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The impact of plant root biomass on the biodegradation and bioaccessibility of hydrocarbons in soil

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Declaration

I hereby declare that this thesis is my own work and has not been submitted in substantially the same form for the award of a higher degree elsewhere. Sections of this thesis containing material resulted from joint research are properly referenced.

Gabriela M. Vázquez Cuevas
Lancaster University, February 2018

Statement of authorship

This thesis has been prepared in the alternative format and is integrated by the following documents:

Chapter 2 is intended for submission as: **Vázquez-Cuevas, G. M.**, Stevens, C. J. and Semple, K. T. (2017) Biodegradation of hydrocarbons in soil from tropical regions. This paper was written by **Miss Vázquez-Cuevas (90 %)** in collaboration with Dr. Stevens (5 %) and Prof. Semple (5 %) who commented on the content and provided recommendations.

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I hereby agree with the above statements:



Prof Kirk T. Semple Dr Carly J. Stevens Miss Gabriela Vázquez-Cuevas

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed throughout the environment, representing a challenge for environmental scientists and decision makers. As soil constitutes a natural sink for contaminants, the control and management of contaminated soil has been the focus of multiple investigations since the last quarter of the 20th century. Within the different approaches that have been developed for this purpose, the use of plants for the remediation of contaminated soil can be considered as a promising technology given its overall low cost and general public acceptance. Although this technology has been studied for over 30 years, the specific mechanisms behind the process have not been fully understood yet. Therefore, the aim of this thesis was to investigate the impact of plant root biomass and components typically found within root exudates on the biodegradation and bioaccessibility of phenanthrene from soil with particular interest in the study of root-induced changes of hydrocarbon bioaccessibility. Results showed that microbial catabolism and biodegradation of ^{14}C-phenanthrene can be promoted by the incorporation of plant roots into the soil, with significant effects most likely to be observed after an adaptation period. Moreover, although chemically assessed bioaccessibility of ^{14}C-phenanthrene was not observed to be affected by root biomass or organic acids typically found within root exudates, the use of high concentrations of citric and malic acid were observed to promote lager desorbable fractions of the hydrocarbon. This thesis contributes evidence supporting the role of root decay, turnover and exudation for the biodegradation of PAHs in contaminated soil as defining mechanisms by which plants can promote the biodegradation of this type of contaminants.

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List of acronyms and abbreviations

2,4-D	2,4-dichlorophenoxyaxetic acid
2,4-DNT	2,4-dinitrotoluene
αCD	α cyclodextrin (cyclohexaamylose)
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BC	Black carbon
βCD	β cyclodextrin (cycloheptaamylose)
CD	Cyclodextrin
CEC	Cation exchange capacity
CFU	Colony forming units
CGTase	Glycosyltransferase enzyme
CMCD	Carboxymethyl-β-cyclodextrin
CW	Coke works
DCM	Dichloromethane
DCVG	DC-voltage gradient
DDT	Dichlorodiphenyltrichloroethane
DOM	Dissolved organic matter
dw	Dry weight
F_{rap}	Rapid desorbing fraction
F_{slow}	Slow desorbing fraction
$F_{very\ slow}$	Very slow desorbing fraction
γCD	γ cyclodextrin (cyclooctamylose)
GC	Gas chromatography
GCD	glycine-β-cyclodextrin

GPA	General purpose agar
HC	Hydrocarbons
HCB	Hexachlorobenzene
HOC	Hydrophobic organic contaminant
HPCD	Hydroxypropyl-β-cyclodextrin
HPLC	High-performance liquid chromatography
IPU	Isoproturon
K_d	Dissociation constant
k_{rap}	Rapid desorption rate constant
K_{oc}	Organic carbon-water partition coefficient
K_{ow}	Octanol-water partition coefficient
k_{slow}	Slow desorption rate constant
$k_{very\ slow}$	Very slow desorption rate constant
LOA	Low molecular weight organic acid
LSC	Liquid scintillation counting
MCD	Methyl-β-cyclodextrin
MGP	Manufactured gas plant
NAPL	Non-aqueous phase liquid
PAH	Polycyclic aromatic hydrocarbon
PCA	Plate count agar
PCB	Polychlorinated biphenyls
PET	Potential evapotranspiration
Phe	Phenanthrene
POM	Polyoxymethylene
RAMEB	Randomly methylated β-cyclodextrin

R:S	Root:shoot
rpm	Revolutions per minute
SFE	Supercritical fluid extraction
SOM	Soil organic matter
SPE	Solid phase extraction
SPME	Solid phase microextraction
TPH	Total petroleum hydrocarbons
whc	Water holding capacity

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Chapter 7

Chapter 1

General introduction

1.1 Soil contamination

Hydrophobic organic contaminants (HOCs) constitute one of the largest groups of soil contaminants, encompassing a range of compounds with low water solubility and high persistency in the environment (Luthy *et al.*, 1997; Semple *et al.*, 2003; Ortega-Calvo *et al.*, 2015). HOCs include polycyclic aromatic compounds, tars, creosotes, and chlorinated compounds contained in solvents and different chemicals including polychlorinated biphenyls (Luthy *et al.*, 1997; Semple *et al.*, 2003). Economic activities represent a significant contributing factor for soil contamination (Cunningham *et al.*, 1993; Wang *et al.*, 2015). Different industries such as the chemical, petrochemical, wood treatment and agriculture are recognised as the primary sources of HOC, introducing them into the environment by intentional application, as well as spillages, leaks, and atmospheric deposition (Lee *et al.*, 2003; Pilon-Smits, 2005; Wang *et al.*, 2015). Once HOCs have reached the soil, their fate will be defined by the combination of different mechanisms including biodegradation, leaching, volatilisation and sequestration (Reid *et al.*, 2000a; Semple *et al.*, 2003; Ortega-Calvo *et al.*, 2015).

1.1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are considered to be a group of critical contaminants (Agnello *et al.*, 2014), and consequently considered as a priority among government environmental agencies around the globe (Wilson & Jones, 1993; Semple *et al.*, 2003). PAHs have been found to be widely distributed across the different environmental compartments including water, soil and air (Samanta *et al.*, 2002; Saleha, 2008). Incomplete combustion of organic materials (Samanta *et al.*, 2002) including wood, coal, oil and petrol (Wild & Jones, 1995) has been recognised as the main contributor of these contaminants into the environment. PAHs are defined as a group of compounds formed by two or more fused benzene rings (Wild & Jones, 1995). Furthermore, a number of these compounds pose a threat to the environment and human wellbeing, including health problems such as genetic mutations and cancer (Wild & Jones, 1995; Samanta *et al.*, 2002; Semple *et al.*, 2003). The fate and behaviour of PAHs will greatly differ depending on their specific physical and chemical properties (Wild & Jones, 1995). Lower molecular weight PAHs such as naphthalene (128.17 g mol^{-1}) are more labile and water soluble than larger molecules

like benzo[a]pyrene (252.31 g mol^{-1}) (Wild & Jones, 1995). Therefore, the extent into which PAHs would be susceptible to be removed from the soil cannot be generalised (Samanta *et al.*, 2002).

1.2 Fate and behaviour of organic contaminants in soil

The fate and behaviour or HOCs in soil is defined by the multiple interactions between the biological, chemical and physical factors of the soil and the contaminant (Semple *et al.*, 2003; Prokop *et al.*, 2016). The role of these complex interactions have been summarised by Semple et al. (2003) as bioaccumulation, degradation, volatilisation, leaching and sequestration. Among these, biological degradation is considered one of the most important mechanisms through which HOCs can be removed from the soil (Liste & Alexander, 1999; Semple *et al.*, 2006). The extent by which a HOC can be degraded by biological processes will be greatly limited by two factors (1) the ability of a given organism to metabolise the contaminant, and (2) the fraction of the contaminant that is bioaccessible to the organism (Semple *et al.*, 2003; Volkering & Breure, 2003).

1.2.1 Bioaccessibility of organic contaminants in soil

Degradability and availability are considered to be the two general conditions that need to be met for bioremediation to occur (Reid *et al.*, 2000a). A bioavailable compound is defined as "that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time" (Semple *et al.*, 2004). Further to this concept, Semple *et al.* (2004) also introduced the definition of bioaccessibility, which in addition to what is bioavailable at a certain time, it also encompasses the fraction of the contaminant that can potentially become bioavailable. Although different concepts, given the close relationship between these, it is not rare to use bioavailability and bioaccessibility interchangeably. More importantly, despite the more regular use of the term "bioavailability", it has been observed that what is normally quantified by the use of chemical and biological assays might be in fact the bioaccessible rather than the bioavailable fraction (Semple *et al.*, 2004). Bearing this in mind and for the purposes of this work, "bioaccessibility" will be used as a collective term of these concepts unless otherwise indicated.

The bioaccessibility of a contaminant is a dynamic process that depends on different factors including the compound's specific chemical and physical properties, as well as soil characteristics and environmental conditions (Semple *et al.*, 2003; Pilon-Smits, 2005). Furthermore, chemical properties of the contaminant including hydrophobicity and volatility will greatly define its fate and behaviour within the environmental compartments (Pilon-Smits, 2005) and ultimately its biodegradation potential (Salt *et al.*, 1998). In addition to the specific characteristics of the contaminants, soil physical, chemical and biological properties are known to have a significant role in the bioaccessibility of PAHs in soil (Wild & Jones, 1995). Similarly, sorption and desorption kinetics have continuously been observed to significantly define the extent by which organic contaminants are bioaccessible (Reid *et al.*, 2000a; Rhodes *et al.*, 2010b). These mechanisms are controlled by a variety of conditions including soil-contaminant contact time, soil organic matter (SOM), soil type, temperature, moisture, and biological activity (Leahy & Colwell, 1990; Hatzinger & Alexander, 1995; Liste & Alexander, 1999; Reid *et al.*, 2000a; Macleod & Semple, 2003; Rhodes *et al.*, 2010b). Among these conditions, increasing soil-contaminant contact times have been proven to be a highly influential factor reducing the bioavailability of HOC in soil (Figure 1) (Hatzinger & Alexander, 1995; Alexander, 2000; Reid *et al.*, 2000a). This phenomenon, also known as "ageing", has been directly associated with a subsequent reduction on the bioaccessibility of hydrocarbons from contaminated soil (Hatzinger & Alexander, 1995; Siciliano & Germida, 1998).

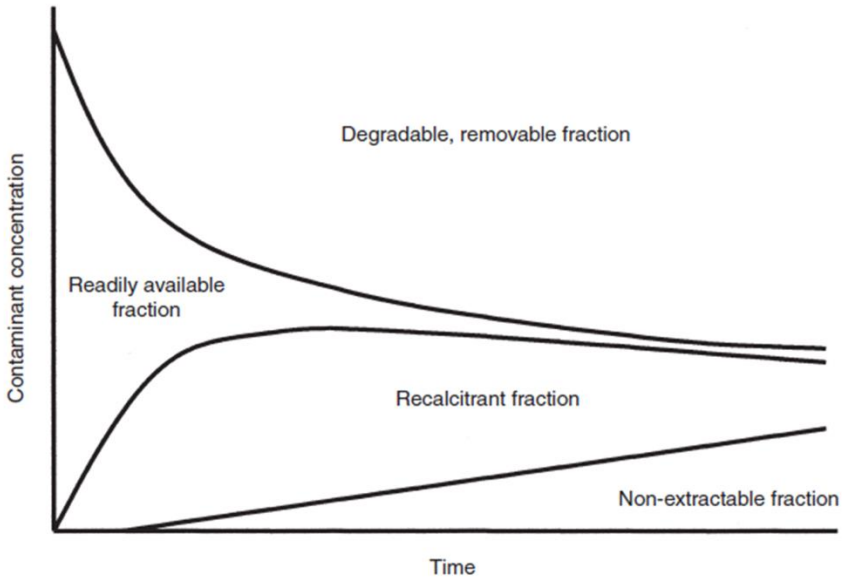

Figure 1. Representation of the influence of ageing time on the bioaccessibility of HOCs in soil (Reproduced with permission from Semple *et al.* (2003)).

Bioaccessible fractions of HOCs are normally assessed through biological or chemical means (Reid *et al.*, 2000a; Semple *et al.*, 2003). Although bioaccessibility can be generalised as the proportion of the contaminant that is available for biological degradation (Vázquez-Cuevas & Semple, 2016), this fraction varies among different types of soil organisms (Reid *et al.*, 2000b; Semple *et al.*, 2004). Therefore, the amount of a contaminant that is available for bacterial biodegradation might not necessarily be the same for higher organisms such as earthworms or plants (Reid *et al.*, 2000a). Considering this, the selection and validation of the appropriate methodology for the assessment of bioaccessibility is likely to have a significant effect on the quantified amount of the contaminant (Kelsey *et al.*, 1997; Reid *et al.*, 2000a). Biological assessment of the bioaccessibility from contaminated soil is normally carried through contaminant's microbial degradation (Semple *et al.*, 2006), or earthworm accumulation (Jonker *et al.*, 2007) assays. A large number of chemical based approaches including mild solvent and aqueous extractions (Reid *et al.*, 2000b; Liste & Alexander, 2002) , and passive sampling (Harwood *et al.*, 2012) have also been applied for the prediction of HOCs bioaccessibility in soil. Ultimately, one of the biggest challenges within the management and risk assessment of contaminated land is the correct use and interpretation of the bioaccessible fractions of organic contaminants (Prokop *et al.*, 2016). Based on this, if bioaccessible fractions of HOCs

are detected, bioremediation protocols would ideally aim to promote a net biodegradation as close as possible to the total bioaccessible fraction (Alexander, 1999). Hence the necessity of a proper quantification and understanding of this fraction and the factors governing the process (Alexander, 1999; Semple *et al.*, 2007).

1.2.2 Remediation of contaminated soil

Based on the type and amount of the contaminant, as well as the specific characteristics of the soil and other environmental factors, an array of approaches might be available for remediation purposes (Cunningham *et al.*, 1996). These can be categorised as thermal, chemical, physical and biological depending on the principle followed by the selected protocol (Cunningham *et al.*, 1996; Tan, 2009). Expensive "engineering-based" approaches aiming for the modification of the physical and chemical properties of the contaminated soil tend to be considered the most effective for the removal of HOC (Pepper *et al.*, 2006). Therefore, these are often used for the treatment of highly contaminated sites (Cunningham *et al.*, 1996). This type of methodologies includes electrokinetic remediation (Mulligan *et al.*, 2001), soil washing and venting (Pepper *et al.*, 2006), thermal desorption (Singh *et al.*, 2009) and chemical extraction, reduction and oxidation (Tan, 2009). On the other hand, remediation of contaminated soil through microbial degradation has also proven to be a feasible methodology for the dissipation of HOC (Cunningham *et al.*, 1996). This approach, also known as bioremediation, has been defined by Alexander (1999) as "the utilisation of microbial processes to destroy chemicals". Protocols derived from this principle include landfarming (Alexander, 1999), biopiles (Jørgensen *et al.*, 2000), bioreactors (Bollag & Bollag, 1995), monitored natural attenuation (Tan, 2009), and phytoremediation (Pilon-Smits, 2005).

1.3 Plant enhanced bioremediation of hydrocarbon contaminated soil

Plants are considered to possess an inherent degrading ability towards HOCs (Cunningham *et al.*, 1995). Consequently, their use as an *in situ* soil remediation technology for the removal of organic contaminants from soil has been extensively studied since the last decade of the 20th century (Cunningham *et al.*, 1996; Pilon-Smits, 2005). Plant enhanced bioremediation, also known as phytoremediation, was originally developed as a response to an increasing demand of an alternative low-cost

in situ remediation technology to substitute the notably more expensive engineering-based *ex situ* approaches normally used around that time (Cunningham *et al.*, 1996; Pilon-Smits, 2005). Phytoremediation was first defined by Cunningham *et al.* (1993) as "the use of green plants to remove, contain, or render harmless environmental contaminants". This definition was further developed by Cunningham *et al.* (1996), who included not only the plants but also "their associated microbiota, soil amendments, and agronomic techniques".

Since those early stages in the development of phytoremediation, the specific beneficial effect of plant roots towards the dissipation of organic contaminants has been studied by a number of authors (Anderson *et al.*, 1993; Pilon-Smits, 2005; Martin *et al.*, 2014). Cunningham *et al.* (1995) first proposed that available contaminants within planted soil would be physically and/or biologically degraded, incorporated into humic fractions, or removed through root uptake. Although the concept of phytoremediation can present some variations from author to author, a highly accepted definition is the one presented by Pilon-Smits (2005) who describes it as "the use of plants and their associated microbes for environmental clean-up". Furthermore, due to the broad array of mechanisms involved within this definition, phytoremediation has been subdivided into categories based on the specific fate of the targeted contaminant (Pilon-Smits, 2005). These include *(1)* phytoextraction or plant accumulation, *(2)* phytovolatilization where either the plants or their associated microbes promote the volatilisation of the contaminant, *(3)* phytodegradation or degradation through plant uptake, and *(4)* rhizodegradation, where plant roots or root-associated microbial populations enhance the biodegradation of the contaminant (Anderson *et al.*, 1993; Cunningham *et al.*, 1996; Salt *et al.*, 1998; Pilon-Smits, 2005).

The applicability of this technology has been successfully proven for a wide number of compounds including both organic and inorganic toxic and carcinogenic substances (Pilon-Smits, 2005). Among these, the use of plants for the dissipation of different HOCs such as crude oil, petrol, toluene, xylene and a variety of polycyclic aromatic hydrocarbons (PAHs) has been consistently reported (Pilon-Smits, 2005). Although potentially useful, this process is considered to be a highly variable and complex (Germida *et al.*, 2002; Martin *et al.*, 2014); where the most obvious limitation of this

solar-driven technology is the capability of the plant to grow in highly contaminated soil (Cunningham *et al.*, 1995; Pilon-Smits, 2005).

The dynamic plant-root-microbe relationship in the closest proximity to the roots (1 - 2 mm) has been identified as the main mechanism through which plants can promote the dissipation of organic contaminants from soil (Anderson *et al.*, 1993; Germida *et al.*, 2002; Pilon-Smits, 2005). Also known as the rhizosphere (Figure 2) (Anderson *et al.*, 1993); this area has been the object of many studies; where multiple investigations have proposed different mechanisms by which plants can promote the biodegradation of hydrocarbons. It is a well acknowledged fact that the soil located in close proximity to the roots and under the direct influence of root exudation consistently presents significantly higher microbial activity than bulk soil (Anderson *et al.*, 1993; Siciliano & Germida, 1998). This phenomenon is temed the "rhizosphere effect", and was first expressed in terms of biodegradation of contaminants by Anderson *et al.* (1993).

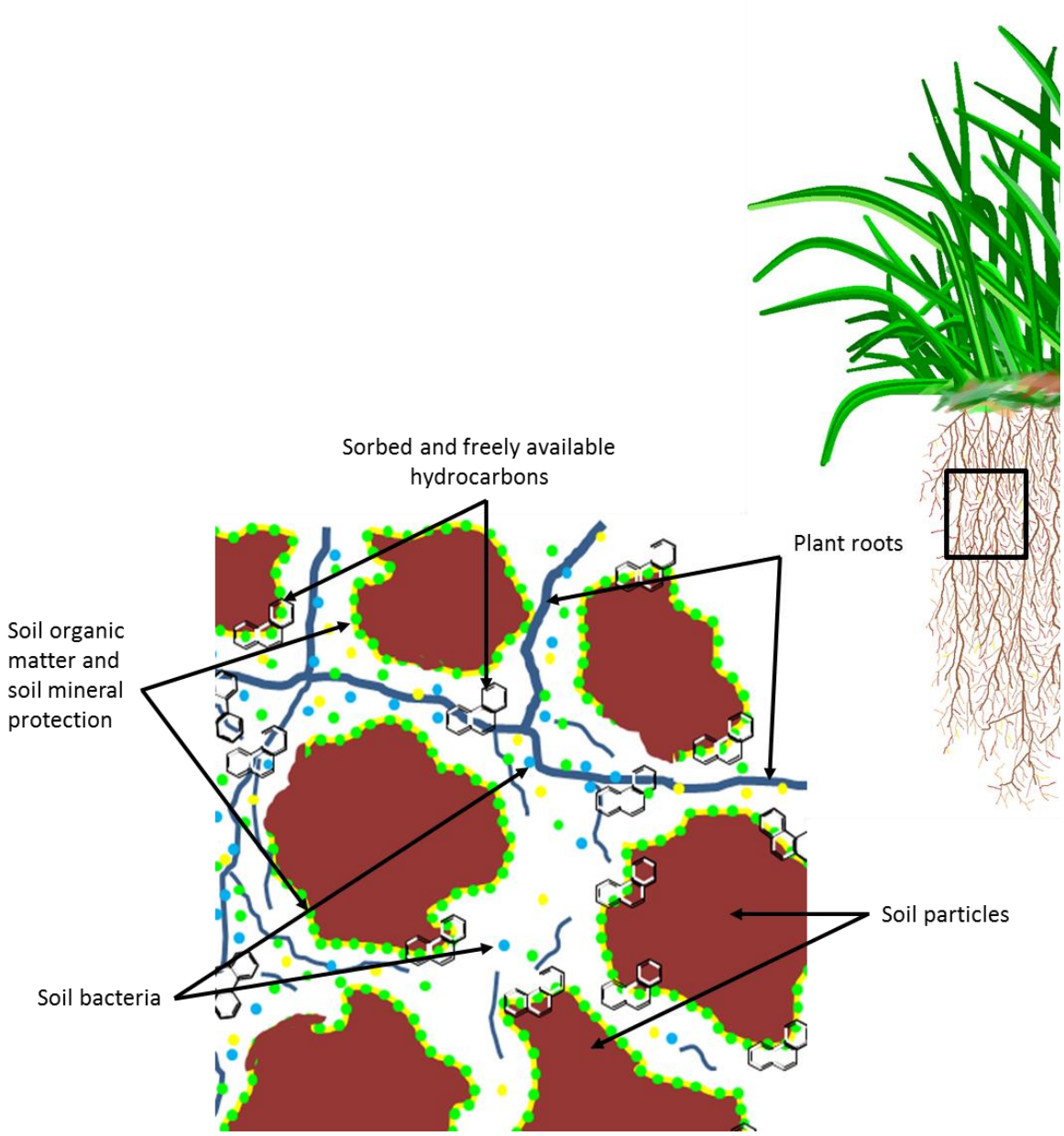

Figure 2. Hypothesised general representation of the rhizosphere effect, where alive and decaying plant roots can promote the mobilisation of sorbed hydrocarbons towards more bioaccessible fraction.

1.3.1 Mechanisms for the biodegradation of PAHs in the rhizosphere

Changes induced by roots on the physical, chemical and biological properties of the soil are believed to be the main factor enhancing the biodegradation of HOCs during phytoremediation (Anderson *et al.*, 1993; Gerhardt *et al.*, 2009). Ultimately, how soil physical and chemical characteristics are modified by the action of the plants will have a synergic effect on the inherent microbiological degrading activity of the soil

(Siciliano & Germida, 1998). These modifications are mainly caused by a dynamic process involving root exudation, decay and turnover (Günther *et al.*, 1996; Miya & Firestone, 2001); where specific patterns and characteristics are expected to vary based on a number of factors including plant species and age, soil type, moisture content, pH and temperature (Siciliano & Germida, 1998; Leigh *et al.*, 2002). Furthermore, individual constituents of these substrates have been associated as a significant contributing factor promoting the higher microbial activity normally attributed to the so called rhizosphere effect (Siciliano & Germida, 1998; Jones *et al.*, 2003; van Hees *et al.*, 2005).

Root-derived substrates are estimated to account for about 20 % of the total photosynthesised products (Schnoor *et al.*, 1995; Hütsch *et al.*, 2002). Furthermore, root exudation (van Hees *et al.*, 2005), decay and turnover (Leigh *et al.*, 2002) constitute important mechanisms by which the photosynthetically fixed carbon is introduced into the soil. Although variable, root exudates have been observed to be composed by a range of organic substrates including sugars, polysaccharides, amino-, organic- and fatty acids, sterols, enzymes, flavonoids and nucleotides (Uren, 2007). In addition, root turnover has also been observed to represent a source of energy and carbon necessary for the stimulation of microbial growth (Leigh *et al.*, 2002; Uren, 2007; Dennis *et al.*, 2010). As presented in Figure 2, there are two main processes that have been identified as possible drivers for the enhancement of the biodegradation of HOCs through the action of plant roots: (*i*) enlargement of the soil microbial populations in the closest proximity with the roots, and (*ii*) enhancement of the bioaccessibility of the contaminants by root-derived substrates.

In the first place, significantly higher root-associated biological activity within the rhizosphere has been associated to an enhancement of the biodegradation of petroleum hydrocarbons and other organic contaminants (Siciliano & Germida, 1998; Pilon-Smits, 2005). This includes the dissipation of single contaminants including phenanthrene (Miya & Firestone, 2001; Corgié *et al.*, 2003) and pyrene (Reilley *et al.*, 1996; Chen *et al.*, 2003), as well as complex mixtures of PAHs (Binet *et al.*, 2000; Joner & Leyval, 2003; Smith *et al.*, 2011) and oil derivatives such as diesel (Kaimi *et al.*, 2006), oil sludge (Muratova *et al.*, 2008), and crude oil (Banks *et al.*, 2003). These results have allowed authors to suggest the enlargement of microbial populations as

the main factor contributing to the enhancement of the biodegradation of contaminants in the presence of plants (Siciliano & Germida, 1998; Pilon-Smits, 2005). Furthermore, in addition to the enhanced microbial populations, these microorganisms would also need to be able of incorporating the contaminant into their metabolic processes. This ability has been observed to be promoted within the rhizosphere through the enhancement of bacterial acclimation towards pyrene (Mueller & Shann, 2007) and stimulating the mineralisation of contaminants with similar chemical characteristics that typical root-derived substances (Siciliano & Germida, 1998; Gerhardt *et al.*, 2009).

In addition to the enhancement of the microbial populations and activity, a higher bioaccessibility of organic contaminants has also been associated with rhizospheric soil (Siciliano & Germida, 1998). More importantly, this is the underlying mechanism by which authors have suggested that root-derived substrates can enhance the biodegradation of HOC (Siciliano & Germida, 1998; Martin *et al.*, 2014). This effect has been mainly observed to be produced by low molecular weight organic acids (LOAs) including citric, malic, oxalic and succinic acid (Ling *et al.*, 2009, 2015, Gao *et al.*, 2010a; b, 2015a; b; Kong *et al.*, 2013).

Despite the advances into the elucidation of the mechanisms supporting higher biodegradation of organic hydrocarbons in the rhizosphere (Agnello *et al.*, 2014; Ouvrard *et al.*, 2014), there is still a general lack knowledge regarding the specific processes enhancing this effect (Pilon-Smits, 2005; Martin *et al.*, 2014). One of the most notorious limitations is the difficulty of separating the direction and magnitude into which the single variables might contribute to the biodegradation process in the rhizosphere (Pilon-Smits, 2005; Agnello *et al.*, 2014; Martin *et al.*, 2014). This includes mechanical-root induced changes such as root penetration and aeriation (Cunningham & Ow, 1996), chemical and biological modification of the soil by the input of substrates from root exudates (Wilson & Jones, 1993; Jones & Darrah, 1994), and the response of the microbial community towards each one of these conditions (Anderson *et al.*, 1993).

Through these mechanisms, it can be hypothesised that basic soil-HOC mechanisms such as the impact of time on its bioaccessibility and biodegradation (Figure 1) can be significantly changed by plant roots (Figure 3). By this, different root-driven

processes, such as exudation, decay and turnover, might be able to retard the effects of ageing through different mechanisms, such as promotion of bacterial activity and enhancement of contaminant bioaccessibility. Consequently, the presence of plant roots in soil might be able to promote a larger degradable fraction of the contaminant and reduce the non-extractable and recalcitrant forms.

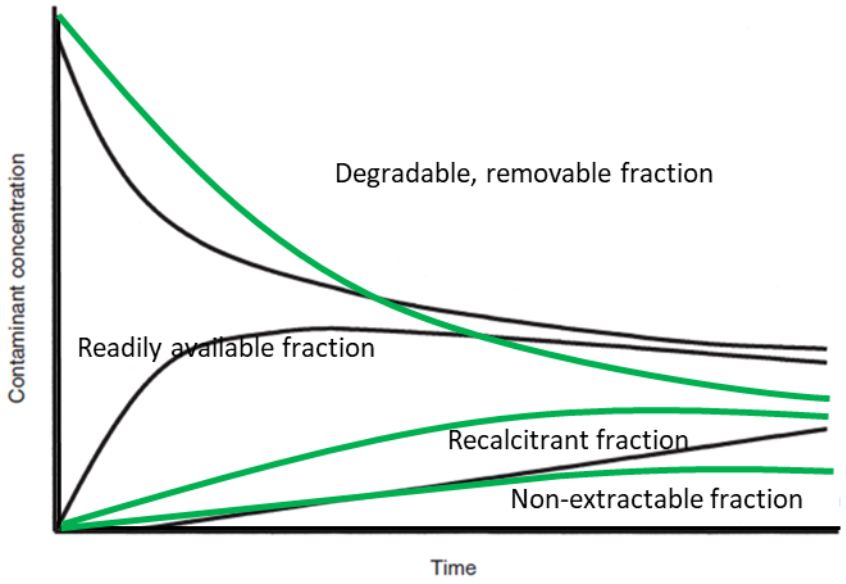

Figure 3. Proposed root-induced changes on the influence of ageing and bioaccessibility of HOCs in soil. Green lines represent the hypothesised behaviour of the bioaccessibility against time, while black lines are the original model presented by Semple *et al.* (2003).

1.4 Aim and objectives

This thesis investigated the effect of plant roots and components typically found within root exudates on the biodegradation and bioaccessibility of phenanthrene from soil and in particular the study of root-induced changes on the bioaccessibility and extractability of phenanthrene across time.

This thesis integrates a series of reviews, methodological and experimental papers addressing the following objectives:

- Review the key factors controlling the biodegradation of hydrocarbons under tropical conditions and the state of the art on the subject in this climatic region.

- Summarize the applicability of hydroxypropyl-β-cyclodextrin (HPCD) for remediation and the assessment of bioaccessibility of contaminants in soil.
- Evaluate the microbial catabolic activity from planted and root amended PAH contaminated soil.
- Assess the effect of simulated rhizosphere conditions on the biodegradation and bioaccessibility of phenanthrene from phenanthrene/diesel contaminated soil.
- Study the effect of two low molecular weight organic acids (as representative exudate substrates) on the desorption, bioaccessibility and biodegradability of phenanthrene from soil.

1.5 Summary of publications

Chapter 2 is intended for submission as: Vázquez-Cuevas, G. M., Stevens, C. J. and Semple, K. T. (2017) Biodegradation of hydrocarbons in soil from tropical regions

Biodegradation of hydrocarbons from contaminated soil depends on a wide range of factors among which environmental conditions play a significant role. As with other aspects of modern science, a significant proportion of the research studying the biodegradation of organic contaminants in soil has been developed under temperate climate conditions. In contrast, soil from tropical regions has been acknowledged to possess certain characteristics that can significantly differ from their temperate counterparts. Soil from the tropics is often characterised by acidic pH values, low organic matter and fertility and high clay content. A review of the main experimental and field conditions used for the study of the biodegradation of hydrocarbon contaminated soil from the tropics showed that a considerable number of physical, chemical and biological characteristics are comparable with temperate-based studies. Nevertheless, temperature was consistently a contrasting factor with respect to the standard values used among temperate studies. It was also observed that a significant amount of the research within this subject has been directed towards complex mixtures of petroleum hydrocarbons under field conditions. This particular characteristic has led to a lack of knowledge regarding the fate, behaviour and suitable

remediation approaches of other types of single and mixed contaminants in laboratory controlled environments.

Chapter 3 is intended for submission as: Vázquez-Cuevas, G. M., Rhodes, A. H., Stevens, C. J. and Semple, K. T. (2017) Cyclodextrins: Their use in the assessment and remediation of contaminated land – A review

The use of cyclodextrins (CDs) is wide and encompasses industries such as the biomedical, pharmaceutical, agricultural and environmental. CDs are formed by glucopyranose units with a structure that confers these molecules a hydrophobic non-polar cavity and a hydrophilic polar exterior. This feature is of particular interest within the environmental sciences as it has been observed to form water soluble inclusion complexes with different hydrophobic organic molecules including HOCs. The removal of this type of contaminants though CD complexation was initially applied for the stabilisation, encapsulation and adsorption of contaminants within waste water treatment and further transferred to other environmental areas. This review summarises the further development of the applicability of CDs on the remediation and assessment of contaminated land. Different types of CD have been proven to promote the biodegradation of a number of contaminants through the solubilisation of these compounds. Furthermore, extractions using CD solutions have also been proven to be an accurate and reproducible methodology for the prediction of the microbiologically degradable fraction of a range of hydrocarbons from contaminated soil.

Chapter 4 has been published as: Yu, L., Vázquez-Cuevas, G. M., Duan, L., and Semple, K. T. (2016). Buffered cyclodextrin extraction or ^{14}C-phenanthene from black carbon amended soil. *Environmental Technology & Innovation*. 6: 177-184

The extractability of buffered HPCD for the evaluation of the bioavailable fractions of ^{14}C-phenanthrene from aged contaminated soil impacted by different types of black carbon was assessed. The efficiency of these extractions to predict the microbiologically accessible ^{14}C-phenanthrene was also evaluated through their comparison against the $^{14}CO_2$ evolved from the same soil. Results showed that black carbon of any type significantly reduced the extractability and mineralisation of ^{14}C-phenanthrene. Data from the effect of the phosphate buffer on HPCD extractability

showed different trends depending on the ageing time. The solution at pH 7 extracted significantly higher amounts of ^{14}C-phenanthrene within the first 25 days of ageing, while pH 8 buffered solutions exhibited this trend only after 50 days of contaminant-soil contact time. Comparisons between the extracted and mineralised fractions of ^{14}C-phenanthrene showed that pH 7 HPCD solutions had a tendency to extract a higher amount that the mineralised fraction. Overall results showed that high pH HPCD solutions might be a feasible tool for the enhancement of the extractability of ^{14}C-phenanthrene from soil affected by sorbents such as black carbon.

Chapter 5 has been published as: Vázquez-Cuevas, G. M., Stevens, C. J. and Semple, K. T. (2018). Enhancement of ^{14}C-phenanthrene mineralisation in the presence of plant-root biomass in PAH-NAPL amended soil. *International Biodeterioration and Biodegradation*. 125: 78-85

Plant tolerance and ability to promote the biodegradation of ^{14}C-phenanthrene from a PAH and diesel as a non-aqueous phase liquid (NAPL) contaminated soil were evaluated. An initial phytotoxicity assessment was performed to evaluate the germination and early growth of nine different plant species towards highly contaminated soil. From this assay four of the more resistant plant species were selected for a further biodegradation assay. Roots (0.5% dry weight) from two grasses (*L. perenne* and *S. bicolor*) and two legumes (*M. sativa* and *S. bicolor*) were mixed into PAH and diesel contaminated soil. Mineralisation of ^{14}C-phenanthrene and microbial numbers from the root amended soil were further investigated. Evolution of $^{14}CO_2$ was significantly higher in soil amended with roots from either species after at least two weeks root-soil incubation time, with a more obvious effect in the absence of diesel. Microbial numbers did not correlate with this enhanced mineralisation and were further observed to decrease over time despite the higher evolution of ^{14}C-phenanthrene into $^{14}CO_2$. Main findings from this experiment suggest that the higher dissipation of PAHs (in this case ^{14}C-phenanthene) from planted soil is not a consequence of larger microbial populations but rather to a microbial adaptation period towards contaminated environments.

Chapter 6 is intended for submission as: Vázquez-Cuevas, G. M., Stevens, C. J. and Semple, K. T. (2017) Bioaccessibility of ^{14}C-phenanthrene from simulated rhizosphere contaminated soil

This study investigated the effect of a simulated rhizosphere soil on the bioavailability and mineralisation of ^{14}C-phenanthrene from aged phenanthrene/diesel contaminated soil. Roots from the grasses *Lolium perenne* (ryegrass) and *Sorghum bicolor* (sorghum), and the legume *Medicago sativa* (alfalfa) were grown and amended into ^{14}C-phenanthrene contaminated soil aged over 1, 25, 50 and 100 days. The effect of the approach used for the introduction of the root amendments (pieces or paste) and their contact time with the soil (1, 21 and 42 days) were further evaluated. Results indicated that the presence of diesel had significant effects in both mineralisation of ^{14}C-phenanthrene. Furthermore, although root amendments did not affect the bioaccessibility of phenanthrene, treatments containing roots showed significantly higher mineralisation extents against the control after 50 days ageing and at least 21 days of root-soil contact time. Moreover, this root-enhanced mineralisation of ^{14}C-phenanthrene was more likely to be observed when soil was contaminated with phenanthrene/diesel rather than just phenanthrene. Results from this investigation suggest that a higher dissipation of phenanthrene from aged contaminated soil might be achieved by a simulated rhizosphere after a microbial adaptation period towards hydrocarbon contaminated environments.

Chapter 7 has been published as: Umeh, A., Vázquez-Cuevas, G. M. and Semple, K. T. (2017) Mineralisation of ^{14}C-phenanthrene in complex PAH-diesel oil mixtures contaminated soil in the presence of *Sorghum bicolor* and *Medicago sativa* mono- and mixed cultures. *Applied Soil Ecology*. *In press*

Mineralisation of ^{14}C-phenanthrene from PAH-diesel contaminated soil planted with single- and mixed cultures of *Medicago sativa* (alfalfa) and *Sorghum bicolor* (sorghum) was evaluated. Simultaneously, phytotoxic effects of highly PAH-diesel contaminated soil over these species and soil microbial numbers were also assessed. Results showed that despite exhibiting diminishing effects at higher concentration of PAHs, mineralisation of ^{14}C-phenanthrene can be significantly enhanced by the action of plants. It was also observed that an acclimation period was required before this plant-induced enhancement became significant. Data from the glasshouse experiment

showed that although seedling emergence and growth were not affected by PAHs and diesel, plant biomass was observed to significantly generally decrease when compared against the control. Root biomass from both plant species has higher in soil contaminated with 100 mg kg^{-1} PAHs was consistently higher in contaminated soil than in the control. Overall results suggest the feasibility of the use of mono- and multi-species phytotoxicity and plant-assisted biodegradation for risk assessment and soil remediation purposes.

