is known that some microbes can produce biosurfactants, enhancing the desorption rates and, consequently, the bioavailability of PAHs (Haritash and Kaushik, 2009). Nevertheless, it is accepted that the availability to microorganisms depends primarily on the speed of desorption that, in turn, depends on the soil type (Hickman and Reid, 2005), compound (Juhász et al., 2005) and aging (Rhodes et al., 2008b; Tang et al., 1998).

Regarding soil properties, higher microbial degradation in soils with lower SOM and clay mineral contents was observed (Hickman and Reid, 2005; Rhodes et al., 2008a, 2008b; Thiele-Bruhn and Brümmer, 2004). In addition, the presence of BC has been shown to reduce mineralization from 70.8% to 17.2% (Rhodes et al., 2008a). On the other hand, a high content of fulvic acids (soluble fraction of organic matter) resulted in a higher rate of PAHs degradation, whereas the sequestration of these compounds occurs mainly in the humin fraction (Bogan and Sullivan, 2003). Other authors concluded that SOM content was not the only factor affecting microbial availability of PAHs: Yang et al. (2009) suggest that soil porosity was an important factor (reduced porosity inhibited the sequestration in soils with higher OC contents). Bogan and Sullivan (2003) suggest that in addition to micropore volume, the surface area may have some influence.

A wide range of biodegradation rates of different naturally contaminated soils can be found in literature, most of them using indigenous microorganisms: the sum of $\sum 16$ PAHs decreased 16 to 72% in four industrial soils and $33 \pm 5.4\%$ to $83.4 \pm 8.7\%$ in two soils from MGP, both after 6 weeks of inoculation with catabolically active microorganisms in addition to indigenous (Hickman et al., 2008); in 10 contaminated amended soils from gasworks and coking plants, the concentration of PAHs ($\sum 15$) decreased 53 to 93% after 74 weeks (Thiele-Bruhn and Brümmer, 2004); in MGP soils, the decrease for the $\sum 15$ PAHs ranged

Table 5
Comparison between microbial degradation assays and several chemical methods.

Method	Compounds	Correlation (r^2)	Relationship	Number of soils; aging ^a	References
BuOH	NP	NS ^b	–	1, 68 d	Kelsey and Alexander (1997)
BuOH	PHE	–	1.05 ± 0.02	1, 120 d	Kelsey et al. (1997)
BuOH	PHE	0.957	1.63	1, 322 d	Reid et al. (2000b)
BuOH	PHE; PYR	0.971; 0.994	0.952; 0.998	1, 47 d	Liste and Alexander (2002)
BuOH	PHE; PYR	NS ^b	–	6, 120 d	Bogan and Sullivan (2003)
BuOH	∑ 15	0.73 ^c	2.41	10	Thiele-Bruhn and Brümmer (2004)
MeOH	∑ 15	0.76 ^c	2.80	10	Thiele-Bruhn and Brümmer (2004)
50% MeOH	PHE	–	1.04 ± 0.07	1, 120 d	Kelsey et al. (1997)
50% EtOH	PHE	–	1.10 ± 0.09	1, 120 d	Kelsey et al. (1997)
50% EtOH	∑ 15	0.85 ^c	0.95	10	Thiele-Bruhn and Brümmer (2004)
SFE	∑ 8	–	0.99 ^d	8	Szolar et al. (2004)
SFE	∑ 13	0.81–0.99	–	4	Cajthaml and Šašek (2005)
SFE	20 PAHs	0.93	–	1	Hawthorne and Grabanski (2000)
SFE	20 PAHs	0.96	–	1	Hawthorne et al. (2001)
XAD2	20 PAHs	0.96	–	1	Hawthorne et al. (2001)
XAD4	NP	NS ^b	–	5, 2d	Patterson et al. (2004)
Tenax	PHE	0.657	0.81 ^d	15, 190 d	Braida et al. (2004)
HPCD	NP	0.917	–	5	Patterson et al. (2004)
HPCD	PHE	0.964	1.07	1, 322 d	Reid et al. (2000b)
HPCD	PHE	0.89	1.11	4, 100 d	Allan et al. (2006)
HPCD	PHE	0.88	0.99	4, 37 d	Hickman and Reid (2005)
HPCD	PHE	0.13–0.95	0.079–1.12	4, 100 d	Rhodes et al. (2008a)
HPCD	PHE	0.95–0.99	0.99–1.08	4, 100 d	Rhodes et al. (2008b)
HPCD	PHE	0.97–1	0.89–1.08 (1.04)	2, 139 d	Doick et al. (2006)
HPCD	PHE	0.96–0.98	1.01–1.05	4, 100 d	Papadopoulos et al. (2007b)
HPCD	PHE	0.89	1.18	4, 100 d	Rhodes et al. (2010)
HPCD	13 PAHs	0.77	0.88 ^d	1	Juhasz et al. (2005)
HPCD	∑ 14 PAHs	0.94–0.98	0.93–1.07	6	Papadopoulos et al. (2007a)
HPCD	16 PAHs	0.94–0.99	0.73–1.4 ^d	6	Hickman et al. (2008)
HPCD	∑ 24 PAHs	0.85–0.96	0.8–1.0 (0.92) ^d	2	Doick et al. (2005)
HPCD	16 PAHs	0.786–0.986	0.835–0.934 ^d	2, 12 m	Stokes et al. (2005)
	20 PAHs	0.986	1.032 ^d	1	
surfactants	∑ 15 PAHs	0.71–0.73 ^c	1.31–1.36	30	Thiele-Bruhn and Brümmer (2004)
persulfate	13 PAHs	0.86	1.06 ^d	1	Juhasz et al. (2005)
persulfate	∑ 16 PAHs	0.95	0.97 ^d	14 ^e	Cuyppers et al. (2000)
SPME	PHE	0.483–0.908 ^c	–	5, 120 d	Yang et al. (2009)

^a Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soils.

^b Not significant.

^c Correlation coefficients (r).

^d Based on remaining fractions.

^e 7 soils + 7 sediments.

from 18 to 30% after 8 weeks of incubation (Papadopoulos et al., 2007a); the PAH (∑ 13) degradation in 4 industrial soils after composting for 8 weeks ranged from 64 to 96% (Cajthaml and Šašek, 2005); but only from 0.3 to 9.6% (∑ 12) in 11 industrial soils that were incubated for 245 days (Bernhardt et al., 2013). Moreover, other authors suggest that naturally contaminated soils may not be biodegradable, especially if they have a high percentage of HMW PAHs. For example Szolar et al. (2004) studied 8 industrial soils and 5 of them showed no biodegradation of the 8 PAHs with 4- and 5-rings studied, after inoculation with PAH-degrading bacteria during 14 weeks. In creosote-contaminated soils, after 16 week incubation with indigenous microbes, 89 to 99% of 3 ring-PAHs were degraded, 21 to 79% of the 4-rings and no degradation of 5- and 6-rings PAHs was observed (Juhasz et al., 2005). In coke works' soils, the biodegraded % after 6 weeks ranged between 0.7 (for BaP) and 53% (for NP) using an inoculum of PAH-degrading bacteria (Stokes et al., 2005). Similarly, in MGP soils only LMW PAHs, especially 2 and 3 ring PAHs, were removed at a great extent during 1 year bioremediation with indigenous microbes, whereas the 5 and 6 ring PAHs were not degraded (Hawthorne and Grabanski, 2000). In another study conducted in MGP soils, the biodegradation ranged from 77% for NP to 6% for dibenzo(a)anthracene, being the relationship with log K_{OW} statistically significant (Papadopoulos et al., 2007a). Other authors also observe that the rate of degradation decreases when the number of rings increases (Li et al., 2005; Sabaté et al., 2006; Szolar et al., 2004). The main reason is believed to be the low desorption rates of HMW PAHs which tend to be recalcitrant in

soils. However, it is known that only a limited number of bacteria can grow in pure cultures of PAHs with 5 or more rings, and therefore the ability of the microbial communities for their degradation is low (Haritash and Kaushik, 2009). Other reason may be due to the biological factors such as the slow transport of HMW PAHs over microbial cell. In addition, it has also been demonstrated that some microorganisms may have active efflux mechanisms which could play an important role in controlling intracellular and membrane concentrations, especially for PAHs with higher K_{OW} (Bugg et al., 2000). Hence, the low biodegradation rates of HMW PAHs may be either due to the low desorption rate from soils or due to the inadaptation of communities to degrade these compounds.

3.1.1. Chemical methods for assessing microbial availability

Based on the principle that microbial availability is first governed by contaminant mass transfer from the solid phase to the aqueous phase, several non-exhaustive extractions have been proposed to predict bioavailability to microbes (Swindell and Reid, 2006). Regarding mild solvent extraction it becomes very difficult to arise any conclusion about the feasibility of these methods due to the small amount of tested compounds, the differences in operational procedures and the fact that most studies were performed in one single spiked soil (Tables 5 and S1). A BuOH extraction with agitation was addressed as the most appropriate solvent for predicting microbial degradation of PHE, when comparing with other solvents (Kelsey et al., 1997). Further studies conducted with spiked soils also found good correlations between BuOH

Table 6
Comparison between earthworm accumulation and several chemical methods.