Chapter 8 is intended for submission as: Vázquez-Cuevas, G. M., Stevens, C. J., Ortega-Calvo, J. J., Lag-Brotons, A. J. and Semple, K. T. (2017) The effect of organic acids on the fate of ^{14}C-phenanthrene from aged contaminated soil

The effect of a range of concentrations of the citric and malic low molecular weight organic acids (LOAs) (0, 0.5, 100, 250, 500 and 1000 mmol l^{-1}) on the extractability, bioavailability and mineralisation of ^{14}C-phenanthrene was evaluated. Desorption of ^{14}C-phenanthrene was observed to have a tri-phasic behaviour characterised by a rapid, slow, and very slow desorbing fractions. Furthermore, organic acids showed to produce significant changes on the desorption of ^{14}C-phenanthrene from soil although the magnitude and direction of these were observed to vary as a function of LOA concentration and soil ageing time, with more evident effects observed from the aged contaminated soil (15 weeks). Although total desorbing fractions of ^{14}C-phenanthrene were consistently larger when LOAs where present at high concentrations, desorption kinetics showed that a significant proportion of desorbed phenanthrene was mobilised towards the very slowly desorbing fraction ($F_{very\ slow}$), which is normally associated with the least biologically accessible proportion of the contaminant. While desorption of ^{14}C-phenanthrene was enhanced by LOAs, this effect was not observed to be reflected on neither a higher bioaccessibility or mineralisation of ^{14}C-phenanthrene. Findings from this study showed that despite inducing a higher desorption, there is no direct evidence suggesting that the incorporation of citric or malic acid at the tested concentrations can promote the microbial utilisation of ^{14}C-phenanthrene from contaminated soil.

Appendix

The following book chapter has also been written and published as a part of the work developed during my PhD:

Vázquez-Cuevas, G. M. and Semple, K. T. (2016). Measurement of hydrocarbon bioavailability in soil. *In*: McGenity, T. J., Timmis, K. N. & Nogales Fernández, B. (eds.) Hydrocarbon and lipid microbiology protocols. Springer protocols handbooks. pp 231-246. Totowa, N.J. Humana Press

The measurement of the bioavailability and bioaccessibility of hydrocarbons from contaminated soil is considered an important factor for the assessment of the biodegradation potential. Due to its importance, a number of methodologies have been developed for the quantification of the bioavailable fraction through the use of biological and chemical approaches. This book chapter summarises the most commonly used methodologies for the evaluation of the bioavailable fraction from contaminated soils and sediments. Furthermore, it presents a detailed protocol for selected methodologies that have proven to provide robust, accurate and reproducible prediction of the bioaccessible fraction of hydrocarbons from contaminated soil.

1.6 References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D. & Esposito, G. 2014. Enhanced phytoremediation: A review of low molecular weight organic acids and surfactants used as amendments. Critical Reviews in Environmental Science and Technology, 44, 2531–2576.

Alexander, M. 1999. Biodegradation and bioremediation. Academic Press.

Alexander, M. 2000. Aging, bioavaiability, and overestimation of risk from environmental pollutants. Environmental Science & Technology, 34, 4259–4265.

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science and Technology, 27, 2630–2636.

Banks, M.K., Kulakow, P., Schwab, P., Chen, Z. & Rathbone, K. 2003. Degradation of crude oil in the rhizosphere of Sorghum bicolor. International journal of phytoremediation, 5, 225–34.

Binet, P., Portal, J.M. & Leyval, C. 2011. Dissipation of 3 ± 6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biology, 32.

Bollag, J.-M. & Bollag, W.B. 1995. Soil Contamination and the Feasibility of Biological Remediation. In: Bioremediation: Science and Applications (eds. Skipper, H.D. & Turco, R.F.), pp. 1–12. Soil Science Society of America, American Society of Agronomy, and Crop Science Society of America.

Chen, Y.C., Banks, M.K. & Schwab, A.P. 2003. Pyrene Degradation in the Rhizosphere of Tall Fescue (Festuca arundinacea) and Switchgrass (Panicum virgatum L.). Environmental Science and Technology, 37, 5778–5782.

Corgié, S.C., Joner, E.J. & Leyval, C. 2003. Rhizospheric degradation of phenanthrene is a function of proximity to roots. Plant and Soil, 257, 143–150.

Cunningham, S.D., Anderson, T.A., Schwab, A.P. & Hsu, F.C. 1996. Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy, 56, 55–114.

Cunningham, S.D., Berti, W.R. & Huang, J.W. 1995. Phytoremediation of contaminated soils. Trends in Biotechnology, 13, 393–397.

Cunningham, S.D. & Ow, D.W. 1996. Promises and Prospects of Phytoremediation. Plant Physiology, 110, 715–719.

Cunningham, S, D., Berti & W, R. 1993. Phytoremediation of contaminated soils: progress and promise. Symposium on bioremediation and bioprocessing, 13, 265–268.

Dennis, P.G., Miller, A.J. & Hirsch, P.R. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology, 72, 313–327.

Gao, Y., Ren, L., Ling, W., Gong, S., Sun, B. & Zhang, Y. 2010a. Desorption of phenanthrene and pyrene in soils by root exudates. Bioresource Technology, 101, 1159–1165.

Gao, Y., Ren, L., Ling, W., Kang, F., Zhu, X. & Sun, B. 2010b. Effects of Low-Molecular-Weight Organic Acids on Sorption–Desorption of Phenanthrene in Soils. Soil Science Society of America Journal, 74, 51.

Gao, Y., Wang, N., Li, H., Hu, X. & Goikavi, C. 2015a. Low-molecular-weight organic acids influence the sorption of phenanthrene by different soil particle size fractions. Journal of Environment Quality, 44, 219.

Gao, Y., Yuan, X., Lin, X., Sun, B. & Zhao, Z. 2015b. Low-molecular-weight organic acids enhance the release of bound PAH residues in soils. Soil and Tillage Research, 145, 103–110.

Gerhardt, K.E., Huang, X.D., Glick, B.R. & Greenberg, B.M. 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Science, 176, 20–30.

Germida, J.J., Frick, C.M. & Farrell, R.E. 2002. Phytoremediation of oil-contaminated soils. Developments in Soil Science, 28, 169–186.

Günther, T., Dornberger, U. & Fritsche, W. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere, 33, 203–215.

Harwood, A.D., Landrum, P.F. & Lydy, M.J. 2012. A comparison of exposure methods for SPME-based bioavailability estimates. Chemosphere, 86, 506–511.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science and Technology, 29, 537–545.

van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L. & Lundström, U.S. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry, 37, 1–13.

Hütsch, B.W., Augustin, J. & Merbach, W. 2002. Plant rhizodeposition-an important source for carbon turnover in soils. J. Plant Nutr. Soil Sci., 165, 397–407.

Joner, E.J. & Leyval, C. 2003. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environmental Science and Technology, 37, 2371–2375.

Jones, D.L. & Darrah, P.R. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil, 166, 247–257.

Jones, D.L.L., Dennis, P.G.G., Owen, a. G.G. & van Hees, P. a. W. a W. 2003. Organic acid behavior in soils - misconceptions and knowledge gaps. Plant and Soil, 248, 31–41.

Jonker, M.T.O., Van Der Heijden, S.A., Kreitinger, J.P. & Hawthorne, S.B. 2007. Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with solid-phase microextraction. Environmental Science and Technology, 41, 7472–7478.

Jørgensen, K.S., Puustinen, J. & Suortti, A.M. 2000. Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. Environmental Pollution, 107, 245–254.

Kaimi, E., Mukaidani, T., Miyoshi, S. & Tamaki, M. 2006. Ryegrass enhancement of biodegradation in diesel-contaminated soil. Environmental and Experimental Botany, 55, 110–119.

Kelsey, J.W., Kottler, B.D. & Alexander, M. 1997. Selective chemiscal extractants to predict bioavaiability of soil-aged organic chemicals. Environmental Science & Technology, 31, 214–217.

Kong, H., Sun, R., Gao, Y. & Sun, B. 2013. Elution of polycyclic aromatic hydrocarbons in soil columns using low-molecular-weight organic acids. Soil Science Society of America Journal, 77, 72–82.

Leahy, J.G. & Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews, 54, 305–315.

Lee, P.H., Doick, K.J. & Semple, K.T. 2003. The development of phenanthrene catabolism in soil amended with transformer oil. FEMS Microbiology Letters, 228, 217–223.

Leigh, M.B., Fletcher, J.S., Fu, X. & Schmitz, F.J. 2002. Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environmental Science and Technology, 36, 1579–1583.

Ling, W., Ren, L., Gao, Y., Zhu, X. & Sun, B. 2009. Impact of low-molecular-weight organic acids on the availability of phenanthrene and pyrene in soil. Soil Biology and Biochemistry, 41, 2187–2195.

Ling, W., Sun, R., Gao, X., Xu, R. & Li, H. 2015. Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil. European Journal of Soil Science, 66, 339–347.

Liste, H.H. & Alexander, M. 1999. Rapid screening of plants promoting phenanthrene degradation. Journal-of-Environmental-Quality, 28, 1376–1377.

Liste, H.H. & Alexander, M. 2002. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere, 46, 1011–1017.

Luthy, G.R., Ainken, R.G., Brusseau, L.M., Cunningham, D.S., Gschwend, M.P., Pignatello, J.J., Reinhard, M., Traina, J.S., Jr, Weber, J.W. & Westall, C.J. 1997. Critical review sequestration of hydrophobic organic contaminants by geosorbents. American Chemical Society, 31, 3341–3347.

Macleod, C.J.A. & Semple, K.T. 2003. Sequential extraction of low concentrations of pyrene and formation of non-extractable residues in sterile and non-sterile soils. Soil Biology and Biochemistry, 35, 1443–1450.

Martin, B.C., George, S.J., Price, C.A., Ryan, M.H. & Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. Science of the Total Environment, 472, 642–653.

Miya, R.K. & Firestone, M.K. 2001. Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environment Quality, 30, 1911–1918.

Mueller, K.E. & Shann, J.R. 2007. Effects of tree root-derived substrates and inorganic nutrients on pyrene mineralization in rhizosphere and bulk soil. Journal of Environment Quality, 36, 120.

Mulligan, C.N., Yong, R.N. & Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. Engineering Geology, 60, 193–207.

Muratova, A.Y., Dmitrieva, T. V., Panchenko, L. V. & Turkovskaya, O. V. 2008. Phytoremediation of oil-sludge-contaminated soil. International Journal of Phytoremediation, 10, 486–502.

Ortega-Calvo, J.J., Harmsen, J., Parsons, J.R., Semple, K.T., Aitken, M.D., Ajao, C., Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R., Peijnenburg, W.J.G.M., Römbke, J., Streck, G. & Versonnen, B. 2015. From bioavailability science to regulation of organic chemicals. Environmental Science and Technology, 49, 10255–10264.

Ouvrard, S., Leglize, P. & Morel, J.L. 2014. PAH Phytoremediation: Rhizodegradation or Rhizoattenuation? International Journal of Phytoremediation, 16, 46–61.

Pepper, I.L., Gerba, C.P. & Brusseau, M.L. 2006. Environmental and Pollution Sience. Elsevier/Academic Press.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39, (At: http://www.annualreviews.org/doi/10.1146/annurev.arplant.56.032604.144214.).

Prokop, Z., Nečasová, A., Klánová, J. & Čupr, P. 2016. Bioavailability and mobility of organic contaminants in soil: new three-step ecotoxicological evaluation. Environmental Science and Pollution Research, 23, 4312–4319.

Reid, B.J., Jones, K.C. & Semple, K.T. 2000a. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environmental Pollution, 108, 103–112.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000b. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science and Technology, 34, 3174–3179.

Reilley, K.A., Banks, M.K. & Schwab, A.P. 1996. Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality, 25, 212–219.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353, (At: http://dx.doi.org/10.1016/j.envpol.2010.01.008.).

Saleha, H. 2008. Literature overview: Microbial metabolism of high molecular weight polycyclic aromatic hydrocarbons. Remediation Journal, 18, 131–161.

Salt, D.E., Smith, R.D. & Raskin, I. 1998. Phytoremediation. Annual Review of Plant Biology, 49, 643–668.

Samanta, S.K., Singh, O. V & Jain, R.K. 2002. Polycyclic aromatic hydrocarbons : environmental pollution and bioremediation. Trends in Biotechnology, 20, 243–248.

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L. & Carreira, L.H. 1995. Phytoremediation of organic and nutrient contaminants. Environmental Science and Technology, 29, 318–323.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science & Technology, 38, 228A–231A.

Semple, K.T., Doick, K.J., Wick, L.Y. & Harms, H. 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. Environmental Pollution, 150, 166–176.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Siciliano, S.D. & Germida, J.J. 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environmental Reviews, 6, 65–79.

Singh, A., Kuhad, R.C. & Ward, O.P. 2009. Biological Remediation of Soil: An Overview of Global Market and Available Technologies. pp. 1–19. Springer, Berlin, Heidelberg.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Saito, H. 2011. Study of PAH dissipation and phytoremediation in soils: Comparing freshly spiked with weathered soil from a former coking works. Journal of Hazardous Materials, 192, 1219–1225.

Tan, K.H. 2009. Environmental Soil Science, Third Edition. CRC Press.

Uren, N.C. 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface (eds. Pinton, R., Varanini, Z. & Nannipieri, P.), pp. 1–21. Marcel Dekker, Inc.

Vázquez-Cuevas, G.M. & Semple, K.T. 2016. Measurement of Hydrocarbon Bioavailability in Soil. In: Hydrocarbon and Lipid Microbiology Protocols - Springer Protocols Handbooks (eds. McGenity, T., Timmis, K. & Nogales, B.), pp. 231–246. Springer, Berlin, Heidelberg.

Volkering, F. & Breure, A.M. 2003. Biodegradation and general aspects of bioavailability. In: PAHs: An ecotoxicological perspective (ed. Douben, P.), p. 391. Wiley.

Wang, C. hui, Wu, S. hua, Zhou, S. lu, Wang, H., Li, B. jie, Chen, H., Yu, Y. na & Shi, Y. xing. 2015. Polycyclic aromatic hydrocarbons in soils from urban to rural areas in Nanjing: Concentration, source, spatial distribution, and potential human health risk. Science of the Total Environment, 527–528, 375–383.

Wild, S.R. & Jones, K.C. 1995. Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventtory and budget. Environmental Pollution, 88, 91–108.

Wilson, S.C. & Jones, K.C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environmental pollution (Barking, Essex : 1987), 81, 229–249.

Chapter 2

Biodegradation of hydrocarbons in soil from tropical environments

Biodegradation of hydrocarbons in soil from tropical environments

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2.1 Abstract

Acidic pH, high temperature, low organic matter content, low fertility and high clay content can be enlisted as the most obvious characteristics identifying the soil from tropical regions. These same characteristics have also an important influence on the biodegradation of hydrocarbons of soil in tropical environments. As with different aspects of modern science, the majority of the research regarding biodegradation of hydrocarbons in soil has been performed under temperate conditions. This situation has led to the assumption of the feasibility of extrapolating protocols and expected results between these two climatic regions. This review focuses on the analysis of the factors influencing the biodegradation and remediation of hydrocarbon contaminated soil in the tropics, focusing on the protocols and outcomes followed for the remediation of hydrocarbon contaminated soil. Overall outcomes from tropical environments showed similar trends to those observed at temperate regions. However, experimental temperature makes it difficult to accurately compare data from temperate and tropical regions. While standard temperature employed in colder regions of the world is about 21 ºC, the observed mean temperature used for the tropical counterpart was 28.61 ºC. The data reviewed in this document shows that a considerable amount of research from tropical regions has been focused on biodegradation of total petroleum hydrocarbons (TPH) from crude oil and its refined forms. In contrast, there is a general absence of knowledge regarding the fate, behaviour and remediation approaches of other forms of hydrocarbons such as aromatic and aliphatic compounds.

Keywords: Biodegradation, hydrocarbons, tropics, contaminated soil

2.2 Introduction

Tropical regions account for about 36 % of the world's surface, encompassing the South of Asia, Central and South America, the Caribbean, major regions of Africa and a small area of Northern Australia and North America (Juo & Franzluebbers, 2003). The most obvious characteristic of these regions is the temperature, with a mean annual value at sea level of 25 – 26 ºC (Juo & Franzluebbers, 2003; Nortcliff, 2010).

Among the different reasons leading to soil contamination, economic activities are often pointed as a primary source of contaminants for all the compartments of the environment, including soil (Wild & Jones, 1995). Although the type and magnitude of the economic activities will differ from country to country, tropical regions are characterized to share two main sources of income: agriculture and oil industry. Agriculture has historically been one of the most profitable activities within the population in the rural areas in tropical countries (Craswell & Lefroy, 2001; Juo & Franzluebbers, 2003; Díaz-Ramírez *et al.*, 2013). Environmental consequences of agriculture are well-known and include soil organic matter (SOM) exploitation (Greenland *et al.*, 1992; Craswell & Lefroy, 2001), depletion of naturally occurring nutrients, and introduction of chemicals into the soil (Greenland *et al.*, 1992). Similarly, the oil industry also constitutes an important economic activity in a vast majority of these regions (Ferreira Do Nascimento *et al.*, 2013; Adams *et al.*, 2014). One of the clearest consequences of this is the common occurrence of contaminated sites as a direct consequence of different oil-related activities such as drilling and the transportation and refining of crude oil (Trindade *et al.*, 2005; Chikere *et al.*, 2009; Chagas-Spinelli *et al.*, 2012; Riveroll-Larios *et al.*, 2015).

Together these two activities have produced a wide range of environmental issues in tropical regions including the introduction of hydrocarbons into the soil (Díaz-Ramírez *et al.*, 2013). Despite of the importance of hydrocarbon contamination of soil, the majority of research and protocols looking at its understanding, control and remediation have been developed focusing on temperate regions (Zech *et al.*, 1997; Arbeli & Fuentes, 2010; Okere & Semple, 2012). The research carried in colder regions of the world has allowed a better comprehension of the fate and behaviour of hydrocarbons in soil, though the extent by which this can be extrapolated to different climatic environments is not completely understood. Moreover, environmental

characteristics have a significant impact on different soil conditions such as microbial populations, biogeochemical transformations and chemical persistence (Alexander, 1999), which will ultimately define the mass transfer process ruling the bioavailability of hydrocarbons (Ramirez & Cutright, 2001).

Although tropical regions represent more than a third of the world's surface, the specific implication of this climate on the biodegradation of hydrocarbons present in the soil has not been consistently assessed or summarized as in temperate regions. This particular situation has been pointed by different authors such as Juo & Franzluebbers (2003) and Bandala & Torres (2012). It has also produced a lack of understanding of the specific dynamics that the tropical attributes might ultimately have on the different soil-hydrocarbon processes (Zech *et al.*, 1997; Ruiz-Marín *et al.*, 2013). Therefore, a proper characterisation of these specific conditions is essential for an adequate planning and execution of any remediation protocol (Alexander, 1999). This review aims to offer a comprehensive overview of the key factors ruling the biodegradation of hydrocarbons from soil in tropical regions. Furthermore, a critical analysis of the state of the art regarding the research of degradation of hydrocarbons in these regions of the world is also presented. Through this, it will be possible to summarize the areas of opportunity regarding the biodegradation processes of soil-hydrocarbons under tropical conditions.

2.3 General classification of tropical soils

Despite the existence of several abiotic factors controlling the biodegradation of hydrocarbons, Alexander (1999) has pointed out that there are three main components that need to be considered in promoting the biodegradation of organic contaminants: temperature, moisture and pH. These, alongside with other abiotic characteristics such as soil texture and nutrient supply are the parameters that will largely define if a specific site is suitable for the implementation of a bioremediation protocol (Admassu & Korus, 1996; Alexander, 1999).

From all the differences that could be noted between temperate and tropical regions, temperature could be considered the most important factor controlling soil processes in the tropics. As a direct consequence of higher temperatures and humidity, the turnover rate of SOM in the tropics is considered to be significantly faster than in

temperate regions (Greenland *et al.*, 1992; Craswell & Lefroy, 2001). More importantly, as many of the tropical soils are characterized by a limited availability of inorganic nutrients, the cycling of SOM often functions as the only reliable way to maintain the minimum required fertility to sustain biological processes (Tiessen *et al.*, 1994; Zech *et al.*, 1997).

Unlike temperature, which is considered to remain relatively constant through the year, precipitation exhibits a wide variation. Based on these two attributes, it is possible to classify the topical regions as: (1) humid, (2) sub-humid, (3) semiarid and (4) arid (Nortcliff, 2010). Overall, humid areas get a constant high annual rainfall (~3500 mm), and can be observed in about 24 % of the tropical regions (Nortcliff, 2010). In contrast, the majority of tropical regions are subject to clearly defined dry and wet seasons (Juo & Franzluebbers, 2003; Nortcliff, 2010), falling under the sub-humid range. These sub-humid areas tend to have strongly weathered soils but not as leached as the humid tropical areas, allowing higher concentrations of macro- and micronutrients (Juo & Franzluebbers, 2003). Finally, semiarid and arid tropics are defined as areas where annual precipitation above the potential evapotranspiration (PET) ranges between 2 and 4.5 months or the two month line respectively (Juo & Franzluebbers, 2003). Rainfall is also considered to be one of the main reasons of the low fertility in tropical soils (Craswell & Lefroy, 2001). There is a close relationship between rainfall and nutrient availability, with a large extent of tropical soil classified as strongly weathered (Juo & Franzluebbers, 2003), infertile and acidic (Craswell & Lefroy, 2001). Even though these conditions are often observed in the humid tropical regions (Juo & Franzluebbers, 2003; Nortcliff, 2010), the same trend can also be observed at the slightly more fertile sub-humid areas (Nortcliff, 2010). This low-fertility characteristic can be considered as a direct output of the leaching processes produced by the common high total annual precipitation of these regions (Juo & Franzluebbers, 2003). Moreover, this constant “washing” of the soil has been acknowledged to produce low concentrations of mineral nutrients such as phosphorus as well as low cation exchange capacity (CEC) (Juo & Franzluebbers, 2003; Nortcliff, 2010). These and other main characteristics of the different types of tropical soils are summarized in Table 1.

2.4 Abiotic factors and the biodegradation of hydrocarbons

2.4.1 Temperature

The effect of temperature on the biodegradation of hydrocarbons in soil can be approached from two different perspectives; (1) changes on the fate and behaviour of the contaminant, and (2) changes on the soil microbial composition and metabolism (Atlas, 1981; Leahy & Colwell, 1990). Different authors have observed that higher temperatures are capable of inducing faster rates of microbial degradation of crude oil hydrocarbons by raising their metabolic activity (Dibble & Bartha, 1979; Margesin & Schinner, 2001; Okere & Semple, 2012). For instance, Yeung *et al.* (1997) observed an optimum biodegradation of hydrocarbons between 30 and 40 ºC. Similarly, Dibble & Bartha, (1979) also reported that the biodegradation of hydrocarbons was significantly higher at temperatures above 20 ºC. Observations like these have led to the assumption that tropical environments should possess certain advantages over temperate regions by presenting steady high temperatures throughout the year. Furthermore, it has also been hypothesized that certain processes, such as bioremediation, can be extrapolated from temperate to tropical climates (Greenland *et al.*, 1992) when soil chemical and structural similarities are observed. Although this could be done, it is important to bear in mind that most laboratory based research studying the fate and behaviour of hydrocarbons in soil uses 21 ºC as a standard temperature.

2.4.2 Moisture

Soil moisture is often considered a limiting factor for overall microbial activity, especially at the soil surface, where extreme values can lead to a reduced microbial metabolic transformation of the contaminants (Alexander, 1999). Either the excess or absence of water in the soil will affect the rates and extents of the diffusion process, which involves nutrients, oxygen (Frick *et al.*, 1999), and contaminants if present (Semple *et al.*, 2003). Although deficient amounts of water in the soil are often considered a major concern, excessive quantities could also inhibit the aerobic microbial processes by inducing anoxic conditions (Alexander, 1999). One of the reasons for this is that the initial microbial catabolic phases of most hydrocarbons require the oxidation of the compound (Leahy & Colwell, 1990). Furthermore,

specific soil physical characteristics controlling moisture content can significantly change by the presence of most contaminants, such as crude oil, which is known to reduce the ability of the soil to incorporate water within its pores (Adams *et al.*, 2008). This effect can even be more obvious after extended dry periods (Adams *et al.*, 2008), which is one of the principal characteristics of sub-humid and arid tropical regions (Nortcliff, 2010). While a minimum water supply should not be a concern when treating humid tropical areas, sites located within sub-humid and semiarid/arid tropics should include a proper irrigation system to avoid excessive dryness during the dry seasons (Margesin & Schinner, 2001).

2.4.3 pH

pH values of non-contaminated tropical soil are generally believed to be within the acidic range. Nonetheless, authors such as Obire & Nwaubeta (2002) have reported that although contro(Osuji & Nwoye, 2007)l soil from Nigeria was acidic, pH of the same soil evolved to neutral values when it was exposed to refined forms of hydrocarbons. This same trend has also been observed by other researchers in Nigeria (Osuji *et al.*, 2006; Osuji & Nwoye, 2007; Ayotamuno *et al.*, 2009b), as well as in other tropical countries including Brazil (Trindade *et al.*, 2005) and Ecuador (Maddela *et al.*, 2015a). Although the effect of extreme pH values on the biodegradation process might be variable, these would be expected to negatively modify the metabolic activity of the microbial populations degrading the contaminants (Leahy & Colwell, 1990). As a result, it is common practice around the globe to use pH buffers when soil with extreme pH is remediated; such is the case of the use of lime on acidic soils (Alexander, 1999). This specific approach has been proven to successfully enhance hydrocarbon degradation in a humid tropical contaminated site of Mexico (Adams *et al.*, 2014) and a sub-humid location in Nigeria (Osuji & Nwoye, 2007). Furthermore, Adams *et al.* (2014) reported that this chemical approach was also effective in the reduction of phytotoxicity.

2.4.4 Texture

Texture of the soil often defines the fate and behaviour of a contaminant though different physical processes such as sorption and desorption (Rhodes *et al.*, 2010b). In the case of tropical soil, predominantly clayey textures (Juo & Franzluebbers, 2003)

would be expected to increase the sorption of hydrocarbons, therefore reducing its bioaccessibility. For instance, this has been confirmed by a higher sorption of pyrene on clayey tropical soil from Colombia when it was compared against soil from a temperate region (Ramirez & Cutright, 2001). To overcome this, a number of amendments have been used aiming to increase the solubility and desorption of hydrocarbons from the soil (Mulligan *et al.*, 2001). Within tropical soils, the enhancement of the solubility of hydrocarbons has been reported by the use of surfactants. Researchers have tested soil washing efficiencies using commercial (Alba *et al.*, 2013; Hernández-Espriú *et al.*, 2013) and natural (Alba *et al.*, 2013) surfactants for the remediation of oil contaminated soil. This approach has produced higher degradation of hydrocarbons and has also been especially useful as a combined technology alongside with electrokinetic remediation (Alba *et al.*, 2013). Furthermore, studies carried out in tropical regions have indicated that despite the importance of the soil texture on the biodegradation, it was the combination of different environmental factors what defined the removal or hydrocarbons from the soil (Martínez & López, 2001; Torres *et al.*, 2007). Such factors included soil's organic matter, bulk density, porosity (Martínez & López, 2001; Adams Schroeder *et al.*, 2002; Torres *et al.*, 2007), cation exchange capacity (CEC) (Hwang *et al.*, 2003), nutrient availability (Adams Schroeder *et al.*, 2002) and type and concentration of the contaminant (Martínez & López, 2001).

2.5 Microbial activity and biodegradation of hydrocarbons

Contamination of soil with crude oil and its refined forms is an important concern for oil producer countries (Merkl *et al.*, 2005; Nwinyi *et al.*, 2014), including those in tropical regions. Petroleum hydrocarbons are constantly considered to be the most widespread contaminant in both soil and water (Margesin & Schinner, 2001). These type of contaminants have also been the object of study in most of the research looking at the bioremediation in extreme environments (Margesin & Schinner, 2001). Although the oil industry has played a key role in the way that bioremediation research has been developed in tropical regions, focusing on the study of complex oil mixtures, the study of single components typically found in oil have also been developed in tropical environments (Wilcke *et al.*, 2003). More importantly, the study of single hydrocarbons rather than a mixture can also represent a tool for the study of

the fate and behaviour of these substances. Nonetheless, research focusing on PAHs in tropical regions of the world, as well as the specific implications of its degradation from the soil under these specific climatic conditions is rare.

2.5.1 Indigenous microbial activity

Microbial degradation is known to be the most important mechanism for the dissipation of organic contaminants from soil. This phenomenon has been observed for several organic contaminants, including different forms of hydrocarbons and pesticides (Atlas, 1981; Alexander, 1999; Semple *et al.*, 2003; Brooijmans *et al.*, 2009). The general assumption when extrapolating the fate and behaviour of organic contaminants from temperate to tropical conditions is that providing that nutrient availability is not a limiting factor (Merkl *et al.*, 2004a), a faster biodegradation of organic contaminants would be expected as a direct consequence of higher and constant temperature and humidity (Alexander, 1999; Frick *et al.*, 1999; Merkl *et al.*, 2004b, 2005; Arbeli & Fuentes, 2010).

The presence of bacteria capable of using organic contaminants is also an imperative factor for the use of bioremediation protocols, functioning as an indicative of the biodegradability potential of a specific site. The absence of these microorganisms has been pointed as the underlying limiting factor for the biodegradation of tropical soil contaminated with crude oil, even above other important conditions such as the intrinsic fate and behaviour of the contaminants (Rizzo *et al.*, 2008a). There are a number of historically well-known genera of bacteria capable of using hydrocarbon as their source of carbon including *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia, and Pseudomonas* spp. (Leahy & Colwell, 1990). As with other aspects of the biodegradation of organic contaminants, the majority of the research regarding the isolation of hydrocarbon-degrading microorganism has been focused on a temperate climate (Nwinyi *et al.*, 2014). Consequently, work on the field of biodegradation of hydrocarbons in tropical environments has been focused towards the assessment of their intrinsic biological activity. So far it has been acknowledged that even in extreme environments, it is possible to find enough hydrocarbon degraders (Margesin & Schinner, 2001). This condition has been observed in tropical environments, with authors reporting the presence of a range of bacterial strains capable of using different organic contaminants

as their sole source of carbon in different regions of the world including Brazil (von der Weid *et al.*, 2007), Indonesia (Chaillan *et al.*, 2004), Mexico (Riveroll-Larios *et al.*, 2015) and Nigeria (Ijah, 1998).

Evaluation of the natural attenuation and catabolic response of the indigenous microbial community are the two main approaches that have been used for the assessment of the intrinsic catabolic response of microorganism on contaminated soil in tropical regions. The feasibility of using natural attenuation for historically hydrocarbon-contaminated soil has been reported for contaminated sites in Brazil (Schneider *et al.*, 2006; Rizzo *et al.*, 2008b) and Mexico (Díaz-Ramírez *et al.*, 2013). More importantly, these results have allowed to identify the factors limiting the bioremediation process including depletion of nutrients (Díaz-Ramírez *et al.*, 2013), and inhibition of the microbial response under highly contaminated conditions (Rizzo *et al.*, 2008b; Ferreira Do Nascimento *et al.*, 2013). These results agree with the high catabolic response that has been observed from recently (Riveroll-Larios *et al.*, 2015; Morais *et al.*, 2016) and weathered oil-contaminated tropical soils (Trindade *et al.*, 2005; Riveroll-Larios *et al.*, 2015). A detailed review of microorganisms, including bacteria, yeast and fungi that have been isolated and identified from hydrocarbon contaminated tropical soil is presented on Table 2.

2.5.2 Bioaugmentation

In addition to the potential use of indigenous bacteria, the application of additional bacterial strains isolated from different tropical soils has successfully promoted the dissipation of oil-contaminated soil under different conditions. For instance, bacterial strains isolated from tropical rhizospheric soil have successfully been used for the remediation of crude oil (Díaz-Ramírez *et al.*, 2008) and diesel (Ruiz-Marín *et al.*, 2013) contaminated sites in Mexico. The assessment of the feasibility of using bacterial bioaugmentation for the biodegradation of single contaminants has also been tested. For instance, the dissipation of anthracene was studied by Somtrakoon *et al.* (2008) on artificially contaminated tropical soils with results showing a negative correlation between the degradation of the PAH and the organic matter content. Combined approaches using both bioaugmentation and biostimulation studying the biodegradation PAHs are also reported with overall positive results. These studies have reported an enhanced dissipation of PAHs from creosote (de Souza Pohren *et al.*,

2016) and diesel (Chagas-Spinelli *et al.*, 2012), as well as the individual biodegradation of phenanthrene and pyrene (Hwang & Cutright, 2002).

Although bacteria are the microorganisms of choice for the research and implementation of bioremediation protocols, fungi have also been observed to degrade hydrocarbons contained in soil. Moreover, the bioremediation potential of soil inoculated with a mixture of fungi has also been reported for tropical soils, although not always with the expected results. In addition to the enhancement of the dissipation of hydrocarbons from oil (Adenipekun & Fasidi, 2005; Pérez-Armendáriz *et al.*, 2010) and diesel contaminated soil (Maddela *et al.*, 2015a), inoculation of contaminated soil with fungi has also shown to reduce the phytotoxicity of the lower molecular weight fractions (Maddela *et al.*, 2015b). Furthermore, the absence of effects due to fungal inocula have been reported (Beraldo De Morais & Tauk-Tornisielo, 2009). Inoculation with fungi and its interaction with indigenous and inoculated bacteria have also been tested for the dissipation of PAHs. Regarding this, results show that mixtures of PAHs (Clemente & Durrant, 2005) and phenanthrene (Amezcua-Allieri *et al.*, 2003; Chávez-Gómez *et al.*, 2003) can successfully be biodegraded by this approach, though inhibition of the biodegradation process can be observed due to competition for resources between bacteria and fungi (Clemente & Durrant, 2005).

2.5.3 Biostimulation

In terms of soil fertility, even if the soil possesses an adequate amount of available nutrients, the biodegradation of organic contaminants tends to either immobilize or deplete its values (Frick *et al.*, 1999; Obire & Nwaubeta, 2002; Adenipekun & Fasidi, 2005). This has already been observed in tropical soil contaminated with refined forms of hydrocarbon, with consistently lower amounts of available phosphorous and total nitrogen than uncontaminated soil (Obire & Nwaubeta, 2002; Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede, 2007). Additionally, nutrient cycling might also be modified by the presence of specific hydrocarbon-degrading microorganisms (Osuji & Nwoye, 2007). For instance Osuji & Nwoye (2007) observed a depletion of nitrates as a consequence of a reduced nitrification process occurring after a crude oil spillage in Nigeria. To prevent the inhibition of the biodegradation due to this, nutrients can be added to contaminated soil, this practice is usually called biostimulation.

An example of biostimulation in tropical regions is presented by Evans *et al.* (2004), where the authors simultaneously analysed the response of the microbial community from a pristine tropical soil to both biostimulation and oil contamination. Results showed that the greatest shift on the microbial community was observed in the nutrient enhanced contaminated soil (Evans *et al.*, 2004). Evans *et al.* (2004) also reported that the availability of inorganic nutrients was the limiting factor for the biodegradation of contaminants. These findings are supported by other authors who have also observed the beneficial effects of bioremediation protocols that include the incorporation inorganic nutrients (Vasudevan & Rajaram, 2001; Rojas-Avelizapa *et al.*, 2005; Ayotamuno *et al.*, 2006).

In addition to amendment with inorganic nutrients, a newer protocol has incorporated the use of organic amendments typically consisting on waste or a by-product of a profitable industry. This type of soil amendments present certain advantages over the inorganic nutrients on tropical soils as they are considered to represent a significant source of nitrogen, phosphorus and organic matter at a lower cost than commercially available fertilizers (Okolo, 2005; Adesodun & Mbagwu, 2008). Different organic amendments including poultry manure (Okolo, 2005; Adesodun & Mbagwu, 2008) and filter cake (Tellechea *et al.*, 2016) have proven to enhance the bioremediation of hydrocarbon contaminated soil in different tropical regions.

2.5.4 Phytoremediation

Phytoremediation is considered a promising remediation approach within tropical regions due to the characteristic constant humid and warm temperatures of these environments typically promoting plant growth (Merkl *et al.*, 2004a). Similar to other remediation protocols, phytoremediation research and development has been systematically based on temperate climate (Merkl *et al.*, 2004a). As such, a considerable number of the most commonly used plant species might not be capable to properly develop under common tropical environmental conditions such as high temperatures and specific rainfall regimes. Consequently, authors have tested the tolerance of plants against contaminants of different tropical plant species, an extensive review of plant species tested under tropical conditions is presented in Table 3.

The majority of the research has been focused towards the screening and validation of indigenous species (Hernández-Valencia & Mager, 2003; Merkl *et al.*, 2004a; b). Other screening and toxicity assays using tropical plant species have also been reported over the years with overall positive results although not all of them have simultaneously been assessed for dissipation of the targeted hydrocarbon. Studies looking at the phytoremediation potential of tropical plant species have included small legumes (Merkl *et al.*, 2005; Sangabriel *et al.*, 2006) and grasses (Merkl *et al.*, 2005; Zavala-Cruz *et al.*, 2005; Brandt *et al.*, 2006; Sangabriel *et al.*, 2006), as well as trees (Sun *et al.*, 2004; De Farias *et al.*, 2009; Pérez-Hernández *et al.*, 2013) and shrubs (Sun *et al.*, 2004).Overall results from these experiments showed that although some physiological detrimental effects have been observed (Merkl *et al.*, 2005; Zavala-Cruz *et al.*, 2005), phytoremediation protocols using adequate plant species are likely to induce an enhancement on the bioremediation of petroleum hydrocarbon contaminated soil (Sun *et al.*, 2004; Merkl *et al.*, 2005; Zavala-Cruz *et al.*, 2005; Sangabriel *et al.*, 2006; Ayotamuno *et al.*, 2009a; De Farias *et al.*, 2009; Pérez-Hernández *et al.*, 2013).

2.6. Conclusions

Although soil from tropical environments varies considerably, the most important factors controlling the biodegradation of hydrocarbons from tropical soil do not seem to greatly differ to what has already been observed under temperate conditions. This makes possible to conclude that when similar soil characteristics are found, it is possible to extrapolate outcomes of temperate bioremediation of contaminated soil to its tropical counterpart. Nevertheless, in order to do this, special attention needs to be paid to the specific and contrasting characteristics between temperate and tropical regions. Some of these factors can be summarized as: high temperatures, acidic pH, low organic matter content, low fertility, high clay content, high moisture content with a tendency to promote anoxic conditions, and poor aeration.

Based on this review, it was possible to define that the mean temperature used for the study of the biodegradation processes in tropical environments was 28.61 ºC (min 25 ºC, max 37 ºC), while the standard temperature in colder regions is usually 21ºC. Similarly, as pH has an important effect on biological, chemical and physical

processes in the soil, acidic values must also be incorporated into the research protocols. Based on the literature presented in this review, the mean pH of the soil used across the different reports is 5.4 (min 3.56, max 7.47).

It is important to note that the vast majority of research referring to bioremediation and biodegradation of hydrocarbons in soil of tropical regions is focused on crude oil contaminated soil. This feature has produced a general lack of understanding on the fate and behaviour of single contaminants under tropical conditions. In addition, a considerable amount of studies is performed on field contaminated soil, where few conditions can be controlled; this can also reduce the level of understanding of the biodegradation processes which can only be isolated under well-known and controlled laboratory conditions. Therefore, in order to achieve a better understanding of these processes in tropical regions, there is a need of further and more detailed research focusing on single contaminants and under controlled laboratory conditions.

One factor that is not regularly mentioned when analysing the available reports on this subject is the regionalisation of the information. Even though most of the consulted studies are published in English, there is still a considerable number of research articles published in their country of origin language, consequently limiting the access to those. Moreover, while a wide range of scholarly journals are available worldwide; there are still a number of local academic journals containing valuable information but with limited accessibility outside of their country of origin. This specific situation was more obvious when reviewing the available information reported in Venezuela.

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Table 1. Primary classification and characteristics of tropical soils

Characteristic	Humid	Sub-humid	Semi-arid	Arid
Precipitation*	> 9.5 months	4.5 - 9.5 months	2 – 4.5 months	< 2 months
pH	< 5.5	> 5.5	5 to 6	5 to 6
Fertility	Low availability of mineral nutrients	Higher level of mineral nutrients than humid tropics	Low	Low
Texture	Clayey	Clayey	Mostly sandy	Sandy
SOM	High on the surface	High (lower than in humid tropics)	Low	Low
CEC ($cmol_c\ kg^{-1}$)	< 20	> 15	NA	NA
Silt:Clay ratio	< 0.15	> 0.15	NA	NA

Obtained from Juo (2003) and Nortcliff (2010). *Time where total precipitation is higher than the potential evapotranspiration (PET) (Juo & Franzluebbers, 2003). NA = Not available

Table 2. Hydrocarbon degrading microorganisms isolated from tropical soil

Name	Contaminant present in soil	Country	
	Bacteria		
Achromobacter insolitus	Refinery soil	India	Janbandhu & Fulekar (2011)
Acinetobacter sp.	Refinery soil	India	Roldan *et al.* (2010) Chaudhary *et al.* (2015)
	Oil sludge	Colombia	
Acinetobacter anitratus	Crude oil and wood processing waste	Nigeria	Nwanna *et al.* (2006)
Acinetobacter baumannii	Oil sludge	Colombia	Roldan *et al.* (2010)
Acinetobacter mallei	Crude oil and wood processing waste	Nigeria	Nwanna *et al.* (2006)
Aerobacter sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Aeromonas sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Aeromicrobium erythreum	Crude oil	Indonesia	Chaillan *et al.* (2004) Chaillan et al. (2004)
Alcaligenes sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Alcaligenes eutrophus	Crude oil	Nigeria	Ilori & Amund (2000)
Alcaligenes faecalis	Crude oil and wood processing waste	Nigeria	Nwanna *et al.* (2006)
Arthrobacter sp.	Gas oil	Nigeria	Akpor *et al.* (2007)
	Kerosene	Nigeria	Akpor *et al.* (2007)
	Motor oil	Nigeria	Akpor *et al.* (2007)
	Crude oil	Nigeria	Onifade & Abubakar (2007)
Azospirillum brasilense	Crude oil	Mexico	Ojeda-Morales *et al.* (2015)
Azospirillum lipoferum	Crude oil	Mexico	Ojeda-Morales *et al.* (2015)
Bacillus sp.	Refinery soil	India	Chaudhary *et al.* (2015)
	Gas oil	Nigeria	Akpor *et al.* (2007)
	Kerosene	Nigeria	Akpor *et al.* (2007)
	Motor oil	Nigeria	Akpor *et al.* (2007)
	Crude oil	Nigeria	Onifade & Abubakar (2007)
	Oil sludge	Colombia	Roldan *et al.* (2010)
.	Diesel oil	Nigeria	Nwinyi *et al.* (2014)
Bacillus amyloliquefaciens	Crude oil and asphalt	Nigeria	Lateef & Oluwafemi (2014)
Bacillus cereus	Crude oil	Ecuador	Maddela *et al.* (2015a)
Bacillus graminis	Crude oil	Indonesia	Widada *et al.* (2002)
Bacillus marisflavi	Crude oil	Thailand	Supaphol *et al.* (2006)
Bacillus salmalaya	Background contamination in agricultural soil	Malaysia	Dadrasnia *et al.* (2016)
Bacillus subtilis	Crude oil contaminated soil	Nigeria	Ilori & Amund (2000)
	Hydrocarbon and asphalt	Nigeria	Lateef & Oluwafemi (2014)
Bacillus thuringiensis	Crude oil	Ecuador	Maddela *et al.* (2015a)
Brevibacterium sp.	Refinery soil	India	Chaudhary *et al.* (2015)
	Diesel oil	Nigeria	Nwinyi *et al.* (2014)
Burkholderia sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Burkholderia kururiensis	Crude oil	Mexico	Ortega-Gonz??lez *et al.* (2013)
Burkholderia thailandensis	Crude oil	Indonesia	Chaillan *et al.* (2004)
Cellulomonas denverensis	Crude oil	Mexico	Lara-Severino *et al.* (2016)
Cellulomonas hominis	Crude oil	Mexico	Ortega-Gonz??lez *et al.* (2013)
Corynebacterium sp.	Motor oil	Nigeria	Akpor *et al.* (2007)

Dietzia sp.	Crude oil	Indonesia	Chaillan *et al.* (2004)
Dietzia cinnamea	Crude oil	Brazil	von der Weid *et al.* (2007)
Flavobacterium sp.	Gas oil	Nigeria	Akpor *et al.* (2007)
Gordonia alkanivorans	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
Gordonia hydrophobica	Crude oil	Indonesia	Chaillan *et al.* (2004)
Gordonia rubripertincta	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
Gordonia terrae	Crude oil	Indonesia	Chaillan *et al.* (2004)
Klebsiella sp.	Kerosene	Nigeria	Akpor *et al.* (2007)
Kocuria rosea	Crude oil	Mexico	Lara-Severino *et al.* (2016)
Kocuria palustris	Crude oil	Mexico	Lara-Severino *et al.* (2016)
Lactobacter sp.	Crude oil	Nigeria	Onifade & Abubakar, (2007)
Lysinibacillus sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Micrococcus sp.	Gas oil	Nigeria	Akpor *et al.* (2007)
	Kerosene	Nigeria	Akpor *et al.* (2007)
	Crude oil	Nigeria	Onifade & Abubakar, (2007)
	Crude oil	Nigeria	Ayotamuno *et al.* (2009b)
Micrococcus roseus	Crude oil	Nigeria	Ayotamuno *et al.* (2009b)
Microbacterium sp.	Oil from the mechanical engineering industry	Nigeria	Salam *et al.* (2014)
Microbacterium arborescens	Oil sludge	Colombia	Roldan *et al.* (2010)
Microbacterium barkeri	Oil sludge	Colombia	Roldan *et al.* (2010)
Microbacterium oxydans	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
	Crude oil	Thailand	Supaphol *et al.* (2006)
Microbacterium testaceum	Crude oil	Mexico	Lara-Severino *et al.* (2016)
Micrococcus luteus	Crude oil contaminated soil	Nigeria	Ilori & Amund (2000)
Micrococcus varians	Crude oil and wood processing waste	Nigeria	Nwanna *et al.* (2006)
Mycobacterium sp.	Diesel	Nigeria	Nwinyi *et al.* (2014)
Mycobacterium sp.	Crude oil	Indonesia	Chaillan *et al.* (2004)
Nocardia sp.	Crude oil	Nigeria	Ayotamuno *et al.* (2009b)
Nocardia farcinica	Crude oil	Mexico	Lara-Severino *et al.* (2016)
Nocardia veterana	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
Ochrobactrum intermedium	Crude oil	Colombia	Roldan *et al.* (2010)
Proteus sp.	Gas oil	Nigeria	Akpor *et al.* (2007)
	Kerosene	Nigeria	Akpor *et al.* (2007)
	Motor oil	Nigeria	Akpor *et al.* (2007)
Pseudomonas sp.	Refinery soil	India	Chaudhary *et al.* (2015)
	Gas oil	Nigeria	Akpor *et al.* (2007)
	Kerosene	Nigeria	Akpor *et al.* (2007)
	Motor oil	Nigeria	Akpor *et al.* (2007)
	Crude oil	Nigeria	Onifade & Abubakar, (2007), Obayori *et al.* (2008)
	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
	Diesel	Nigeria	Nwinyi *et al.* (2014)
Pseudomonas aeruginosa	Gas oil	Nigeria	Ilori & Amund (2000; Akpor *et al.* (2007); Obayori *et al.* (2008)
	Motor oil	Nigeria	Akpor *et al.* (2007))
	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
	Oil sludge	Colombia	Roldan *et al.* (2010)
	Diesel	Mexico	Ruiz-Marín *et al.* (2013)
Pseudomonas capacia	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
Pseudomonas fluorescens	Oil sludge	Colombia	Roldan *et al.* (2010)

	Diesel	Mexico	Ruiz-Marín *et al.* (2013)
Pseudomonas luteola	Diesel	Mexico	Ruiz-Marín *et al.* (2013)
Pseudomonas oleovorans	Crude oil	Thailand	Supaphol *et al.* (2006)
Pseudomonas panipatensis	Refinery soil	India	Gupta *et al.* (2008)
Ralstonia insidiosa	Crude oil	Mexico	Ortega-González *et al.* (2013)
Ralstonia pickettii	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
Rhodococcus sp.	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
Rhodococcus equi	Crude oil and saline production water	Brazil	Alvarez *et al.* (2008)
Serratia sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Serratia marcescens	Motor oil	Nigeria	Akpor *et al.* (2007)
	Crude oil	Mexico	Ortega-González *et al.* (2013)
Sphingomonas sp.	Refinery soil	India	Chaudhary *et al.* (2015)
Sphingomonas cloacae	Crude oil	Thailand	Widada *et al.* (2002)
Sphingomonas paucimobilis	Diesel	Mexico	Ruiz-Marín *et al.* (2013)
Sphingobacterium sp.	Refinery soil	India	Janbandhu & Fulekar (2011)
Stenotrophomonas sp.	Refinery soil	India	Chaudhary *et al.* (2015)
	Crude oil	Brazil	Alvarez *et al.* (2008)
Stenotrophomonas acidaminiphila	Oil sludge	Colombia	Roldan *et al.* (2010)
Stenotrophomonas maltophilia	Oil sludge	Colombia	Roldan *et al.* (2010)
Websiella sp.	Gas oil	Nigeria	Akpor *et al.* (2007)
	Fungi		
Alternaria tenuis	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
Amorphotheca resinae	Crude oil	Indonesia	Chaillan *et al.* (2004)
Articulosporium inflata	Crude oil	Nigeria	Onifade & Abubakar (2007)
Aspergillus clavatus	Crude oil	Indonesia	Chaillan *et al.* (2004)
Aspergillus flavipes	Crude oil	Indonesia	Oudot *et al.* (1993)
Aspergillus fumigatus	Crude oil	Indonesia	Oudot *et al.* (1993); Chaillan *et al.* (2004)
Aspergillus niger	Crude oil	Indonesia	Chaillan *et al.* (2004)
Aspergillus sydowii	Crude oil	Indonesia	Oudot *et al.* (1993); Chaillan *et al.* (2004)
Aspergillus terreus	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
	Crude oil	Indonesia	Chaillan *et al.* (2004)
Cunninghamella echinulata	Crude oil	Indonesia	Chaillan *et al.* (2004)
Eupenicillium javanicum	Crude oil	Indonesia	Oudot *et al.* (1993)
Eupenicillium ochrosalmoneum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Fusarium decemcellulare	Crude oil	Indonesia	Chaillan *et al.* (2004)
Fusarium oxysporum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Geomyces sp.	Crude oil	Ecuador	Maddela *et al.* (2015a)
Geomyces pannorum	Crude oil	Ecuador	Maddela *et al.* (2015a)
Graphium putredinis	Crude oil	Indonesia	Oudot *et al.* (1993)
	Crude oil	Indonesia	Chaillan *et al.* (2004)
Lentinus subnudus	Crude oil	Nigeria	Adenipekun & Fasidi (2005)
Neosartorya fischeri	Crude oil	Indonesia	Chaillan *et al.* (2004)
Paecilomyces variotii	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium sp.	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
Penicillium citrinum	Crude oil	Indonesia	Oudot *et al.* (1993)
Penicillium janthinellum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium montanense	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium oxalicum	Crude oil	Indonesia	Chaillan *et al.* (2004)

Penicillium pinophilum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium restrictum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium simplicissimum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Penicillium verruculosum	Crude oil	Indonesia	Chaillan *et al.* (2004)
Talaromyces bacillisporus	Crude oil	Indonesia	Oudot *et al.* (1993)
Talaromyces flavus	Crude oil	Indonesia	Chaillan *et al.* (2004)
Talaromyces helicus	Crude oil	Indonesia	Oudot *et al.* (1993)
Trichoderma viride	Crude oil	Mexico	Chávez-Gómez *et al.* (2003)
Zoopage mitospora	Crude oil	Nigeria	Onifade & Abubakar (2007)
	Yeast		
Candida ernobbi	Diesel	Brazil	Miranda *et al.* (2007)
Candida fukuyamaensis	Crude oil	Indonesia	Chaillan *et al.* (2004)
Candida palmioleophila	Crude oil	Indonesia	Chaillan *et al.* (2004)
Candida tropicalis	Crude oil	Thailand	Palittapongarnpim *et al.* (1998)
Candida viswanathii	Crude oil	Indonesia	Chaillan *et al.* (2004)
Candida xestobii	Crude oil	Indonesia	Chaillan *et al.* (2004)
Hansenula sp.	Crude oil	Nigeria	Ayotamuno *et al.* (2009b)
Pichia guilliermondii	Crude oil	Indonesia	Chaillan *et al.* (2004)
Rhodotorula aurantiaca	Diesel	Brazil	Miranda *et al.* (2007)
Yarrowia lipolytica	Crude oil	Indonesia	Chaillan *et al.* (2004)

Table 3. Plant species tested for phytoremediation in tropical soil

Species	Contaminant in soil	Country	Reference
	Cyperaceae family		
Cyperus sp	Crude oil	Mexico	Rivera-Cruz *et al.* (2004)
Cyperus articulates	Crude oil	Mexico	Rivera-Cruz *et al.* (2004)
Cyperus laxus	Crude oil	Mexico	Gallegos Mart??nez *et al.* (2000)
Pycreus polystachyos	Crude oil	Malaysia	Idris *et al.* (2016)
	Fabaceae family		
Calopogonium mucunoides	Crude oil	Venezuela	Merkl *et al.* (2004a, 2005)
Centrosema brasilianum	Crude oil	Venezuela	Merkl *et al.* (2004a, 2005)
Cicer arietinum	Fuel oil	Mexico	Sangabriel *et al.* (2006)
Clitora ternatea	Fuel oil	Mexico	Sangabriel *et al.* (2006)
Desmodium glabrum	Crude oil	Venezuela	Merkl *et al.* (2004a)
Erythrina crista-galli	Crude oil	Brazil	De Farias *et al.* (2009)
Erythrina variegata	Crude oil	Venezuela	Sun *et al.* (2004)
Haematoxylum campechianum	Crude oil	Mexico	Pérez-Hernández *et al.* (2013)
Inga jinicuil	Crude oil	Mexico	Chan-Quijano *et al.* (2012)
Mimosa camporum Benth.	Crude oil	Venezuela	Merkl *et al.* (2004a)
Mimosa orthocarpa Spruce	Crude oil	Venezuela	Merkl *et al.* (2004a)
Phaseolus coccineus	Fuel oil	Mexico	Sangabriel *et al.* (2006)
Prosopis pallida	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
Stylosanthes capitata			Merkl *et al.* (2004a, 2005)
Vigna unguiculata	Crude oil	Nigeria	Jidere *et al.* (2012)
	Malvaceae family		
Guazuma ulmifolia	Crude oil	Mexico	Chan-Quijano *et al.* (2012)
Thespesia populnea	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
	Meliaceae family		
Cedrela odorata	Crude oil	Mexico	Pérez-Hernández *et al.* (2013) Chan-Quijano *et al.* (2012)
Swietenia macrophylla	Crude oil	Mexico	Pérez-Hernández *et al.* (2013)
Swietenia macrophylla	Crude oil	Mexico	Chan-Quijano *et al.* (2012)
	Poaceae family		
Brachiaria brizantha	Crude oil	Venezuela	Hernández-Valencia & Mager (2003)
Brachiaria brizantha	Fuel oil	Mexico	Sangabriel *et al.* (2006)
Brachiaria brizantha	Crude oil	Venezuela	Merkl *et al.* (2004a, 2005)
Brachiaria humidicola	Crude oil	Mexico	Zavala-Cruz *et al.* (2005)
Brachiaria hybrid	Fuel oil	Mexico	Sangabriel *et al.* (2006)
Brachiaria mutica	Crude oil	Mexico	Zavala-Cruz *et al.* (2005)
Brachiaria mutica	Crude oil	Mexico	Rivera-Cruz *et al.* (2004)
Cenchrus ciliaris	Crude oil	Venezuela	Sun *et al.* (2004)
Chloris barbata	Crude oil	Malaysia	Idris *et al.* (2016)
Cyperus aggregatus	Crude oil	Venezuela	Merkl *et al.* (2005)
Echinochloa polystachya	Crude oil	Mexico	Zavala-Cruz *et al.* (2005)
Echinochloa polystachya	Crude oil	Mexico	Rivera-Cruz *et al.* (2004)
Echinochloa polystachya	Crude oil	Mexico	Rivera-Cruz *et al.* (2004)
Eleusine indica	Crude oil	Venezuela	Merkl *et al.* (2005)
Panicum máximus	Crude oil	Venezuela	Hernández-Valencia & Mager (2003)
Panicum maximum	Crude oil	Venezuela	Merkl *et al.* (2004a)
Panicum maximum	Fuel oil	Mexico	Sangabriel *et al.* (2006)

Paspalum scrobiculatum	Crude oil	Malaysia	Idris *et al.* (2016)
Paspalum vaginatum	Crude oil	Malaysia	Idris *et al.* (2016)
Vetiveria zizanioides	Crude oil	Venezuela	Brandt *et al.* (2006)
Zea mays	Crude oil	Nigeria	Jidere *et al.* (2012)
Other families			
Byrsonima crassifolia (Malpighiaceae)	Crude oil	Mexico	Chan-Quijano *et al.* (2012)
Casuarina equisetifolia (Casuarinaceae)	Diesel fuel	United States	Sun *et al.* (2004)
Cordia subcordata (Boraginaceae)	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
Myoporum sandwicense (Scrophulariaceae)	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
Nerium oleander (Apocynaceae)	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
Psidium guajava (Myrtaceae)	Crude oil	Mexico	Chan-Quijano *et al.* (2012)
Scaevola sericea (Goodeniaceae)	Diesel fuel	United States	Jones *et al.* (2004); Sun *et al.* (2004)
Tabebuia rosea (Bignoniaceae)	Crude oil	Mexico	Pérez-Hernández *et al.* (2013), Chan-Quijano *et al.* (2012)

2.7 References

Adams, R.H., Guzmán-Osorio, F.J. & Domínguez-Rodríguez, V.I. 2014. Field-scale evaluation of the chemical-biological stabilization process for the remediation of hydrocarbon-contaminated soil. International Journal of Environmental Science and Technology, 11, 1343–1352.

Adams, R.H., Guzman Osorio, F.J. & Cruz, J.Z. 2008. Water repellency in oil contaminated sandy and clayey soils. International Journal of Environmental Science and Technology, 5, 445–454.

Adams Schroeder, R.H., Dominguez Rodríguez, V.I. & Vinalay Carrillo, L. 2002. Evaluation of microbial respiration and ecotoxicity in contaminated soils representative of the pretroleum-producing region of southeastern Mexico. Terra Latinoamericana, 60, 253–265.

Adenipekun, C.O. & Fasidi, I.O. 2005. Bioremediation of oil-polluted soil by Lentinus subnudus, a Nigerian white-rot fungus. African Journal of Biotechnology, 4, 796–798.

Adesodun, J.K. & Mbagwu, J.S.C. 2008. Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresource Technology, 99, 5659–5665.

Admassu, W. & Korus, R.A. 1996. Engineering of bioremediation processes: needs and limitations. In: Bioremediation (eds. Crawford, R.L. & Crawford, D.L.), pp. 13–34. Cambridge University Press, Cambridge.

Akpor, O.B., Igbinosa, O.E. & Igbinosa, O.O. 2007. Studies on the effect of petroleum hydrocarbon on the microbial and physico-chemicals characteristics of soil. African Journal of Biotechnology, 6, 1939–1943.

Alba, G.I., Cuevas, M.C. & Bustos, E. 2013. Comparing the electroremediation of gleysol soil contaminated with hydrocarbons with triton X-114 washing and bioremediation with solid cultures employing agroindustrial residues. International Journal of Electrochemical Science, 8, 6365–6376.

Alexander, M. 1999. Biodegradation and bioremediation. Academic Press.

Alvarez, V.M., Santos, S.C.C.D.S., Casella, R.D.C., Vital, R.L., Sebastin, G.V. & Seldin, L. 2008. Bioremediation potential of a tropical soil contaminated with a mixture of crude oil and production water. Journal of Microbiology and Biotechnology, 18, 1966–1974.

Amezcua-Allieri, M.A., Lead, J.R., Meléndez-Estrada, J. & Rodríguez-Vázquez, R. 2003. Phenanthrene Removal in a Selected Mexican Soil by the Fungus Penicillium frequentans: Role of C:N Ratio and Water Content. Soil and Sediment Contamination, 12, 387–399.

Arbeli, Z. & Fuentes, C.L. 2010. Microbial degradation of pesticides in tropical soils. In: (ed. Dion, P.), pp. 251–274.

Atlas, R.M.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological reviews, 45, 180–209.

Ayotamuno, J.M., Kogbara, R.B. & Agoro, O.S. 2009a. Biostimulation supplemented with phytoremediation in the reclamation of a petroleum contaminated soil. World Journal of Microbiology and Biotechnology, 25, 1567–1572.

Ayotamuno, M.J., Kogbara, R.B., Ogaji, S.O.T. & Probert, S.D. 2006. Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt, Nigeria. Applied Energy, 83, 1249–1257.

Ayotamuno, J.M., Okparanma, R.N. & Araka, P.P. 2009b. Bioaugmentation and composting of oil-field drill-cuttings containing polycyclic aromatic hydrocarbons (PAHs). Journal of Food, Agriculture and Environment, 7, 658–664.

Bandala, E.R. & Torres, L.G. 2012. Remediation of soils and aquifers in tropical and subtropical regions. Physics and Chemistry of the Earth, 37–39, 1.

Beraldo De Morais, E. & Tauk-Tornisielo, S.M. 2009. Biodegradation of oil fefinery residues using mixed- culture of microorganisms isolated from a landfarming. Brazilian Archives of Biology and Technology, 52652, 1571–1578.

Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C. & Broll, G. 2006. Potential of vetiver (Vetiveria zizanioides (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. International Journal of Phytoremediation, 8, 273–284.

Brooijmans, R.J.W., Pastink, M.I. & Siezen, R.J. 2009. Hydrocarbon-degrading bacteria: The oil-spill clean-up crew. Microbial Biotechnology, 2, 587–594.

Chagas-Spinelli, A.C.O., Kato, M.T., de Lima, E.S. & Gavazza, S. 2012. Bioremediation of a tropical clay soil contaminated with diesel oil. Journal of Environmental Management, 113, 510–516.

Chaillan, F., Le Flèche, A., Bury, E., Phantavong, Y.H., Grimont, P., Saliot, A. & Oudot, J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Research in Microbiology, 155, 587–595.

Chan-Quijano, J.G., Ochoa-Gaona, S., Pérez-hernández, I., Gutiérrez-Aguirre, M.A. & Saragos-Méndez, J. 2012. Germinación y sobrevivencia de especies arbóreas que crecen en suelos contaminados por hidrocarburos Germination and survival of tree species growing in soils contaminated by hydrocarbons. Teoría y Praxis, 12, 102–119.

Chaudhary, P., Sahay, H., Sharma, R., Pandey, A.K., Singh, S.B., Saxena, A.K. & Nain, L. 2015. Identification and analysis of polyaromatic hydrocarbons (PAHs)—biodegrading bacterial strains from refinery soil of India. Environmental Monitoring and Assessment, 187.

Chávez-Gómez, B., Quintero, R., Esparza-García, F., Mesta-Howard, A.M., Zavala Díaz De La Serna, F.J., Hernández-Rodríguez, C.H., Gillén, T., Poggi-Varaldo, H.M., Barrera-Cortés, J. & Rodríguez-Vázquez, R. 2003. Removal of phenanthrene from

soil by co-cultures of bacteria and fungi pregrown on sugarcane bagasse pith. Bioresource Technology, 89, 177–183.

Chikere, C.B., Okpokwasili, G.C. & Chikere, B.O. 2009. Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. African Journal of Biotechnology, 8, 2535–2540, (At: http://www.academicjournals.org/AJB.).

Clemente, A.R. & Durrant, L.R. 2005. Biodegradation of PAHs in soil by two deuteromycete fungi. Contaminated Soils, Sediments and Water, 9, 13–20.

Craswell, E.T. & Lefroy, R.D.B. 2001. The role and function of organic matter in tropical soils. Nutrient Cycling in Agroecosystems, 61, 7–18.

Dadrasnia, A., Usman, M.M., Wei, K.S.C., Velappan, R.D., Jamali, H., Mohebali, N. & Ismail, S. 2016. Native soil bacterial isolate in Malaysia exhibit promising supplements on degrading organic pollutants. Process Safety and Environmental Protection, 100, 264–271.

Díaz-Ramírez, I., Escalante-Espinosa, E., Adams Schroeder, R., Fócill-Monterrubio, R. & Ramírez-Saad, H. 2013. Hydrocarbon Biodegradation Potential of Native and Exogenous Microbial Inocula in Mexican Tropical Soils. In: Biodegradation of Hazardous and Special Products (eds. Chamy, R. & Rosenkrans, F.). InTech.

Díaz-Ramírez, I.J., Escalante-Espinosa, E., Favela-Torres, E., Gutiérrez-Rojas, M. & Ramírez-Saad, H. 2008. Design of bacterial defined mixed cultures for biodegradation of specific crude oil fractions, using population dynamics analysis by DGGE. International Biodeterioration and Biodegradation, 62, 21–30.

Dibble, J.T. & Bartha, R. 1979. Effect of environmental parameters on the biodegradation of oil sludge. Applied and Environmental Microbiology, 37, 729–739.

Evans, F.F., Rosado, A.S., Sebastián, G. V., Casella, R., Machado, P.L.O.A., Holmström, C., Kjelleberg, S., Van Elsas, J.D. & Seldin, L. 2004. Impact of oil contamination and biostimulation on the diversity of indigenous bacterial communities in soil microcosms. FEMS Microbiology Ecology, 49, 295–305.

De Farias, V., Maranho, L.T., De Vasconcelos, E.C., Da Silva Carvalho Filho, M.A., Lacerda, L.G., Azevedo, J.A.M., Pandey, A. & Soccol, C.R. 2009. Phytodegradation potential of Erythrina crista-galli L., Fabaceae, in petroleum-contaminated soil. Applied Biochemistry and Biotechnology, 157, 10–22.

Ferreira Do Nascimento, T.C., Santos Oliveira, F.J. & Pessoa De França, F. 2013. Biorremediación de un suelo tropical contaminado con residuos aceitosos intemperizados. Revista Internacional de Contaminacion Ambiental, 29, 21–28.

Frick, C.M., Germida, J.J., Farrell, R.E. & Germida, J.J. 1999. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Technical Seminar on Chemical Spills, 105a–124a.

Gallegos Martínez, M., Gómez Santos, A., González Cruz, L., Montes De Oca García, M.A., Yúñez Trujillo, L., Zermeño Eguña Lis, J.A. & Gutiérrez-Rojas, M. 2000.

Diagnostic and resulting approaches to restore petroleum-contaminated soil in a Mexican tropical swamp. Water Science and Technology, 42, 377–384.

Greenland, D.J., Wild, A. & Adams, D. 1992. Organic matter dynamics in soils of the tropics - from myth to complex reality. Myths and science of soils in the tropics, 17–33.

Gupta, S.K., Kumari, R., Prakash, O. & Lal, R. 2008. Pseudomonas panipatensis sp. nov., isolated from an oil-contaminated site. International Journal of Systematic and Evolutionary Microbiology, 58, 1339–1345.

Hernández-Espriú, A., Sánchez-León, E., Martínez-Santos, P. & Torres, L.G. 2013. Remediation of a diesel-contaminated soil from a pipeline accidental spill: Enhanced biodegradation and soil washing processes using natural gums and surfactants. Journal of Soils and Sediments, 13, 152–165.

Hernández-Valencia, I. & Mager, D. 2003. Uso de Panicum maximum y Brachiaria brizantha para fitorremediar suelos contaminados con un crudo de petróleo liviano. Bioagro, 15, 149–155.

Hwang, S. & Cutright, T.J. 2002. Biodegradability of aged pyrene and phenanthrene in a natural soil. Chemosphere, 47, 891–899.

Hwang, S., Ramirez, N. & Cutright, T.J. 2003. The role of soil properties in pyrene sorption and desorption. 65–80.

Idris, M., Abdullah, S.R.S., Titah, H.S., Latif, M.T., Abasa, A.R., Husin, A.K., Hanima, R.F. & Ayub, R. 2016. Screening and identification of plants at a petroleum contaminated site in Malaysia for phytoremediation. Journal of Environmental Science and Management, 19, 27–36.

Ijah, U.J.J. 1998. Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. Waste Management, 18, 293–299.

Ilori, M.O.N. & Amund, D.I. 2000. Degradation of anthracene by bacteria isolated from oil polluted tropical soils. Zeitschrift fur Naturforschung - Section C Journal of Biosciences, 55, 890–897.

Janbandhu, A. & Fulekar, M.H. 2011. Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. Journal of Hazardous Materials, 187, 333–340.

Jidere, C.M., Akamigbo, F.O.R. & Ugwuanyi, J.O. 2012. Phytoremediation potentials of cowpea (Vigina unguiculata) and maize (Zea mays) for hydrocarbon degradation in organic and inorganic Manure-Amended tropical typic paleustults. International Journal of Phytoremediation, 14, 362–373.

Jones, R.K., Sun, W.H., Tang, C.-S. & Robert, F.M. 2004. Phytoremediation of petroleum hydrocarbons in tropical coastal soils. II. Microbial response to plant roots and contaminant. Environmental Science and Pollution Research iIternational, 11, 340–346.

Juo, A.S.R. & Franzluebbers, K. 2003. Tropical soils : properties and management for sustainable agriculture. Oxford University Press.

Lara-Severino, R. del C., Camacho-López, M.A., Casanova-González, E., Gómez-Oliván, L.M., Sandoval-Trujillo, Á.H., Isaac-Olivé, K. & Ramírez-Durán, N. 2016. Haloalkalitolerant Actinobacteria with capacity for anthracene degradation isolated from soils close to areas with oil activity in the state of Veracruz, Mexico. International Microbiology, 19, 15–26.

Lateef, B.S. & Oluwafemi, S.O. 2014. Fluorene biodegradation potentials of Bacillus strains isolated from tropical hydrocarbon-contaminated soils. African Journal of Biotechnology, 13, 1554–1559.

Leahy, J.G. & Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews, 54, 305–315.

Maddela, N.R., Masabanda, M. & Leiva-Mora, M. 2015a. Novel diesel-oil-degrading bacteria and fungi from the Ecuadorian Amazon rainforest. Water Science and Technology, 71, 1554–1561.

Maddela, N.R., Scalvenzi, L., Pérez, M., Montero, C. & Gooty, J.M. 2015b. Efficiency of Indigenous Filamentous Fungi for Biodegradation of Petroleum Hydrocarbons in Medium and Soil: Laboratory Study from Ecuador. Bulletin of Environmental Contamination and Toxicology, 95, 385–394.

Margesin, R. & Schinner, F. 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56, 650–663.

Martínez, V.E. & López, F. 2001. Efecto de hidrocarburos en las propiedades físicas y químicas de suelo arcilloso. Terra Latinoamericana.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2004a. Phytoremediation in the tropics-the effect of crude oil on the growth of tropical plants. Bioremediation Journal, 8, 177–184.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2004b. Phytoremediation of petroleum-contaminated soils in the tropics - Pre-selectlon of plant species from eastern Venezuela. Journal of Applied Botany and Food Quality, 78, 185–192.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2005. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water, Air, and Soil Pollution, 165, 195–209.

Miranda, R.D.C., De Souza, C.S., Gomes, E.D.B., Lovaglio, R.B., Lopes, C.E. & Sousa, M.D.F.V.D.Q. 2007. Biodegradation of diesel oil by yeasts isolated from the vicinity of Suape Port in the State of Pernambuco -Brazil. Brazilian Archives of Biology and Technology, 50, 147–152.

Morais, D., Pylro, V., Clark, I.M., Hirsch, P.R. & Tótola, M.R. 2016. Responses of microbial community from tropical pristine coastal soil to crude oil contamination. PeerJ, 4, e1733.

Mulligan, C.N., Yong, R.N. & Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. Engineering Geology, 60, 193–207.

Nortcliff, S. 2010. Soils of the Tropics. pp. 1–15. Springer, Berlin, Heidelberg.

Nwanna, I.E.M., George, G.O. & Olusoji, I.M. 2006. Growth study on chrysene degraders isolated from polycyclic aromatic hydrocarbon polluted soils in Nigeria. African Journal of Biotechnology, 5, 823–828.

Nwinyi, O.C., Kanu, I.A., Tunde, A. & Ajanaku, K.O. 2014. Characterization of diesel degrading bacterial species from contaminated tropical ecosystem. Brazilian Archives of Biology and Technology, 57, 789–796.

Obayori, O.S., Ilori, M.O., Adebusoye, S.A., Oyetibo, G.O. & Amund, O.O. 2008. Pyrene-degradation potentials of Pseudomonas species isolated from polluted tropical soils. World Journal of Microbiology and Biotechnology, 24, 2639–2646.

Obire, O. & Nwaubeta, O. 2002. Effects of refined petroleum hydrocarbon on soil physicochemical and bacteriological characteristics. Journal of Applied Sciences and Environmental Management, 17, 47–67.

Ojeda-Morales, M.E., Domínguez-Domínguez, M., Hernández-Rivera, M.A. & Zavala-Cruz, J. 2015. Biosurfactant production by strains of azospirillum isolated from petroleum-contaminated sites. Water, Air, and Soil Pollution, 226.

Okere, U. V. & Semple, K.T. 2012. Biodegradation of PAHs in “Pristine” soils from different climatic regions. Journal of Bioremediation & Biodegradation, s1, 1–11.

Okolo, J.C. 2005. Enhancing crude oil degradation in a sandy soil: Effects of addition of poultry manure. Global Journal of Environmental Sciences, 4, 47–53.

Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede, T.O. 2007. The Effects of Spent Engine Oil on Soil Properties and Growth of Maize (Zea mays L .) No of seedlings that survived x 100 No of seedlings contaminat ed. Journal of Applied Sciences and Environmental Management, 11, 147–152.

Onifade, A.K. & Abubakar, F.A. 2007. Characterization of hydrocarbon-hegrading microorganisms isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. Research Journal of Microbiology, 2, 149–155, (At: http://www.scialert.net/abstract/?doi=jm.2007.149.155. Accessed: 25/1/2018).

Ortega-González, D.K., Zaragoza, D., Aguirre-Garrido, J., Ram??rez-Saad, H., Hern??ndez-Rodr??guez, C. & Jan-Roblero, J. 2013. Degradation of benzene, toluene, and xylene isomers by a bacterial consortium obtained from rhizosphere soil of Cyperus sp. grown in a petroleum-contaminated area. Folia Microbiologica, 58, 569–577.

Osuji, L.C. & Nwoye, I. 2007. An appraisal of the impact of petroleum hydrocarbons on soil fertility : the Owaza experience. African Journal of Agricultural Research, 2, 318–324.

Osuji, L.C., Udoetok, I.A. & Ogali, R.E. 2006. Attenuation of petroleum hydrocarbons by weathering: A case study. Chemistry and Biodiversity, 3, 422–433.

Oudot, J., Dupont, J., Haloui, S. & Roquebert, M.F. 1993. Biodegradation potential of hydrocarbon-assimilating tropical fungi. Soil Biology and Biochemistry, 25, 1167–1173.

Palittapongarnpim, M., Pokethitiyook, P., Upatham, E.S. & Tangbanluekal, L. 1998. Biodegradation of crude oil by soil microorganisms in the tropic. Biodegradation, 9, 83–90.

Pérez-Armendáriz, B., Martínez-Carrera, D., Calixto-Mosqueda, M., Joel, A. & Rodríguez-Vázquez, R. 2010. Filamentous fungi remove weathered hydrocarbons from polluted soil of tropical México. Revista Internacional de Contaminacion Ambiental, 26, 193–199.

Pérez-Hernández, I., Ochoa-Gaona, S., Adams Schroeder, R.H., Rivera-Cruz, M.C. & Geissen, V. 2013. Tolerance of four tropical tree species to heavy petroleum contamination. Water, Air, and Soil Pollution, 224.

Ramirez, N. & Cutright, T.J. 2001. Sorption-desorption of pyrene for Colombia and New Mexico soils. Polycyclic Aromatic Compounds, 18, 273–292.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Rivera-Cruz, M.D.C., Ferrera-Cerrato, R., Sánchez-García, P., Volke-Haller, V., Fernández-Linares, L. & Rodríguez-Vázquez, R. 2004. Descontaminación de suelos con petróleo crudo mediante microorganismos autóctonos y pasto alemán [echinochloa polystachya (H.B.K.) hitchc.]. Agrociencia, 38, 1–12.