Method	Compound	Correlation (r^2)	Relationship	Number of soils; aging ^a	References
BuOH	PHE		0.81 ± 0.15	1, 120 d	Kelsey et al. (1997)
BuOH	PYR	0.98	–	1, 222 d	Khan et al. (2011)
BuOH	PYR	0.8567	3.477	6, 120 d	Sun and Li (2005)
BuOH	PYR, BaA	NS ^c	–	1, 240 d	Johnson et al. (2002)
BuOH	ANT, FLA, PYR	$0.933\text{--}0.96^b$	–	1, 203 d	Tang and Alexander (1999)
BuOH	\sum 5 PAHs	0.54	–	1, 6 m	Gomez-Eyles et al. (2010)
BuOH	\sum 12 PAHs	0.53	–	10	Gomez-Eyles et al. (2012)
MeOH	ANT	0.992^b	–	1, 203 d	Tang and Alexander (1999)
PrOH	ANT, FLA, PYR	$0.943\text{--}0.992^b$	–	1, 203 d	Tang and Alexander (1999)
1% BuOH	\sum 16	–	3.2	1	Bergknut et al. (2007)
50%MeOH	PHE	–	1.52 ± 0.55	1, 120 d	Kelsey et al. (1997)
50% MeOH	\sum 16 PAHs	–	125	1	Bergknut et al. (2007)
1% MeOH	\sum 16 PAHs	–	4.0	1	Bergknut et al. (2007)
35%EtOH	PHE	–	1.49 ± 0.43	1, 120 d	Kelsey et al. (1997)
95%EtOH	\sum 4 PAHs	0.922	–	6, 335 d	Tang et al. (2002)
SFE	PYR	0.9076	5.128	6, 120 d	Sun and Li (2005)
SFE	PHE; PYR	$0.43\text{--}0.45^b$	–	6, 56 d	Bielská et al. (2013)
SFE	16 PAHs	–	$0.8\text{--}2.1$	3	Kreitinger et al. (2007)
Tenax	PYR	0.8855	–	6, 120 d	Li et al. (2007)
Tenax	\sum 12(+CBs)	0.7594	0.28	10	Ten Hulscher et al. (2003)
HPCD	PHE	NS ^c	–	4, 37 d	Hickman and Reid (2005)
HPCD	PYR	0.98	–	n = 1, 222d	Khan et al. (2011)
HPCD	\sum 5 PAHs	0.51	–	1, 6 m	Gomez-Eyles et al. (2010)
HPCD	\sum 12 PAHs	0.10	–	10	Gomez-Eyles et al. (2012)
HPCD	\sum 16 PAHs	–	24.8	1	Bergknut et al. (2007)
SPME	\sum 12 PAHs	0.46	–	10	Gomez-Eyles et al. (2012)
SPME	\sum 16 PAHs	–	1.2	1	Bergknut et al. (2007)
POM-SPE	\sum 12 PAHs	0.46	10	10	Gomez-Eyles et al. (2012)
C18	\sum 4 PAHs(3–5)	0.873	–	6, 335 d	Tang et al. (2002)
C18	15 PAHs	$0.47\text{--}0.87$	$0.49\text{--}4.4$	25	Krauss and Wilcke (2001)
SPMD	\sum 16 PAHs	–	2.4	1	Bergknut et al. (2007)
TECAM	NP; PHE; PYR; BaA	$0.970\text{--}0.933$	$0.033\text{--}0.61$	10, 150 d	Tao et al. (2008b)
TECAM	NP; PHE; PYR; BaP	$0.591\text{--}0.824$	$0.84\text{--}1.22$	18	Tao et al. (2009)

^a Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soil.

^b Correlation coefficients (r).

^c Not significant.

extractions and PHE or PYR biodegradation, but others did not found any significant correlation (Table 5). Yet, a significant correlation was observed for the \sum 15 PAHs in industrial soils, not only for BuOH but also using other solvents. Regarding the relationship between the two approaches (Table 5), using either pure solvents or mixtures showed correlations close to 1 for PHE and PYR in spiked soils, but a slight overestimation was observed in a soil that was aged for a longer period than the others. Similarly, BuOH or MeOH extractions showed an overestimation for the \sum 15 PAHs in industrial soils, but using 50% EtOH the relationship was close to 1. Other studies, also using naturally contaminated samples but reporting results for individual compounds instead of the sum, found that this overestimation increased with K_{OW} of compounds (Juhász et al., 2005; Latawiec and Reid, 2009). Even

more, Juhász et al. observed a significant underestimation of LMW PAHs (being lower when using pure solvents) and an overestimation of HMW PAHs (being higher using pure solvents) in cresote contaminated soils. Macleod and Semple (2003) used a sequential extraction and concluded that the first step (50% MeOH) underestimated the availability of PYR in aged soils, whereas the second step (BuOH) gave an overestimation.

The use of SWE was tested to predict PHE biodegradation in 2 dissimilar soils at 3 different contact times, but results are not very consistent (Latawiec et al., 2008). Overall, the authors did not find significant differences between SWE, at 160 °C (10 min), and biodegradation, although two of the six comparisons made showed incoherent results. Further, these authors used the same method to extract several

Table 7
Comparison between plant accumulation and several chemical methods.

Method	Compound	Correlation (r^2)	Relationship	Number of soils; aging ^a	References
BuOH	ANT	$0.892\text{--}0.929$	–	1, 203 d	Tang and Alexander (1999)
BuOH	NP; PHE; PYR; BaP	–	163–277	15	Tao et al. (2008a)
BuOH	\sum 5 PAHs	0.93	–	1, 6 m	Gomez-Eyles et al. (2010)
BuOH	\sum 12 PAHs	0.38	10–1000	10	Gomez-Eyles et al. (2012)
MeOH	ANT	$0.927\text{--}0.943$	–	1, 203 d	Tang and Alexander (1999)
PrOH	ANT	$0.921\text{--}0.945$	–	1, 203 d	Tang and Alexander (1999)
50%MeOH	NP; PHE; PYR; BaP	–	3.6–38	15	Tao et al. (2008a)
Tenax	\sum 5 PAHs	0.92	0.633	1	Cofield et al. (2008)
HPCD	\sum 5 PAHs	0.97	–	1, 6 m	Gomez-Eyles et al. (2010)
HPCD	\sum 12 PAHs	0.26	10–1000	10	Gomez-Eyles et al. (2012)
SPME	\sum 12 PAHs	0.27	–	10	Gomez-Eyles et al. (2012)
POM	\sum 12 PAHs	0.16	–	10	Gomez-Eyles et al. (2012)
TECAM	NP; PHE; PYR; BaP	$0.798\text{--}0.925$	$0.82\text{--}1.25$	15	Tao et al. (2008a)

^a Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soil.

PAHs from spiked and naturally contaminated soils and found that availability to microorganisms was underestimated (as high as around 70% for 3 ring PAHs) (Latawiec and Reid, 2009). However, the extraction efficiency decreased as the log K_{OW} increased, being the extractions analogous to the bioavailable fraction.

Very good linear correlations have been obtained using SFE either for the \sum 13 PAHs or for individual compounds in naturally contaminated soils (Table 5). Yet, the conditions applied were different for each study and few dissimilar soils were tested. Hawthorne and Grabanski (2000), for instance, obtained the best results with the mildest sequential SFE condition (60 min, at 120 bar and 50 °C) but, in another study, Hawthorne et al. (2001) used a single extraction and found that 20 min extraction (200 bar and 50 °C) successfully simulated a 147-day field bioremediation for the 20 PAHs. Other study conducted by Cajthaml and Šašek (2005) concluded that the best compromise between F_{rap} and biodegradation of \sum 13 PAHs in 4 dissimilar soils was achieved by changing the pressure to 300 bar. These authors observed differences in the coefficients of correlation of PAHs groups, with r^2 ranging from 0.74 to 1.0 for 3-ring PAHs, from 0.80 to 1.0 for 4-ring and from 0.27 to 0.99 for 5- and 6-ring PAHs. There is also an increased underestimation as the molecular weight increases. On the other hand, Hawthorne et al. (2001), stated that the method tends to overestimate availability of middle molecular weight PAHs (PYR-CRY). Szolar et al. (2004) found that the first two phases of a sequential SFE roughly estimates biodegradation, even though it does so with a tendency for underestimation. In spite of all this, it could be a promising method even though it is not easy to implement, since few laboratories have the required technology.

The PAH extraction using XAD2 during 2.2 days correlated well with a 147-day field bioremediation of MGP soils (Table 5). However, the authors found some differences between compounds and stated that, regarding middle molecular weight PAHs (PYR-CRY), the method tends to overestimate availability, as observed for SFE. Another study found poor correlations between NP mineralization and a 20 h extraction with XAD4 in spiked soils (Table 5). Even though the SPE using Tenax showed to provide an estimation of the amount available for biodegradation in sediment samples, few studies were conducted in soils (Table 1). Bernhardt et al. (2013) concluded that residual concentration of \sum 12PAHs in industrial soils after biodegradation was similar to the non-extracted concentration, but the relationship was not shown. Similar results were observed by Braida et al. (2004), which calculated the relationship between the PHE fraction resistant to desorption and the one resistant to biotransformation after 30 days, in 15 spiked soils (Table 5). However, this study emphasizes that the decline in mineralization of aged PAHs, at late times, is rate-limited by desorption. The authors also stated that other organisms capable of producing larger amounts of surfactants would be less dependent of desorption rate. In fact, another study conducted by Li et al. (2005) found that the fraction of \sum 12 PAHs resistant to desorption was greater than the fraction resistant to biodegradation, but no correlations were calculated. These authors suggest several explanations: the production of biosurfactants by microorganisms that enhance the solubilization of compounds; microorganisms are able to “use” contaminants directly from soil particles; and they may have access to contaminants in the film layer. These authors also observed that the resistance to both desorption and biodegradation increases with ring size.

HPCD is considered a good estimator of PAH bioavailability to microorganisms, since it conceptually mimics the mass transfer process governing microbial bioavailability, mainly due to the ability of microorganisms to produce surfactants. Indeed, several studies found strong correlations and slopes close to 1 between HPCD extractions (around 20 h, corresponding to the F_{rap}) and microbial degradation of PHE, in dissimilar spiked soils (Table 5). Though, Rhodes et al. (2010) calculated the desorption kinetics of PHE and concluded that the F_{rap} occurred from 0 to 6 h. Deviations to the predictability using HPCD may derive from the presence of BC, since the increase content affected the linear

correlations and the relationship: both decreases (Table 5) as the BC content increases from 0 to 5% (Rhodes et al., 2008a). Other authors (Doick et al., 2006; Papadopoulos et al., 2007b) tested the method to predict the availability of PHE in co-contaminated spiked samples and found that it still provided a good estimation for PHE. The estimation of biodegradation using HPCD also proved to be applicable to dissimilar naturally contaminated soils, considering several PAH compounds (Table 5). Even considering each one of the 24 PAHs investigated, Doick et al. (2005) stated that the indigenous microflora has degraded a quantity equal to that extracted by the HPCD. However, several other studies refers differences between compounds, generally with a slight overestimation (slope around 2) of the biodegradation endpoint for HMW PAHs probably due to the desorption limitation of these compounds (Juhász et al., 2005; Papadopoulos et al., 2007a). In the specific study of Hickman et al. (2008), results are given by ring classes for two different types of soil, and it was observed that slopes were more variable for HMW PAHs: for 2- and 3-ring the slopes were between 0.75 and 0.98; for 4-ring they were 0.98 and 1.38, whereas for 5- and 6-ring they were 0.78 and 1.80. Similarly, correlations were better for LMW PAHs: the r^2 was 0.95 and 0.97 for 2- and 3-ring; 0.93 and 0.97 for 4-ring; and 0.83 and 0.98 for 5- and 6-ring, respectively. Sabaté et al. (2006) observed low correlations for BaA and CRY, and other HMW PAHs were not either biodegraded nor extracted by HPCD.

Regarding other solubilizing agents, Latawiec and Reid (2009) used Brij 700 and did not find consistent results across different PAHs in spiked and naturally contaminated soils, but an underestimation was generally observed. Thiele-Bruhn and Brümmer (2004) tested Genapol UDD 88 and SynperonicLF7RA 30 for the extraction of 15 PAHs from 30 industrial soils and found good results (Table 5) with a slightly overestimation, but the behavior was similar for all individual groups.

A 3 h persulfate oxidation was successfully used to predict the percentage of total PAHs biodegradable (21 days), as well as for 2–4 ring compounds ($r^2 > 0.82$), in several historically contaminated soils and sediments (Cuyper et al., 2000). However, for 5 ring PAHs the method could not predict biodegradation, since compounds were better oxidized than biodegraded. Yet, when comparing the residual PAH concentration after biodegradation with the residual concentration after persulfate oxidation, results are much better (Table 5). The correlations found by the PAHs group were good in all cases ($r^2 > 0.91$) and the slopes were close to one (1.02, 0.99 and 0.91 for 2 + 3-, 4- and 5 + 6-ring, respectively). Similar results were obtained by Juhász et al. (Table 5), being the $r^2 > 0.85$ for each group, with a slight overestimation (1.3) of PAHs with 5 + 6 ring plus CRY, but only one soil type was tested. These better correlations observed when comparing residual fractions are likely to be due to the low removal fractions, which give a high uncertainty of results.

Due to the working principles it is not expected that biomimetic methods could be a good predictor of biodegradation. Even so, the single study that has used SPME (Table 1), concluded that PHE degradation was significantly related to C_{free} (Table 5) up to 144 h of biodegradation (being the highest observed for 1–3 h). Since the estimated residue fraction of PHE in soil was higher than the measured residue fraction, it's suggested that bacteria are able to degrade PHE in the sorbed form and not just the fraction desorbed.

3.2. Bioaccumulation in earthworms

Earthworms (*Lumbricidae*) are normally used to estimate the potential exposure of soil biota. The major reasons for their usage in standard toxicity tests and what makes them appropriate test organisms are the fact that they live in intimate contact with soil, they uptake contaminants directly from soils (soil solution or ingestion), they show a high degree of pollutant accumulation, and they are of easy handling (Lanno et al., 2004). The main problems concern the pH and other soil property dependence and the limited concentration ranges tolerated for some substances. Ecologically, their importance relies on the fact

that they are of extreme importance in terrestrial food chains, they are the largest part of soil biomass (in several soils), and they are essential to the cycling of nutrients, thus having a key role in several soil services (Jager et al., 2000; Ma et al., 1998).

EqPT has been successfully applied to derive ecological screening benchmarks for PAHs in sediment invertebrates; however the applicability of this theory to terrestrial organisms can be questioned. For example, Jager et al. (2003) found that BSAFs obtained in field samples were up to two orders of magnitude lower than the predicted by EqPT and Kreitinger et al. (2007) observed values 3 to 11-folds lower than predicted. Overestimations between 10 and 10,000 of predicted concentration by EqPT were observed for 13 PAHs in worms (Jonker et al., 2007) and between 3 and 11 for 16 PAHs (Kreitinger et al., 2007), both using MGP soils. On the other hand, Krauss et al. (2000) found that EqPT was applicable to predict the bioaccumulation of Σ 20 PAHs in urban soils.

The deviations to EqPT, in addition to physical–chemical factors as explained previously (section 2.2), can be due to the species related differences. Differences between species, which may be related to their behavior, have been highlighted: residues of PAHs were lower in *Lumbricus rubellus* than in *Eisenia andrei*, on average by a factor of 2, but as high as 17 for BghiP (Jager et al., 2003). One of the assumptions of EqPT is that the uptaking of HOCs by organisms occurs via passive diffusion from soil solution through outer membrane. However, earthworms can also access contaminants from solid phase, through gut uptake, being the importance of this route dependent on the species and the compound. Moreover, Jonker et al. (2007) suggest that compounds can be transferred directly from soil solids to worm tissue by contact between the two phases, however this mechanism is not well studied. It is believed that pore water is the major route of exposure for compounds with $\log K_{ow} < 5$, but soil ingestion is very important for more hydrophobic compounds (Ma et al., 1998). Consequently, these authors concluded that EqPT was applicable to predict the bioaccumulation LMW PAHs in field contaminated soils, but not for the HMW.

Other factors that may cause a deviation in EqPT are biotransformation, active excretion and reproduction. These are mechanism that may be used for depuration of contaminants, explaining differences in metabolic fate of compounds and, consequently, the differences in bioaccumulation rates (Ma et al., 1998; Sijm et al., 2000). For instance, BSAFs were found to be higher for PCBs than for PAHs with similar K_{ow} , probably due to a higher metabolic transformation rate of PAHs (Krauss et al., 2000). Moreover, the elimination rate of PAHs in earthworms decreases with K_{ow} , due to a decrease in metabolic transformation, even though not as strong as in other HOCs such as chlorobenzenes (Jager et al., 2000; Ma et al., 1998).

Literature data on earthworm's uptake and accumulation is difficult to compare due to differences in experimental conditions and even presentation of results. Jager et al. (2000) obtained BSAFs for *E. andrei* of 7.3 (kg_{oc}/kg_{lip}) for PHE, 8.2 for FLA, 3.9 for PYR and 2.4 for BaP, in a freshly spiked soil with different concentrations. However, the same authors obtained an average of 0.23 (kg_{oc}/kg_{lip}) (Σ 14) in field contaminated soils using the same species (Jager et al., 2003). Kreitinger et al. (2007) obtained values ranging from 0.004 (kg_{oc}/kg_{lip}) for NP to 0.227 for BaA in 4 MGP soils using *Eisenia fetida*. Ma et al. (1998) found BSAF values (Σ 11PAHs) for *L. rubellus* ranging from 0.03 (kg_{oc}/kg_{lip}) to 0.26, with an average of 0.1, in 12 naturally contaminated soils. Parrish et al. (2006) calculated BSAF values for Σ 4 PAHs of 0.011 (kg_{oc}/kg_{lip}) for *E. fetida* and 0.007 for *Lumbricus terrestris*, in MGP soils.

Other studies present the % of PAHs accumulated by earthworms and, similarly, a wide range of results were observed. For example, the study of Sun and Li (2005) refers that *E. fetida* accumulated between 0.87 and 3.6% of total PYR in dissimilar soils aged for 120 days, whereas Khan et al. (2011), for a similar aging period, presented percentages ranging between 11 and 25% (with higher percentages corresponding to lower contamination levels). Kelsey and Alexander (1997), for a similar aging period, reported 3.3% of PHE accumulated by *E. fetida* and for

ANT an uptake percentage of 13.7 after 203 days aging was reported by Tang and Alexander (1999). Regarding field contaminated soils, Bergknut et al. (2007) found that *E. fetida* accumulated 0.04% of the total PAHs (Σ 22) in a MGP soil. Kreitinger et al. (2007) similarly observed an average value of 0.06% for the same species, compounds and contamination source. Also in MGP soils, only FLA, PYR, BaA and CRY contained detected levels in earthworms (*E. fetida* and *L. terrestris*) among 12 PAHs analyzed (Parrish et al., 2006).