Riveroll-Larios, J., Escalante-Espinosa, E., Fócil-Monterrubio, R.L. & Díaz-Ramírez, I.J. 2015. Biological Activity Assessment in Mexican Tropical Soils with Different Hydrocarbon Contamination Histories. Water, Air, & Soil Pollution, 226, 353.

Rizzo, A.C.L., Da Cunha, C.D., Santos, R.L.C., Santos, R.M., Magalhães, H.M., Leite, S.G.F. & Soriano, A.U. 2008a. Preliminary identification of the bioremediation limiting factors of a clay bearing soil contaminated with crude oil. Journal of the Brazilian Chemical Society, 19, 169–174.

Rizzo, A.C.L., Santos, R.M., Soriano, A.U., Landa, F. & Keite, S.G.F. 2008b. Monitored natural attenuation of tropical petroleum-bearing soils. In: International UFZ-Deltares/TNO Conference on Soil-Water Systems.

Rojas-Avelizapa, N., Olvera-Barrera, E. & Fernández-Linares, L. 2005. Feasibility study of bioremediation of a drilling-waste-polluted soil: Stimulation of microbial activities and hydrocarbon removal. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 40, 2189–2201.

Roldan, F., Maldonado, C., Guevara, C. & Cubillos, A.M. 2010. Natural attenuation of oily sludge used for the maintenance of an unpaved road in Arauca (Colombia). Bioremediation Journal, 14, 81–91.

Ruiz-Marín, A., Zavala-Loria, J.C., Canedo-López, Y. & Cordova-Quiroz, A. V. 2013. Tropical bacteria isolated from oil-contaminated mangrove soil: bioremediation by natural attenuation and bioaugmentation. Revista Mexicana de Ingeniería Química, 12, 553–560.

Salam, L.B., Obayori, O.S. & Olatoye, N.O. 2014. Biodegradation of anthracene by a novel actinomycete, Microbacterium sp. isolated from tropical hydrocarbon-contaminated soil. World Journal of Microbiology and Biotechnology, 30, 335–341.

Sangabriel, W., Ferrera-Cerrato, R., Trejo-Aguilar, D., Mendoza-López, M.R., Cruz-Sánchez, J.S., López-Ortiz, C., Delgadillo-Martínez, J. & Alarcón, A. 2006. Tolerancia y capacidad de fitorremediación de combustóleo en el suelo por seis especies vegetales. Revista Internacional de Contaminacion Ambiental, 22, 63–73.

Schneider, R.P., Morano, S.C., Gigena, M.A.C., Missawa, S.K., Rocha, R.C.S., Da Silva, L.R., Ellert, N., Kataoka, S., Katsuragi, C., Da Silva Rosa, C. & De Oliveira Filho, L.C. 2006. Contamination levels and preliminary assessment of the technical feasibility of employing natural attenuation in 5 priority areas of Presidente Bernardes Refinery in Cubatão, São Paulo, Brazil. Environmental Monitoring and Assessment, 116, 21–52.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Somtrakoon, K., Suanjit, S., Pokethitiyook, P., Kruatrachue, M., Lee, H. & Upatham, S. 2008. Enhanced biodegradation of anthracene in acidic soil by inoculated Burkholderia sp. VUN10013. Current Microbiology, 57, 102–106.

de Souza Pohren, R., de Oliveira Leite, D.A.N., de Franceschi de Angelis, D. & Vargas, V.M.F. 2016. Performance of simulated bioremediation in real samples of soils contaminated with PAHs. Water, Air, and Soil Pollution, 227, 1–14.

Sun, W.H., Lo, J.B., Robert, F.M., Ray, C. & Tang, C.-S. 2004. Phytoremediation of petroleum hydrocarbons in tropical coastal soils. I. Selection of promising woody plants. Environmental Science and Pollution Research International, 11, 260–266.

Supaphol, S., Panichsakpatana, S., Trakulnaleamsai, S., Tungkananuruk, N., Roughjanajirapa, P. & O'Donnell, A.G. 2006. The selection of mixed microbial inocula in environmental biotechnology: Example using petroleum contaminated tropical soils. Journal of Microbiological Methods, 65, 432–441.

Tellechea, F.R.F., Martins, M.A., da Silva, A.A., da Gama-Rodrigues, E.F. & Martins, M.L.L. 2016. Use of sugarcane filter cake and nitrogen, phosphorus and potassium fertilization in the process of bioremediation of soil contaminated with diesel. Environmental Science and Pollution Research, 23, 18027–18033.

Tiessen, H., Cuevas, E. & Chacon, P. 1994. The role of soil organic matter in sustaining soil fertility. Nature, 371, 783–785.

Torres, L.G., Climent, M., Saquelares, J., Bandala, E.R., Urquiza, G. & Iturbe, R. 2007. Characterization and treatability of a contaminated soil from an oil exploration zone. International Journal of Environmental Science and Technology, 4, 311–322.

Trindade, P.V.O., Sobral, L.G., Rizzo, A.C.L., Leite, S.G.F. & Soriano, A.U. 2005. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: A comparison study. Chemosphere, 58, 515–522.

Vasudevan, N. & Rajaram, P. 2001. Bioremediation of oil sludge-contaminated soil. Environment International, 26, 409–411.

von der Weid, I., Marques, J.M., Cunha, C.D., Lippi, R.K., dos Santos, S.C.C., Rosado, A.S., Lins, U. & Seldin, L. 2007. Identification and biodegradation potential of a novel strain of Dietzia cinnamea isolated from a petroleum-contaminated tropical soil. Systematic and Applied Microbiology, 30, 331–339.

Widada, J., Nojiri, H., Kasuga, K., Yoshida, T., Habe, H. & Omori, T. 2002. Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. Applied Microbiology and Biotechnology, 58, 202–209.

Wilcke, W., Amelung, W., Krauss, M., Martius, C., Bandeira, A. & Garcia, M. 2003. Polycyclic aromatic hydrocarbon (PAH) patterns in climatically different ecological zones of Brazil. Organic Geochemistry, 34, 1405–1417.

Wild, S.R. & Jones, K.C. 1995. Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventtory and budget. Environmental Pollution, 88, 91–108.

Yeung, P.Y., Johnson, R.L. & Xu, J.G. 1997. Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. Journal of Environment Quality, 26, 1511.

Zavala-Cruz, J., Gavi-Reyes, F., Adams-Schroeder Randy Howard, Ferrera-Cerrato, R., Palma-López, D.J. & Vaquera-Huerta, Humberto Domínguez-Ezquivel, J.M. 2005. Derrames de petróleo en suelos y adaptación de pastos tropicales en El Activo Cinco Presidentes, Tabasco, México. Terra Latinoamericana, 23, 293–302.

Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, T.M., Miltner, A. & Schroth, G. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma, 79, 117–161.

Chapter 3

Cyclodextrins: Use in the assessment and remediation of contaminated soil – A review

Cyclodextrins: Use in the assessment and remediation of contaminated soil – A review

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3.1 Abstract

Cyclodextrins (CDs) are defined as macrocyclic oligosaccharides with a hydrophobic-non polar internal cavity and a hydrophilic-polar exterior. This unique structure allows CDs to form inclusion complexes with a wide variety of guest molecules, including many hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbons (PAHs), agrochemicals, explosives and chlorinated organic compounds. Their structure has allowed the development of the use of CDs in the assessment and remediation of contaminated soil. The determination of pollutants bioavailability in soil and sediments has been considered as one of the main parameters defining overall remediation potential of contaminated soil. Over the years, CD has proven to offer a reliable prediction of this biodegradable fraction, being hydroxypropyl-β-cyclodextrin (HPCD) the type of CD most commonly used for this purpose. Moreover, CDs have also been used as enhancers for the remediation of contaminated soil under different protocols. Remediation of contaminated soil has also been observed to be improved by CDs due to its solubilizing properties alongside with the formation of inclusion complexes. Through these processes, the enhancement of the bioavailability of certain contaminants has been consistently observed by the addition of CDs. CDs have been shown to be an effective tool for both the assessment and remediation of contaminated land under a variety of circumstances ranging from laboratory single-contaminant spiked soil up to soil from contaminated sites containing complex mixtures of pollutants.

Keywords: Inclusion complexes, cyclodextrin, bioavailability, soil contamination, soil remediation, organic contaminants

3.2 Introduction

Cyclodextrins (CDs) were first discovered in 1891, they can be defined as "crystalline, homogeneous, non-hygroscopic substances" formed from glucopyranose units (Szejtli, 1998; Crini & Morcellet, 2002). The main applications in which CDs are involved include (1) biomedical and pharmaceutical research; (2) food and flavour formulation; (3) cosmetics manufacture; (4) forestry and agricultural industries; (5) adhesive, coating and polymer production; (6) bioconversion and fermentation processes and; (7) textile industries (Table 1). Currently, CDs play a major role in environmental science largely due to their unique ability to form water-soluble inclusion complexes with many hydrophobic organic molecules. For example, different types of CDs are commonly used in water treatment processes to increase the stabilisation, encapsulation and adsorption of contaminants (Crini, 2005). CDs have also been investigated as alternatives to surfactants for increasing contaminant solubility and transport in soils through *ex-situ* soil washing or *in-situ* soil flushing (Mccray & Brusseau, 1999). Furthermore, several studies have shown that CD-inclusion complexes with hydrophobic organic contaminants (HOCs) can enhance the rate of biodegradation of such compounds (Wang *et al.*, 1998; Ramsay *et al.*, 2005; Bernhardt *et al.*, 2013). More recently, CDs have been shown to be a suitable extractant for predicting the biodegradation of organic contaminants in soil (Bekers *et al.*, 1991; Loftsson & Brewster, 1996; Uekama *et al.*, 1998). The structural and chemical characteristics of CDs, as well as industrial uses and applications have been reviewed extensively; consequently only a brief overview of CD chemistry is provided in this review.

The main aims of this review are to summarize the applications of CDs in soil science from an environmental perspective with reference to their use in: (1) prediction of bioavailability of contaminants in soil, and (2) enhancement of soil remediation technologies, in both cases focusing mainly on organic pollutants.

3.3 Cyclodextrin chemistry

3.3.1 Structure

CDs can also be called cycloamyloses, cyclomaltoses, Schardinger dextrins, cycloglucopyranoses, cycloglucans and cyclomaltooligoses (Szejtli, 1998; Crini & Morcellet, 2002). The three most well-known and used types of CDs are: α (cyclohexaamylose, αCD), β (cycloheptaamylose, βCD), and γ cyclodextrin (cyclooctamylose, γCD), which are composed of 6, 7 and 8 α-1,4-glycoside bonds (glucose units), respectively, where the number of glucose units defines the dimension and size of the cavity (Li & Purdy, 1992). These CDs are often referred to as first generation, parent or native CDs given that these were the only ones known during the early stages of CD research and characterization (Szejtli, 1998; Crini & Morcellet, 2002). Table 2 summarizes the major physical and chemical properties of each one of the parent CD molecules.

CDs have the shape of a truncated cone or torus (Table 2) due to a "$^4C_1$ chair conformation" of the glucose units (Li & Purdy, 1992; Szejtli, 1998). Secondary hydroxyl groups (C_2 and C_3) are located on the wider edge or "secondary face"' of the molecule whereas primary hydroxyl groups (C_6) are aligned on the narrower edge or "primary face" (Li & Purdy, 1992; Szejtli, 1998). Moreover, intramolecular hydrogen bonding between C_2 and C_3 hydroxyl groups of adjacent glucose units provides rigidity (Szejtli, 1998). The specific arrangement of C_6 hydroxyl groups opposite the hydrogen-bonded C_2 and C_3 hydroxyl groups forces the oxygen bonds into close proximity within the central cavity leading to an electron rich-hydrophobic interior, meaning that non-bonding electron pairs of the glucose units are directed towards the interior of the cavity producing a high electron density (Szejtli, 1998). As a result, CDs possess a hydrophobic, non-polar interior cavity and a hydrophilic, polar exterior (Table 2). Detailed information on the properties of CDs has been summarised in various reviews (Li & Purdy, 1992; Shieh & Hedges, 1996; Hedges, 1998; Szejtli, 1998; Schneiderman & Stalcup, 2000; Singh *et al.*, 2002)**.**

3.3.2 Production

CDs are naturally formed during the intramolecular bioconversion or biodegradation of starch, which is a biologically inert biopolymer formed by amylopectin and

amylose with just glucose as carbohydrate source (Del Valle, 2004). This conversion occurs via the enzyme 1,4-α-D-glucan 4-α-D-(1,4-α-D-glucano)-transferase (cyclizing), also referred to as CD glucosyltransferase, cyclomaltodextrin glucanotransferase or cyclomaltodextrin glycosyltransferase enzyme (CGTase) (Biwer *et al.*, 2002; Del Valle, 2004; Crini, 2005; Qi & Zimmermann, 2005). CGTase is produced by a number of microorganisms including *Bacillus macerans*, *Klebsiella oxytoca* and *Bacillus circulans* (Biwer *et al.*, 2002; Del Valle, 2004; Crini, 2005; Qi & Zimmermann, 2005). As a consequence of its growing demand for industrial purposes, artificial CDs also started to be commercially synthesised from starch, i.e. CDs produced by the amination or etherification of primary or secondary glucose units (Del Valle, 2004; Leemhuis *et al.*, 2010). In this case, an enzyme produces non-reducing cyclic dextrins from starch, amylase or other polysaccharides by catalysing different transglycosylation steps (Biwer *et al.*, 2002). This process occurs when the CGTase breaks the linear α-glucans structure in between the donor and acceptor's site (also known as cleavage site) to be then transferred to the end of its own fragment to form the CDs characteristic and stable cyclic structure (Leemhuis *et al.*, 2010). CGTases are specialized, and specific strains of bacteria will produce a distinct proportion of CDs. Therefore, different approaches have been used to identify the strain that is needed, as well as the optimal conditions to produce certain types of CDs (Leemhuis *et al.*, 2010). A review of specific strains associated with CD specificity is presented by Leemhuis *et al.* (2010) and Chen *et al.* (2012).

Overall, CDs industrial production is often limited by two factors (Leemhuis *et al.*, 2010): (1) CGTases inhibition, leading to a reduced CD conversion efficiency of about 50%, and (2) CGTase mixed production of α-, β- and γCD, forcing companies to include a purification step. Therefore, the use of alternative approaches has led to the research and development of more "active" enzymes through methods such as directed evolution and site-directed mutagenesis (Leemhuis *et al.*, 2010). Generally, industrial CD production usually involves the use of a complex series of fractionation, filtration and purification via "solvent" or "non-solvent" processes (Biwer *et al.*, 2002). The "solvent" process involves using an organic agent to precipitate a specific type of CD, producing CD with purity above 99 %. On the other hand, "non-solvent" process do not need any complexing agent and produces a mixture of CDs (Qi & Zimmermann, 2005). CDs may also be modified to improve the solubility of these

new CD's derivatives. This process is commonly achieved by substituting various functional groups (i.e. alkyl, hydroxyalkyl, amino or thio, glucosyl, methyl, hydroxyethyl, hydroxypropyl and acetyl) on the primary or secondary face of the molecule. The degree of chemical substitution generally determines the solubility of the CD derivative in aqueous media, which is often different from that of the parent CD, and most chemically modified CDs are able to achieve a 50 % (w/v) concentration in water (Szejtli, 1998). In addition, CD modification also changes the cavity volume, often improving molecular stability (Del Valle, 2004).

3.3.3 Complex formation

The main interest in CDs lies in their ability to form inclusion complexes with a wide range of compounds such as straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, as well as aromatics and polar compounds such as halogens, oxyacids and amines (Del Valle, 2004). The interior cavity of each CD molecule provides a relatively hydrophobic 'microenvironment' into which appropriately sized guest molecules become entrapped resulting in the formation of an inclusion complex (Figure 1). The difference in CD cavity size provides the basis for selectivity (Shieh & Hedges, 1996), being more frequently formed in a CD:guest ratio of 1:1, 1:2, and 2:1 (Szejtli, 1998). Normally, αCD complexes low molecular weight molecules with aliphatic side chains, βCD does it with aromatic and heterocyclic compounds, and γCD can host greater molecular weight complexes than the other two such as macrocycles (Shieh & Hedges, 1996; Szejtli, 1998; Del Valle, 2004). These complexes can be formed either in solution or in crystalline state, where water is typically the solvent of choice (Del Valle, 2004). Overall, it has been observed that the formation of an inclusion complex has many profound effects on the physico-chemical properties of the guest molecule such as solubility enhancement, stabilization against oxidation, visible or UV light and heat, control of volatility and sublimation, physical isolation, chromatographic separation and odour/taste modification (Szejtli, 1998).

A variety of non-covalent forces, such as Van Der Waals, hydrogen bonding, dipole-dipole interaction, London dispersion forces and steric interactions, are responsible for the formation of inclusion complexes, but without the formation of formal chemical bonds (Szejtli, 1998; Del Valle, 2004). The main driving force in the complexation

process involves the release of high energy (enthalpy rich) water molecules which occupy the CD cavity in aqueous solution (Del Valle, 2004). In this process, water molecules are displaced by more hydrophobic guest molecules to attain an apolar-apolar association (Szejtli, 1998; Del Valle, 2004). The inclusion of guest molecules within the host CD is not fixed or permanent but is rather a dynamic equilibrium (Figure 1).The association of the CD and guest molecule, as well the dissociation of the formed CD:guest complex is governed by their thermodynamic equilibrium and is described in detail elsewhere (Del Valle, 2004).

Each year, CDs and their formation of inclusion complexes are the subject of several research articles and scientific abstracts, many of which demonstrate their involvement in various environmentally relevant subject areas. In addition, numerous inventions have been described which involve the use of CDs, and over 1000 patents or patent applications have been made since the beginning of the 21st century (Del Valle, 2004). The unique ability of CDs to form inclusion complexes with a number of guest molecules, namely HOCs, allows them to be utilised as commercial multipurpose technological tools in many aspects of contaminated land assessment and remediation and is discussed further in this review.

3.4 Cyclodextrins for the assessment of contaminated land

Traditionally soil contamination has been assessed by the determination of total concentrations of the targeted pollutant by exhaustive extractions using harsh organic solvents (Kelsey *et al.*, 1997; Cachada *et al.*, 2014). However, such methods have showed little relevance for the assessment of bioavailable or bioaccessible contaminant fractions within soils (Hatzinger & Alexander, 1995; Semple *et al.*, 2004; Cachada *et al.*, 2014). As a consequence, over the last few decades considerable effort has been directed towards developing non-exhaustive chemical techniques for the measurement of putative contaminant bioavailability in the assessment of contaminated land with relevance to risk and remediation (Kelsey *et al.*, 1997).

The use of this type of approach is not new, and different methodologies have been used over the past decades for the prediction of the bioavailable fraction of contaminants in both soil and sediment. These assays typically involve a previous validation of a mild chemical extraction against the measurement of bioavailability

using test organisms e.g. earthworms. Based on these validation assays, multiple authors have found a close positive correlation between these two measurements, especially using *n*-butanol in the case of PAHs (Kelsey *et al.*, 1997; Liste & Alexander, 2002). This approach is considered to be limited due to a variety of factors, including the nature of the extraction, the type of solvent used and their impact on physical and chemical soil properties (Semple *et al.*, 2003). To overcome this, multiple attempts have been made to validate a range of non-exhaustive extraction techniques for the assessment of bioavailable contaminants in soil. These include: (1) solid phase extraction (SPE), (2) Tenax (Cornelissen *et al.*, 1997, 1998a; Cuypers *et al.*, 2002; Liste & Alexander, 2002), (3) XAD resin (Huesemann *et al.*, 2003; Lei *et al.*, 2004), (4) solid phase microextraction (SPME) (Van Der Wal *et al.*, 2004), (5) supercritical fluid extraction (SFE) (Hawthorne & Grabanski, 2000; Hawthorne *et al.*, 2000, 2002), (6) C18 membranes (Tang *et al.*, 2002), (7) persulphate oxidation (Cuypers *et al.*, 2000, 2001), and (8) CD extraction (Reid *et al.*, 2000b; Stokes *et al.*, 2005).

Specifically, the use of CD extractions for the prediction of PAH biodegradation was first studied by Reid *et al.* (1998), who demonstrated that PAH extractability with hydroxypropyl-β-cyclodextrin (HPCD) was closely related to PAH mineralisation by microorganisms. In this case, HPCD was used to solubilise phenanthrene, pyrene and benzo[a]pyrene up to 6.25 times above their respective solubility limits (Reid *et al.*, 1998). In a further study, Reid *et al.*, (1998) investigated the extractability of soil-associated phenanthrene (50 mg kg^{-1}) using an excess of HPCD in aqueous solution overtime. It was observed that the amount of phenanthrene rapidly (20 h) exchanged (via the aqueous phase) into the CD solution provided a good 1:1 correlation with the amount of compound degraded by catabolically active microorganisms. Following this, Reid *et al.* (2000) tested, optimised and validated this method for phenanthrene, benzo[a]pyrene and pyrene using a range of HPCD/contaminant concentrations, extraction times and soil types. Furthermore, linear correlations were calculated between the amounts of mineralised phenanthrene and total residual phenanthrene concentrations assessed by dichloromethane (r^2 = 0.986), *n*-butanol (r^2 = 0.957) and HPCD (r^2 = 0.964) (Reid *et al.*, 2000b). HPCD extraction gave an intercept of 0.162 and a slope of 0.977, indicating good agreement with the amount of mineralisation either at high (minimal ageing) or low (extensive ageing) extractability (Reid *et al.*,

2000b). According to Semple *et al.* (2003), the HPCD extraction technique is the one that more closely mimics the mass transfer mechanisms that govern the bioavailability of non-polar organic contaminants. It thereby provides a more relevant and reliable extraction method for determining the microbial bioavailability of organic contaminants, namely PAHs, in soil. One example of this is the work conducted by Hickman & Reid (2005), who evaluated the ability of an extraction using HPCD to predict earthworm (*Lumbricus rubellus*) accumulation and microbial mineralisation (*Pseudomonas* sp.) of soil-associated phenanthrene in a range of soil types, indicating no relationship between earthworm accumulation and phenanthrene extractability using either HPCD or water. In both cases earthworm accumulation was overestimated, in contrast, HPCD provided a sound prediction of microbial mineralisation in all soils tested (slope = 0.99, r^2 = 0.88), confirming the feasibility of the use of HPCD extractions for the prediction of microbially bioavailable phenanthrene in soil (Hickman & Reid, 2005).

3.4.1 Prediction of bioavailability

Most investigations to date have focussed on the use of HPCD extraction to predict microbial biodegradation of HOCs in laboratory spiked soils. Nevertheless, investigations with HPCD extractions have also shown an ability to predict the biodegradability of PAHs in soil from different locations, including contaminated harbour sediments (Cuypers *et al.*, 2002) and contaminated coke plant soils (Stokes *et al.*, 2005). Similarly, HPCD extractability has been shown to predict the mineralisable fraction of phenanthrene in laboratory spiked soil amended with transformer oil (Doick *et al.*, 2003), cable insulating oil (Dew *et al.*, 2005), combination of PAHs (Doick *et al.*, 2006; Papadopoulos *et al.*, 2007) and diesel (Swindell & Reid, 2007). An overview of recent research regarding the use of CDs for the prediction of bioavailability of organic contaminants in soil and sediments is presented in Table 3. Although the determination of microbial mineralisation and earthworm accumulation are standard procedures for evaluating bioavailability of pollutants, it is not all-encompassing (Semple *et al.*, 2003, 2004) given the specific constrains of each approach. It should therefore be borne in mind that such measurements do not necessarily determine contaminant bioavailability but rather evaluate an interaction endpoint between a specific organism and the contaminants associated with soils or sediments (Semple *et al.*, 2003, 2004). Bernhardt *et al.* (2013) looked at the

correlation between the porous polymer Tenax and HPCD extractions with the degradation of PAHs and petroleum hydrocarbons in soil. In the case of PAHs, the authors found that both procedures showed a good correlation. For petroleum hydrocarbons, it was observed that HPCD extraction presented a higher slope than Tenax extractions (Bernhardt *et al.*, 2013). It was concluded that HPCD extractions could have the potential to be used as tools to predict the extent of hydrocarbon biodegradation (Bernhardt *et al.*, 2013). An early attempt to establish the applicability of CD extraction for the prediction of PAH bioavailability in aged samples from contaminated sites was reported by Cuypers *et al.* (2002). In this study, two sediment samples were sequentially extracted with aqueous solution of HPCD (followed by a solid phase extraction SPE) and the non-ionic surfactant Triton-X; PAH removal during extraction was compared with PAH removal during biodegradation and solid phase extraction (Cuypers *et al.*, 2002). It was observed that HPCD-SPE extraction produced a better prediction of the readily bioavailable fraction of the PAHs (Cuypers *et al.*, 2002). Later, Stokes *et al.* (2005) compared HPCD extractability and microbial mineralisation in aged field-contaminated soils from a former coke plant works containing a broad spectrum of contaminants including PAHs (Figure 2). It was observed that HPCD extraction correlated strongly with biodegradation ($r^2 = 0.99$), suggesting that HPCD mimicked the processes controlling mineralisation and represented a direct measure of microbial availability. Moreover, Johnsen *et al.* (2006) used the HPCD extraction to estimate the bioaccessibility of PAHs at a motorway site. Here, a similar method to that of Cuypers *et al.* (2002) was used whereby soil samples were repeatedly extracted with HPCD solutions obtaining the desorbable quantities of PAHs.

As it can be observed, several attempts have been made to correlate the amount of the contaminant desorbed against its biodegradable fraction. For example, the HPCD extraction of soil PAHs, as a direct estimate of their bioaccessibility, indicated that only 1-5% of the PAHs were accessible to soil bacteria (Johnsen *et al.*, 2006). Similarly, Sabaté *et al.* (2006) calculated the bioavailable fraction for several target PAHs in aged creosote-contaminated soil using CD. It was found that the amount of extractable PAHs corresponded to the desorbing fraction and was presumed as the bioavailable fraction (Sabaté *et al.*, 2006). Khan *et al.* (2012) also evaluated the bioavailability of phenanthrene using different extraction techniques, including HPCD

among other organic solvents such as methanol, *n*-hexane, isopropyl alcohol and a mixture of acetone and dichloromethane. They concluded that the use of HPCD extractions offered a better relation with the bioavailability of phenanthrene of both aged and freshly spiked soil (Khan *et al.*, 2012a). Papadopoulos *et al.* (2007) compared the results from HPCD extractions against indigenous microbial mineralisation of ^{14}C-phenanthrene in the presence of pyrene and benzo(a)pyrene over a range of concentrations and ageing periods. The results showed a 1:1 correlation between the ^{14}C-phenanthrene extracted using HPCD and the mineralised, showing that HPCD extractions can be considered as a reliable tool to predict the bioavailability of the tracer for microbial consumption.

Although HPCD has been the CD of choice for the prediction of bioavailability of organic contaminants, research has also been developed to study the performance of different CDs. Stroud *et al.* (2009) studied the feasibility of using β- and αCD to predict biodegradation of ^{14}C-phenanthrene and ^{14}C-hexadecane (respectively) under single, co-contaminant and multiple contaminant conditions in aged soil over 75 days. Overall results showed that β-CD produced a more reliable prediction of ^{14}C-phenanthrene under single- and multiple contaminant conditions., no significant differences were observed between the mineralized and extracted (Stroud *et al.*, 2009). Different factors mimicking the heterogeneous nature of soil have also been assessed, validating the use of HPCD for the prediction of bioavailable contaminants. For example, the impact of black carbon (BC) on the bioavailability of phenanthrene in soil has been studied by Rhodes *et al.* (2008). For this, soil was amended with different proportions of activated charcoal and bioavailability was assessed through mineralisation at different contact times (Rhodes *et al.*, 2008a). It was observed that relationship between HPCD extractability capacity and mineralisation was affected at high BC proportions (> 0.5 %), while at lower BC percentages (0.1 %) the HPCD was still producing a reliable assessment of bioavailability (r^2 = 0.67, slope = 0.95) (Rhodes *et al.*, 2008a).

CD extractions have also been used in the assessment of herbicides and pesticides, among other organic compounds. Sopeña *et al.* (2012) used HPCD to assess the bioaccessibility of the herbicide isoproturon (IPU) under the influence of biochar using a ^{14}C tracer. Results showed that IPU's bioavailability was effectively predicted by the aqueous mild extraction, even in the presence of BC. On the other hand, Hartnik *et al.*

(2008) assessed the capacity of HPCD extractions to estimate uptake of α-cypermethrin and chlorfenvinphos (insecticides) on *E. fetida*. Authors found that by using a 3.5:1 HPCD:soil ratio, the extractions mimicked worm's uptake. Song *et al.* (2011) studied the feasibility of assessing the bioavailability of aged chlorobenzene contaminated soil using different extractants. To do this, Tenax, HPCD and *n*-butanol were compared against *E. fetida* accumulation. In this case, HPCD did not show to have better results than Tenax or *n*-butanol (Song *et al.*, 2011).

3.4.1.1 Desorption kinetics

Other related subject areas have found extractions with HPCD a useful tool. The sorption - desorption process has been extensively investigated and reviewed (Means *et al.*, 1980; Pignatello & Xing, 1996; Cornelissen *et al.*, 1998b, 2000), being one the of most important processes influencing the fate and behaviour of organic contaminants in soil and sediment. More importantly, these type of studies have also been considered as an alternative for the estimation of the availability of hydrophobic organic contaminants (HOCs) in soil and sediment (Cachada *et al.*, 2014). The desorption of PAHs from soil is generally described in terms of an initial rapid phase followed by a phase of much slower transformation or release (Cornelissen *et al.*, 1998a). Such "biphasic" behaviour has also been observed for biodegradation. Hence, it has been postulated that the rapidly desorbing fraction of PAHs may provide a direct measure of the microbially degradable component of HOC-contamination (Cornelissen *et al.*, 1998a). Consequently, several studies have investigated the similarity between contaminant desorption kinetics, often calculated using a first-order / two or three compartment mathematical model, and microbial degradation (Carmichael *et al.*, 1997; Cornelissen *et al.*, 1998a; b; Doick *et al.*, 2006; Rhodes *et al.*, 2010b). Most of these investigations have utilised a polymeric sorbent such as Tenax for the study of desorption kinetics of PAH. However, a single HPCD extraction has also showed to remove only the rapidly desorbing/degradable fraction (Doick *et al.*, 2006; Sabaté *et al.*, 2006), and may subsequently provide a reasonably good tool for estimating the extent of contaminant bioavailability/bioaccessibility. Rhodes *et al.* (2010) for instance, studied the relationship between desorption kinetics of ^{14}C-phenanthrene using HPCD extractions and the readily degradable/mineralised PAH fraction. Regarding this last aim, the authors found a good linear correlation between phenanthrene mineralisation and the rapidly desorbing fraction (r = 0.89),

concluding that HPCD extractions could represent a tool to estimate mass-transfer limited biodegradation in soil (Rhodes *et al.*, 2010b).

3.5 Remediation applications

Over the last few decades, the use of solubilizing agents to desorb HOC's from soils has been used as an approach for the remediation of contaminated sites. These agents are selected due to its capability of enhancing the partition processes, to be then extracted from the matrix (Cachada *et al.*, 2014). The ability of CDs to enhance the contaminant solubility and its transport has significant implications for the bioaccessibility and biodegradation of such compounds in soil. It is well known that a limited aqueous solubility and large sorption potentials of many HOCs often reduce their chemical and biological availability in soils. Several studies imply that CDs have the potential to enhance the rate and magnitude of biodegradation for a variety of HOCs. CDs have also been studied as an alternative for the enhancement of specific remediation technologies, such as electrokinetic processes or photodegradation, among others. Different types of CD have been proved to be an effective tool for the solubilisation of organic contaminants in soil. An extensive review of research focussing on the use of CD for the enhancement of different soil-remediation approaches is presented in Table 4 and summarized in the following sections.

3.5.1 Cyclodextrin enhanced bioremediation

It is postulated that CDs promote a more rapid catabolism of contaminants in soils by enhancing the mass transfer processes from soil surfaces to microbial cells present in the aqueous phase (Wang *et al.*, 1998). Wang *et al.* (1998) were among the first to investigate the effects of CDs on the biodegradation of PAHs using phenanthrene as a model contaminant, where experiments were performed using analytical and technical grade HPCD at varying concentrations. The results showed that analytical grade HPCD significantly increased the solubility of phenanthrene and subsequently its biodegradation rate (Wang *et al.*, 1998). The authors observed differences between these results of those using technical grade HPCD where biodegradation rate increased to a lesser extent; this was attributed to the presence of a biodegradable impurity which competed with phenanthrene as a carbon source. Bardi *et al.* (2000) also found that βCD (1 % w/v) significantly enhanced the degradative activity of a

natural microbial soil population towards both aliphatic (dodecane and tetracosane) and aromatic (naphthalene and anthracene) hydrocarbons, although the effects on naphthalene and anthracene were more pronounced when present at concentrations higher than their aqueous solubility. More recently, Wang *et al.* (2005) investigated the influence of HPCD on the bioavailability of pyrene from aqueous media. Results indicated that 14% of pyrene was biodegraded in the presence of HPCD, concluding the feasibility of the use its use for the biodegradation of pyrene (Wang *et al.*, 2005).

To date, there are few studies investigating the impact of CDs in the biodegradation of HOCs in soil, with earlier research focusing on the study of different conditions allowing the enhancement of HOCs bioavailability using CDs (recent studies are summarized in Table 4). For example, Reid *et al.* (2004) demonstrated that CDs have the potential to enhance the biodegradation of PAHs in soil. Here, the authors performed a variety of analyses to determine the effect of HPCD on the extractability and biodegradability of phenanthrene. A significant finding of this study was that in soils treated with HPCD, the extent of phenanthrene loss over time was significantly more than in control soils, suggesting that the presence of CD in soils may inhibit or retard the sequestration of phenanthrene by retaining a larger portion of the compound in a more microbially available phase (Reid *et al.*, 2004). In a different approach, Garon *et al.* (2004) assessed the potential of bioaugmentation using a variety of fungal species in combination with the addition of various CDs. This to test biodegradation of fluorene within soil slurries; results showed an enriched biodegradation when maltosyl-cyclodextrin was used. Notably, most of these studies have been restricted to either single or mixed low molecular weight PAHs e.g. fluorene or phenanthrene. In contrast, Allan *et al.* (2007) investigated the effects of CD on the biodegradation of field-aged PAHs and phenols in complex manufactured gas plant (MGP) soil. In this study, the amendment of soils with HPCD (100 g l^{-1}) significantly enhanced the biodegradation of most PAHs and phenolic compounds; such findings were attributed to the abatement of limited PAH bioaccessibility (Allan *et al.*, 2007).

Other types of CD have also been tested although not as extensively as HPCD. For example, Fava *et al.* (1998) concluded that two CDs (HPCD and γCD) were biodegradable additives capable of enhancing the bioavailability and biodegradation of polychlorinated biphenyls (PCBs) in an aged contaminated soil treated under both slurry and fixed phase aerobic conditions. Moreover, Fava *et al.* (2003) also tested the

ability of randomly methylated β-cyclodextrin (RAMEB), a technical grade mixture of methylated CDs, as an enhancer of PCB bioavailability in aged contaminated soil. The authors observed significant increase of PCB's biodegradation, with a positive correlation between the PCB removal and RAMEB concentration (Fava *et al.*, 2003). Fava & Ciccotosto (2002) also applied RAMEB in slurry and solid phase aerobic microcosms to improve the biodegradation of PCBs in three different pristine soils amended with PCB-containing transformer oil and an exogenous biodegrading aerobic co-culture. The results obtained showed that RAMEB strongly enhanced the biological removal of PCBs from the three spiked soils with dose-dependent effects. Molnár *et al.* (2005) also studied the role of RAMEB in the bioremediation of soils contaminated with transformer oil using bench scale reactors and field experiments. Their results supported the findings from other authors with a significantly enhanced bioremediation of the transformer oil-contaminated soils (Molnár *et al.*, 2005). This effect was the result of an increased bioavailability of the pollutants and enhanced catabolic response of the indigenous microorganisms (Molnár *et al.*, 2005).

3.5.2 Cyclodextrin enhanced soil flushing

Soil flushing is a remediation technology that involves the transfer of soil contaminants from solid to aqueous phases. However, the removal of organic contaminants from soil or sediment using water as a flushing agent is relatively ineffective due to their low aqueous solubility. In order to significantly enhance removal efficiency, the addition of solubilising agents in washing solutions has been shown to be an effective and common practice. Previous research has examined the possibility of enhancing the removal of low solubility and highly sorptive compounds through the addition of anionic or non-ionic surfactants, such as rhamnolipids (Zhang *et al.*, 1997), X-100 (Guha *et al.*, 1998; Cuypers *et al.*, 2002) or Tween 80 (Yuan *et al.*, 2006). In a similar way, CDs have been proposed as an alternative to surfactants for the remediation of contaminated land (Wang & Brusseau, 1993, 1995; Brusseau *et al.*, 1994; Wang *et al.*, 1998, 2005; Mccray & Brusseau, 1999).

The application of CDs has some inherent advantages over conventional surfactant-based technologies: (i) Surfactants rely upon critical micelle concentrations, whereas CDs act through the formation of inclusion complexes (Cuypers *et al.*, 2002). As a consequence, *in-situ* remediation may be compromised by surfactant dilution in

ground and surface waters (Brusseau *et al.*, 1994); (ii) Surfactants often possess high affinities for soil and are largely affected by precipitation, emulsion formation or sorption. In contrast, CDs are non-surface reactive, making them more suitable for field application (Wang & Brusseau, 1993; Boving *et al.*, 1999; Ko *et al.*, 1999); (iii) CDs are expected to exhibit relatively stable physical and chemical properties over wide range conditions; (iv) CDs are relatively biodegradable (Fenyvesi *et al.*, 2005), and have been shown to exhibit no reproductive or developmental toxicity (Barrow *et al.*, 1995),resulting on reduced health-related and environmental regulatory concerns.

The prospect of enhancing contaminant solubility through the application of CDs has been demonstrated for a wide range of chemicals, including PAHs, agrochemicals and chlorinated organics (Table 4). For instance, the apparent aqueous solubility of PAHs in aqueous CD solutions has been observed by many authors to increase linearly with the concentration of CD (Wang & Brusseau, 1993; Brusseau *et al.*, 1994; Mccray & Brusseau, 1999). In addition to HPCD, other CDs have been evaluated for potential use as solubilising agents in subsurface remediation applications. For example, carboxymethyl-β-cyclodextrin (CMCD), or a combination of CMCD and HPCD have also been studied to evaluate its feasibility for simultaneously remediate organic contaminants and heavy metals i.e. “mixed wastes” (Wang & Brusseau, 1995; Luthy *et al.*, 1997; Chatain *et al.*, 2004). Wang & Brusseau (1995) found that CMCD simultaneously increased the apparent aqueous solubility of selected organic compounds (anthracene, trichlorobenzene, biphenyl and dichlorodiphenyltrichloroethane (DDT)), and also complexed with cadmium (II) using glass beads as a model sorbent. In a later study, Brusseau *et al.* (1997) investigated the ability of CMCD and HPCD for the simultaneous removal of heavy metals and low polarity organic contaminants from three dissimilar aged contaminated soils using a column elution technique. The authors concluded that ageing had no significant impact on the desorption/elution of either contaminant when using CDs as solubility enhancing agents (Brusseau *et al.*, 1997).