Regarding the effects of soil properties, Sun and Li (2005) observed that percentages of PYR accumulated in earthworms were dependent of soil properties, with a decrease in accumulation following the increase of SOM, in spiked soils. In addition, these authors suggest that the clay content may also have some influence on accumulation. Tang et al. (2002) tested different soils and found that the availability of ANT, PYR, CRY and BaP to *E. fetida* varied, being the greatest uptake observed for soil with low content of SOM, but any trend was evident for other soils. Jager et al. (2003) stated that SOM is not the only property controlling sorption and bioaccumulation. Besides, these authors observed that soils with a higher content of OC and clay, a low pH and low PAH level, showed higher BSAF values. Hickman and Reid (2005) observed that there were little differences in earthworm (*L. rubellus*) accumulation of PHE between dissimilar spiked soils.

Aging showed an effect on earthworm's accumulation for PHE and PYR (Chung and Alexander, 1999; Sun and Li, 2005), in dissimilar soils. On the other hand, Khan et al. (2011) found that the amount of PYR accumulated by earthworms suffered little changes with aging, for high contamination levels, but great differences were observed in low contamination levels. The effect of concentration has also been studied and, regarding PHE, the assimilated percentage was higher for lower concentrations, although contradictory results were found for PYR (Chung and Alexander, 1999; Khan et al., 2011). However, it should be noted that in low contaminated soils biological factors (e.g.: digestible organic matter) become important (Barthe et al., 2008).

3.2.1. Chemical methods for assessing bioavailability to earthworms

Extractions using either pure solvents or mixtures showed significant correlations with earthworm's accumulation, (mainly for 3- and 4-ring PAHs in spiked soils) in all except one study (Table 6). The use of 95% EtOH provided good results even when looking to individual compounds (ANT, CRY, PYR, BaP): $r^2 \geq 0.844$ (Tang et al., 2002). Gomez-Eyles et al. (2010) also calculated the correlations for the individual PAHs, which were higher than 0.63 using a 50 s extraction with pure BuOH. In another study, these authors found similar results for 12 PAHs in industrial soils, for both total concentrations (Table 6) or individual compounds ($r^2 = 0.66$ for 4 ring PAHs), but in this case 5- and 6-ring PAHs were also tested and correlations were low (0.48). These authors attempted to improve the relationships by estimating the C_{org} based on EqPT calculations but without success.

Regarding the relationship between the mild solvent extraction and earthworm accumulation, results were not consistent. An underestimation of PHE, PYR, CRY and BaA predictions, in spiked soils, was observed by some authors (Johnson et al., 2002; Kelsey et al., 1997; Liste and Alexander, 2002). However, overpredictions were observed in most studies and, in addition to values presented in Table 6, they were also referred in other studies (Gomez-Eyles et al., 2012; Khan et al., 2011; Krauss et al., 2000; Tang et al., 2002). Even though some conclusions are very hard to reach due to the variability of results and methods used, it seems that overestimations are higher when dealing with naturally contaminated samples and that decreases when lowering the percentage of solvent (Bergknut et al., 2007; Gomez-Eyles et al., 2012). However, there is a lack of comparisons between the patterns of PAHs in organism and mild-solvent extractions and few studies tested dissimilar soils, which makes it difficult to arise to conclusions on the method's predictability.

SFE was used to predict availability to earthworms in three studies (Table 2): two focused on PHE and PYR availability in dissimilar spiked

soils and the other on 16 PAHs present in several MGP soils. In the latter, the prediction of PAHs uptake by *Aporrectodea caliginosa* provided better results (up to a factor of 2.1) when adjusting the EqPT for the F_{rap} based on mild SFE, yet no correlations are given (Table 6).

SPE extraction with XAD4 was used in only one study (Bogan et al., 2005; Table 2) and its authors stated that, for MGP soils with high desorption fractions and uptake by earthworms, the two approaches agreed positively, but not in samples with low to intermediate mobility (based on resin assays). Some authors suggest that a rapid desorbing process assisted with Tenax should be a good predictor of the earthworm uptake, based on findings obtained for benthic organisms in sediment samples (Ten Hulscher et al., 2003). Indeed, Ten Hulscher et al. found significant correlations between concentration accumulated by *L. rubellus* (normalized to the lipid content) and the amount of PAHs (plus chlorobenzenes) desorbed to Tenax (normalized to OC), even though an underestimation of prediction was observed (Table 6). Other study also found good correlations for PYR in spiked soils, but in this case its authors related the \ln BSAF and desorption percentage (\ln), after 12 days (Li et al., 2007). Gomez-Eyles et al. (2010) compared the profile of individual PAHs in Tenax extraction with the worm tissues, observing that the chemical method overestimated bioaccumulation of 2- and 3-ring PAHs whereas 4-ring compounds were underestimated, for both aged and fresh spikes.

In spite of the study conducted by Khan et al. (2011) that found a very good correlation between HPCD extractions and earthworm accumulation for PYR, the other studies indicate poor or no correlation (Table 6). Moreover, HPCD extractions systematically overpredicted earthworm accumulation in spiked and naturally contaminated soils (Bergknut et al., 2007; Gomez-Eyles et al., 2012; Khan et al., 2011). Gomez-Eyles et al. (2012) estimated C_{org} based on EqPT calculations but results were not improved (either correlations or relationship). Tween-80 was tested by Bergknut et al. (2007) and, considered this surfactant a lipophilic phase, it underpredicted the earthworm lipid normalized concentration by a factor of 0.64.

Some authors concluded that the prediction using non-exhaustive extractions was only slightly improved when compared with total extractions, especially if comparing compound profiles. In general non-exhaustive methods show higher percentages of LMW PAHs, whereas the percentage of HMW PAHs in total extractions is higher, being closer to what is observed in earthworms. This is most likely to be due to mechanisms of biotransformation or the importance of other routes of uptake rather than soil solution, as explained before.

Theoretically, the application of biomimetic methods, with or without the use of EqPT calculations, would be a best approach to predict PAH accumulation in earthworms. Despite the fewer studies conducted, comparing with non-exhaustive extractions, these were mostly performed in dissimilar naturally contaminated samples (Table 2). For example, Gomez-Eyles et al. (2012) used SPME and POM-SPE to predict the bioavailability of 12 PAHs in naturally contaminated soils, and in spite of low correlations found for total PAHs (Table 6), they were better when looking to each group, with the exception of HMW PAHs. The r^2 was 0.67 for 3-ring, 0.8 for 4-ring and 0.31 for 5 + 6-ring PAHs in the case of SPME and of 0.51 for 3-ring, 0.7 for 4-ring and 0.2 for 5 + 6-ring PAHs, in the case of POM-SPE. This study, along with the one conducted by Jonker et al. (2007), that also tested heterogeneous sets of naturally contaminated soils, concluded that either SPME or POM-SPE predicted bioaccumulation by a factor of 10, i.e. both under- or over-predicted within this factor. However, these authors suggest that this could be a promising method, since current methods (total extractions using or not EqPT model) overpredict accumulation by a factor of 10–10,000. It is also suggested that the estimation of C_{org} could be improved by an accurate determination of BCF for key species instead of assuming that BCF values are equal to K_{ow} (Jonker et al., 2007).

Krauss and Wilcke (2001) used C18 disks and derived a model (for individual 20 PAHs) based on $\log K_{disk}$ and $\log K_{ow}$ values which could predict the BSAFs for dissimilar urban soils (Table 6).

When directly comparing K_{disks} with BSAF values, the latter were underestimated for HMW PAHs, corroborating the hypothesis of the existence of other uptake routes such as gut. The other study that used C18 (Table 6) also showed good correlations for individual compounds ($r^2 \geq 0.770$), but an underestimation was observed for all compounds, being higher for BaP.

SPMD was only used in one study and one tested soil, giving a slight overestimation (Tables 2 and 6). TECAMs were tested in two studies, and good correlations were generally observed (Tables 2 and 6). In a first study, Tao et al. (2008b), performed a direct comparison between the concentration in TECAM and in the earthworm (without lipid normalization) and observed that the slope of linear relationship decreased with compound hydrophobicity being as low as 0.033 for BaP (Table 6). According to the authors, the general underestimation may be due to differences in exposure routes and activity of earthworms in soil (e.g. ingestion and excretion of contaminants, or due to disturbance of soils caused by earthworms). However, in a further study using field contaminated soils (Tao et al., 2009) a relationship of about 1:1 (Table 6) was found, although in this case a lipid normalization was performed.

3.3. Bioaccumulation assays using plants

Root uptake of HOCs by plants is an important pathway for their transfer into the food chain (Bogolte et al., 2007; Gao and Collins, 2009). Moreover, it is important to understand the availability of these compounds to plants in order to predict the potential for phytoremediation (Ahn et al., 2005). Direct relationship between PAH concentration in soil and plants has been observed, suggesting a pathway from soil to roots. As for the other organisms, this uptake will depend on the plant species, contaminant type and soil properties.