In addition to enhancing the solubilisation of contaminants, CDs have also shown to facilitate the transport of HOCs in soils. For instance, the transport of anthracene, pyrene and 2,4,4-trichlorobiphenyl through soil has been shown to be significantly enhanced by the presence of CDs in column experiments (Brusseau *et al.*, 1994). Morillo *et al.* (2001) reported on the effect of βCD on the adsorption and mobility of

the pesticide 2,4-dichlorophenoxyaxetic acid (2,4-D), also through soil columns. It was found that 2,4-D mobility was enhanced by the presence of β-cyclodextrin; leading to the complete desorption of the herbicide (Morillo *et al.*, 2001). However, the authors stated that, in contrast, βCD tended to adsorb on soil surfaces when it was applied prior to the flushing process, acting as a sequestering agent of 2, 4-D.

Previous research has focussed on the use of CD for the solubilisation of individual compounds often from laboratory-spiked soil. However, evidence also suggests that CDs can enhance the solubility of contaminants contained within "real" field-contaminated soils and sediments. For example, Mccray & Brusseau (1999) conducted an *in-situ* field study successfully demonstrating that elution with HPCD was able to remove significant amounts of multi-component non-aqueous phase liquids (NAPLs) from an aquifer in comparison to water-based elution methods. Viglianti *et al.* (2006) used column extraction experiments to evaluate the ability of three types of CDs (βCD, HPCD and methyl- β –cyclodextrin (MCD)) to extract three PAHs (anthracene, phenanthrene and pyrene) from soil collected at a former manufacturing gas plant. The authors concluded that CD had great potential as a solubility enhancing agent for the extraction of PAHs from aged-contaminated soil (Viglianti *et al.*, 2006). In contrast, Doick *et al.* (2005) observed that the addition of HPCD to soil did not significantly increase the extractability of PAHs (fluoranthene and benzo[a]pyrene) or PCBs (28 and 52) after 36 days. Such findings were attributed to the strong bound residue formation of PAHs with soil during extensive ageing within lysimeters (12 years) prior to experimental work (Doick *et al.*, 2005a).

3.5.3 Cyclodextrin enhanced electrokinetic remediation

Electrokinetic remediation is a technology that has been demonstrated to effectively remove heavy metals and organic contaminants from soils with low or moderate permeability (Acar & Alshawabkeh, 1993; Ko *et al.*, 1999). This technology involves the installation of electrodes in contaminated soil followed by the application of a low DC-voltage gradient (DCVG). When this electric current is applied to soil, pollutants migrate towards the appropriate electrode via electromigration, electroosmosis and electrophoresis (Ko *et al.*, 1999). In order to efficiently utilise electrokinetic processes for soil and sediment remediation, methodologies to increase contaminant mobility may need to be incorporated into electrokinetic operations. (Ko *et al.*, 1999) initiated

research into a new process termed “solubility enhanced electrokinetic remediation” for the removal of HOCs from contaminated-low permeability subsurface systems. This process combines the beneficial effects of enhanced contaminant solubilisation, using appropriate facilitating agents, with the accelerated transport (i.e. electroosmotic flow) gained from electrokinetic processes (Ko *et al.*, 1999). To date, different types of CD have been proven to be an effective tool for the solubilisation of organic contaminants in soil (Table 4), and have previously been included in state of the art reviews by different authors, providing either an overall analysis (Yeung & Gu, 2011; Gomes *et al.*, 2012; Reddy, 2013),

Ko *et al.* (2000) conducted electrokinetic column experiments to study the removal of phenanthrene from kaolin, a model clay soil, using HPCD as a solubility enhancing agent. Electrokinetic columns using HPCD solutions showed greater phenanthrene removal, efficiency of which was found to be positively correlated upon HPCD concentration (Ko *et al.*, 2000). The results obtained from this study showed that an electrokinetic process combined with HPCD flushing and pH buffering may be a good remediation alternative for the removal of HOCs from low permeability subsurface soils. Over the past twenty years, advances in the development of electrokinetic remediation processes have been reached (Table 4). For example, Maturi & Reddy (2006) conducted bench-scale electrokinetic experiments to study the simultaneous removal of organic compounds (phenanthrene) and heavy metals (nickel) from kaolin using HPCD at low (1 %) and high (10 %) concentrations. It was concluded that sustained electroosmotic flow with higher CD concentrations and maintaining low soil pH near the cathode ought to increase the removal efficiency of both phenanthrene and nickel respectively. Similarly, Khodadoust *et al.* (2006) used this same approach to increase the solubility, desorption and removal of 2,4-dinitrotoluene (2,4-DNT) using HPCD solutions (1 % and 2 % w/v) from kaolin and glacial till (silt). Authors observed greater current and electro-osmotic flow when using HPCD flushing solutions, resulting on 94 % removal from kaolin whereas only 20% removal occurred in the glacial till due to the strong retention of 2,4-DNT by soil organic matter (Khodadoust *et al.*, 2006).

CMCD has also been studied for the removal of naphthalene and 2,4-DNT from soil (Jiradecha *et al.*, 2006). Findings from this study again demonstrated that CD efficiently enhanced the removal of naphthalene (83 %) and 2,4-DNT (89 %)

(Jiradecha *et al.*, 2006). The combined use of CMCD and electrokinetics showed improvement in both total removal and rates as compared to CMCD or electrokinetics alone (Jiradecha *et al.*, 2006). Results also showed that the electrical potential applied in order to generate electroosmotic flow was more significant for the enhancement of the remediation process than the concentration of CMCD used; while the application of a higher voltage resulted in an increased removal rate whereas elevated CMCD concentration (> 2 g l^{-1}) appeared to have little effect (Jiradecha *et al.*, 2006). Moreover, the effectiveness of CD when compared against other electrokinetic enhancers has been tested by different authors. For example, Yuan *et al.* (2006) investigated the behaviour of simulated and real hexachlorobenzene (HCB) contaminated soils in an electrokinetic system enhanced by Tween 80 or β-cyclodextrin. It was found that the removal of HCB with βCD was much higher than that with Tween-80 in the electrokinetic system (Yuan *et al.*, 2006). In the same way as it has been observed in the case of research of other remediation technologies, the majority of the aforementioned enhanced-electrokinetic studies were performed using artificial laboratory spiked soils. However, Reddy *et al.* (2006) conducted a series of bench-scale electrokinetic experiments using two different surfactants (5 % Igepal CA-720 and 3 % Tween 80), a co-solvent (20 % n-butylamine) and a CD (10 % HPCD) to assess the extent of removal of PAHs from a manufacturing gas plant soil. PAHs were found to solubilise in the surfactant-HPCD enhanced systems more efficiently even at low concentrations as compared to the co-solvent system, resulting in significant migration towards the cathode (Reddy *et al.*, 2006). Overall, Ipegal CA-720 surfactant yielded the highest PAH removal efficiency; however, no significant removal of heavy metals was observed for any of the flushing solutions tested due to strong adsorption/precipitation under high pH conditions. Another field-contaminated soil experiment was carried by Wan *et al.* (2011), in this case focusing on the removal of TPHs. The authors studied both the removal efficiency as well as soil respiration after the electrokinetic remediation process, concluding that the approach using both CD and specific strains of bacteria represent an effective protocol for the remediation of oil-contaminated soil (Wan *et al.*, 2011). On this basis, the application of CD solutions as facilitating agents in enhanced electrokinetic systems, for the remediation of contaminated soils, may be limited.

3.6 Conclusions

Research suggests that CDs have great potential for use in both chemical and biological remediation-enhancement techniques. The versatility of CDs, and modified CD derivatives has been demonstrated by their involvement in a range of remediation applications. However, their low cost, biocompatibility and effective degradation make βCDs particularly a useful tool for bioremediation processes. Furthermore, recent biotechnological advancements have resulted in dramatic improvements in the efficient manufacture of CDs making highly purified CD and derivatives more commercially available for use in remediation-enhancement techniques.

Overall results show that solubility of a wide variety of contaminants contained in soil can be enhanced by the addition of CDs. Moreover, the type and amount of CD needed will depend on several factors, resulting on the need of trial assays to establish the working parameters prior the use of CDs as a part of a remediation strategy.

Table 1. Applications of CDs within different industries

Industry	Example	Description	References
Biomedical and pharmaceutical	Substrates for soft tissue engineering drug delivery e.g. morphine, ibuprofen, insulin and mitomycine. Regulation of constant bioavailability of opioid peptides Anti-HIV1 (Human Immunodeficiency Virus, type 1) activity	• Solubilisation and stabilisation of compounds • Improvement of absorption/permeability i.e. increase of drug bioavailability • Intermediate drug production	Bekers *et al.* (1991); Eastburn & Tao (1994); Loftsson & Brewster (1996); Uekama *et al.* (1998); Loftsson *et al.* (1999); Bhardwaj *et al.* (2000); Loethen *et al.* (2007)
Food	Removal of bitter components e.g. naringin from grapefruit, theobromine from cocoa Physical and chemical stabilisation of flavours, vitamins, colorants, fats, etc.	• Removal and masking of undesirable components • Conserves expensive flavours • Encapsulates bacteriocides, flavours and others sensitive ingredients • Gelling and thickening agent • Extend product shelf-life	Eastburn & Tao (1994); Hedges (1998); Mar Sojo *et al.* (1999); Szente & Szejtli (2004)
Cosmetic	Dental products e.g. toothpaste Paper towels and tissues Sunscreen Perfumes and air-fresheners	• Increases availability of antimicrobial properties • Suppresses fragrances • Enhances skin penetration of topical formulations	Loftsson *et al.* (1999); Cal & Centkowska (2008)
Analytical Chemistry	Component of HPLC, GC, electrophoresis Reagent in spectrometric analyses, e.g. UV-visible spectrophotometric analysis, fluorescence method	• Ligands in stationary phases • Solution additives (mobile phases) • Separation of isomers • Increases electrons mobilization of guest molecules	Li & Purdy (1992); Schneiderman & Stalcup (2000); Mosinger *et al.* (2001)
Environmental	Manipulation of the germination time of certain grains Water treatment Remediation of contaminated land	• Bioavailability assessment and enhancement • Adsorbents in wastewater treatment • Additives in pesticides • Control of plant growth • Increasing bioavailability of natural antioxidants in crops • Encapsulation and stabilization of insecticides and herbicides	Saenger (1980); Szejtli (1998); Baudin *et al.* (2000); Reid *et al.* (2000); Crini (2005); Lucas-Abellán *et al.* (2008); Bernhardt *et al.* (2013)

Table 2. Characteristics of αCD, βCD and γCD (Saenger, 1980; Crini & Morcellet, 2002)

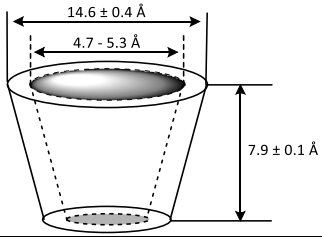
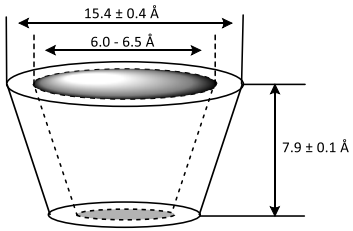
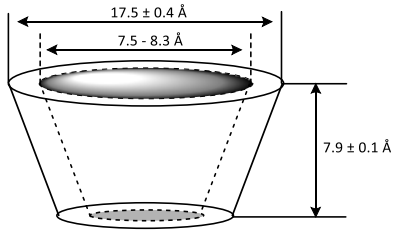
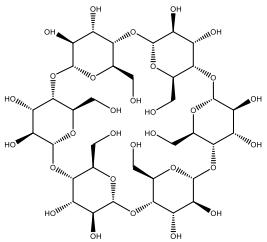
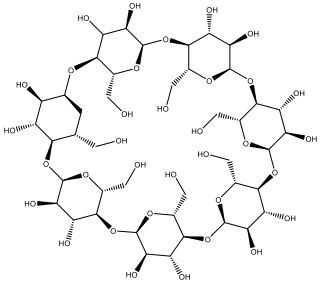
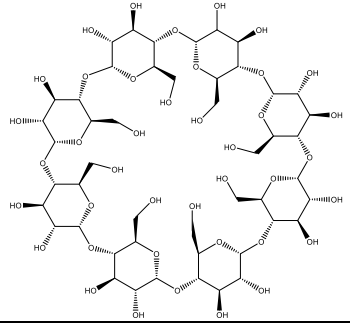
	α - CD	β - CD	γ - CD
Geometric dimensions			
Structural formula			
Volume (Å)	174	262	472
Number of glucose units	6	7	8
Molecular weight (anhydrous)	972	1135	1297
pK_a (25 °C)	12.33	12.20	12.08
Diffusion constant at 40°C	3.443	3.223	3.000
Solubility in water (g 100ml^{-1}) at: 25°C 45°C	 12.8 - 14.5 29.0	 1.8 - 1.85 4.5	 25.6 - 23.2 58.5

Table 3. Recent research regarding the use of CD for the prediction of bioavailability of contaminants in soil (2010 to date)

Contaminant	Type of CD and concentration used	Overall outcome	Reference
PAHs			
Anthracene	HPCD – 60 mM	Anthracene extracted with HPCD did not correlate with the bioavailable fraction	Castro-Silva *et al.* (2015)
Benzo[a]pyrene	HPCD – 60 mM	Under specific conditions, the HPCD non-desorbable fraction can be potentially bioavailable	Duan *et al.* (2015)
Phenanthrene	HPCD – 50 mM	A strong linear relationship was observed between the phenanthrene extracted and degraded in bulk soil and humin fractions, while humic acids produced a higher degradation than the predicted by the HPCD extraction	Gao *et al.* (2014)
		HPCD extractions showed to be a more reliable method for the prediction of phenanthrene bioavailability in soil compared with organic solvents extraction in bot aged and freshly spiked soil	Khan *et al.* (2012)
		^{1}H NMR showed to have a better correlation than HPCD extractable phenanthrene when compared against earthworm toxicity	McKelvie *et al.* (2010)
		HPCD extractions showed to be a feasible tool for the prediction of the extent of phenanthrene bioaccessibility	Ogbonnaya *et al.* (2014)
		HPCD extractions fitted to a first-order three-compartment modelling showed to be an accurate predictor to estimate the biodegradation of phenanthrene in soil limited by mass-transfer processes	Rhodes *et al.* (2010)
		HPCD extractability was significantly affected by the presence of activated charcoal, producing an inadequate prediction of phenanthrene bioavailability	Rhodes *et al.* (2012)
		Extractability of phenanthrene with HPCD is highly influenced by the presence of Al and Cu	Obuekwe & Semple (2013)

Phenanthrene	HPCD – 50 mM	Authors report that the bioavailable fractions of phenanthrene predicted by HPCD extractions of aged soil tend to be overestimated when compared the bioavailability observed using self-dying reporter bacterium	Shin & Nam (2014)
Pyrene	HPCD – 50 mM	Earthworm uptake and HPCD extractability of pyrene under different ageing times presented a high correlation, suggesting the feasibility of the use of HPCD extractions to predict the bioavailability of pyrene in soil	Khan *et al.* (2011)
Fluorene and fluoranthene	HPCD – 10 mM	Given the higher aqueous solubility of fluorene, the use of HPCD to predict its bioavailability does not appear to be viable	Morillo *et al.* (2014)
Phenanthrene and pyrene	HPCD – 70 mM	A 3-step sequential extraction including the use of HPCD offered a reliable method to predict phenanthrene bioavailability in soil, but presenting limitations when used for pyrene	Ma *et al.* (2012)
Phenanthrene, anthracene and pyrene	HPCD – 50 mM	Acidification of soil increases the fraction of PAHs that can be extracted with HPCD	Mahanty & Kim (2016)
Mixture of 6 PAHs	HPCD – 60 mM	PAHs extracted by HPCD did not reflect the amount of hydrocarbons uptake neither by earthworm or ryegrass, showing greater differences for higher molecular weight PAHs	Gomez-Eyles *et al.* (2010)
Mixture of 7 PAHs	HPCD – 50 mM	PAH degradation was not strongly correlated with the bioavailability assessed through HPCD extraction	Crampon *et al.* (2014)
Mixture of 7 PAHs	HPCD – 50 mM MCD CMCD pCMCD	Extractability (used as a measure of bioaccessibility) using different CDs, surfactants and *n*-butanol. Authors suggest that pCMCD can produce more reliable predictions of bioaccessibility than HPCD	Crampon *et al.* (2016)
Mixture of 10 PAHs	HPCD – 70 mM	HPCD was effectively used to represent the bioavailable fractions of PAHs before and after the application of a bioremediation protocol	Gong *et al.* (2015)
Mixture of 14 PAHs	HPCD – 40 mM	HPCD extractions of PAHs from an industrial contaminated soil estimated a low bioavailability, agreeing with a lack of natural biodegradation observed in the site	Mahmoudi *et al.* (2013)

Mixture of 15 PAHs	HPCD – 40 mM	For the majority of the studied PAHs, there was a consistent relationship, suggesting that results from enhanced natural attenuation can be predicted using HPCD extractions	Juhasz *et al.* (2014)
Petroleum hydrocarbon mixture	HPCD – 50 mM	CD did not showed to be a viable predictor of bioavailability of hydrocarbons in sediments. Extractions presented to be concentration-dependant, not reflecting the hydrocarbon uptake pattern exhibited by worms	Muijs & Jonker (2011)
Soil contaminated with diesel, coal ash or coal tar containing a mixture of 16 PAHs	HPCD – 50 mM	Results showed that HPCD extractions did not properly predict the bioavailability of high molecular weight PAHs	Wu *et al.* (2013)
Mixture of PAHs and lindane			
Phenanthrene and lindane	HPCD – 50 mM	HPCD extractability of pyrene was negatively related to the total organic carbon content	Šmídová *et al.* (2012)
Pyrene and lindane	HPCD – 50 mM	HPCD extractions showed to be a viable method for the prediction of microbial accessibility of both pyrene and lindane	Šmídová *et al.* (2012)
Soil from contaminated sites			
Gasworks site soil	HPCD – 60 mM	PAH bioavailable concentrations were measured using CD extractions in the experiment assessing the effects of biochar and earthworm on the bioavailability of PAHs	Gomez-Eyles *et al.* (2011)
Soil from former manufacturing gas plant, wood preservation and tram yard sites containing a mixture of 16 PAHs	HPCD – 40 mM	HPCD predicted and measured biodegradation presented a high correlation	Rostami & Juhasz (2013)
Hydrocarbon contaminated soil from former oil refinery	HPCD – 40 mM	Correlation between microcosm biodegradation assays and HPCD extractions ranged depending on the hydrocarbon fraction showing the need of validation assays for particular hydrocarbons	Dandie *et al.* (2010)

Others			
4-chlorophenol	HPCD – 10 %	A strong correlation between HPCD extractions and and microbial community analyses showed that the HPCD method can be considered as a reliable way to predict 4-chlorophenol bioavailability in soil	Magdolna Nagy *et al.* (2013)
Diuron	HPCD – 50 mM	The amount of diuron extracted with HPCD presented a close correlation when compared with microbial degradation ad different ageing times up to 120 days	Villaverde *et al.* (2013)
Epoxiconazole	HPCD – 50 mM	Concentrations of epoxiconazole extracted with HPCD were found accurately simulate the amount uptake by earthworms	Nélieu *et al.* (2016)
Hexachlorobenzene	HPCD – 50 mM	HPCD extractions exhibited a strong correlation with results from an earthworm bioavailability assay regardless the presence of biochar	Song *et al.* (2012)
Isoproturon	HPCD – 50 mM	Authors observed significant correlations between the isoproturon extracted by HPCD and the amount mineralized by indigenous soil bacteria, suggesting that HPCD extractions can proved an accurate prediction of isoproturon bioavailability either in the presence or absence of black carbon	Sopeña *et al.* (2012)

CMCD = carboxymethyl-β-cyclodextrin, GCD = glycine-β-cyclodextrin, HPCD = hydroxypropyl-β-cyclodextrin, MCD = methyl- β –cyclodextrin, RAMEB = randomly methylated β-cyclodextrin, αCD = α-cyclodextrin. βCD = β-cyclodextrin

Table 4. Research regarding the use of CD for remediation of contaminated soil

Contaminant	Type of CD	Overall results	Reference
CD-enhanced bioremediation			
Phenanthrene	HPCD	HPCD significantly increased the biodegradation of phenanthrene in aged soil (up to 322 days)	Reid *et al.* (2004)
	HPCD and βCD	Biodegradation of phenanthrene in a NAPL-water system had a positive strong correlation with the type (HPCD had a better performance) and concentration (up to 3.6 mM)	Gao *et al.* (2013)
Pyrene	HPCD	HPCD produced a change in the rate of pyrene biodegradation by modifying the hydrophobicity and emulsifying activity of the microorganisms used	Zhang *et al.* (2012)
Naphtalene	HPCD	HPCD (10 g L^{-1}) produced an enhancement of naphthalene degradation under anaerobic=nitrate reducing conditions	Dou *et al.* (2011
Fluorene and pyrene	HPCD	Dissipation of fluorene was not increased by the addition of HPCD. Contrastingly, pyrene CD was observed to significantly increase the biodegradation rate of pyrene	Madrid *et al.* (2016)
Mixture of PAHs	MCD	Results from an MCD (10% w/w) bioaugmentation assay showed that biodegradation of total PAHs was significantly enhanced by the use of MCD.	Sun *et al.* (2012)
	MCD	MCD (10% w/w) increased the biodegradation of PAHs up to 20% when compared against the control	Sun *et al.* (2013)
Former military site contaminated with diesel and engine oil	RAMEB	RAMEB was able to mobilize a significantly higher fraction of the contaminants than the control. Authors also observed a higher removal of humic substances	Fenyvesi *et al.* (2010)
Diuron	HPCD	Diuron biodegradation from soil was improved (up to 22%) by the addition of HPCD	Villaverde *et al.* (2013a)
	HPCD	Biodegradation of durion from soil using HPCD (50 mg kg^{-1}) and a two-species bacterial consortium showed to have a removal above 80%, suggesting the feasibility of the approach to treat durion contaminated soil	Villaverde *et al.* (2012)
n-hexadecane	αCD and βCD	β –cyclodextrin (2.5 mM) showed the higher biodegradation of n-hexadecane than both control and α-cyclodextrin treatment	Sivaraman *et al.* (2010)

PCBs	RAMEB andβCD	β-cyclodextrin (1% w/w) used as a surfactant to remove PCBs from soil showed to produce an enhanced removal of the contaminant on a phytoremediation assay	Chen *et al.* (2010)
CD-enhanced solubilisation			
Phenanthrene and naphthalene	HPCD and βCD	PAHs solubility was significantly enhanced by HPCD (50 g L^{-1}). HPCD also showed a higher removal capacity than βCD	Badr *et al.* (2004)
	HPCD	Although HPCD produced similar results than conventional surfactants (e.g. Tuween 80), HPCD showed to be more effective in the removal of sorbed contaminants sue to its negligible sorption to the solid phase	Ko *et al.* (1999)
Mixture of HOCs (including PAHs)	HPCD	HPCD significantly increased the solubilisation and transport properties of all HOCs during a soil flushing process	Brusseau *et al.* (1994)
Soil from a coking plant contaminated with PAHs and metals	MCD	MCD was used as an initial stage of a clean-up strategy to enhance ex situ soil washing of coking plant soil. MCD (100 g L-1) showed to enhance the extraction of PAHs and metals	Sun *et al.* (2013)
Metals	CMCD, HPCD, MCD and βCD	Extraction of arsenic, copper and iron from mining soil using CDs was only enhanced by the use of CMCD (10 mM)	Chatain *et al.* (2004)
TeCP	CMCD, HPCD, MCD and βCD	Solubilisation of 2,3,4,6 tetrachlorophenol from soil was significantly increased by the use of CMCD (10 mM)	Chatain *et al.* (2004)
Norflurazon	αCD and βCD	Both αCD and βCD increased the mobilisation of sorbed norflurazon from soil	Villaverde *et al.* (2005)
	βCD	βCD (0.01 M) increased the desorption of norflurazon. The efficiency of βCD was observed to be negatively correlated with the norflurazon-soil contact time	Villaverde (2007)
RDX	HPCD and MCD	Extraction of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine from spiked soil was enhanced up to 2.4 times than the amounts recovered by water	Hawari *et al.* (1996)
PCP	HPCD	Use of HPCD (5 mmol L-1) increased the aqueous concentration of PCP in soil extract	Hanna *et al.* (2005)
CD-enhanced electrokinetic remediation			
Mixture of PAHs	HPCD	Efficiency of HPCD as a flushing agent to enhance electrokinetic remediation of sediments was compared against Nonidet P40, Poloxamer 407 and Tween 80. HPCD resented lower removal efficiency than Nonidet P40 and Poloxamer 407	Hahladakis *et al.* (2013)

Phenanthrene and HCB	HPCD	Both contaminants showed to be removed by the combined HPCD/ultrasonication protocol. Moreover, phenanthrene presented higher a higher desorption efficiency than hexachlorobenzene (HCB)	Pham *et al.* (2010)
HCB	βCD	Authors studied the electrokinetic-Fenton remediation process in soil contaminated with hexachlorobenzene (HCB) showed to be an effective flushing solution, increasing the efficiency of the process	Oonnittan *et al.* (2009)
	βCD	Electrokinetic-Fenton remediation was applied for the removal of hexachlorobenzene from kaolin. CD was observed to reduce the mobilisation of the contaminant. βCD used to enhance the solubility of HCB	Oonnittan *et al.* (2009)
	MCD	A higher mobilisation of hexachlorobenzene (HCB) was observed as MCD concentrations were higher. Authors concluded that MCD could be considered a suitable solubilising agent for long-term field applications	Wan *et al.* (2009)
	HPCD	Sediment from a contaminated site containing hexachlorobenzene (HCB) and zinc was treated at a pilot scale. Although mobilisation of contaminants was observed, removal of both HCB and zinc was considered to be negligible.	Li *et al.* (2009)
	HPCD	Authors tested the effect of HPCD (5 and 20%, w/v) on desorption and electrokinetic remediation of hexachlorobenzene (HCB) and heavy metals. Results showed that the mobilisation of HCB was significantly enhanced at the highest HPCD concentration	Li *et al.* (2010)
Atrazine	GCD	Authors studied the feasibility of using GCD in the electrokinetic remediation of atrazine contaminated soil. Addition of GCD (10 g L-1) into the flushing solution was found to provide a significant enhancement of removal efficiency	Wang *et al.* (2012)
Mixture of 6 heavy metals	HPCD	Efficiency of HPCD as a flushing agent to enhance electrokinetic remediation of sediments was compared against Nonidet P40, Poloxamer 407 and Tween 80. Non-enhanced treatment was the only one presenting removal capacity for all metals	Hahladakis *et al.* (2013)
Chlorobenzenes	βCD	The use of βCD showed to inhibit the removal efficiency of a variety of chlorobenzenes including TeCB, i-TeCB, and TCB due to a lower solubility of the βCD-chlorobenzene inclusion complex than chlorobenzenes alone	Yuan *et al.* (2006)

CMCD = carboxymethyl-β-cyclodextrin, GCD = glycine-β-cyclodextrin, HPCD = hydroxypropyl-β-cyclodextrin, MCD = methyl-β-cyclodextrin, RAMEB = randomly methylated β-cyclodextrin, αCD = α-cyclodextrin. βCD = β-cyclodextrin

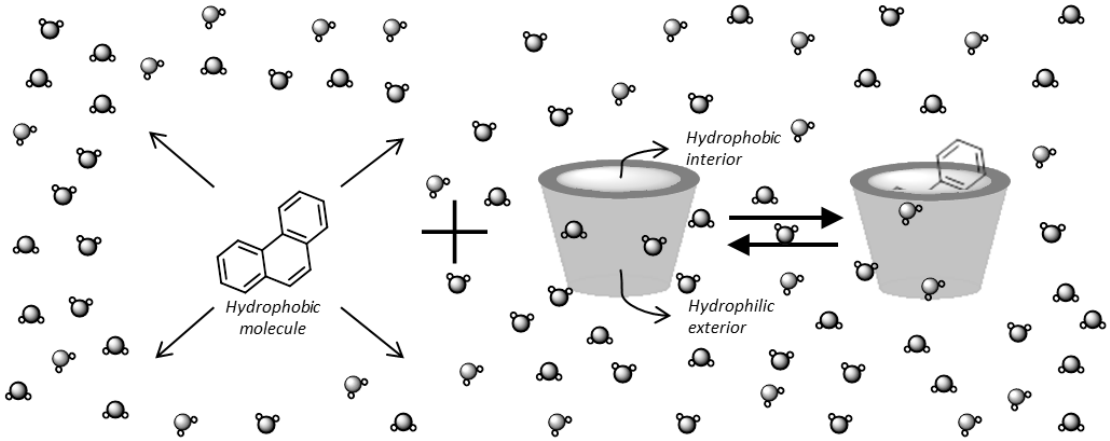


Figure 1. Schematic representation of CD inclusion complex formation; the small circles represent water molecules. Adapted from Szejtli (1998).

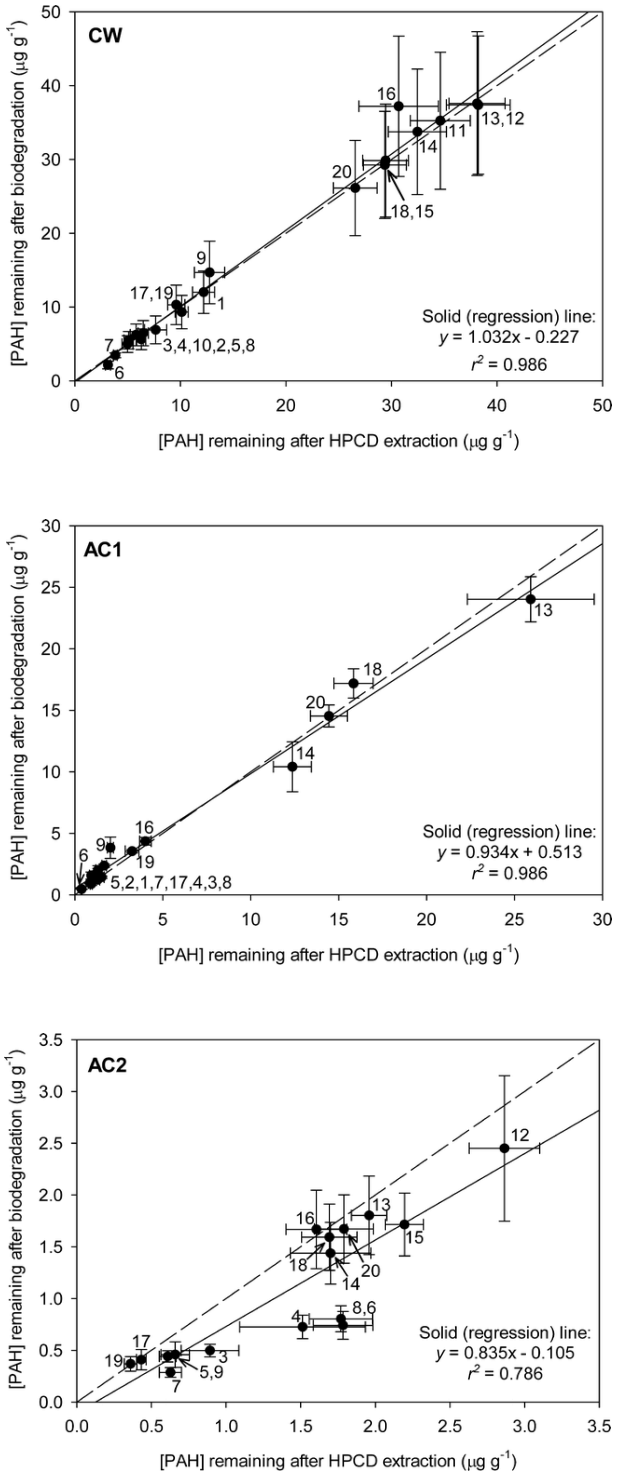


Figure 2. Correlation between the concentration of polycyclic aromatic hydrocarbons (PAHs) remaining in the coke works (CW) soil and in artificially contaminated soils (AC 1 - 2) after extraction with HPCD and after six weeks of biodegradation using results from 16 PAHs. Taken from Stokes *et al.* (2005).

3.7 References

Acar, Y.B. & Alshawabkeh, A.N. 1993. Principles of electrokinetic remediation. Environmental Science & Technology, 27, 2638–2647.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2007. Cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and phenols in contaminated soil slurries. Environmental Science & Technology, 41, 5498–5504.

Badr, T., Hanna, K. & De Brauer, C. 2004. Enhanced solubilization and removal of naphthalene and phenanthrene by cyclodextrins from two contaminated soils. Journal of Hazardous Materials, 112, 215–223.

Bardi, L., Mattei, A. & Steffan, S. 2000. Hydrocarbon degradation by a soil microbial population with ??-cyclodextrin as surfactant to enhance bioavailability. Enzyme and Microbial Technology, 27, 709–713.

Barrow, P.C., Olivier, P. & Marzin, D. 1995. The reproductive and developmental toxicity profile of beta-cyclodextrin in rodents. Reproductive Toxicology, 9, 389–398.

Baudin, C., Pean, C., Perly, B. & Gosselin, P. 2000. Inclusion of organic pollutants in cyclodextrins and derivatives. International Journal of Environmental Analytical Chemistry, 77, 233–242.

Bekers, O., Beijnen, J.H., Tank, M.J.T.K., Bult, A. & Underberg, W.J.M. 1991. Effect of cyclodextrins on the chemical stability of mitomycins in alkaline solution. Journal of Pharmaceutical and Biomedical Analysis, 9, 1055–1060.

Bernhardt, C., Derz, K., Kördel, W. & Terytze, K. 2013. Applicability of non-exhaustive extraction procedures with Tenax and HPCD. Journal of Hazardous Materials, 261, 711–717.

Bhardwaj, R., Dorr, R.T. & Blanchard, J. 2000. Approaches to reducing toxicity of parenteral anticancer drug formulations using cyclodextrins. PDA Journal of Pharmaceutical Science and Technology, 54, 233–239.

Biwer, A., Antranikian, G. & Heinzle, E. 2002. Enzymatic production of cyclodextrins. Applied Microbiology and Biotechnology, 59, 609–617.

Boving, T.B., Wang, X. & Brusseau, M.L. 1999. Cyclodextrin-enhanced solubilization and removal of residual-phase chlorinated solvents from porous media. Environmental Science & Technology, 33, 764–770.

Brusseau, M.L., Wang, X. & Hu, Q. 1994. Enhanced transport of low-polarity organic compounds through soil by cyclodextrin. Environmental Science & Technology, 28, 952–956.

Brusseau, M.L., Wang, X. & Wang, W.-Z. 1997. Simultaneous elution of heavy metals and organic compounds from soil by cyclodextrin. Environmental Science & Technology, 31, 1087–1092.

Cachada, A., Pereira, R., da Silva, E.F. & Duarte, A.C. 2014. The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges. Science of the Total Environment, 472, 463–480.

Cal, K. & Centkowska, K. 2008. Use of cyclodextrins in topical formulations: Practical aspects. European Journal of Pharmaceutics and Biopharmaceutics, 68, 467–478.

Carmichael, L.M., Christman, R.F. & Pfaender, F.K. 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. Environmental Science & Technology, 31, 126–132.

Castro-Silva, C., Ruiz-Valdiviezo, V.M., Rivas-Rivera, S.G., Sosa-Trinidad, A.R., Luna-Guido, M., Delgado-Balbuena, L., Marsch, R. & Dendooven, L. 2015. Bioavailability and dissipation of anthracene from soil with different alkalinity and salinity. Journal of Environmental Biology, 36, 229–234.

Chatain, V., Hanna, K., De Brauer, C., Bayard, R. & Germain, P. 2004. Enhanced solubilization of arsenic and 2,3,4,6 tetrachlorophenol from soils by a cyclodextrin derivative. Chemosphere, 57, 197–206.

Chen, Y., Tang, X., Cheema, S.A., Liu, W. & Shen, C. 2010. β-cyclodextrin enhanced phytoremediation of aged PCBs-contaminated soil from e-waste recycling area. Journal of Environmental Monitoring, 12, 1482.

Chen, B., Yuan, M. & Qian, L. 2012. Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. Journal of Soils and Sediments, 12, 1350–1359.

Cornelissen, G., Van Noort, P.C.M. & Covers, H.A.J. 1998a. Mechanism of slow desorption of organic compounds from sediments: A study using model sorbents. Environmental Science and Technology, 32, 3124–3131.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M.A. & Van Noort, P.C.M. 1998b. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environmental Science and Technology, 32, 966–970.

Cornelissen, G., Rigterink, H., Van Noort, P.C.M. & Govers, H.A.J. 2000. Slowly and very slowly desorbing organic compounds in sediments exhibit Langmuir-type sorption. Environmental Toxicology and Chemistry, 19, 1532–1539.

Cornelissen, G., vanNoort, P.C.M. & Govers, H. a J. 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with Tenax(R) and effects of contact time and solute hydrophobicity. Environmental Toxicology And Chemistry, 16, 1351–1357.

Crampon, M., Bodilis, J., Le Derf, F. & Portet-Koltalo, F. 2016. Alternative techniques to HPCD to evaluate the bioaccessible fraction of soil-associated PAHs and correlation to biodegradation efficiency. Journal of Hazardous Materials, 314, 220–229.

Crampon, M., Bureau, F., Akpa-Vinceslas, M., Bodilis, J., Machour, N., Le Derf, F. & Portet-Koltalo, F. 2014. Correlations between PAH bioavailability, degrading bacteria, and soil characteristics during PAH biodegradation in five diffusely contaminated dissimilar soils. Environmental Science and Pollution Research, 21, 8133–8145.

Crini, G. 2005. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Progress in Polymer Science (Oxford), 30, 38–70.

Crini, G. & Morcellet, M. 2002. Synthesis and applications of adsorbents containing cyclodextrins. Journal of Separation Science, 25, 789–813.