Root uptake has been shown to be a passive and diffusive process and it is known that the composition of plant root tissues, particularly its lipid content, controls the uptake of HOCs. For this reason, the lipid content of a plant is normally included in plant uptake models (Gao and Collins, 2009; Zhang and Zhu, 2009). EqPT calculations have been applied to predict concentration in plants (C_{plant}), assuming that uptake occurs mainly through pore water. Hence, the estimation of C_{plant} can be carried out as described for other organisms ($C_{plant} = RCF * C_{free}$), where RCF is the root concentration factor. As for BCF, it has been shown that the relationship between the \log RCF and $\log K_{ow}$ is linear (Gao and Collins, 2009). However, other studies suggest that assuming RCF, specifically lipids-water partition coefficients (K_{lip}) equal to K_{ow} , may underestimate the K_{lip} , especially for more hydrophobic compounds (Zhang and Zhu, 2009). These authors suggest that both plant lipids and carbohydrates regulate PAH uptake and the affinity of PAHs for lipids is 1.64 orders of magnitude higher than for carbohydrates. Nevertheless, roots of species such as ryegrass contain 98 times more carbohydrates than lipids and, therefore, this process should be considered. As a result of these recent findings, improvements on EqPT calculations have been suggested (Gomez-Eyles et al., 2012). These authors suggest the separate calculation of C_{plant} for these two major root components: lipids and carbohydrates (ch): $C_{plant, lip} = C_{free, K_{lip}}$; $C_{plant, ch} = C_{free, K_{ch}}$. However, this approach requires knowing the species specific relationships between K_{lip} and K_{ch} with K_{ow} .

Other important processes involved in the uptake of contaminants by plants are the sorption of PAHs to the cell wall and the production of root exudates (Ling et al., 2009; Zhang and Zhu, 2009). Exudates, such as low molecular weight acids, are capable of disrupting the sequestering soil matrix and as a consequently increase the availability of PAHs. On the other hand, exudates may increase the microbial community and therefore the biodegradation process (Ling et al., 2009).

Differences in uptake between species were observed by Tang et al. (1998), for instance, which reported a % of ANT uptake of 0.62% by wheat and 0.35% by barley after a 200 day aging. As for other organisms differences between compound uptake have been observed. For

example, in MGP soils, 2 plant species were tested and the % dissipation after one year was between 1.5 for indeno(1,2,3-cd)pyrene and almost 90% for 3- and 4-ring PAHs (Cofield et al., 2008). In a coke oven site soil, where profiles were dominated by HMW PAHs no significant differences in the total PAHs concentration were observed after the third year of phytoremediation (Ahn et al., 2005). On the other hand, in agricultural soils the uptake by wheat roots showed little differences between the four PAHs analyzed: 0.16% (for PHE) and 0.32% (for BaP) (Tao et al., 2008a).

3.3.1. Chemical methods for assessing phytoavailability

In general good linear relationships have been found between mild solvent extractions and accumulation by different plant species in spiked soils (Table 7). Yet, the study of Gomez-Eyles et al. (2010) found lower correlations for individual compounds ($r^2 > 0.57$) than for the total, which wasn't significant for PHE. Tao et al. (2008a) also stated that good linear relationships were observed for field contaminated samples, but no values were provided. On the other hand, the study of Gomez-Eyles et al. (2012), also conducted in naturally contaminated samples, showed very low correlations, being similar even when looking for individual groups. Even when accumulation data was correlated with the concentrations predicted by using EqPT calculations the r^2 were similar, except for the 4-ring group which rises to 0.54. Regarding the relationship between the two approaches, it was observed that, for a 2 month aging period BuOH extractions underestimated the accumulation of 2- and 3-ring PAHs and overestimated for 4-ring PAHs (Gomez-Eyles et al., 2010). On other studies, very high overestimation factors were found either for individual PAHs (2-, 3-, 4- and 5-ring) or for the \sum 12 compounds naturally contaminated soils (Table 7). Using 50%MeOH, lower overestimation factors were recorded, in comparison with those obtained with BuOH, decreasing with the increase of MW.

Cofield et al. (2008) concluded that a 24 h Tenax extraction was a predictor of the labile fraction of LMW changing during phytoremediation, especially for 3-ring (Table 7). Yet, for the 4-ring PAHs there is an underestimation of availability (lability) and for the 5 ring compounds no trends were observed. Gomez-Eyles et al. (2010) compared the profiles of Tenax extractions and plant tissues, and in spite of a significant difference between PAHs profiles (slightly underestimated for 3- and 4-ring and overestimated for 2-ring PAHs) they were closer than the observed values for earthworms.

Gomez-Eyles et al. state that HPCD could be a good predictor of accumulation of PAHs in ryegrass roots for total PAHs (Table 7), despite the correlation not being significant for PHE when considering individual compounds. This method seems to underestimate concentrations of 2- and 3-ring and overestimate 4-ring PAHs in root tissues after an aging period of 2 months. In another study using industrial soils, these authors concluded that HPCD extractions largely overpredict the plant accumulation in naturally contaminated soils (Table 7), and very low correlations were observed (the highest $r^2 = 0.43$ for 4-ring PAHs). Similarly to BuOH extractions, correlating accumulation data with the concentrations predicted by EqPT does not improve the relationships.

Gomez-Eyles et al. (2012) studied the ability of SPME fibers to predict the accumulation in plants, and found that measurements were closer to the 1:1 line than other methods. However, correlations were very low even when looking at individual groups (ranging from 0.07 for 3-ring PAHs to 0.25 for 5 + 6-ring PAHs). Similar results were observed using POM-SPE, with correlations for individual groups ranging from 0.13 for 5 + 6-ring PAHs to 0.27 for 4-ring PAHs. Moreover, a trend to underestimate accumulation using both methods was observed, which could be due to both: a) the presence of soil particles in roots or b) the production of exudates by soil roots.

Lipid-containing passive samplers such as TECAMs are expected to be a good surrogate of plant uptake, since it is passive and diffuse processes and root lipids have an important role on the uptake of HOCs. Yet, only one study used these membranes to predict root uptake

(Table 3). Concentrations in TECAMs were 40 times higher than in roots, and although these values become closer after lipid normalization, they were still high (maximum of 7.1). The authors suggested that relating the amounts of PAHs uptaken by TECAMs to the amounts uptaken by roots provides better results (Table 7). Gomez-Eyles et al. (2012) suggested that the better correlation obtained by TECAM, may be because they are deploying samplers that were buried close to the plant roots.

3.4. Ecotoxicological assays/molecular biology methods

Toxicity testing, with whole soils and ecologically relevant soil organisms, indirectly measures (through several different endpoints) the biological availability of contaminants in soils. The advantages are both the fact that they only respond to the bioavailable fraction of contaminants and also that they reflect the site-specific effects of contaminants (mixtures and metabolites). However, the non-selectivity of these tests may also be a drawback, especially important in urban soils, for instance, due to the presence of multiple contaminants. In ideal conditions a chemical-specific test should be used concomitantly to identify the contaminants of potential concern present in the different environmental matrices or test substrates. Recently, the use of metabolomics has been suggested, which may distinguish between organic and inorganic contaminant exposure (Brown et al., 2010). This method identifies the metabolic responses of earthworms to a sub-lethal exposure using ^1H nuclear magnetic resonance (NMR) (Brown et al., 2010). Genotoxic assays using solid-phase, aqueous or solvent extracts of soil have been also used to assess the effect of organic contamination in soils (Alexander and Alexander, 2000).

Table 4 presents an overview of existing studies comparing ecotoxicological assays with chemical assays. Alexander and Alexander (2000) used BuOH extraction and a bacterial genotoxicity assay for testing soils spiked with BaP, with good results ($r = 0.691$). These authors also observed that, excluding soils with $<0.7\%$ OC, significant negative correlations were found between bioavailability, %OC and CEC, but not with clay or surface area. Kreitinger et al. (2007) observed that a mild SFE extraction (normalized to OC) was related to acute toxicity of \sum 16 PAHs to *E. fetida* in 16 industrial soils. Similarly, Čvančarová et al. (2013) concluded that the F_{rap} , estimated by sequential SFE, correlated with the toxicity to *E. fetida*. Yet, results of the other toxicity tests were unreliable due to potentially toxic elements present in soil samples. Cofield et al. (2008) concluded that a 24 h Tenax has the potential to predict the toxicity response of several bioassays (nematode and earthworm survival, and lettuce emergence) to the \sum 5 PAHs in a MGP soil, with an r^2 between 0.8 and 0.94, with exception of microbial respiration ($r^2 = 0$). Brown et al. (2010) used ^1H NMR metabolomics to monitor *E. fetida* responses to PHE and compared results with total and HPCD-extracted ($r^2 = 0.64$, slope = 1.2) concentrations. The authors did not find any differences between the two extraction methods but, only one freshly spiked soil was used.

The use of passive samplers to estimate C_{free} has been proposed to complement toxicity tests, since these tests are often performed at high concentrations and the saturation of aqueous phase may occur, which could lead to underestimation of results (ter Laak et al., 2006a). Styrišhave et al. (2008) found a strong correlation (value not shown) between toxicity of PYR to the springtail *Folsomia candida* (generation of reproductive effect concentrations, EC_{50}) and estimated pore water concentrations in spiked soils. Jonker et al. (2007) observed that using C_{free} determined by SPME allowed to correctly predicted *E. fetida* mortality (\sum 13 PAHs in MPG soils) in 87% of the cases. The approach consisted in converting predicted individual PAH concentration in worm lipids to concentrations on a molar basis (mmol/kg lip). Further, concentrations were summed and compared to critical body residues from literature (between 50 and 200 mmol/kg lip).