Cuypers, C., Clemens, R., Grotenhuis, T. & Rulkens, W. 2001. Prediction of petroleum hydrocarbon bioavailability in contaminated soils and sediments. Soil and Sediment Contamination, 10, 459–482.

Cuypers, H., Grotenhuis, T., Joziasse, J. & Rulkens, W. 2000. Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments. Environmental Science and Technology, 34, 2057–2063.

Cuypers, C., Pancras, T., Grotenhuis, T. & Rulkens, W. 2002. The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere, 46, 1235–1245.

Dandie, C.E., Weber, J., Aleer, S., Adetutu, E.M., Ball, A.S. & Juhasz, A.L. 2010. Assessment of five bioaccessibility assays for predicting the efficacy of petroleum hydrocarbon biodegradation in aged contaminated soils. Chemosphere, 81, 1061–1068.

Dew, N.M., Paton, G.I. & Semple, K.T. 2005. Prediction of [3-14C]phenyldodecane biodegradation in cable insulating oil-spiked soil using selected extraction techniques. Environmental Pollution, 138, 316–323.

Doick, K.J., Burauel, P., Jones, K.C. & Semple, K.T. 2005. Effect of cyclodextrin and transformer oil amendments on the chemical extractability of aged [14C]polychlorinated biphenyl and [14C]polycyclic aromatic hydrocarbon residues in soil. Environmental Toxicology and Chemistry, 24, 2138–2144.

Doick, K.J., Clasper, P.J., Urmann, K. & Semple, K.T. 2006. Further validation of the HPCD-technique for the evaluation of PAH microbial availability in soil. Environmental Pollution, 144, 345–354.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Dou, J., Li, S., Cheng, L., Ding, A., Liu, X. & Yun, Y. 2011. The enhancement of naphthalene degradation in soil by hydroxypropyl-β- cyclodextrin. In: Procedia Environmental Sciences, pp. 26–31.

Duan, L., Naidu, R., Liu, Y., Palanisami, T., Dong, Z., Mallavarapu, M. & Semple, K.T. 2015. Effect of ageing on benzo[a]pyrene extractability in contrasting soils. Journal of Hazardous Materials, 296, 175–184.

Eastburn, S.D. & Tao, B.Y. 1994. Applications of modified cyclodextrins. Biotechnology Advances, 12, 325–339.

Fava, F., Bertin, L., Fedi, S. & Zannoni, D. 2003. Methyl-β-cyclodextrin-enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils. Biotechnology and Bioengineering, 81, 381–390.

Fava, F. & Ciccotosto, V.F. 2002. Effects of randomly methylated-β-cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with a transformer oil. Applied Microbiology and Biotechnology, 58, 393–399.

Fava, F., Di Gioia, D. & Marchetti, L. 1998. Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Biotechnology and Bioengineering, 58, 345–355.

Fenyvesi, É., Csabai, K., Molnár, M., Leitgi, L. & Gruiz, K. 2010. Cyclodextrins for the enhancement of soil remediation technologies. In: Construction for a Sustainable Environment - Proceedings of the International Conference of Construction for a Sustainable Environment.

Fenyvesi, E., Gruiz, K., Verstichel, S., De Wilde, B., Leitgib, L., Csabai, K. & Szaniszlo, N. 2005. Biodegradation of cyclodextrins in soil. Chemosphere, 60, 1001–1008.

Gao, H., Ma, J., Xu, L. & Jia, L. 2014. Hydroxypropyl-β-cyclodextrin extractability and bioavailability of phenanthrene in humin and humic acid fractions from different soils and sediments. Environmental Science and Pollution Research, 21, 8620–8630.

Gao, H., Xu, L., Cao, Y., Ma, J. & Jia, L. 2013. Effects of hydroxypropyl-β-cyclodextrin and β-cyclodextrin on the distribution and biodegradation of phenanthrene in NAPL-water system. International Biodeterioration and Biodegradation, 83, 105–111.

Garon, D., Sage, L., Wouessidjewe, D. & Seigle-Murandi, F. 2004. Enhanced degradation of fluorene in soil slurry by Absidia cylindrospora and maltosyl-cyclodextrin. Chemosphere, 56, 159–166.

Gomes, H.I., Dias-Ferreira, C. & Ribeiro, A.B. 2012. Electrokinetic remediation of organochlorines in soil: Enhancement techniques and integration with other remediation technologies. Chemosphere, 87, 1077–1090.

Gomez-Eyles, J.L., Collins, C.D. & Hodson, M.E. 2010. Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability. Environmental Pollution, 158, 278–284.

Gomez-Eyles, J.L., Sizmur, T., Collins, C.D. & Hodson, M.E. 2011. Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environmental Pollution, 159, 616–622.

Gong, X., Xu, X., Gong, Z., Li, X., Jia, C., Guo, M. & Li, H. 2015. Remediation of PAH-contaminated soil at a gas manufacturing plant by a combined two-phase partition system washing and microbial degradation process. Environmental Science and Pollution Research, 22, 12001–12010.

Guha, S., Jaffé, P.R. & Peters, C.A. 1998. Solubilization of PAH mixtures by a nonionic surfactant. Environmental Science & Technology, 32, 930–935.

Hahladakis, J.N., Calmano, W. & Gidarakos, E. 2013. Use and comparison of the non-ionic surfactants Poloxamer 407 and Nonidet P40 with HP-β-CD cyclodextrin, for the enhanced electroremediation of real contaminated sediments from PAHs. Separation and Purification Technology, 113, 104–113.

Hanna, K., Chiron, S. & Oturan, M.A. 2005. Coupling enhanced water solubilization with cyclodextrin to indirect electrochemical treatment for pentachlorophenol contaminated soil remediation. Water Research, 39, 2763–2773.

Hartnik, T., Jensen, J. & Hermens, J.L.M. 2008. Nonexhaustive β-cyclodextrin extraction as a chemical tool to estimate bioavailability of hydrophobic pesticides for earthworms. Environmental Science & Technology, 42, 8419–8425.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

Hawari, J., Paquet, L., Zhou, E., Halasz, A. & Zilber, B. 1996. Enhanced recovery of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from soil: Cyclodextrin versus anionic surfactants. Chemosphere, 32, 1929–1936.

Hawthorne, S.B. & Grabanski, C.B. 2000. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. Environmental Science & Technology, 34, 4103–4110.

Hawthorne, S.B., Grabanski, C.B., Martin, E. & Miller, D.J. 2000. Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: Recovery, selectivity and effects on sample matrix. Journal of Chromatography A, 892, 421–433.

Hawthorne, S.B., Poppendieck, D.G., Grabanski, C.B. & Loehr, R.C. 2002. 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. Environmental Science & Technology, 36, 4795–4803.

Hedges, A.R. 1998. Industrial applications of cyclodextrins. Chemical Reviews, 98, 2035–2044.

Hickman, Z.A. & Reid, B.J. 2005. Towards a more appropriate water based extraction for the assessment of organic contaminant availability. Environmental Pollution, 138, 299–306.

Huesemann, M.H., Hausmann, T.S. & Fortman, T.J. 2003. Assessment of bioavailability limitations during slurry biodegradation of petroleum hydrocarbons in aged soils. Environmental Toxicology and Chemistry / SETAC, 22, 2853–2860.

Jiradecha, C., Urgun-Demirtas, M. & Pagilla, K. 2006. Enhanced electrokinetic dissolution of naphthalene and 2,4-DNT from contaminated soils. Journal of Hazardous Materials, 136, 61–67.

Johnsen, A.R., De Lipthay, J.R., Reichenberg, F., Sørensen, S.J., Andersen, O., Christensen, P., Binderup, M.L. & Jacobsen, G.S. 2006. Biodegradation, bioaccessibility, and genotoxicity of diffuse polycyclic aromatic hydrocarbon (PAH) pollution at a motorway site. Environmental Science & Technology, 40, 3293–3298.

Juhasz, A.L., Aleer, S. & Adetutu, E.M. 2014. Predicting PAH bioremediation efficacy using bioaccessibility assessment tools: Validation of PAH biodegradation-bioaccessibility correlations. International Biodeterioration and Biodegradation, 95, 320–329.

Kelsey, J.W., Kottler, B.D. & Alexander, M. 1997. Selective chemiscal extractants to predict bioavaiability of soil-aged organic chemicals. Environmental Science & Technology, 31, 214–217.

Khan, M.I., Cheema, S.A., Shen, C., Zhang, C., Tang, X., Malik, Z., Chen, X. & Chen, Y. 2011. Assessment of pyrene bioavailability in soil by mild hydroxypropyl-β-cyclodextrin extraction. Archives of Environmental Contamination and Toxicology, 60, 107–115.

Khan, M.I., Cheema, S.A., Shen, C., Zhang, C., Tang, X., Shi, J., Chen, X., Park, J. & Chen, Y. 2012. Assessment of phenanthrene bioavailability in aged and unaged soils by mild extraction. Environmental Monitoring and Assessment, 184, 549–559.

Khodadoust, A.P., Reddy, K.R. & Narla, O. 2006. Cyclodextrin-Enhanced Electrokinetic Remediation of Soils Contaminated with 2,4-Dinitrotoluene. Journal of Environmental Engineering, 132, 1043–1050.

Ko, S.O., Schlautman, M.A. & Carraway, E.R. 1999. Partitioning of hydrophobic organic compounds to sorbed surfactants. 2. Model development/predictions for surfactant-enhanced remediation applications. Environmental Science & Technology, 32, 2776–2781.

Ko, S.O., Schlautman, M.A. & Carraway, E.R. 2000. Cyclodextrin-enhanced electrokinetic removal of phenanthrene from a model clay soil. Environmental Science & Technology, 34, 1535–1541.

Leemhuis, H., Kelly, R.M. & Dijkhuizen, L. 2010. Engineering of cyclodextrin glucanotransferases and the impact for biotechnological applications. Applied Microbiology and Biotechnology, 85, 823–835.

Lei, L., Suidan, M.T., Khodadoust, A.P. & Tabak, H.H. 2004. Assessing the bioavailability of PAHs in field-contaminated sediment using XAD-2 assisted desorption. Environmental Science & Technology, 38, 1786–1793.

Li, S. & Purdy, W.C. 1992. Cyclodextrins and their applications in analytical chemistry. Chemical Reviews, 92, 1457–1470.

Li, T., Yuan, S., Wan, J., Lin, L., Long, H., Wu, X. & Lu, X. 2009. Pilot-scale electrokinetic movement of HCB and Zn in real contaminated sediments enhanced with hydroxypropyl-β-cyclodextrin. Chemosphere, 76, 1226–1232.

Li, T., Yuan, S., Wan, J. & Lu, X. 2010. Hydroxypropyl-??-cyclodextrin enhanced electrokinetic remediation of sediment contaminated with HCB and heavy metals. Journal of Hazardous Materials, 176, 306–312.

Liste, H.H. & Alexander, M. 2002. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere, 46, 1011–1017.

Loethen, S., Kim, J.M. & Thompson, D.H. 2007. Biomedical applications of cyclodextrin based polyrotaxanes.

Loftsson, T. & Brewster, M.E. 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. Journal of Pharmaceutical Sciences, 85, 1017–1025.

Loftsson, T., Leeves, N., Bjornsdottir, B., Duffy, L. & Masson, M. 1999. Effect of cyclodextrins and polymers on triclosan availability and substantivity in toothpastes in vivo. Journal of Pharmaceutical Sciences, 88, 1254–1258.

Lucas-Abellán, C., Fortea, I., Gabaldón, J.A. & Núñez-Delicado, E. 2008. Encapsulation of quercetin and myricetin in cyclodextrins at acidic pH. Journal of Agricultural and Food Chemistry, 56, 255–259.

Luthy, G.R., Ainken, R.G., Brusseau, L.M., Cunningham, D.S., Gschwend, M.P., Pignatello, J.J., Reinhard, M., Traina, J.S., Jr, Weber, J.W. & Westall, C.J. 1997. Critical review sequestration of hydrophobic organic contaminants by geosorbents. American Chemical Society, 31, 3341–3347.

Ma, L., Zhang, J., Han, L., Li, W., Xu, L., Hu, F. & Li, H. 2012. The effects of aging time on the fraction distribution and bioavailability of PAH. Chemosphere, 86, 1072–1078.

Madrid, F., Rubio-Bellido, M., Villaverde, J., Tejada, M. & Morillo, E. 2016. Natural attenuation of fluorene and pyrene in contaminated soils and assisted with hydroxypropyl-β-cyclodextrin. Effect of co-contamination. Science of the Total Environment, 571, 42–49.

Magdolna Nagy, Z., Gruiz, K., Molnár, M. & Fenyvesi, É. 2013. Comparative evaluation of microbial and chemical methods for assessing 4-chlorophenol biodegradation in soil. Periodica Polytechnica: Chemical Engineering, 57, 25–35.

Mahanty, B. & Kim, C.G. 2016. Effect of soil acidification on n-hexane extractable PAH fractions. European Journal of Soil Science, 67, 60–69.

Mahmoudi, N., Slater, G.F. & Juhasz, A.L. 2013. Assessing limitations for PAH biodegradation in long-term contaminated soils using bioaccessibility assays. Water, Air, and Soil Pollution, 224.

Mar Sojo, M., Nuñez-Delicado, E., García-Carmona, F. & Sánchez-Ferrer, A. 1999. Cyclodextrins as activator and inhibitor of latent banana pulp polyphenol oxidase. Journal of Agricultural and Food Chemistry, 47, 518–523.

Maturi, K. & Reddy, K.R. 2006. Simultaneous removal of organic compounds and heavy metals from soils by electrokinetic remediation with a modified cyclodextrin. Chemosphere, 63, 1022–1031.

Mccray, J.E. & Brusseau, M.L. 1999. Cyclodextrin-enhanced in situ flushing of multiple-component immiscible organic liquid contamination at the field scale: Analysis of dissolution behavior. Environmental Science & Technology, 33, 89–95.

McKelvie, J.R., Wolfe, D.M., Celejewski, M., Simpson, A.J. & Simpson, M.J. 2010. Correlations of Eisenia fetida metabolic responses to extractable phenanthrene concentrations through time. Environmental Pollution, 158, 2150–2157.

Means, J.C., Wood, S.G., Hassett, J.J. & Banwart, W.L. 1980. Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environmental Science & Technology, 14, 1524–1528.

Molnár, M., Leitgib, L., Gruiz, K., Fenyvesi, E., Szaniszló, N., Szejtli, J. & Fava, F. 2005. Enhanced biodegradation of transformer oil in soils with cyclodextrin--from the laboratory to the field. Biodegradation, 16, 159–168.

Morillo, E., Pérez-Martínez, J.I. & Ginés, J.M. 2001. Leaching of 2,4-D from a soil in the presence of β-cyclodextrin: Laboratory columns experiments. Chemosphere, 44, 1065–1069.

Morillo, E., Sánchez-Trujillo, M.A., Villaverde, J., Madrid, F. & Undabeytia, T. 2014. Effect of contact time and the use of hydroxypropyl-β-cyclodextrin in the removal of fluorene and fluoranthene from contaminated soils. Science of the Total Environment, 496, 144–154.

Mosinger, J., Tománková, V., Němcová, I. & Zýka, J. 2001. Cyclodextrins in analytical chemistry. Analytical Letters, 34, 1979–2004.

Muijs, B. & Jonker, M.T.O. 2011. Assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments. Environmental Science & Technology, 45, 3554–3561.

Nélieu, S., Delarue, G., Ollivier, E., Awad, P., Fraillon, F. & Pelosi, C. 2016. Evaluation of epoxiconazole bioavailability in soil to the earthworm Aporrectodea icterica. Environmental Science and Pollution Research, 23, 2977–2986.

Obuekwe, I.S. & Semple, K.T. 2013. Impact of Al and Fe on the development of phenanthrene catabolism in soil. Journal of Soils and Sediments, 13, 1589–1599.

Ogbonnaya, O.U., Adebisi, O.O. & Semple, K.T. 2014. The impact of biochar on the bioaccessibility of 14 C-phenanthrene in aged soil. Environ. Sci.: Processes Impacts, 16, 2635–2643, (At: http://xlink.rsc.org/?DOI=C4EM00396A.).

Oonnittan, A., Shrestha, R.A. & Sillanpää, M. 2008. Effect of cyclodextrin on the remediation of hexachlorobenzene in soil by electrokinetic Fenton process. Separation and Purification Technology, 64, 314–320.

Papadopoulos, A., Paton, G.I., Reid, B.J. & Semple, K.T. 2007. Prediction of PAH biodegradation in field contaminated soils using a cyclodextrin extraction technique. Journal of Environmental Monitoring, 9, 516.

Pham, T.D., Shrestha, R.A. & Sillanpaa, M. 2010. Removal of hexachlorobenzene and phenanthrene from clayey soil by surfactant- and ultrasound-assisted electrokinetics. Journal of Environmental Engineering-Asce, 136, 739–742.

Pignatello, J.J. & Xing, B. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environmental Science & Technology, 30, 1–11.

Qi, Q. & Zimmermann, W. 2005. Cyclodextrin glucanotransferase: From gene to applications. Applied Microbiology and Biotechnology, 66, 475–485.

Ramsay, J.A., Robertson, K., VanLoon, G., Acay, N. & Ramsay, B.A. 2005. Enhancement of PAH biomineralization rates by cyclodextrins under Fe(III)-reducing conditions. Chemosphere, 61, 733–740.

Reddy, K.R. 2013. Electrokinetic remediation of soils at complex contaminated sites: Technology status, challenges, and opportunities. Coupled Phenomena in Environmental Geotechnics, 131–147.

Reddy, K.R., Ala, P.R., Sharma, S. & Kumar, S.N. 2006. Enhanced electrokinetic remediation of contaminated manufactured gas plant soil. Engineering Geology, 85, 132–146.

Reid, B.J., Semple, K.T. & Jones, K.C. 1998. Prediction of bioavailability of persistent organic pollutants by a novel extraction technique. Contaminated Soil ’98, Proceedings of the International FZK/TNO Conference on Contaminated Soil, 6th, Edinburgh, May 17-21, 1998, 2, 889–890.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science & Technology, 34, 3174–3179.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2004. Influence of hydroxypropyl-β-cyclodextrin on the extraction and biodegradation of phenanthrene in soil. Environmental Toxicology and Chemistry, 23, 550–556.

Rhodes, A.H., Carlin, A. & Semple, K.T. 2008. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science & Technology, 42, 740–745.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Rhodes, A.H., Riding, M.J., McAllister, L.E., Lee, K. & Semple, K.T. 2012. Influence of activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil. Environmental Science & Technology, 46, 12445–12451.

Rostami, I. & Juhasz, A.L. 2013. Bioaccessibility-based predictions for estimating PAH biodegradation efficacy - Comparison of model predictions and measured endpoints. International Biodeterioration and Biodegradation, 85, 323–330.

Sabaté, J., Viñas, M. & Solanas, A.M. 2006. Bioavailability assessment and environmental fate of polycyclic aromatic hydrocarbons in biostimulated creosote-contaminated soil. Chemosphere, 63, 1648–1659.

Saenger, B.W. 1980. Cyclodextrin Inclusion Compounds in Research and Industry. 8, 344–362.

Schneiderman, E. & Stalcup, A.M. 2000. Cyclodextrins: A versatile tool in separation science. In: Journal of Chromatography B: Biomedical Sciences and Applications, pp. 83–102.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science & Technology, 38, 228A–231A.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Shieh, W.J. & Hedges, A.R. 1996. Properties and applications of cyclodextrins. Journal of Macromolecular Science - Pure and Applied Chemistry, 33, 673–683.

Shin, D. & Nam, K. 2014. Potential use of a self-dying reporter bacterium to determine the bioavailability of aged phenanthrene in soil: Comparison with physicochemical measures. Journal of Hazardous Materials, 265, 1–7.

Singh, M., Sharma, R. & Banerjee, U.C. 2002. Biotechnological applications of cyclodextrins. Biotechnology Advances, 20, 341–359.

Sivaraman, C., Ganguly, A. & Mutnuri, S. 2010. Biodegradation of hydrocarbons in the presence of cyclodextrins. World Journal of Microbiology and Biotechnology, 26, 227–232.

Šmídová, K., Hofman, J., Ite, A.E. & Semple, K.T. 2012. Fate and bioavailability of 14C-pyrene and 14C- lindane in sterile natural and artificial soils and the influence of aging. Environmental Pollution, 171, 93–98.

Song, Y., Wang, F., Bian, Y., Kengara, F.O., Jia, M., Xie, Z. & Jiang, X. 2012. Bioavailability assessment of hexachlorobenzene in soil as affected by wheat straw biochar. Journal of Hazardous Materials, 217–218, 391–397.

Song, Y., Wang, F., Yang, X., Liu, C., Kengara, F.O., Jin, X. & Jiang, X. 2011. Chemical extraction to assess the bioavailability of chlorobenzenes in soil with different aging periods. Journal of Soils and Sediments, 11, 1345–1354.

Sopeña, F., Semple, K., Sohi, S. & Bending, G. 2012. Assessing the chemical and biological accessibility of the herbicide isoproturon in soil amended with biochar. Chemosphere, 88, 77–83.

Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C. & Semple, K.T. 2005. Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl-β-cyclodextrin extraction technique. Environmental Toxicology and Chemistry, 24, 1325.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2009. Predicting the biodegradation of target hydrocarbons in the presence of mixed contaminants in soil. Chemosphere, 74, 563–567.

Sun, M., Luo, Y., Christie, P., Jia, Z., Li, Z. & Teng, Y. 2012. Methyl-β-cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and associated microbial activity in contaminated soil. Journal of Environmental Sciences, 24, 926–933.

Sun, M., Luo, Y., Teng, Y., Jia, Z., Li, Z. & Deng, S. 2013. Remediation of polycyclic aromatic hydrocarbon and metal-contaminated soil by successive methyl-β-cyclodextrin-enhanced soil washing-microbial augmentation: A laboratory evaluation. Environmental Science and Pollution Research, 20, 976–986.

Swindell, A.L. & Reid, B.J. 2007. The influence of a NAPL on the loss and biodegradation of 14C-phenanthrene residues in two dissimilar soils. Chemosphere, 66, 332–339.

Szejtli, J. 1998. Introduction and general overview of cyclodextrin chemistry. Chemical Reviews, 98, 1743–1754.

Szente, L. & Szejtli, J. 2004. Cyclodextrins as food ingredients. Trends in Food Science and Technology, 15, 137–142.

Tang, J., Liste, H.H. & Alexander, M. 2002. Chemical assays of availability to earthworms of polycyclic aromatic hydrocarbons in soil. Chemosphere, 48, 35–42.

Uekama, K., Hirayama, F. & Irie, T. 1998. Cyclodextrin drug carrier systems. Chemical Reviews, 98, 2045–2076 ST–Cyclodextrin Drug Carrier Systems.

Del Valle, E.M.M. 2004. Cyclodextrins and their uses: A review. Process Biochemistry, 39, 1033–1046.

Viglianti, C., Hanna, K., De Brauer, C. & Germain, P. 2006. Removal of polycyclic aromatic hydrocarbons from aged-contaminated soil using cyclodextrins: Experimental study. Environmental Pollution, 140, 427–435.

Villaverde, J. 2007. Time-dependent sorption of norflurazon in four different soils: Use of β-cyclodextrin solutions for remediation of pesticide-contaminated soils. Journal of Hazardous Materials, 142, 184–190.

Villaverde, J., Pérez-Martínez, J.I., Maqueda, C., Ginés, J.M. & Morillo, E. 2005. Inclusion complexes of α- and γ-cyclodextrins and the herbicide norflurazon: I. Preparation and characterisation. II. Enhanced solubilisation and removal from soils. Chemosphere, 60, 656–664.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M., Laiz, L., Saiz-Jimenez, C., Sanchez-Trujillo, M.A. & Morillo, E. 2012. Enhanced mineralization of diuron using a cyclodextrin-based bioremediation technology. Journal of Agricultural and Food Chemistry, 60, 9941–9947.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M. & Morillo, E. 2013a. Effect of hydroxypropyl-β-cyclodextrin on diuron desorption and mineralisation in soils. Journal of Soils and Sediments, 13, 1075–1083.

Villaverde, J., Rubio-Bellido, M., Posada-Baquero, R., Madrid, F. & Morillo, E. 2013b. Hydroxypropyl-β-cyclodextrin-based extraction for diuron bioaccessibility in an artificially contaminated soil. International Journal of Environmental Analytical Chemistry, 93, 1620–1627.

Van Der Wal, L., Jager, T., Fleuren, R.H.L.J., Barendregt, A., Sinnige, T.L., Van Gestel, C.A.M. & Hermens, J.L.M. 2004. Solid-phase microextraction to predict bioavailability and accumulation of organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil. Environmental Science & Technology, 38, 4842–4848.

Wan, C., Du, M., Lee, D.J., Yang, X., Ma, W. & Zheng, L. 2011. Electrokinetic remediation and microbial community shift of β-cyclodextrin-dissolved petroleum hydrocarbon-contaminated soil. Applied Microbiology and Biotechnology, 89, 2019–2025.

Wan, J., Yuan, S., Chen, J., Li, T., Lin, L. & Lu, X. 2009. Solubility-enhanced electrokinetic movement of hexachlorobenzene in sediments: A comparison of cosolvent and cyclodextrin. Journal of Hazardous Materials, 166, 221–226.

Wang, X. & Brusseau, M.L. 1993. Solubilization of some low-polarity organic compounds by hydroxypropyl- β-cyclodextrin. Environmental Science & Technology, 27, 2821–2825.

Wang, X. & Brusseau, M.L. 1995. Simultaneous complexation of organic compounds and heavy metals by a modified cyclodextrin. Environmental Science & Technology, 29, 2632–2635.

Wang, J.M., Maier, R.M. & Brusseau, M.L. 2005. Influence of hydroxypropyl-β-cyclodextrin (HPCD) on the bioavailability and biodegradation of pyrene. Chemosphere, 60, 725–728.

Wang, J.M., Marlowe, E.M., Miller-Maier, R.M. & Brusseau, M.L. 1998. Cyclodextrin-enhanced biodegradation of phenanthrene. Environmental Science & Technology, 32, 1907–1912.

Wang, G., Xu, W., Wang, X. & Huang, L. 2012. Glycine-β-cyclodextrin–enhanced electrokinetic removal of atrazine from contaminated soils. Environmental Engineering Science, 29, 406–411.

Wu, G., Kechavarzi, C., Li, X., Sui, H., Pollard, S.J.T. & Coulon, F. 2013. Influence of mature compost amendment on total and bioavailable polycyclic aromatic hydrocarbons in contaminated soils. Chemosphere, 90, 2240–2246.

Yeung, A.T. & Gu, Y.-Y. 2011. A review on techniques to enhance electrochemical remediation of contaminated soils. Journal of Hazardous Materials, 195, 11–29.

Yuan, S., Tian, M. & Lu, X. 2006. Electrokinetic movement of hexachlorobenzene in clayed soils enhanced by Tween 80 and β-cyclodextrin. Journal of Hazardous Materials, 137, 1218–1225.

Zhang, Y., Maier, W.J. & Miller, R.M. 1997. Effect of rhamnolipids on the dissolution, bioavailability, and biodegradation of phenanthrene. Environmental Science & Technology, 31, 2211–2217.

Zhang, Z.X., Zhu, Y.X., Li, C.M. & Zhang, Y. 2012. Investigation into the causes for the changed biodegradation process of dissolved pyrene after addition of hydroxypropyl-β-cyclodextrin (HPCD). Journal of Hazardous Materials, 243, 139–145.

Chapter 4

Buffered cyclodextrin extraction of ^{14}C-phenanthrene from black carbon amended soil

Buffered cyclodextrin extraction of ^{14}C-phenanthrene from black carbon amended soil

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4.1 Abstract

The presence of black carbon (BC) in soil drastically reduced the mineralisation of ^{14}C-phenanthrene and its extractability by hydroxypropyl-β-cyclodextrin (HPCD) extractions. This study also tested the effects of pH on the HPCD extraction of ^{14}C-phenanthrene in soils with BC. Extractions using 60 mM HPCD solutions prepared in deionized water (pH 5.89) and phosphate buffers (pH 7 and 8) were conducted on ^{14}C-phenanthrene spiked soils amended with three different types of BC (1% dry weight) after 1, 25, and 50 d of ageing. Biodegradation assays using a *Pseudomonas* sp. strain were also carried out. Results showed that after 1 and 25 d, HPCD at pH 7 extracted significantly more ^{14}C-phenanthrene ($p < 0.05$) from BC-amended soils than the other two solutions, while HPCD at pH 8 extracted statistically similar ($p > 0.05$) amounts of phenanthrene compared to the un-buffered solution. At 50 d, HPCD at pH 8 generally extracted more ^{14}C-phenanthrene from all treatments. It was proposed that higher pH promoted the dissolution of soil organic matter (SOM), leading to a greater solubility of phenanthrene in the solvent phase and enhancing the extractive capability. Although correlations between extractability and biodegradability of ^{14}C-phenanthrene in BC-amended soils were poor, increasing pH was demonstrated a viable approach to enhancing HPCD extractive capability from the ^{14}C-PAH from soil.

Keywords: Black carbon, phenanthrene, hydroxypropyl-β-cyclodextrin extraction (HPCD), mineralisation, pH

4.2 Introduction

Massive consumption of fossil fuels and combustion of biomass in modern world has dramatically increased the input of black carbon (BC) into the environment (Schmidt & Noack, 2000). BC is a group of heterogeneous carbon material possessing strong sorptive capabilities and recalcitrance to chemical and biological transformation (Semple *et al.*, 2013). It is mainly produced by incomplete combustion of fossil fuels or biomass (Schmidt & Noack, 2000; Rhodes *et al.*, 2008a; Xia *et al.*, 2010; Semple *et al.*, 2013). BC is ubiquitously distributed across the environmental compartments including soil, where it impacts the fate and behaviour of a range of contaminants such as hydrophobic organic contaminants (HOCs) (Yang & Sheng, 2003; Lohmann *et al.*, 2005). Moreover, commercially produced BC (e.g. activated carbon) has also been proposed and piloted as a tool for contaminated land remediation (Cho *et al.*, 2007). Nevertheless, there is still a lack of understanding regarding the implications of BC on the bioaccessibility of soil organic contaminants and risk assessment of contaminated land (Semple *et al.*, 2013).

In the presence of BC, the rate and extent of biodegradation of polycyclic aromatic hydrocarbons (PAHs) can be dramatically reduced (Rhodes *et al.*, 2008a, 2010a; Xia *et al.*, 2010). Furthermore, extractability of PAHs from contaminated soil by hydroxypropyl-β-cyclodextrin (HPCD) has been shown to be influenced by the presence of BC (Rhodes *et al.*, 2008a; Towell *et al.*, 2011a). Importantly, HPCD is acknowledged as a well-established mimetic method to assess the bioaccessibility of organic contaminants in soils (Reid *et al.*, 2000b; Doick *et al.*, 2006; Brand *et al.*, 2009). However, the HPCD extraction has been shown to underestimate the mineralisation of phenanthrene in soils amended with 0.1 % or more of activated carbon (AC), thereby interfering with the reliability of this technique (Rhodes *et al.*, 2008a). Rhodes *et al.* (2008) and Xia *et al.* (2010) attributed such incompatibility between HPCD extractability and biodegradability to the direct mineralisation of BC-associated phenanthrene by microorganisms, which HPCD extraction was not able to access. Although the mechanism involved in direct microbial uptake of sorbed substances has been reported by Alexander (1999), this explanation is still questionable considering that the uptake of organic substances by soil microorganisms predominantly takes place in the aqueous phase (Cerniglia, 1993; Johnsen *et al.*, 2005). It is also possible that other microbial processes (e.g. biosurfactant production)

could promote the desorption of the BC-associated target chemicals, while water as the solvent of HPCD solution used in these researches was not capable of displacing target compounds from sorption sites on BC particles (Jonker & Koelmans, 2002).

As has been previously suggested by Semple *et al.* (2013), it is important to find a reliable chemical method to estimate the bioaccessibility of HOCs in soils with BC given the growing input of BC to soil from anthropogenic sources and the application of commercially produced BC as a strategy for the remediation of contaminated systems. For this purpose, a potential approach is to modify HPCD extraction methodology by integrating a buffer of higher pH into the solvent to achieve a greater displacement capacity for target compounds, as increasing pH promotes the dissolution of SOM (Grybos *et al.*, 2009) which contributes to greater aqueous solubility of organic pollutants (Chiou *et al.*, 1986). This was also demonstrated by Reid *et al.* (2000) who observed enhanced extractive capability of HPCD solution prepared in phosphate buffer of pH 8 for phenanthrene. Therefore, this study aims to investigate the effects of phosphate buffers of higher pH values on the extractive capability of HPCD solutions for phenanthrene (a) in soils amended with different types of commercially produced BC, (b) after different periods of soil-contaminant interactions. Parallel biodegradation assays with a phenanthrene-degrading inoculum (*Pseudomonas* sp.) will also be conducted to measure the microbially accessible fraction of the PAHs in the soil.

4.3 Materials and methods

4.3.1 Chemicals

Unlabelled phenanthrene was obtained from Sigma Aldrich Co, Ltd. UK. [9-14C] Phenanthrene was purchased from American Radiolabelled Chemicals, Inc., USA. Liquid scintillation cocktail (Goldstar) and sample oxidation cocktails (Carbotrap and Carbocount) were obtained from Meridian Biotechnologies Ltd, UK. Hydroxypropyl-β-cyclodextrin (HPCD) was purchased from Acros Organics, Belgium. General purpose grade agar (GPA) was obtained from Fisher Scientific, UK. Activated carbon (Colorsorb P3-1, Aquasorb CP2 and Aquasorb BP2) was obtained from Jacobi Carbons, UK.

4.3.2 Soil collection and characterization

Pristine soil was collected (A horizon; 5 – 20 cm) from Myerscough Agricultural College in Lancashire, UK, and passed through a 2mm sieve to remove stones and roots. General soil properties are presented in Table 1. Particle size was analysed through laser diffraction (Hydro 2000MU, Malvern Instruments Ltd., UK). Soil organic matter content (dry weight basis) was determined by mass loss on ignition (450 °C for 24 h). Total carbon and nitrogen content (%) were assessed using an Elementar Vario EL III elemental analyser (Hanau, Germany).

4.3.3 BC amendment and soil spiking

Prior to BC amendment, the soil was rehydrated with deionized water to field moisture content (30 – 35 % dry weight basis). Subsequently, soil treatments with 1 % (dry weight basis) of three different types of BC (designated as P3-1, CP 2 and BP 2, properties presented in Table 2) were prepared by blending specific quantities of BC with each treatment using a stainless spoon Rhodes *et al.* (2008). A treatment without BC was also prepared as a control. Immediately after BC amendment, soils were spiked with ^{12}C-/^{14}C-phenanthrene using acetone as carrier (3.75 ml per 300 g dry soil at 0.8 mg ml^{-1} for ^{12}C- and 6666.67 Bq ml^{-1} for ^{14}C-phenanthrene) as described by Doick *et al.* (2003), to achieve a ^{12}C-phenanthrene concentration of 10 mg kg^{-1} and ^{14}C-phenenthrene-associated radioactivity of 64 – 78 kBq kg^{-1} dry soil. Unspiked control soils were also prepared for each BC treatment. As mineralisation of phenanthrene by both indigenous and inoculated microorganism has been shown to be equally efficient and dependent solely on the available amount of phenanthrene (Doick *et al.*, 2005b; Semple *et al.*, 2006), the soil samples were not sterilised after spiking and were incubated in sealed amber glass jars at room temperature (21 ± 1°C) for 1, 25, and 50 d.

4.3.4 Preparation of phenanthrene-degrading inoculum

Prior to the mineralisation assay, a phenanthrene-degrading inoculum of *Pseudomonas* sp. was cultured in a mixture of minimal basal salts solution (MBS) containing phenanthrene solution (0.1 ml l^{-1}) as the sole C-source (Reid *et al.*, 2001) on an IKA Labortechnik KS501 digital orbital shaker at 100 rpm at room temperature (21 ± 1 °C). On the fourth day of incubation (late exponential phase of growth), the

inoculum was concentrated by centrifugation at 10,000 *x g* for 30 minutes (Hettich Zentrifugen, Rotanta 460, UK). The supernatant was then discarded and the cell pellet washed and re-suspended with fresh MBS. A second centrifugation was subsequently carried out to ensure the removal of any residual phenanthrene, obtaining a final cell density of approximately 10^8 cells ml^{-1}.

4.3.5 Mineralisation of ^{14}C-phenanthrene

Mineralisation assays were conducted in "respirometers", which were modified 250 ml Schott bottles as described by Reid *et al.* (2001). After 1, 25 and 50 d of soil incubation, the respirometers (n = 3) were set up with 10 ± 0.2 g soil (wet weight), 25 ml of MBS, and 5 ml of concentrated inoculum (10^5 – 10^6 cells per g soil) (Doick & Semple, 2003). Uninoculated respirometers and soil incubations with no ^{14}C-activity (n = 3) were also set up for each treatment. The respirometers were then incubated on an IKA Labortechnik KS501 digital orbital shaker at 100 rpm for 14 days at room temperature (21 ± 1 °C). During this period of time, $^{14}CO_2$ generated from microbial degradation of ^{14}C-phenanthrene was trapped in 7 ml glass scintillation vials suspended from the Teflon lined-lid containing 1 ml of NaOH (1 M). The vials were replaced every 24 h, after which 5 ml Goldstar scintillation cocktail was subsequently added to each of the sampled vials and the ^{14}C-associated activity was quantified by liquid scintillation counting (LSC, Canberra Packard Tri-Carb2250CA) after a > 12h storage in the dark to avoid chemo-luminescence.

4.3.6 Extraction of ^{14}C-phenanthrene with hydroxypropyl-β-cyclodextrin (HPCD) solutions

Three different HPCD solutions (60 mM) were prepared in deionised water (pH 5.89), and phosphate buffers of pH 7 and 8 respectively. The buffers of pH 7 and 8 were prepared by combining K_2HPO_4 (0.2 M) and KH_2PO_4 (0.2 M) solutions at ratios of 1.6:1 and 17.9:1 respectively. The extraction assays were carried out after 1, 25 and 50 days of ageing, following the methodology described by Reid *et al.* (2000). In brief, soil (1.25 ± 0.1 g wet weight) from each treatment was weighed into 35 ml Teflon centrifuge tubes with 25 ml of each HPCD solution (n = 3). The tubes were then placed onto an orbital shaker (IKA Labortechnik KS501 digital) at 100 rpm for 22 h in darkness at room temperature (21 ± °C). Subsequently, the tubes were

centrifuged at 3000 *x g* for 1 h (Hettich Zentrifugen, Rotanta 460, UK) and 5 ml of supernatant was then mixed with 15 ml Goldstar scintillation cocktail. The samples were assessed by LSC as described previously.

4.3.7 Statistical analysis

Following blank-correction, statistical analysis of the results was carried out with the Statistical Package for the Social Sciences (SPSS Version 22 for Mac). The statistical significance of BC addition, BC type and ageing period to phenanthrene biodegradability and phenanthrene extractability by HPCD solutions, as well as the statistical significance of pH to HPCD extractive capability, was determined using a linear model (ANOVA, Tukey Test) and/or Student t-test at 95 % confidence level ($p < 0.05$).

4.4 Results and discussion

4.4.1 Mineralisation of ^{14}C-phenanthrene in soils

^{14}C-Phenanthrene catabolism was drastically reduced in all BC-treated soils at all time points. Compared to soil without BC, the fastest rates (the highest yield of $^{14}CO_2$ per day during mineralisation assays) and extents and of ^{14}C-phenanthrene mineralisation decreased by more than 99 % at 1 and 25 d, and more than 93 % after 50 d of soil incubation (Tables 3, 4). The fastest rates of phenanthrene mineralisation did not exceed 0.10 % per d at 1 and 25 d and were less than 0.3 % per d at 50 d in BC-amended soils (Table 3). At 1 d, only 0.15 %, 0.07 %, and 0.11 % of ^{14}C-PAH was mineralised in soils amended with P3-1, CP 2, and BP 2 respectively, while 63.20 % of the ^{14}C-phenanthrene was mineralised in soil without BC. Furthermore, influences of BC type on biodegradation were observed. At 25 d contact time, soil amended with CP 2 yielded significantly less ($p < 0.05$) $^{14}CO_2$ than the other two BC-amended soils, while significantly more ^{14}C-phenanthrene ($p < 0.05$) was mineralised in soil with P3-1 at 50 d than the other two BC-treated soils. Overall, these results were in agreement with previous studies by Rhodes *et al.* (2008, 2010). These trends have been attributed to the strong sorptive capacity of BC (Cornelissen *et al.*, 2005; Rhodes *et al.*, 2012). Consequently, the aqueous concentration and biodegradation of target compound was reduced, as the microbial uptake of organic substances mainly takes place in soil

aqueous phase (Cerniglia, 1993; Johnsen *et al.*, 2005). Moreover, a fraction of ^{14}C-phenanthrene may have become inaccessible to microorganisms due to entrapment in collapsed pores on BC particles (Sander & Pignatello, 2007; Semple *et al.*, 2013). However, the extent to which ^{14}C-phenanthrene mineralisation was inhibited in BC-amended soils was much greater than those observed by Rhodes *et al.* (2008, 2010). At least 6 % of spiked ^{14}C-phenanthrene was mineralised in each soil treatment in research by Rhodes *et al.* (2008) by all treatments (0 – 5% AC dry weight), while Rhodes *et al.* (2010) only obtained biodegradation extents lower than 1 % in soils treated with 5 % AC. It appears that the types of BC used in this study possessed greater sorptive capacity than those used in studies by Rhodes *et al.* (2008, 2010). The BC type also influenced the rates and extents of mineralisation of ^{14}C-phenanthrene in the present study (Tables 3, 4). These variations may be attributed to the specific properties of each BC such as surface heterogeneity and functional groups, pore volume, activation and production methods, source material, as well as processing temperature (Burgess & Lohmann, 2004; Chun *et al.*, 2004; Yang *et al.*, 2004; Xia *et al.*, 2010; Semple *et al.*, 2013).

After 50 d ageing, all BC-amended soils yielded greater ^{14}C-phenanthrene mineralisation compared to 1 and 25 d. Unlike BC-amended soils, biodegradation of ^{14}C-phenanthrene in soil without BC decreased significantly ($p < 0.05$) over time (Table 4). Apparently, ageing effect, where biodegradability of HOCs diminishes over time (Hatzinger & Alexander, 1995), was absent in BC-amended soils. Similar results were also obtained by Rhodes *et al.* (2008), who attributed such findings to sorptive attenuation, where soil organic matter (SOM) competes for limited sorption sites on BC particles and blocks them from the spiked chemical, thus lowering sorptive capacity of BC for target substances (Jonker *et al.*, 2004; Rhodes *et al.*, 2008a). Such competitive sorption has also been observed by other researchers; for example, Chun et al. (2004) found that organic chemicals with larger molecular sizes covered the binding sites on BC particles and blocked them from smaller molecules.

4.4.2 HPCD extraction of ^{14}C-phenanthrene in soils

Addition of BC also led to drastic reduction in the extractability of the ^{14}C-PAH by HPCD solutions in each soil treatment at all soil–contaminant contact times (Table 5). Compared to the soil without BC, ^{14}C-activity extracted by unbuffered aqueous HPCD

solution decreased by more than 99 % at 1 and 25 d, while 93 % – 98 % less ^{14}C-phenanthrene was extracted by the unbuffered solution after 50 d of ageing. Extractions with buffered HPCD solutions were also strongly influenced by the presence of BC, but the phosphate buffer also resulted in changes in amounts of ^{14}C-phenanthrene extracted by HPCD. At 1 and 25 d, HPCD at pH 7 extracted 1.03 % – 1.56 % of spiked phenanthrene from all BC-treated soils (Table 5), which was statistically higher ($p < 0.05$) than the amounts extracted by the other two solutions. Further increase in pH of HPCD solution to 8 led to statistically similar ($p > 0.05$) yield of extracted ^{14}C-activity from all BC-amended soils compared to its aqueous counterpart at 1 and 25 d (Table 5). However, after 50 days of soil incubation, HPCD at pH 8 extracted significantly more ($p < 0.05$) ^{14}C-activity than the other two solutions in soils amended with CP 2 and BP 2, while the amounts of ^{14}Cphenanthrene extracted by HPCD at pH 7 were statistically similar ($p > 0.05$) to the values from extractions using aqueous HPCD solution for all BC-amended soils (Table 5).

HPCD is a well-established non-exhaustive extraction technique to measure microbial bioaccessibility of numerous HOCs in soils under different conditions (Cuypers *et al.*, 2002; Dew *et al.*, 2005; Doick *et al.*, 2005b, 2006; Hickman & Reid, 2005; Allan *et al.*, 2006; Hickman *et al.*, 2008; Stroud *et al.*, 2008; Dandie *et al.*, 2010). The HPCD molecules are able to separate organic compounds from water solution, thus mimicking microbial uptake of organic substances and driving mass-transfer of targeted compounds from soil matrix to dissolved phase (Wang & Brusseau, 1995; Jones *et al.*, 1996; Bosma *et al.*, 1997; Reid *et al.*, 2000b; Semple *et al.*, 2003). However, in presence of BC, sorption to of the ^{14}C-PAH resulted in reduction of dissolved phenanthrene for HPCD molecules to separate. Moreover, Jonker & Koelmans (2002) suggested that water, as the solvent of aqueous HPCD solution, was not capable of displacing BC-associated phenanthrene molecules from binding sites on BC particles. In the present study, buffered solutions of pH 7, at 1 and 25 d, as well as that of pH 8, at 50 d, enhanced the extractive capability of HPCD solution in soils amended with BC. Additionally, the buffered extracts from each soil treatment at each time point were highly coloured. This was consistent with the observations made by Reid *et al.* (2000) and was indicative of the existence of dissolved organic matter in the extracts (De Haan *et al.*, 1982; Reid *et al.*, 2000b). The promotion of dissolution of SOM by phosphate buffers at higher pH has been reported in previous studies

(Lerch *et al.*, 1997; Di Corcia *et al.*, 1999; Crescenzi *et al.*, 2000). It was suggested that the deprotonation under basic conditions brought by phosphate buffers attenuated the association between SOM and soil minerals, thus increasing the amount of dissolved organic matter (DOM) (Lerch *et al.*, 1997). Although other researchers also demonstrated the ability of phosphate to inhibit the sorption of phenanthrene in soils (Luo *et al.*, 2011), the effects of phosphate itself in this research on the release of phenanthrene from soil are considered minimal given the amount of phosphate used and its contact time with spiked soils. DOM subsequently contributed to a greater solubility of phenanthrene (Chiou *et al.*, 1986), so that there were more PAH molecules in the aqueous phase for HPCD molecules to separate.

Interestingly, increases in pH did not always result in increases of extraction using HPCD solutions, as a biphasic feature of increasing pH was identified in BC amended soils at 1 and 25 d, and was absent after 50 days of ageing. This observation reflects the complex interactions between soil, BC, phenanthrene and HPCD solutions. It is therefore postulated that extensive sorption of both SOM and ^{14}C-phenanthrene to BC particles was achieved shortly after BC amendment and PAH spiking, while at 1 and 25 d, HPCD at pH 8 dissolved so much BC-associated SOM that sorption sites on BC particles were exposed to phenanthrene. Consequently, greater sorption of phenanthrene to BC was facilitated. At 50 d, however, greater amount of SOM was attached to BC particles and phenanthrene molecules partitioned deeper into BC. As a result, buffer of pH 8, which was able to dissolve more SOM, released more BC- and SOM-bound phenanthrene than buffer of pH 7 (Figure 1).

A simple comparison between HPCD extraction assays and mineralisation assays conducted in BC-amended soils was carried out by calculating the ratios of extraction to mineralisation. The results indicated that in most cases aqueous HPCD extracted underestimated mineralisation of ^{14}C-phenanthrene (Table 6). However, the extents to which biodegradation was underestimated were not as great as those reported by Rhodes *et al.* (2008) except for few cases (Table 6). Such findings suggest that the differences between HPCD extractive capability and biodegradability of phenanthrene in soils with BC may not be as great as they were previously observed. The amounts of phenanthrene extracted by HPCD in pH 7 were 3 to 15 times greater those degraded by microorganisms at 1 and 25 d, and mildly deviated from the degraded amounts at 50 d (Table 6). HPCD in pH 8 provided mixed results in ratios of

extraction to mineralisation in all BC-treated soils, but the deviations of extractability from biodegradability were not as great as those demonstrated by aqueous HPCD and HPCD in pH 7. These findings have two implications. Firstly, the mechanism which was direct degradation of BC-associated phenanthrene proposed in previous studies may not be actually involved in mineralisation assays. Secondly, increasing pH enhances the extractive capability of HPCD and could improve this method in predicting microbial accessibility of phenanthrene in soils with BC after substantial ageing period, as the ratios of HPCD extraction in buffers were approach 1 compared to those of aqueous HPCD (Table 6). However, due to the size of the data acquired in the current study, further verification of these findings are required to optimise this modification of HPCD extraction under various conditions including different pH values, and soil and BC types. Besides, the order of BC amendment and PAH spiking should also be considered as the faster and greater binding of PAH with BC particles in pre-amended soils may occur, thus bringing differences to the results obtained.

4.5 Conclusion

Addition of BC significantly reduced mineralisation and extraction of ^{14}C-phenanthrene after different periods of soil–contaminant interactions, where variations brought by BC type were identified among soil treatments. Introduction of phosphate buffers produced varying effects to the extractive capability of HPCD solutions, as HPCD at pH 7 extracted significantly more phenanthrene at 1 and 25 d and HPCD at pH 8 yielded more extracted ^{14}C-activity at 50 d. A biphasic feature of increasing pH on HPCD extractive capability was observed at 1 and 25 d but not at 50 d. Overall, these findings reflected the complex interactions between SOM, BC, HPCD, and phenanthrene. Aqueous HPCD extractions did not always underestimate biodegradation of phenanthrene in BC-amended soil, rejecting previously proposed mechanism for the incompatibility between mineralisation and HPCD extraction. More studies should be carried out to find out whether presence of BC indeed leads to underestimation of biodegradation by HPCD extraction. If yes, increasing pH of HPCD solution, as it has been demonstrated in this study, is a viable approach to modifying this technique for better prediction of the bioaccessibility of organic contaminants in soils with BC.

Table 1. Physical-chemical properties of soil used in this study. Errors are shown as 1 SEM (n = 3)

Soil Properties		**Parameter Value**
pH (in dH_2O)		5.36 ± 0.01
Organic matter (%)		9.15 ± 0.06
Nitrogen (%)		0.20 ± 0.02
Carbon (%)		2.24 ± 0.01
Particle size*	Clay	23.42%
	Silt	75.26%
	Sand	1.27%
	Soil texture	Silt loam

* Analysis of particle size by laser diffraction reflected the distribution of particles with diameter < 1 mm, using total surface area as baseline.

Table 2. Properties of black carbon used for soil amendments

Activated carbon	Source	Activation method	Processing Temperature (°C)	Surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})	Mean particle diameter (μm)
P3-1	Wood	Chemical activation	700	1150	Not provided	Not applicable[a]
BP 2	Coal	Steam activation	850-950	1000	1.56	21
CP 2	Coconut shell	Steam activation	850-950	950	0.55	21

[a] Particle size of this grade was expressed as distribution of powder size: <150 μm = 95 -100%, <75 μm = 85 – 95%, <45μm = 65 – 85%.

Table 3. Fastest rates of ^{14}C-phenanthrene mineralisation in soils amended with 0 % black carbon and 1% P3-1, CP 2, and BP 2 at 1, 25 and 50 days of soil–phenanthrene interactions. Values are the % $^{14}CO_2$ per d mean (n = 3) ± standard error of the mean (SEM). Values in the same column followed by the same letter, or row followed by the same number are statistically similar (student t-test and ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	Black carbon treatment			
	0 % BC	1 % P3-1	1 % CP2	1 % BP2
1	26.13 ± 1.73^{a1}	0.03 ± 0.01^{a2}	0.02 ± 0.00^{a2}	0.03 ± 0.00^{a2}
25	13.78 ± 1.43^{b1}	0.05 ± 0.01^{a2}	0.02 ± 0.01^{a2}	0.10 ± 0.01^{b3}
50	3.85 ± 0.16^{c1}	0.25 ± 0.00^{b2}	0.04 ± 0.01^{a3}	0.06 ± 0.01^{c3}

Table 4. Total extents of ^{14}C-phenanthrene mineralised by microorganisms in soils amended with 0 % black carbon and 1 % P3-1, CP 2, and BP 2 after 1, 25, and 50 days of soil–phenanthrene interactions. Values are the % mean (n = 3) ± standard error of the mean (SEM). Values in the same column followed by the same letter, or row followed by the same number are statistically similar (student t-test and ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	Black carbon treatment			
	0 % BC	1 % P3-1	1 % CP2	1 % BP2
1	63.20 ± 0.52^{a1}	0.15 ± 0.03^{a2}	0.07 ± 0.01^{a2}	0.11 ± 0.02^{a2}
25	38.79 ± 1.01^{b1}	0.24 ± 0.04^{a2}	0.09 ± 0.04^{ab3}	0.29 ± 0.02^{b2}
50	21.29 ± 0.98^{c1}	1.46 ± 0.00^{b2}	0.21 ± 0.03^{b3}	0.38 ± 0.06^{b3}

Table 5. ^{14}C-Phenanthrene extracted by HPCD solutions from soils amended with 0% black carbon and 1% P3-1, CP2, and BP2 after 1, 25 and 50 days of soil–phenanthrene interactions. Values are the % mean (n = 3) ± standard error of the mean (SEM). At each time point, values in the same column followed by the same letter are statistically similar; values in the same column generated from the extraction assays with the same HPCD solution followed by the same Greek letter are statistically similar; values in the same row followed by the same number are statistically similar (student t-test and ANOVA Tukey test, n = 3, $p < 0.05$).

Ageing period (days)	HPCD solution	Black carbon treatment			
		0% BC	1% P3-1	1% CP2	1% BP2
1	dH_2O	$74.16 \pm 0.39^{a\alpha 1}$	$0.08 \pm 0.08^{a\alpha 2}$	$0.08 \pm 0.02^{a\alpha 2}$	$0.10 \pm 0.10^{a\alpha 2}$
	pH 7	$74.96 \pm 0.80^{a\alpha 1}$	$1.37 \pm 0.07^{b\alpha 2}$	$1.03 \pm 0.21^{b\alpha 3}$	$1.10 \pm 0.14^{b\alpha 3}$
	pH 8	$72.70 \pm 1.46^{a\alpha 1}$	$0.05 \pm 0.05^{a\alpha 2}$	$0.11 \pm 0.08^{a\alpha 2}$	$0.11 \pm 0.11^{a\alpha 2}$
25	dH_2O	$14.29 \pm 1.05^{a\beta 1}$	$0.01 \pm 0.01^{a\alpha 2}$	$0.06 \pm 0.06^{a\alpha 2}$	$0.02 \pm 0.02^{a\alpha 2}$
	pH 7	$24.71 \pm 1.35^{b\beta 1}$	$1.56 \pm 0.17^{b\alpha 2}$	$1.11 \pm 0.09^{b\alpha 3}$	$1.01 \pm 0.17^{b\alpha 3}$
	pH 8	$31.69 \pm 0.06^{c\beta 1}$	$0.20 \pm 0.12^{a\alpha 2}$	$0.20 \pm 0.13^{a\alpha\beta 2}$	$0.13 \pm 0.10^{a\alpha 2}$
50	dH_2O	$3.76 \pm 0.35^{a\gamma 1}$	$0.24 \pm 0.15^{a\alpha 2}$	$0.05 \pm 0.01^{a\alpha 2}$	$0.08 \pm 0.06^{a\alpha 2}$
	pH 7	$8.90 \pm 0.89^{b\gamma 1}$	$0.52 \pm 0.07^{a\beta 2}$	$0.09 \pm 0.07^{a\beta 3}$	$0.05 \pm 0.04^{a\beta 3}$
	pH 8	$12.55 \pm 0.28^{c\gamma 1}$	$0.64 \pm 0.33^{a\alpha 2}$	$0.61 \pm 0.12^{b\beta 2}$	$0.44 \pm 0.06^{b\alpha 2}$

Table 6. Ratios of the amounts of extracted ^{14}C-activity to that of mineralised ^{14}C-activity in BC-amended soils.

HPCD solution	Ageing period (days)	BC treatment		
		1% P3-1	1% CP2	1% BP2
dH2O	1	0.55	1.14	0.85
	25	0.05	0.68	0.07
	50	0.26	0.06	0.42
pH 7	1	9.30	15.18	9.73
	25	6.50	12.36	3.49
	50	0.48	0.75	1.15
pH 8	1	0.31	1.61	1.01
	25	0.83	2.22	0.45
	50	0.67	2.73	1.78

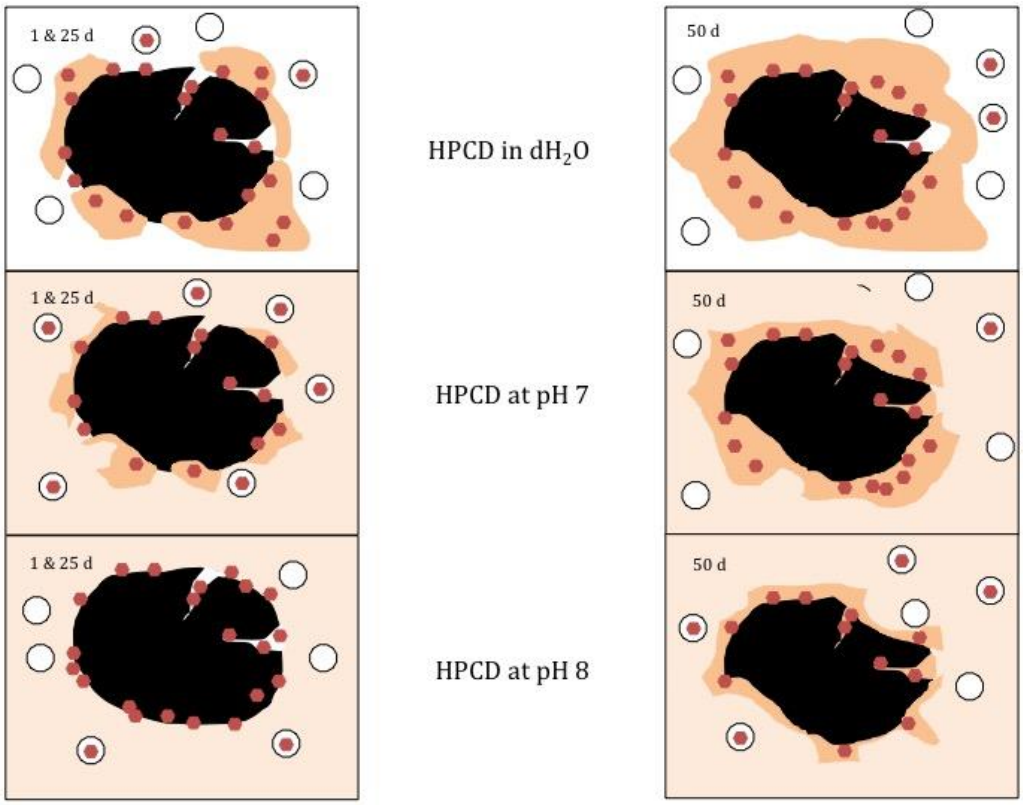

Figure 1. Proposed mechanism for the biphasic feature of increasing pH on extractive capability of HPCD solutions at 1 and 25 d, and the absence of this feature at 50 d. At 1 and 25 d, HPCD at pH 8 dissolved large quantity of BC-associated SOM and exposed sorption sites on BC to phenanthrene, leading to greater sorption of phenanthrene to BC particles. At 50 d, more SOM and phenanthrene was attached to BC, HPCD at pH was more capable of dissolving SOM and therefore released more BC- and SOM- bound phenanthrene.

4.6 References

Alexander, M. 1999. Biodegradation and bioremediation. Academic Press.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2006. Prediction of mono- and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction. Environmental Pollution, 144, 562–571.

Bosma, T.N.P., Middeldorp, P.J.M., Schraa, G. & Zehnder, A.J.B. 1997. Mass transfer limitation of beiotransformation: Quantifying bioavailability. Environmental Science & Technology, 31, 248–252.

Brand, E., Peijnenburg, W., Goenenberg, B., Vink, J., Lijzen, J., ten Hulscher, D., Jonker, C., Romkens, P. & Roex, E. 2009. Towards implementation of bioavailability measurements in the Dutch regulatory framework. National Institute for Public Health and the Environment.

Burgess, R.M. & Lohmann, R. 2004. Role of black carbon in the partitioning and bioavailability of organic pollutants. Environmental Toxicology and Chemistry, 23, 2531–2533.

Cerniglia, C.E. 1993. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3, 351–368.

Chiou, C.T., Malcolm, R.L., Brinton, T.I. & Kile, D.E. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environmental Science & Technology, 20, 502–508.

Cho, Y.-M., Smithenry, D.W., Ghosh, U., Kennedy, A.J., Millward, R.N., Bridges, T.S. & Luthy, R.G. 2007. Field methods for amending marine sediment with activated carbon and assessing treatment effectiveness. Marine Environmental Research, 64, 541–55.

Chun, Y., Sheng, G., Chiou, G.T. & Xing, B. 2004. Compositions and sorptive properties of crop residue-derived chars. Environmental Science & Technology, 38, 4649–4655.

Di Corcia, A., Caracciolo, A.B., Crescenzi, C., Giuliano, G., Murtas, S. & Samperi, R. 1999. Subcritical water extraction followed by liquid chromatography mass spectmmetry for determining terbuthylazine and its metabolites in aged and incubated soils. Environmental Science & Technology, 33, 3271–3277.

Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A. & Van Noort, P.C.M. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environmental Science & Technology, 39, 6881–6895.

Crescenzi, C., Di Corcia, A., Nazzari, M. & Samperi, R. 2000. Hot phosphate-buffered water extraction coupled on-line with liquid chromatography/mass spectrometry for analyzing contaminants in soil. Analytical Chemistry, 72, 3050–5.

Cuypers, C., Pancras, T., Grotenhuis, T. & Rulkens, W. 2002. The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere, 46, 1235–1245.

Dandie, C.E., Weber, J., Aleer, S., Adetutu, E.M., Ball, A.S. & Juhasz, A.L. 2010. Assessment of five bioaccessibility assays for predicting the efficacy of petroleum hydrocarbon biodegradation in aged contaminated soils. Chemosphere, 81, 1061–1068.

Dew, N.M., Paton, G.I. & Semple, K.T. 2005. Prediction of [3-14C]phenyldodecane biodegradation in cable insulating oil-spiked soil using selected extraction techniques. Environmental Pollution, 138, 316–323.

Doick, K.J., Clasper, P.J., Urmann, K. & Semple, K.T. 2006. Further validation of the HPCD-technique for the evaluation of PAH microbial availability in soil. Environmental Pollution, 144, 345–354.

Doick, K.J., Dew, N.M. & Semple, K.T. 2005. Linking catabolism to cyclodextrin extractability: Determination of the microbial availability of PAHs in soil. Environmental Science & Technology, 39, 8858–8864.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Doick, K.J. & Semple, K.T. 2003. The effect of soil:water ratios on the mineralisation of phenanthrene: LNAPL mixtures in soil. FEMS Microbiology Letters, 220, 29–33.

Grybos, M., Davranche, M., Gruau, G., Petitjean, P. & Pédrot, M. 2009. Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. Geoderma, 154, 13–19.

De Haan, H., De Boer, T., Kramer, H.A. & Voerman, J. 1982. Applicability of light absorbance as a measure of organic carbon in humic lake water. Water Research, 16, 1047–1050.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

Hickman, Z.A. & Reid, B.J. 2005. Towards a more appropriate water based extraction for the assessment of organic contaminant availability. Environmental Pollution, 138, 299–306.

Hickman, Z.A., Swindell, A.L., Allan, I.J., Rhodes, A.H., Hare, R., Semple, K.T. & Reid, B.J. 2008. Assessing biodegradation potential of PAHs in complex multi-contaminant matrices. Environmental Pollution, 156, 1041–1045.

Johnsen, A.R., Wick, L.Y. & Harms, H. 2005. Principles of microbial PAH-degradation in soil. Environmental Pollution, 133, 71–84.

Jones, K.C., Alcock, R.E., Johnson, D.L., Semple, K.T. & Woolgar, P.J. 1996. Organic chemicals in contaminated land : analysis, significance and research priorities. Land Contamination and Reclamation, 4, 189–198.

Jonker, M.T.O., Hoenderboom, A.M. & Koelmans, A.A. 2004. Effects of sedimentary sootlike materials on bioaccumulation and sorption of polychlorinated biphenyls. In: Environmental Toxicology and Chemistry, pp. 2563–2570.

Jonker, M.T.O. & Koelmans, A.A. 2002. Extraction of polycyclic aromatic hydrocarbons from soot and sediment: Solvent evaluation and implications for sorption mechanism. Environmental Science & Technology, 36, 4107–4113.

Lerch, R.N., Thurman, E.M. & Kruger, E.L. 1997. Mixed-mode sorption of hydroxylated atrazine degradation products to sell: A mechanism for bound residue. Environmental Science & Technology, 31, 1539–1546.

Lohmann, R., Macfarlane, J.K. & Gschwend, P.M. 2005. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York Harbor sediments. Environmental Science & Technology, 39, 141–148.

Luo, L., Zhang, S., Zhang, L. & Christie, P. 2011. Effect of phosphate on phenanthrene sorption in soils. Journal of Colloid and Interface Science, 353, 275–280.

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D. & Semple, K.T. 2001. A simple 14C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. FEMS Microbiology Letters, 196, 141–146.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science & Technology, 34, 3174–3179.

Rhodes, A.H., Carlin, A. & Semple, K.T. 2008. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science & Technology, 42, 740–745.

Rhodes, A.H., McAllister, L.E., Chen, R. & Semple, K.T. 2010. Impact of activated charcoal on the mineralisation of 14C-phenanthrene in soils. Chemosphere, 79, 463–469.

Rhodes, A.H., Riding, M.J., McAllister, L.E., Lee, K. & Semple, K.T. 2012. Influence of activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil. Environmental Science & Technology, 46, 12445–12451.

Sander, M. & Pignatello, J.J. 2007. On the reversibility of sorption to black carbon: Distinguishing true hysteresis from artificial hysteresis caused by dilution of a competing adsorbate. Environmental Science & Technology, 41, 843–849.

Schmidt, M.W.I. & Noack, A.G. 2000. Black carbon in soils and sediments' Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles, 14, 777–793.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Semple, K.T., Riding, M.J., McAllister, L.E., Sopena-Vazquez, F. & Bending, G.D. 2013. Impact of black carbon on the bioaccessibility of organic contaminants in soil. Journal of Hazardous Materials, 261, 808–816.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2008. Linking chemical extraction to microbial degradation of 14C-hexadecane in soil. Environmental Pollution, 156, 474–481.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Wang, X. & Brusseau, M.L. 1995. Simultaneous complexation of organic compounds and heavy metals by a modified cyclodextrin. Environmental Science & Technology, 29, 2632–2635.

Xia, X., Li, Y., Zhou, Z. & Feng, C. 2010. Bioavailability of adsorbed phenanthrene by black carbon and multi-walled carbon nanotubes to Agrobacterium. Chemosphere, 78, 1329–1336.

Yang, Y., Chun, Y., Shang, G. & Huang, M. 2004. pH-dependence of pesticide adsorption by wheat-residue-derived black carbon. Langmuir, 20, 6736–6741.

Yang, Y. & Sheng, G. 2003. Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. Environmental Science & Technology, 37, 3635–3639.

Chapter 5

Enhancement of ^{14}C-phenanthrene mineralisation in the presence of plant-root biomass in PAH-NAPL amended soil

Enhancement of ^{14}C-phenanthrene mineralisation in the presence of plant-root biomass in PAH-NAPL amended soil

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5.1 Abstract

The study of the interactions between plants and soil microorganisms has been described in terms of bioremediation over the past 30 years. This subject has been summarized as the process where organic contaminants can be removed from the soil through the interaction between roots and catabolic microbial populations. This study assessed the tolerance of different plant species against PAH-NAPL contaminated soil and the feasibility of the use of their roots to promote the biodegradation of ^{14}C-phenanthene. Toxicity results showed that seedling germination was not affected by the presence of PAHs. Furthermore, mineralisation of ^{14}C-phenanthrene was significantly enhanced by the addition of roots after at least two weeks incubation. Moreover, bacterial numbers did not show a significant relationship with ^{14}C-phenanthrene mineralisation. Results suggest that the higher mineralisation of ^{14}C-phenanthrene might be the result of an increased bioavailability of the contaminant and the contribution of carbon and nutrients from root decay and turnover, rather to an increase on the bacterial numbers as is usually assumed.

Keywords: Plant-enhanced, mineralisation, PAHs, diesel, roots, NAPL

5.2 Introduction

Soil bioremediation can be promoted by different biological mechanisms through the action of microorganisms, soil invertebrates and plants. Where plants are involved, this is commonly known as phytoremediation or plant-assisted remediation, which has been defined as the use of higher plants to treat or stabilise contaminated soil (Wenzel,

2009). A key partnership in the removal of pollutants from soil involves plants and plant roots enhancing microbial degradation in soil, though the mechanisms in which plants stimulate microbial degradation is not fully understood (Anderson *et al.*, 1993; Pilon-Smits, 2005). The specific mechanisms of the biodegradative processes are likely to vary between plant, microbial species and pollutant type due to a number of physico-chemical and environmental factors (Pilon-Smits, 2005)(Pilon-Smits, 2005).

Several plant species have been tested to examine their potential for phytoremediation of organic contaminants under specific soil conditions (Muratova *et al.*, 2008; Rezek *et al.*, 2008). So far, grasses such as fescue (*Festuca* sp.) and ryegrass (*Lolium* sp.), along with legumes (e.g. *M. sativa*) have shown to be suitable options to clean up organic contaminants from the soil. These two functional groups are often selected due to their extensive fibrous root structure, which increases the soil-rhizosphere contact surface and penetration capacity (Aprill & Sims, 1990; Pilon-Smits, 2005), and their role on the fixation of nitrogen from the atmosphere into the soil (Wenzel, 2009), respectively. Moreover, it is also needed to take into consideration that these species should be able to germinate and grow in highly contaminated soil, and in most cases under nutrient limiting conditions (Wenzel, 2009; Moubasher *et al.*, 2015). It is generally agreed that a preliminary and effective way to test the phytoremediation potential of specific plant species is through phytotoxicity tests (Trapp & Karlson, 2001; Muratova *et al.*, 2008). These types of assays use germination and early growth assessments as endpoints to determine suitability for growth in contaminated soil. For remediation of PAHs, phytotoxicity assays have become a useful tool to assess plant tolerance to specific soil conditions (Rorison & Rorbinson, 1986; Baek *et al.*, 2004).

Phytotoxicity assays are usually done alongside with the quantification of initial and final concentrations of the targeted pollutant (e.g. Aprill & Sims (1990); Günther *et al.* (1996); Joner *et al.* (2001); Miya & Firestone (2001); Corgié *et al.* (2003); Olson *et al.* (2007); Rezek *et al.* (2008); Thompson *et al.* (2008); Smith *et al.* (2011) for either single or mixtures of PAHs). Furthermore, other approaches to test the effect of plants on the removal of organic contaminants from soil have not been explored as extensively as the initial/final concentration assessment. Among these, only a small number of studies have been reported where ^{14}C-labelled pollutants were used to test the potential of plant-associated microbial communities to mineralize hydrocarbons.

This approach offers a more realistic and accurate assessment of the fate and behaviour of a specific pollutant, avoiding taking into account other sources of carbon being simultaneously used by the soil microorganisms. For example, Yoshitomi & Shann (2001) used exudates from corn (*Zea mays* L.) to look at the mineralisation of ^{14}C-pyrene in rhizospheric and root-amended soil over 80 days; they found no significant differences between these two conditions, suggesting that the catabolic enhancing factor was root exudates and not the whole root system. Mueller & Shann (2007) studied the ^{14}C-pyrene mineralisation in soil amended with red maple root extracts and the influence of inorganic nutrient addition. In this case, rates of mineralisation were greater when rhizosphere soil was used; however, these were inhibited by inorganic nutrient amendments (Mueller & Shann, 2007). Moreover, Oyelami *et al.* (2013) studied not only the effect of plant species in the mineralisation of ^{14}C-phenanthrene, but they also looked at how species diversity, composition and soil fertility could enhance the degradation of ^{14}C-phenanthrene in soil. The authors found that one of the most important elements was the soil fertility, leaving the plant type and composition as secondary factors (Oyelami *et al.*, 2013)(Oyelami et al., 2013).

The hypothesis for this investigation was that the presence of plant roots in PAH-NAPL contaminated soil enhances the mineralisation of ^{14}C-phenanthrene. To address this, the aims were (1) to assess the tolerance of different plant species against highly contaminated soil, and (2) to use the roots from the most resistant species as soil amendment to promote the biodegradation of ^{14}C-phenanthrene, assessed through its mineralisation. Results from this study will allow assessing in a more accurate and controlled manner any possible PAH underestimations often misinterpreted as pollutant dissipation within plant-enhanced bioremediation assays. Additionally, findings will contribute to the understanding of the influence of root decay and turnover on the catabolic response of microbial communities in PAH-NAPL contaminated soil.

5.3 Material and methods

5.3.1 Phytotoxicity testing

5.3.1.1 Soil spiking procedure

A clay loam soil with an organic matter content of 2.7 % was collected from Myerscough, Preston, U.K., a complete characterisation is described elsewhere (Towell *et al.*, 2011a). Following wet sieving (25% moisture content) through a 2 mm mesh, soil was spiked with a molecularly balanced mixture of phenanthrene (Phe), benzo[a]anthracene (B[a]A), and benzo[a]pyrene (B[a]P) to deliver final total concentrations of 100 and 300 mg kg^{-1}, and 0.1% (w/w) diesel using acetone:toluene (1:1) as a carrier. Soil preparation and spiking procedure followed the methods described by Doick *et al.* (2003) for the introduction of PAHs into wet soil using a stainless-steel spoon and a soil inoculum approach.

5.3.1.2 Seedling emergence and early growth

The seedling emergence and early growth studies included 9 plant species (Table 1) using 8 different soil treatments (n = 3) and 4 controls (Table 2) with a total of 324 experimental units in a completely randomised arrangement. Methodology followed the guidelines 208 (OECD, 2006) and OCSPP 850.4100 (U. S. Environmental Protection Agency, 2012), both focused on the study of ecological effects of chemicals in the environment. Given that these guidelines were originally developed for pesticide testing, minor modifications were made based on specific phytoremediation assays (Banks *et al.*, 2003; Gao & Zhu, 2004; Phillips *et al.*, 2006; Smith *et al.*, 2006; Muratova *et al.*, 2009b). In short, 50 g of spiked soil was placed into 9 mm diameter pot with a disc of filter paper at the bottom of each to prevent soil loss from the draining holes. Further, petri dishes were placed under each pot to control any leachate and prevent cross-contamination. In each pot, 10 seeds of the appropriate species (Table 1) were sown at equal distances from each other on soil surface. The pots were placed in a glasshouse to complete a 4-week growing period at ~25 ºC with a 16/8 h photoperiod. At an initial stage, pots were covered with a petri dish to promote germination and watered daily from the top to prevent seed dryness, recording the daily germination rate. Once 50% of the control seedlings of each species emerged, the seedling emergence test was considered finished, and the early

growth assessment started. As the different plant species had different germination times, pots from each species was considered to be part of an independent experiment. The endpoints of the growth test were assessed weekly over a period of 21 days after 50 % emergence of the control pots as survival. During the growth test, plants were watered from the bottom. At the end of the assay above and below ground material was harvested and oven dried for 72 h at 60 ºC (Langer et al., 2010).

5.3.2 Rhizo-enhanced ^{14}C-phenanthrene mineralisation in soil

5.3.2.1 Root amendments and soil preparation

Based on the results from the germination and early growth test (Section 7.4.1), four of the species were identified as resistant to the PAH-NAPL mixture due to none or minimal detrimental effects when compared against the control treatments at any of the PAHs concentrations. Seeds of these species; two grasses (*Sorghum bicolor* L., *Lolium perenne* L.) and two legumes (*Glycine max* L. and *Medicago sativa* L.), were sown into non contaminated soil from the same location as the one used for the phytotoxicity stage. Seeds were grown in a glasshouse under standard growing conditions (U. S. Environmental Protection Agency, 2012) for 21 days after seed emergence. After the growing period, roots were harvested and cleaned by three consecutive washes with sterile deionized water (Miya & Firestone, 2001). Each wash consisted on mixing the roots with 100 ml sterile deionized water for 30 minutes on an orbital shaker (Muratova *et al.*, 2003) at 70 rpm. Finally, the clean roots were blotted dry, cut into 1-2 mm pieces, and ground. The ground root tissue was mixed with distilled water (Mueller & Shann, 2007) forming an homogeneous paste and added to the ^{12}C spiked soil according to the treatments shown in Table 3 to get a final root amendment content of 0.5 % (dry weight based). Additionally, control soil without ^{12}C PAHs were also included, these had as a main objective to allow a clear visualisation of how the absence of PAHs affect the mineralisation process under the different tested conditions.