4. Problems and challenges faced by chemical methods for assessing availability

The determination of biodegradation or bioaccumulation does not necessarily measure the bioavailability of contaminants but rather the integration of complex interactions that occur. Therefore, even when significant correlations between available fractions assessed by chemical methods and bioavailability are found, the findings usually do not provide a 1:1 relationship. There are several factors that may affect this relationship, being the most important ones the biotransformation or metabolism of HOCs (effects on bioaccumulation measures) and the access to contaminants through other routes of exposure rather than aqueous phase.

In the case of biodegradation, since contaminants are biodegraded one can assume that depletive methods (bioaccessibility) would be a more reliable approach. Yet, if desorption is rate-limiting these methods, especially the ones based on the measurement of the F_{rap} , will underestimate biodegradation. Moreover, for species able to produce surfactants or that can access the sorbed phase, chemical methods based on uptake from aqueous phase will also tend to underestimate the availability. Therefore, the use of solubilizing agents such as HPCD provides more reliable prediction as shown by several studies.

Based on EqPT, it is expected that, for higher organisms, such as plants or earthworms, biomimetic methods could be feasible (e.g. TECAMS for plants and SPME for earthworms). However, the application of this theory to terrestrial organisms has been questioned. Moreover, for earthworms, which may have complex accumulation mechanisms or may access contaminants from both solid and aqueous phase, the determination of the contaminants in soil solution may not be the most appropriate estimation of bioavailability. If substances are biotransformed it is likely that chemical methods that measure pore water concentration will overestimate. On the other hand, if organisms have other routes of exposure, they will underestimate. In addition, the estimation of bioconcentration factors based on K_{OW} when applying EqPT models may not be adequate. The solution could be the inclusion, in the prediction models, not only of specific properties of the contaminants (e.g. lipids–water partition coefficients – K_{lip}), but also organism's specific uptake and detoxification mechanisms for key species.

Other problems are related to the complexity of soil as matrix (mechanisms of sorption/desorption, heterogeneity). A high variability of results (for both bioassays and chemical methods) has been observed for different soils. Moreover, many studies were conducted in spiked soils, which may not simulate real contaminated samples, especially due to the quality of SOM, sources and aging. For example, it is known that PAHs in naturally contaminated soils such as MGP sites are recalcitrant and the presence of soot or BC can seriously affect contaminant desorption. Therefore, in addition to physicochemical properties of soils and compounds, the origin of contamination may play a role in chemical and biological availability. In order to overcome the resulting overestimation given when using generic K_{OC} on EqPT models, the use of site specific values has been suggested (Gomez-Eyles et al., 2012).

The behavior of individual PAHs is very different and they should be treated separately or at least by ring size. However, some studies do not consider PAHs individually or by groups, but rather its sum. In addition, little is known for HMW PAHs, mainly because most of spiked samples target only LMW PAHs, and normally they are strongly sorbed in field contaminated samples, showing in general lower chemical availability. Yet, it shouldn't be ignored that bacteria communities could be inadapted to degrade these compounds. On the other hand, for higher organisms, other routes of exposure (rather than pore water) and lower biotransformation can have a stronger influence on bioaccumulation rates of HMW PAHs. For example, Bergknut et al. (2007) observed that PAH composition in earthworms was different from that of the chemical methods (either non-exhaustive extraction or biomimetic methods), but similar to the soil (total extraction), i.e. with a higher percentage of HMW PAHs. This has important implications on a risk

assessment basis, since HMW PAHs are carcinogenic and mutagenic and the effects of the long term exposition to soils contaminated with HMW PAHs are still not clear.

In order to include chemical availability in risk assessment it is necessary to have consistent results (reproducibility and good correlations) for different types of soils, but not necessarily a 1:1 relationship. A systematic overprediction can be seen as the worst-case scenario and ideally a prediction factor should be obtained (for individual or groups of compounds and for a target species) as a function of soil properties. In order to understand and to compare results a standardization of methods, especially for bioaccessibility which is operational defined is needed. Moreover, little attention has also been given to the uncertainty of analytical measurements, especially when dealing with very low concentrations.

It's very likely that it will not be possible to replace bioassays by chemical methods to assess availability, at least on a short time basis. However, the importance of chemical methods is beyond the potential replacement of bioassays. Chemical availability can help to understand the bioavailability process and the behavior of PAHs in soils, being a useful basis for producing descriptive models. Bioaccessibility may be more important to demonstrate the virtual absence of bioavailability when contaminants are strongly sorbed (Reichenberg and Mayer, 2006). Moreover, the potentially available concentrations are much more conservative, despite being more realistic than the current approach. Nevertheless, in spite of the overestimation of risks on a short term, F_{rap} could become available under changing conditions (Brand et al., 2013).

Data about chemical availability can be used as a screening tool (with or instead of total extractions) since they can be a faster and initial approach to estimate availability. Chemical methods can be also used in combination with ecotoxicological tests, to find out which are the contaminants responsible for a given toxicity data. More specifically, Loibner et al. (2006) suggest two different approaches for the inclusion of chemical availability data in risk assessment. The first is the use of bioaccessible data in the refined screening phase (Tier 2), by directly comparing the concentrations obtained in the non-exhaustive extractions with soil screening levels. This approach is based on the fact that soil screening levels are obtained using freshly spiked soils and therefore no aging is likely to occur. However, to use this direct comparison it is necessary that the non-exhaustive method used extracts around 100% of the freshly spiked concentrations. The second approach involves the use of biomimetic methods in a higher tier (Tier 3) and compares the results with the water quality objectives. Yet, this approach should be held with care and the uncertainty should be taken into account since the sensitivity of aquatic and terrestrial species may not be comparable. Moreover, the applicability of aquatic bioassays to assess soil ecotoxicity has been discussed (Antunes et al., 2010).

Brand et al. (2013) suggest the inclusion of both type of methods (non-exhaustive and biomimetic) in the Tier 3 of the risk assessment, using a similar approach to the one described by Loibner et al. (2006). Measuring chemical availability is suggested in samples that show concentrations higher than intervention values, which further directly relates the freely available concentration with risk levels for water, or the bioaccessible fraction with risk levels in soil.

5. Conclusions

Different studies are often based on different concepts of bioavailability. Different methods can be related to a specific availability type and the selection of a method depends on the purpose of the study. There is a need of a "weight-of-evidence" approach to select methods. In general, non-exhaustive extractions and biomimetic methods give a better prediction of available concentrations than exhaustive extractions. Even though some methods overpredict risks, they are an improvement over total extractions and to the current approach of EqPT and they may help to understand bioavailability. Yet, in spite of the suggestions to include chemical availability in risk assessment, both on Tier

2 and Tier 3, there are still some issues to be clarified about data interpretation of the and how to compare results with existing guidelines.

Bioavailability is compound and species specific, and therefore to find a chemical method to predict bioavailability will be difficult. One of the most important problems is biotransformation or metabolism of PAHs and how they affect bioaccumulation measures. Therefore, the potential of chemical methods to predict PAHs microbial mineralization in soils is higher, due to the complexity of accumulation mechanisms in earthworms and plants, which are not taken into account by chemical methods. Yet, the EqPT models may be improved by determining species-specific lipids–water partition coefficients and site-specific carbon–water partition coefficient. Future work will also require accurate kinetic of uptake and release data for key species, compound and species-specific bioconcentration factors. Regarding bioaccessibility, the experimental details need to be clarified and standardized. After having consistent results for some methods, they can be further chosen based on their reproducibility, speed, practicability and costs. Some chemical methods are also time-consuming, expensive and of relatively low precision.

The use of laboratory spiked soils may be an initial approach to understand contaminants behavior but they are not the most appropriate regarding how to understand their behavior in naturally contaminated samples. In spite most of chemical methods being able to respond to aging effects, soil properties and compounds behavior, no consistent data was obtained in some cases. Due to the low number of studies that tested a heterogeneous number of naturally PAHs contaminated soils, there is a need to test chemical methods in these conditions. In addition, due to the different behaviors of individual PAHs, there is a need to perform studies (both in spiked and naturally contaminated soils) with the 16 individual PAHs or at least with a representative compound of each group. Finally, since several factors may play an important role in controlling availability, including the quality of organic matter, the predictability of chemical methods should be tested in several dissimilar soils and not only in few soil types as most studies performed until presently.

Acknowledgments

This work was supported by FCT (Fundação para a Ciência e Tecnologia), through an individual research grant attributed to A. Cachada (SFRH/BD/38418/2007) and by the Territorial Cooperation Program INTERREG IV B through the research project ORQUE SUDO (SOE3/P2/F591). The authors wish to thank Pedro Faria for the English revisions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.11.038>.

References

- Ahn S, Werner D, Luthy RG. Physicochemical characterization of coke-plant soil for the assessment of polycyclic aromatic hydrocarbon availability and the feasibility of phytoremediation. *Environ Toxicol Chem* 2005;24:2185–95.
- Alexander M. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 2000;34:4259–65.
- Alexander RR, Alexander M. Bioavailability of genotoxic compounds in soils. *Environ Sci Technol* 2000;34:1589–93.
- Allan JJ, Semple KT, Hare R, Reid BJ. Prediction of mono- and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction. *Environ Pollut* 2006;144:562–71.
- Antunes SC, Pereira JL, Cachada A, Duarte AC, Goncalves F, Sousa JP, et al. Structural effects of the bioavailable fraction of pesticides in soil: suitability of elutriate testing. *J Hazard Mater* 2010;184:215–25.
- Barthe M, Pelletier É, Breedveld GD, Cornelissen G. Passive samplers versus surfactant extraction for the evaluation of PAH availability in sediments with variable levels of contamination. *Chemosphere* 2008;71:1486–93.
- Bergknut M, Sehlin E, Lundstedt S, Andersson PL, Haglund P, Tysklind M. Comparison of techniques for estimating PAH bioavailability: uptake in *Eisenia fetida*, passive samplers and leaching using various solvents and additives. *Environ Pollut* 2007;145:154–60.
- Bernhardt C, Derz K, Kördel W, Terytze K. Applicability of non-exhaustive extraction procedures with Tenax and HPCD. *J Hazard Mater* 2013;261:711–7.
- Bielská L, Smídová K, Hofman J. Supercritical fluid extraction of persistent organic pollutants from natural and artificial soils and comparison with bioaccumulation in earthworms. *Environ Pollut* 2013;176:48–54.
- Bogan BW, Sullivan WR. Physicochemical soil parameters affecting sequestration and mycobacterial biodegradation of polycyclic aromatic hydrocarbons in soil. *Chemosphere* 2003;52:1717–26.
- Bogan BW, Beardsley KE, Sullivan WR, Hayes TD, Soni BK. Effect of volatile hydrocarbon fractions on mobility and earthworm uptake of polycyclic aromatic hydrocarbons from soils and soil/lampblack mixtures. *Environ Toxicol Chem* 2005;24:181–9.
- Bogolte BT, Ehlers GAC, Braun R, Loibner AP. Estimation of PAH bioavailability to *Lepidium sativum* using sequential supercritical fluid extraction — a case study with industrial contaminated soils. *Eur J Soil Biol* 2007;43:242–50.
- Braida VJ, White JC, Pignatello JJ. Indices for bioavailability and biotransformation potential of contaminants in soils. *Environ Toxicol Chem* 2004;23:1585–91.
- Brand E, Lijzen J, Peijnenburg W, Swartjes F. Possibilities of implementation of bioavailability methods for organic contaminants in the Dutch soil quality assessment framework. *J Hazard Mater* 2013;261:833–9.
- Brown SAE, McKelvie JR, Simpson AJ, Simpson MJ. ¹H NMR metabolomics of earthworm exposure to sub-lethal concentrations of phenanthrene in soil. *Environ Pollut* 2010;158:2117–23.
- Bugg T, Foght JM, Pickard MA, Gray MR. Uptake and active efflux of polycyclic aromatic hydrocarbons by *Pseudomonas fluorescens* LP6a. *Appl Environ Microbiol* 2000;66:5387–92.
- Cachada A, Pato P, Rocha-Santos T, da Silva EF, Duarte AC. Levels, sources and potential human health risks of organic pollutants in urban soils. *Sci Total Environ* 2012;430:184–92.
- Cai Q-Y, Mo C-H, Wu Q-T, Katsoyiannis A, Zeng Q-Y. The status of soil contamination by semivolatile organic chemicals (SVOCs) in China: a review. *Sci Total Environ* 2008;389:209–24.
- Cajthaml T, Šásek V. Application of supercritical fluid extraction (SFE) to predict bioremediation efficacy of long-term composting of PAH-contaminated soil. *Environ Sci Technol* 2005;39:8448–52.
- Chung N, Alexander M. Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. *Environ Sci Technol* 1999;33:3605–8.
- Cofield N, Banks MK, Schwab AP. Liability of polycyclic aromatic hydrocarbons in the rhizosphere. *Chemosphere* 2008;70:1644–52.
- Cui X, Mayer P, Gan J. Methods to assess bioavailability of hydrophobic organic contaminants: principles, operations, and limitations. *Environ Pollut* 2013;172:223–34.
- Cuyper C, Grotenhuis T, Joziassse J, Rulkens W. Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments. *Environ Sci Technol* 2000;34:2057–63.
- Čvančarová M, Křesinová Z, Cajthaml T. Influence of the bioaccessible fraction of polycyclic aromatic hydrocarbons on the ecotoxicity of historically contaminated soils. *J Hazard Mater* 2013;254–255:116–24.
- Doick KJ, Dew NM, Semple KT. Linking catabolism to cyclodextrin extractability: determination of the microbial availability of PAHs in soil. *Environ Sci Technol* 2005;39:8858–64.
- Doick KJ, Clasper PJ, Urmann K, Semple KT. Further validation of the HPCD-technique for the evaluation of PAH microbial availability in soil. *Environ Pollut* 2006;144:345–54.
- Ehlers GAC, Loibner AP. Linking organic pollutant (bio)availability with geosorbent properties and biomimetic methodology: a review of geosorbent characterisation and (bio)availability prediction. *Environ Pollut* 2006;141:494–512.
- Ehlers LJ, Luthy RG. Peer reviewed: contaminant bioavailability in soil and sediment. *Environ Sci Technol* 2003;37:295A–302A.
- Gao Y, Collins CD. Uptake pathways of polycyclic aromatic hydrocarbons in white clover. *Environ Sci Technol* 2009;43:6190–5.
- Ghosh U, Hawthorne SB. Particle-scale measurement of PAH aqueous equilibrium partitioning in impacted sediments. *Environ Sci Technol* 2010;44:1204–10.
- Gomez-Eyles JL, Collins CD, Hodson ME. Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability. *Environ Pollut* 2010;158:278–84.
- Gomez-Eyles JL, Jonker MTO, Hodson ME, Collins CD. Passive samplers provide a better prediction of PAH bioaccumulation in earthworms and plant roots than exhaustive, mild solvent, and cyclodextrin extractions. *Environ Sci Technol* 2012;46:962–9.
- Guha S, Jaffé PR, Peters CA. Bioavailability of mixtures of PAHs partitioned into the micellar phase of a nonionic surfactant. *Environ Sci Technol* 1998;32:2317–24.
- Gustafsson Ö, Haghseta F, Chan C, MacFarlane J, Gschwend PM. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environ Sci Technol* 1997;31:203–9.
- Haritash AK, Kaushik CP. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *J Hazard Mater* 2009;169:1–15.
- Hawthorne SB, Grabanski CB. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. *Environ Sci Technol* 2000;34:4103–10.
- Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. PAH release during water desorption, supercritical carbon dioxide extraction, and field bioremediation. *Environ Sci Technol* 2001;35:4577–83.
- Hawthorne SB, Poppendieck DG, Grabanski CB, Loehr RC. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ Sci Technol* 2002;36:4795–803.
- Hickman ZA, Reid BJ. Towards a more appropriate water based extraction for the assessment of organic contaminant availability. *Environ Pollut* 2005;138:299–306.
- Hickman ZA, Swindell AL, Allan JJ, Rhodes AH, Hare R, Semple KT, et al. Assessing biodegradation potential of PAHs in complex multi-contaminant matrices. *Environ Pollut* 2008;156:1041–5.

- Jager T, Sánchez FAA, Muijs B, van der Velde EG, Posthuma L. Toxicokinetics of polycyclic aromatic hydrocarbons in *Eisenia andrei* (Oligochaeta) using spiked soil. *Environ Toxicol Chem* 2000;19:953–61.
- Jager T, Baerseman R, Dijkman E, de Groot AC, Groenendijk EA, de Jong A, et al. Availability of polycyclic aromatic hydrocarbons to earthworms (*Eisenia andrei*, Oligochaeta) in field-polluted soils and soil-sediment mixtures. *Environ Toxicol Chem* 2003;22:767–75.
- Johnsen AR, de Liphay JR, Reichenberg F, Sørensen SJ, Andersen O, Christensen P, et al. Biodegradation, bioaccessibility, and genotoxicity of diffuse polycyclic aromatic hydrocarbon (PAH) pollution at a motorway site. *Environ Sci Technol* 2006;40:3293–8.
- Johnson DL, Jones KC, Langdon CJ, Pearce TG, Semple KT. Temporal changes in earthworm availability and extractability of polycyclic aromatic hydrocarbons in soil. *Soil Biol Biochem* 2002;34:1363–70.
- Jonker MTO, van der Heijden SA, Kreitinger JP, Hawthorne SB. Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with solid-phase microextraction. *Environ Sci Technol* 2007;41:7472–8.
- Juhasz AL, Waller N, Stewart R. Predicting the efficacy of polycyclic aromatic hydrocarbon bioremediation in creosote-contaminated soil using bioavailability assays. *Bioremed J* 2005;9:99–114.
- Kelsey JW, Alexander M. Declining bioavailability and inappropriate estimation of risk of persistent compounds. *Environ Toxicol Chem* 1997;16:582–5.
- Kelsey JW, Kottler BD, Alexander M. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ Sci Technol* 1997;31:214–7.
- Khan M, Cheema S, Shen C, Zhang C, Tang X, Malik Z, et al. Assessment of pyrene bioavailability in soil by mild hydroxypropyl- β -cyclodextrin extraction. *Arch Environ Contam Toxicol* 2011;60:107–15.
- Krauss M, Wilcke W. Biomimetic extraction of PAHs and PCBs from soil with octadecyl-modified silica disks to predict their availability to earthworms. *Environ Sci Technol* 2001;35:3931–5.
- Krauss M, Wilcke W, Zech W. Availability of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to earthworms in urban soils. *Environ Sci Technol* 2000;34:4335–40.
- Kreitinger JP, Quiones-Rivera A, Neuhauser EF, Alexander M, Hawthorne SB. Supercritical carbon dioxide extraction as a predictor of polycyclic aromatic hydrocarbon bioaccumulation and toxicity by earthworms in manufactured-gas plant site soils. *Environ Toxicol Chem* 2007;26:1809–17.
- Lanno R, Wells J, Conder J, Bradham K, Basta N. The bioavailability of chemicals in soil for earthworms. *Ecotoxicol Environ Saf* 2004;57:39–47.
- Latawiec AE, Reid BJ. Beyond contaminated land assessment: on costs and benefits of bioaccessibility prediction. *Environ Int* 2009;35:911–9.
- Latawiec AE, Swindell AL, Reid BJ. Environmentally friendly assessment of organic compound bioaccessibility using sub-critical water. *Environ Pollut* 2008;156:467–73.
- Li J, Pignatello JJ, Smets BF, Grasso D, Monserrate E. Bench-scale evaluation of in situ bioremediation strategies for soil at a former manufactured gas plant site. *Environ Toxicol Chem* 2005;24:741–9.
- Li J, Sun H, Zhang Y. Desorption of pyrene from freshly-amended and aged soils and its relationship to bioaccumulation in earthworms. *Soil Sediment Contam Int J* 2007;16:79–87.
- Li X, Zhu Y, Wu T, Zhang S, Christie P. Using a novel petroselinic acid embedded cellulose acetate membrane to mimic plant partitioning and in vivo uptake of polycyclic aromatic hydrocarbons. *Environ Sci Technol* 2010;44:297–301.
- Ling W, Ren L, Gao Y, Zhu X, Sun B. Impact of low-molecular-weight organic acids on the availability of phenanthrene and pyrene in soil. *Soil Biol Biochem* 2009;41:2187–95.
- Liste H-H, Alexander M. Butanol extraction to predict bioavailability of PAHs in soil. *Chemosphere* 2002;46:1011–7.
- Loibner A, Jensen J, Ter Laak T, Celis R, Hartnik T. Sorption and ageing of soil contamination. In: Jensen John, Mesman Miranda, editors. *Ecological risk assessment of contaminated land*; 2006. p. 19–29. [711701047. RIVM].
- Ma W-c, van Kleunen A, Immerzeel J, de Maagd PG-J. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. *Environ Toxicol Chem* 1998;17:1730–7.
- Macleod CJA, Semple KT. Sequential extraction of low concentrations of pyrene and formation of non-extractable residues in sterile and non-sterile soils. *Soil Biol Biochem* 2003;35:1443–50.
- MacRae JD, Hall KJ. Comparison of methods used to determine the availability of polycyclic aromatic hydrocarbons in marine sediment. *Environ Sci Technol* 1998;32:3809–15.
- Mayer P, Tolls J, Hermens JLM, Mackay D. Peer reviewed: equilibrium sampling devices. *Environ Sci Technol* 2003;37:184A–91A.
- Papadopoulos A, Paton GI, Reid BJ, Semple KT. Prediction of PAH biodegradation in field contaminated soils using a cyclodextrin extraction technique. *J Environ Monit* 2007a;9:516–22.
- Papadopoulos A, Reid BJ, Semple KT. Prediction of microbial accessibility of carbon-14-phenanthrene in soil in the presence of pyrene or benzo[a]pyrene using an aqueous cyclodextrin extraction technique. *J Environ Qual* 2007b;36:1385–91.
- Parrish ZD, White JC, Isleyen M, Gent MPN, Iannucci-Berger W, Eitzer BD, et al. Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species. *Chemosphere* 2006;64:609–18.
- Patterson CJ, Semple KT, Paton GI. Non-exhaustive extraction techniques (NEETs) for the prediction of naphthalene mineralisation in soil. *FEMS Microbiol Lett* 2004;241:215–20.
- Reichenberg F, Mayer P. Two complementary sides of bioavailability: accessibility and chemical activity of organic contaminants in sediments and soils. *Environ Toxicol Chem* 2006;25:1239–45.
- Reid BJ, Jones KC, Semple KT. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. *Environ Pollut* 2000a;108:103–12.
- Reid BJ, Stokes JD, Jones KC, Semple KT. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. *Environ Sci Technol* 2000b;34:3174–9.
- Rhodes AH, Carlin A, Semple KT. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. *Environ Sci Technol* 2008a;42:740–5.
- Rhodes AH, Dew NM, Semple KT. Relationship between cyclodextrin extraction and biodegradation of phenanthrene in soil. *Environ Toxicol Chem* 2008b;27:1488–95.
- Rhodes AH, McAllister LE, Semple KT. Linking desorption kinetics to phenanthrene biodegradation in soil. *Environ Pollut* 2010;158:1348–53.
- Sabaté J, Viñas M, Solanas AM. Bioavailability assessment and environmental fate of polycyclic aromatic hydrocarbons in biostimulated creosote-contaminated soil. *Chemosphere* 2006;63:1648–59.
- Semple KT, Morriss AWJ, Paton GI. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *Eur J Soil Sci* 2003;54:809–18.
- Semple KT, Doick KJ, Jones KC, Burael P, Craven A, Harms H. Peer reviewed: defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environ Sci Technol* 2004;38:228A–31A.
- Sijm D, Kraaij R, Belfroid A. Bioavailability in soil or sediment: exposure of different organisms and approaches to study it. *Environ Pollut* 2000;108:113–9.
- Stokes JD, Wilkinson A, Reid BJ, Jones KC, Semple KT. Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl- β -cyclodextrin extraction technique. *Environ Toxicol Chem* 2005;24:1325–30.
- Styrishave B, Mortensen M, Henning Krogh P, Andersen O, Jensen J. Solid-phase microextraction (SPME) as a tool to predict the bioavailability and toxicity of pyrene to the springtail, *Folsomia candida*, under various soil conditions. *Environ Sci Technol* 2008;42:1332–6.
- Sun H-W, Li J-G. Availability of pyrene in unaged and aged soils to earthworm uptake, butanol extraction and SFE. *Water Air Soil Pollut* 2005;166:353–65.
- Swindell AL, Reid BJ. Comparison of selected non-exhaustive extraction techniques to assess PAH availability in dissimilar soils. *Chemosphere* 2006;62:1126–34.
- Szolar OHJ, Rost H, Hirmann D, Hasinger M, Braun R, Loibner AP. Sequential supercritical fluid extraction (SSFE) for estimating the availability of high molecular weight polycyclic aromatic hydrocarbons in historically polluted soils. *J Environ Qual* 2004;33:80–8.
- Tang J, Alexander M. Mild extractability and bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ Toxicol Chem* 1999;18:2711–4.
- Tang J, Carroquino MJ, Robertson BK, Alexander M. Combined effect of sequestration and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ Sci Technol* 1998;32:3586–90.
- Tang J, Liste H-H, Alexander M. Chemical assays of availability to earthworms of polycyclic aromatic hydrocarbons in soil. *Chemosphere* 2002;48:35–42.
- Tao Y, Zhang S, Wang Z, Christie P. Predicting bioavailability of PAHs in soils to wheat roots with triolein-embedded cellulose acetate membranes and comparison with chemical extraction. *J Agric Food Chem* 2008a;56:10817–23.
- Tao Y, Zhang S, Wang Z, Ke R, Shan X-q, Christie P. Biomimetic accumulation of PAHs from soils by triolein-embedded cellulose acetate membranes (TECAMs) to estimate their bioavailability. *Water Res* 2008b;42:754–62.
- Tao Y, Zhang S, Wang Z, Christie P. Predicting bioavailability of PAHs in field-contaminated soils by passive sampling with triolein embedded cellulose acetate membranes. *Environ Pollut* 2009;157:545–51.
- Ten Hulscher TEM, Postma J, den Besten PJ, Stroomborg GJ, Belfroid A, Wegener JW, et al. Tenax extraction mimics benthic and terrestrial bioavailability of organic compounds. *Environ Toxicol Chem* 2003;22:2258–65.
- ter Laak TL, Agbo SO, Barendregt A, Hermens JLM. Freely dissolved concentrations of PAHs in soil pore water: measurements via solid-phase extraction and consequences for soil tests. *Environ Sci Technol* 2006a;40:1307–13.
- ter Laak TL, Barendregt A, Hermens JLM. Freely dissolved pore water concentrations and sorption coefficients of PAHs in spiked, aged, and field-contaminated soils. *Environ Sci Technol* 2006b;40:2184–90.
- Thiele-Bruhn S, Brümmer GW. Fractionated extraction of polycyclic aromatic hydrocarbons (PAHs) from polluted soils: estimation of the PAH fraction degradable through bioremediation. *Eur J Soil Sci* 2004;55:567–78.
- van der Heijden SA, Jonker MTO. PAH bioavailability in field sediments: comparing different methods for predicting in situ bioaccumulation. *Environ Sci Technol* 2009;43:3757–63.
- Yang Y, Hunter W, Tao S, Gan J. Microbial availability of different forms of phenanthrene in soils. *Environ Sci Technol* 2009;43:1852–7.
- Yeom IT, Ghosh MM, Cox CD. Kinetic aspects of surfactant solubilization of soil-bound polycyclic aromatic hydrocarbons. *Environ Sci Technol* 1996;30:1589–95.
- You J, Harwood AD, Li H, Lydy MJ. Chemical techniques for assessing bioavailability of sediment-associated contaminants: SPME versus Tenax extraction. *J Environ Monit* 2011;13:792–800.
- Zhang M, Zhu L. Sorption of polycyclic aromatic hydrocarbons to carbohydrates and lipids of ryegrass root and implications for a sorption prediction model. *Environ Sci Technol* 2009;43:2740–5.