5.3.2.2 ^{14}C-Phenanthrene mineralisation in soil

The ^{12}C-PAHs spiked and root amended soil was stored in amber jars at 21 ± 1 ºC in the dark over 28 days to simulate ageing. To test how the root amendment affected ^{14}C–phenanthrene mineralisation rates over the time, three contact times were selected

to conduct a respirometric assay following the method described by Doick & Semple (2003) and Semple *et al.* (2006). At 1, 14 and 28 d after ^{12}C spiking and root amendment, 10 g (dry wt) incubated soil from each treatment (Table 3) was placed into 250 ml modified Teflon lined screw cap-Schott bottles (respirometers) in triplicate. Each respirometer also contained 30 ml of sterilized minimal basal salts (MBS) medium (Fenlon *et al.*, 2007) to give a soil:liquid ratio of 1:3 (Doick & Semple, 2003), and incorporated a suspended 7 ml glass scintillation vial containing 1 ml 1 M NaOH solution which served as a $^{14}CO_2$ trap. At every time point, each respirometer was spiked with $^{12/14}C$-phenanthrene (10 mg kg $^{-1}$ / 56 Bq g $^{-1}$, dry wt). Respirometers were incubated at 21 ± 1 ºC in the dark onto an orbital shaker at 100 rpm for 14 days. The ^{14}C-activity in the $^{14}CO_2$ traps was assessed every 24 h by replacing the vial containing NaOH and adding 5 ml Goldstar liquid scintillation cocktail. After storage in the dark for 24 h, the ^{14}C-activity was quantified using a Canberra Packard Tricarb 2250CA liquid scintillation analyser. An analytical blank (not contaminated soil without ^{14}C-phenanthrene) was also set up to establish the level of background activity. The endpoints of this assay were the lag phase (defined as the period of time before mineralisation reached 5 %), maximum rate (determined by the amount of $^{14}CO_2$ produced at any 24 h period of time), and the maximum extent of mineralisation after 14 days (Fenlon *et al.*, 2007).

5.3.2.3 Microbial enumeration

Total heterotrophic and PAH degrading bacteria for each treatment was quantified by assessing the number of colony forming units (CFUs) following standard serial dilutions. Plates were prepared using plate count agar (PCA), and general purpose agar (GPA) amended with phenanthrene (0.2 %) and nutrients respectively, and incubated at 21 ± 1 ºC in the dark. Microbial enumeration was assessed before and after the mineralisation experiment as recommended by Oyelami *et al.* (2013).

5.3.3 Statistical analysis

Statistical analysis was done using IBM SPSS Statistics software version 21. In all cases, normality of the residuals was verified using the Shapiro-Wilk test with a 95 % confidence interval. In cases where data did not show a normal distribution, different data transformations were tested before using a non-parametric test. Data with normal distribution was analysed using an ANOVA analysis of variance and Tukey HSD post

hoc test. When data was not normally distributed and no transformation was possible, Kruskal-Wallis mean rank of the group's analysis was used. Paired *t*-tests were used to test differences between time points.

5.4 Results

5.4.1 Phytotoxicity test

5.4.1.1 Seedling emergence and early growth as survival

The impacts of the chemicals on seedling emergence were investigated (Figure 1). It was found that the germination of *F. rubra*, *C. angustifolium* and *P. lanceolata* in the growth control (C_g) was less than 50%. Therefore, these have not been included in any further analysis. Overall, the number of seedlings that germinated after 7 d was not affected by the presence of PAHs or the other amendments. Only *T. repens* displayed significant differences across the different treatments (F = 3.546, p = 0.005), presenting a significantly lower germination in soil without (T1, p = 0.040) and with (C_d, p = 0.026) diesel (Figure 1).

None of the species showed significant differences in survival within the different treatments ($p > 0.05$). Nonetheless, the lowest survival at the end of the experiment was presented by *T. repens* and *A. millefolium* when sown in the diesel control (C_d), suggesting the sensitivity of these two species towards diesel. Moreover, *S. bicolor* displayed the highest overall survival, being the only species to reach 100 %.

5.4.1.2 Biomass

For both above and belowground biomass (dry wt), out of the six tested species, only *M. sativa* and *G. max* were not significantly affected ($p > 0.05$) by any of the treatments (SI 1). Furthermore, shoot and root biomass of *L. perenne*, *S. bicolor* and *T. repens* showed significant differences across the soil conditions. *L. perenne* had significantly higher shoot biomass when PAHs were present in the soil (F = 3.3776, p = 0.003), while only one treatment (T1) significantly enhanced root biomass (F = 6.534, $p < 0.001$). *S. bicolor* aboveground biomass was significantly reduced by the presence of PAHs (F = 5.663, $p < 0.001$), while the root biomass was promoted when grown on contaminated soil (F = 2.758, p = 0.019). *A. millefolium* only showed

significant differences regarding root biomass, displaying a significant reduction (F = 5.250, p = 0.001) on treatments with higher PAH concentrations.

Root/shoot ratio, was affected in three of the six tested species (SI 2). *L. perenne* showed an increased ratio in the treatment containing 100 mg kg^{-1} and diesel (T1), and only diesel (C_d) when compared against the growth control (F = 5.999, $p < 0.001$). Similarly, *S. bicolor* (F = 3.395, p = 0.006) and *T. repens* (F = 2.766, p = 0.019) also presented significantly higher ratios in the presence of contaminants.

5.4.2 Mineralisation of ^{14}C-phenanthrene in soil

The mineralisation of ^{14}C-phenanthrene was measured in soils under different conditions (Figures 2 – 5 and SI 3). For the treatment in which soil was spiked with the mixture of PAHs only (Treatment A, Figure 2), there were significand differences at each time point. Nonetheless, specific effects were different throughout the incubation time: one day after spiking and root amendment (Figure 2a), *L. perenne* amended soil showed a significantly shorter lag phase than the rest of the treatments (F = 13.851, p = 0.001), while significant higher total extents (F = 19.473, $p < 0.001$) and maximum rates of mineralisation (F = 5.531, p = 0.016) were observed in soil without root amendments. After 14 d of incubation (Figure 2b), lag phases were significantly faster (F = 39.337, $p < 0.001$) and total extents higher (F = 42.555, $p <$ 0.001) in the soils containing root amendments. In the soils incubated for 28 d, trends observed after 14 days continued; the lag phase (F = 49.468, $p < 0.001$) was significantly shorter in the *L. perenne* amendment compared the non-amended treatment (Figure 2c). Maximum rates (F = 69.700, $p < 0.001$) and total extents of mineralisation (F = 85.097, $p < 0.001$) were also significantly higher in all root-amended treatments compared to the non-amended soil.

When 0.1% (dry wt) activated charcoal (Treatment B) was added to the PAH mixture, statistically significant differences were observed at each of the three sampling times (Figure 3). One day after spiking, *S. bicolor* amendment had a negative effect on the ^{14}C-phenanthrene mineralisation; this is reflected in a longer lag phase than the rest of the treatments (F = 3.973, p = 0.004). Additionally, although the maximum rates of mineralisation were similar across treatments (F = 2.960, p = 0.090), total extents of mineralisation from the treatment amended with *G. max* and the rootless soil were

significantly higher (F = 15.653, p = 0.001) than the grass-amended treatments (*L. perenne* and *S. bicolor*). After 14 days of incubation (Figure 3b), lag phases were longer in the absence of roots compared to the root-amended treatments (F = 12.586, p = 0.003). At this same time point, significant increases in the maximum rates of mineralisation (F = 8.863, p = 0.008) were observed when soil was amended with *G. max* roots. The total extents of mineralisation were also significantly increased in the presence of roots from all of the species (F = 124.072, $p < 0.001$). After 28 days of incubation (Figure 3c), significant differences were found for the maximum rates and total extents of mineralisation ($p < 0.05$). The maximum rates in soils containing *L. perenne* were significantly higher than the other treatments (F = 25.505, $p < 0.001$), while total extents were enhanced in the presence of roots from any species compared to the non-amended soil in all the cases (F = 65.824, $p < 0.001$).

To observe the effect that a NAPL can have on the mineralisation of ^{14}C-phenanthrene (Treatment C), 0.1% (w/w) diesel was added to the PAH mixture (Figure 4). By the first and second sampling times of this treatment (Figure 4a-b), lag phases were significantly shorter in the treatments amended with grass roots after 1 day (F = 23.017, p = 0.002), and on not root-amended soil after 14 d (F = 6.832, p = 0.011). In contrast, maximum rates and total extents of mineralisation remained unaffected in the other treatments ($p > 0.05$). By the end of the 28 d incubation (Figure 4c), the lag phases were significantly shorter in the *L. perenne* condition (F = 12.199, p = 0.003) compared to the rest of the treatments. Both maximum rates (F = 24.143, $p < 0.001$), and total extents of mineralisation (F = 26.117, $p < 0.001$) were significantly greater in all root-amended treatments compared to the control, with the highest values measured in the *L. perenne* condition.

The effect of possible interactions between PAHs, diesel and activated charcoal was also measured (Figure 5). At the first time point (Figure 5a), *M. sativa* and *S. bicolor* amended treatments showed significantly shorter lag phases (F = 19.154, p = 0.003). Moreover, total extents of mineralisation were significantly reduced by the presence of roots of any species (F = 21.273, p = 0.002), while maximum rates of mineralisation were not affected by the addition of roots. After 14 days (Figure 5b), the lag phases were significantly different (F = 14.017, $p < 0.001$), being shorter in both the soil amended with *L. perenne*, as well as the non-amended soil. Maximum

rates were also influenced by the treatments; exhibiting highest maximum rates when roots of any of the species were added to the soil (F =11.381, p = 0.001). In contrast, total extents of mineralisation were unaffected by the different treatments. After 28 days incubations, all mineralisation endpoints: lag phases (F = 17.077, p < 0.001), maximum rates (F = 47.729, p < 0.001), and total extents (F = 11.832, p = 0.001) were significantly enhanced by the addition of any of the roots (Figure 5c).

Controls measuring the mineralisation of ^{14}C-phenanthrene in the absence of ^{12}C-PAHs were also assessed in pristine soil, as well as in soils containing activated charcoal and diesel amendments (SI 4). Overall, controls behaved in a similar way after 1 and 14 d incubation. Most significant differences were observed after 14 d, where soil without root amendment often displayed shorter lag phases, especially when *L. perenne* was added. Such are the cases of the pristine soil (F = 13.645, p = 0.002), soil with activated charcoal (F = 246.58, p < 0.001), and solvent amended soil (F = 22.061, p < 0.001). However, after 28 d incubation, maximum rates of mineralisation were significantly faster when *L. perenne* was used as amendment in pristine (F = 4.592, p = 0.033), diesel (F = 3.935, p = 0.023), and the mixture of activated charcoal and diesel controls (F = 4.618, p = 0.035). Total extents of mineralisation were also significantly higher in three of the conditions by the addition of roots from *L. perenne*, with the exception of the control amended with a mixture of diesel and activated charcoal (F = 1.106, p = 0.417). Lag phases presented differences in all treatments, with significantly shorter values in the absence of roots for the pristine soil (F = 160.66, p < 0.001), and individual amendments of diesel (F = 11.683, p = 0.001) and activated charcoal (F = 227.18, p < 0.001).

5.4.3 Enumeration of bacteria

Microbial numbers were significantly influenced by all treatments and conditions (p < 0.05). Both heterotrophic and phenanthrene degrading bacteria (CFU g^{-1}soil dw) significantly increased after the addition of ^{14}C-phenanthrene at all time points (p < 0.01), with the exception of the phenanthrene degrading bacteria at the last sampling time (28 d).

The numbers of heterotrophic bacteria in soil before the mineralisation assay were influenced by the different contaminant conditions, as well as by the root amendments

at the different time points (SI 5). One day after spiking, the control pristine soil showed that root amendments increased the numbers of heterotrophic bacteria (F = 131.952, $p < 0.001$), especially by *G. max*. This same effect was also observed in treatments containing diesel (Treatments C and D). After 14 days, heterotrophic bacterial numbers were significantly reduced compared to the first time point (t = 5.774, $p < 0.001$). CFUs from pristine soil displayed the largest reduction, especially when roots from *L. perenne* and *G. max* were present. Furthermore, all soil conditions displayed the highest microbial numbers in the presence of either one of the grass species (*L. perenne* or *S. bicolor*). Finally, after 28 days incubation, heterotrophic bacterial numbers were not significantly different to those measured after 14 days (t = -1.846, p = 0.068).

Numbers of phenanthrene degrading bacteria were also significantly influenced by the different soil conditions and root amendments ($p < 0.05$). One day after spiking, roots from legumes (*M. sativa* and *G. max*) increased the CFUs in the grass- and non-amended treatments with the exception of the diesel and solvent controls. After 14 days incubation, most soil conditions excluding the mixture of PAHs, activated charcoal and diesel (Treatment D), contained significantly higher numbers of phenanthrene degrading bacteria in the presence of roots ($p < 0.05$). After 28 days incubation, the same trend was observed with decreased microbial numbers in the absence of plant species and an enhanced presence of bacteria in soil amended with *M. sativa* roots ($p < 0.05$).

Overall, heterotrophic bacteria from "respirometers" were significantly decreased over time ($p < 0.05$). Initially, all treatments contained significantly higher bacterial numbers ($p < 0.05$) in the presence of plant roots, exhibiting consistently higher values when *M. sativa* was used. After 14 days, the opposite trend was observed, with the exception of the treatments containing activated charcoal (Treatments B and D), where root-amended soil presented increased microbial numbers but without specific pattern regarding plant identity. This effect was also observed in the control amended only with diesel (Treatment G). After the 28 d sampling, root amendments showed to significantly enhance three out of the nine conditions. This trend was observed on the treatment containing PAHs and activated charcoal (Treatment B, F = 376.55, p = 0.001), the one including a mixture of PAHs, diesel and activated charcoal (Treatment

D, $F = 22.089$, $p = 0.002$), and the control for diesel (Treatment G, $F = 17.493$, $p = 0.004$). In the rest of the conditions, as observed before, root amendments reduced the heterotrophic CFUs.

The phenanthrene degrading bacteria from "respirometers" was only affected by the plant amendments in the first two sampling points (1 and 14 d). In both cases, *L. perenne* roots consistently produced a significantly larger number of CFUs ($p < 0.05$). At the final sampling point of the mineralisation assay (28 d), treatments without root amendments presented significantly fewer bacteria than any of the root amended treatments ($p < 0.05$). Overall results also showed that *L. perenne* and *M. sativa* amendments produced the highest CFUs numbers across the different soil conditions.

5.5 Discussion

5.5.1 Phytotoxicity of PAH-NAPL contaminated soil

Different phytotoxicity parameters can be used to assess the ability of a plant to germinate and grow in contaminated soil (Maila & Cloete, 2002). For instance, seedling emergence cannot always be used as a reliable endpoint of phytotoxicity, as seedlings typically obtain nutrients from internal materials at the earliest stages of germination. Nevertheless, when diesel is included, the low molecular weight fractions have been shown to have inhibitory effects on the germination of some species (Henner *et al.*, 1999). In the current study, these detrimental effects were only observed for *T. repens*. The overall lack of toxicity has also been observed previously by Smith *et al.* (2006), who found that germination of different species (including *T. repens*) was unaffected by the presence of these fractions. The absence of phytotoxic effects of hydrocarbons (especially PAHs) on germination/seedling emergence of different species has been also reported by multiple authors, including Maliszewska-Kordybach & Smreczak (2000) and Hamdi *et al.* (2012) and Hamdi et al. (2012), who also attributed this absence of toxicity to the low bioavailability of these hydrophobic chemicals.

The phytotoxicity assessment, based on seedling survival, showed that *S. bicolor* was the most suitable species to be grown in a PAH-NAPL amended soil. Previous studies have shown similar results; for example, (Muratova *et al.*, 2009b) observed an

increased survival of *S. bicolor* at 10 mg kg^{-1} phenanthrene compared to the control uncontaminated soil. This result gains importance considering that *S. bicolor* has also been found to enhance the bioremediation process of crude oil contaminated soil (Banks *et al.*, 2003). As a consequence, this species would be able to both germinate and grow in contaminated soil while promoting the remediation of contaminated soil.

Although contaminated soil is generally assumed as toxic for most plant species, there are some reported cases where PAHs have been found to have enhancing effects towards plant growth. For instance, Maliszewska-Kordybach & Smreczak (2000)

observed this trend, reflected on increased root growth of certain grass species. The authors described this behaviour as a feedback control mechanism, which in this case is reflected as an enhanced growth of plants as a response to be exposed to PAHs (Maliszewska-Kordybach & Smreczak, 2000). Overall, the lack of consensus regarding the effect of organic contaminants on germination and growth of plant species could be attributed to the natural variability on seed viability, as well as the specific characteristics of the soil used for the test. These factors have already been suggested by different authors such as Maliszewska-Kordybach & Smreczak (2000), who concluded that soils with sandy textures had greater phytotoxic effects on most of the tested plant species. Additionally other soil properties including organic carbon content, cation-exchange capacity, or specific surface area will also play an important role on the fate and behaviour of organic pollutants (Chung & Alexander, 2002). In the present study, overall seedling survival remained unaffected by the presence of PAHs in *L. perenne, S. bicolor, M. sativa* and *G.max*. The lack of phytotoxic effects of the mixture of PAHs might be the result of the combination of two main factors. First, these four species presented a higher PAH and NAPL tolerance, which has also been reported before (Banks *et al.*, 2003; Muratova *et al.*, 2003; Gao & Zhu, 2004; Phillips *et al.*, 2006). And second, the soil used has been defined as high-content silt and clay soil, which might be producing a higher rate and extent of the pollutant's sequestration hence reducing its bioavailability.

5.5.2 Effect of root amendments in the mineralisation of ^{14}C-phenanthrene

When root biomass from different plant species was added to PAHs contaminated soil, the impact on the mineralisation of ^{14}C-phenanthrene became significantly higher

after 2 weeks incubation for soil containing only with PAHs and after 4 weeks in soil contaminated with a mixture of PAHs and diesel, reaching in some cases total extents of mineralisation close to 100 %. In all cases, studies reporting this same trend have been consistently reported, especially using *L. perenne* and *M. sativa*. These two species have been found to be effective enhancers of the dissipation of PAHs from soil by several authors such as Binet *et al.* (2011), Joner *et al.* (2001) or Phillips *et al.* (2009). Other authors have also observed that higher levels of hydrocarbon biodegradation can be achieved by the use of plants –as a whole or a part of them (Miya & Firestone, 2001; Rentz *et al.*, 2005; Smith *et al.*, 2011; Sun *et al.*, 2011). Moreover, when ^{14}C-isotopes were used, similar results have also been observed. For instance, Reilley *et al.* (1996) consistently found higher mineralisation of ^{14}C-pyrene in simulated rhizospheric soil. Additionally, the temporal factor observed in this study has also been observed by other authors, reporting an initial inhibition on the mineralisation of ^{14}C-pyrene, followed by an increased biodegradation after 100 days of incubation (Mueller & Shann, 2007). This same temporal behaviour was also observed by Macleod & Semple (2002), who observed that microbes required an adaptation period in order to biodegrade ^{14}C-pyrene. In this case, authors suggested that this behaviour could indicate that period of microbial growth was necessary before mineralisation started (Macleod & Semple, 2002).

Even though the specific processes producing this enhanced dissipation of hydrocarbons from soil are not completely understood, the overall consensus is that this effect is due to increases in the numbers of pollutant degrading microbes when plants are introduced into contaminated soil – termed the "rhizosphere effect" (Aprill & Sims, 1990; Günther *et al.*, 1996; Radwan *et al.*, 1998; Binet *et al.*, 2000; Chiapusio *et al.*, 2007; Fan *et al.*, 2008). However, measurement of bacterial numbers in this study did not show a significant positive relationship with ^{14}C-phenanthrene mineralisation for either heterotrophic or phenanthrene-degrading bacteria. Nonetheless, a similar decreasing trend has been reported previously by Nichols *et al.* (1997), concluding that this effect could be the result of an environment with limited amounts of accessible carbon and nutrients. Another reason for this trend could be related to a priming effect, which can be characterized by a fast increase of the microbial populations after a change in the system (soil spiking and amendment) followed by a reduction of the microbial numbers after every time point (Kuzyakov,

2002). *M. sativa* was the only species that produced higher CFUs of phenanthrene degrading bacteria after 14 and 28 days incubation, but with no visible pattern among treatments. This specific behaviour produced by *M. sativa* has previously been reported by Phillips *et al.* (2006), who observed a positive correlation between the mineralisation of ^{14}C-phenanthrene and microbial numbers from soil planted with *M. sativa*. Given the overall findings, the observed higher ^{14}C-mineralisation from soil amended with roots could be related to a putative increase of the bioavailability of the contaminant, but this was not measured directly in this study. However, a similar conclusion has also been proposed by Joner *et al.* (2002), who suggested that rhizospheric soil could produce a change in surface adsorption processes controlling the fate of hydrocarbons in soil (Reilley *et al.*, 1996; Joner *et al.*, 2002). Furthermore, authors have hypothesized that substances produced by roots due to exudation, decay and turnover might also be a contributing factor within the biodegradation process (Rentz *et al.*, 2005; Mueller & Shann, 2007).

5.6 Conclusions

The phytotoxicity findings reported in this study show that the grasses (*L. perenne* and *S. bicolor*) and legumes (*M. sativa* and *G. max*) are the most suitable for plant-enhanced biodegradation, as these are capable of germinating and growing in contaminated soil without showing detrimental effects. When these four species were tested to assess the effect of root tissue on the mineralisation of ^{14}C-phenanthrene, all species produced enhanced mineralisation after two and four weeks of root-soil contact time. This suggests that root amended soil has greater potential to mineralise ^{14}C-phenanthrene than soils lacking plants or root biomass.

Microbial numbers of both heterotrophs and phenanthrene degrading bacteria did not appear to have a significant relationship with the mineralisation parameters. The total CFUs were reduced over time despite the increasing extents in the mineralisation of ^{14}C-phenanthrene. Results from the present study suggest that the increased mineralisation of ^{14}C-phenanthrene might be the result of (1) the enhancement of the bioavailability of the contaminant as well as (2) the contribution of readily available carbon and nutrients from root exudation, decay and turnover, rather than to an increase on the number of bacteria capable to degrade PAHs as is generally assumed.

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Table 1. Test plant species

Family	Species
Pocacea	*Lolium perenne**
	Festuca rubra
	*Sorghum bicolor**
Fabaceae	*Medicago sativa**
	Trifolium repens
	*Glycine max**
Asteraceae	*Achillea millefolium*
Onagraceae	*Chamerion angustifolium*
Plantaginaceae	*Plantago lanceolata*

*Species used for both phytotoxicity and mineralisation assay

Table 2. Summary of treatments for the phytotoxicity assay

Treatment	Characteristics
T1	100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel
T2	100 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal
T3	300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel
T4	300 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal
T5	100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal
T6	300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal
T7	100 mg kg^{-1} Σ PAH
T8	300 mg kg^{-1} Σ PAH
C_d	Diesel control: 0.1% (w/w) diesel
C_{ac}	Activated charcoal control: 0.1 % (dry weight) activated charcoal
C_s	Solvent control: 10 ml kg^{-1} 1:1 toluene:acetone
C_g	Growth control: Clean soil

Table 3. Summary of treatments for the ^{14}Cphenanthrene mineralisation assay

Treatment	Characteristics
A	100 mg kg^{-1} Σ PAH
B	100 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal
C	100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel
D	100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal
E	Pristine soil
F	0.1 % (dry weight) activated charcoal
G	0.1% (w/w) diesel
H	0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal
I	1:1 Toluene : Acetone solution

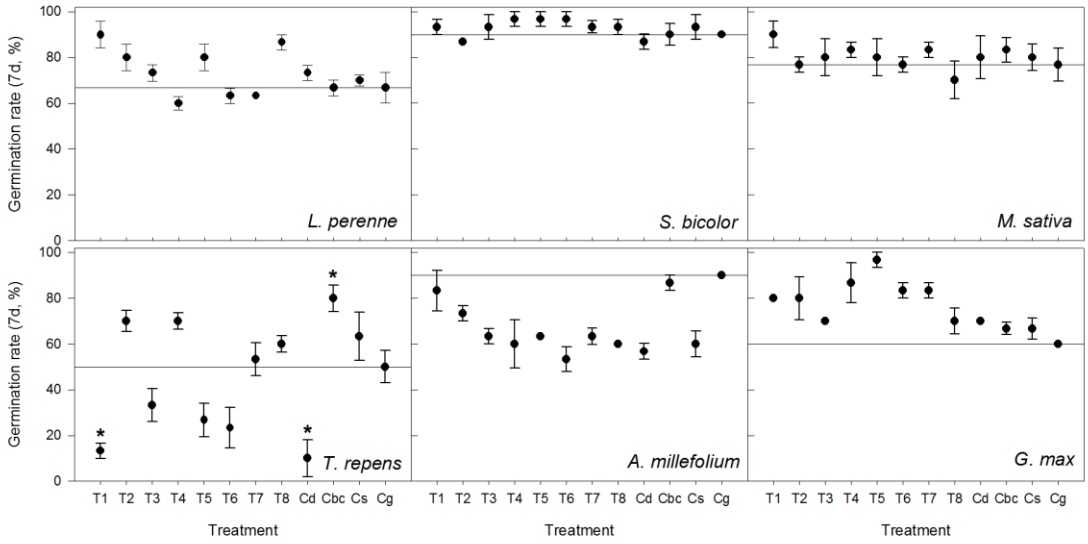

Figure 1. Germination rate estimated at the 7th day after sowing as the percentage of germinated seeds among the seeds sown (Muratova et al., 2008). T1 = 100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel, T2 = 100 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal, T3 = 300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel, T4 = 300 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal, T5 = 100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal, T6 = 300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal, T7 = 100 mg kg^{-1} Σ PAH, T8 = 300 mg kg^{-1} Σ PAH, C_d = Diesel control: 0.1% (w/w) diesel, C_{ac} = Activated charcoal control: 0.1 % (dry weight) activated charcoal, C_s = Solvent control: 10 ml kg^{-1} 1:1 toluene:acetone, C_g = Growth control: Clean soil. Marked boxes (*) represent significant differences against the growth control (C_g) of each species. Solid lines represent the mean value of the growth control (C_g) used as baseline for the analysis of variance (ANOVA) test. Error bars represent the standard error of the mean (n = 3).

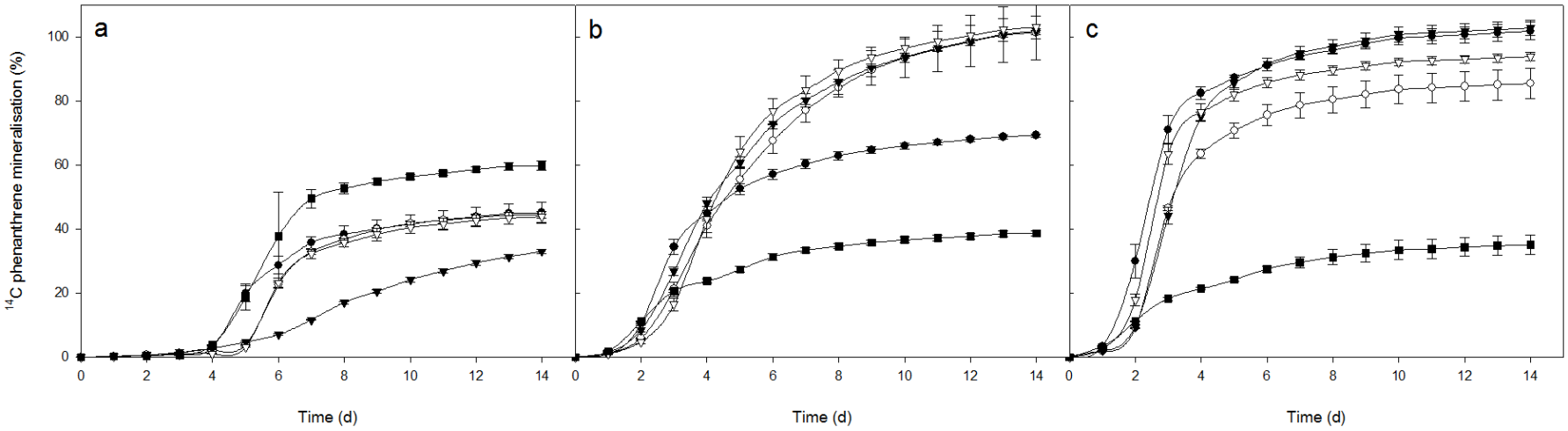

Figure 2. Mineralisation of ^{14}C-phenanthrene in soil spiked with 100 mg kg^{-1} ΣPAH, Root amendments: (●) *L. perenne*, (○) S. *bicolor*, (▼) *M. sativa*, (▽) *G. max*, and (■) without roots after 1 (a), 14 (b), and 28 (c) days ageing. Error bars represent the standard error of the mean (n = 3).

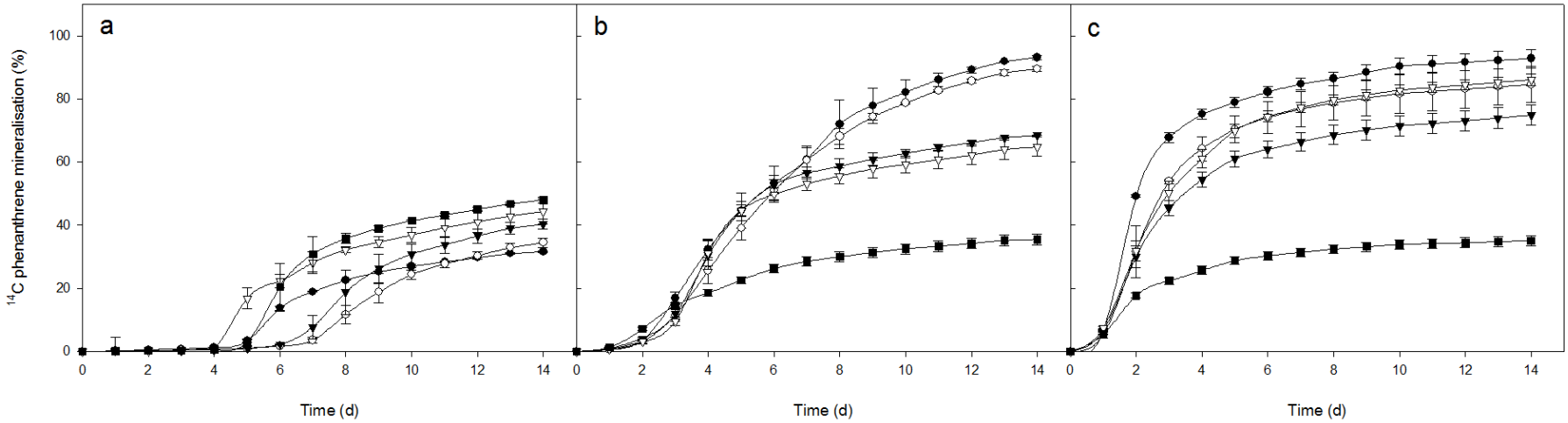

Figure 3. Mineralisation of ^{14}C-phenanthrene in soil spiked with 100 mg kg^{-1} ΣPAH and 0.1% activated charcoal. Root amendments: (●) *L. perenne*, (○) *S. bicolor*, (▼) *M. sativa*, (▽) *G. max*, and (■) without roots after 1 (a), 14 (b), and 28 (c) days ageing. Error bars represent the standard error of the mean (n = 3).

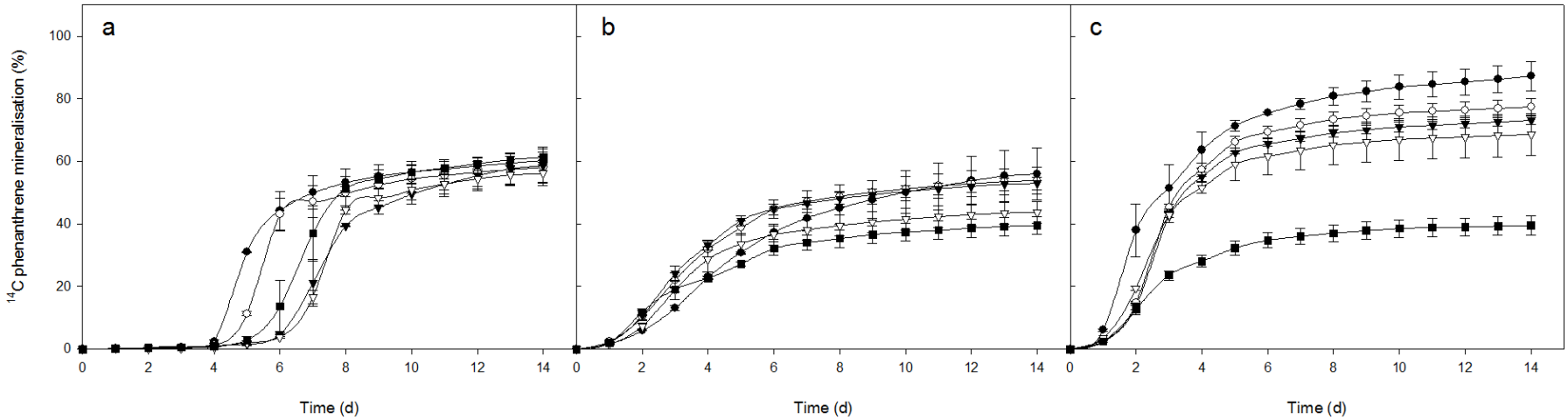

Figure 4. Mineralisation of ^{14}C-phenanthrene in soil spiked with 100 mg kg^{-1} ΣPAH and 0.1% diesel. Root amendments: (●) *L. perenne*, (○) *S. bicolor*, (▼) *M. sativa*, (▽) *G. max*, and (■) without roots after 1 (a), 14 (b), and 28 (c) days ageing. Error bars represent the standard error of the mean (n = 3).

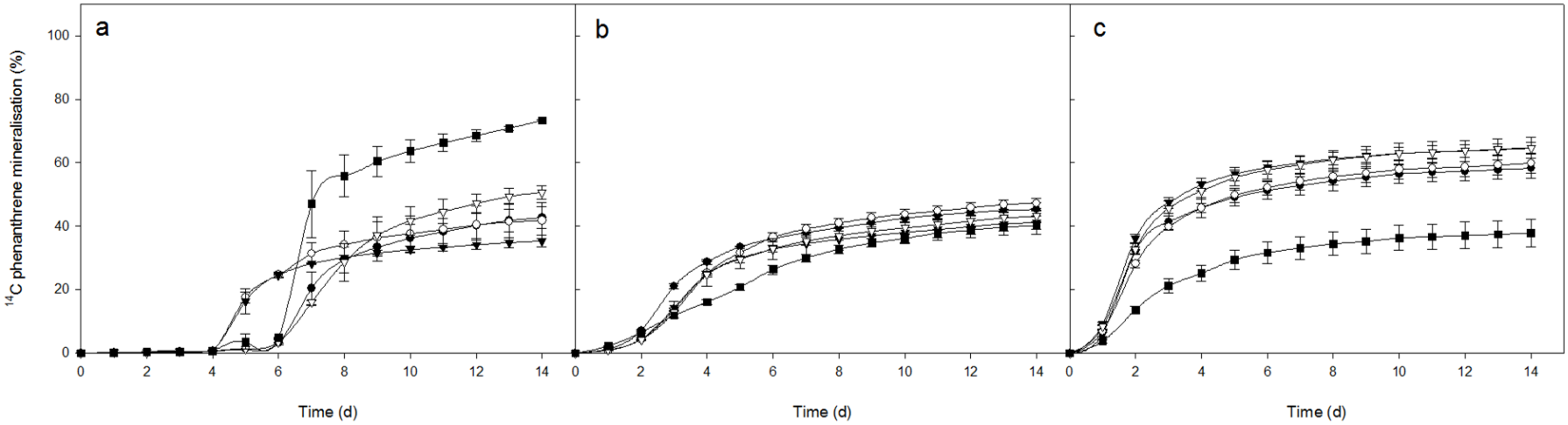

Figure 5. Mineralisation of ^{14}C-phenanthrene in soil spiked with 100 mg kg^{-1} ΣPAH, 0.1% activated charcoal (dw) and 0.1% diesel (w/w). Root amendments: (●) *L. perenne*, (○) *S. bicolor*, (▼) *M. sativa*, (▽) *G. max*, and (■) without roots after 1 (a), 14 (b), and 28 (c) days ageing. Error bars represent the standard error of the mean (n = 3).

5.7 References

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science & Technology, 27, 2630–2636.

Aprill, W. & Sims, R.C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere, 20, 253–265.

Baek, K.-H., Kim, H.-S., Oh, H.-M., Yoon, B.-D., Kim, J. & Lee, I.-S. 2004. Effects of crude oil, oil components, and bioremediation on plant growth. Journal of Environmental Science and Health, Part A- Toxic/Hazardous Substances & Environmental Engineering, 39, 2465–2472.

Banks, M.K., Kulakow, P., Schwab, a P., Chen, Z. & Rathbone, K. 2003. Degradation of crude oil in the rhizosphere of Sorghum bicolor. International Journal of Phytoremediation, 5, 225–34.

Binet, P., Portal, J.M. & Leyval, C. 2000. Dissipation of 3 ± 6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biology and Biochemistry, 32, 2011–2017.

Chiapusio, G., Pujol, S., Toussaint, M.L., Badot, P.M. & Binet, P. 2007. Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (Lolium perenne L. and Trifolium pratense L.) in three spiked soils. Plant and Soil, 294, 103–112.

Chung, N. & Alexander, M. 2002. Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil. Chemosphere, 48, 109–115.

Corgié, S.C., Joner, E.J. & Leyval, C. 2003. Rhizospheric degradation of phenanthrene is a function of proximity to roots. Plant and Soil, 257, 143–150.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Doick, K.J. & Semple, K.T. 2003. The effect of soil:water ratios on the mineralisation of phenanthrene: LNAPL mixtures in soil. FEMS Microbiology Letters, 220, 29–33.

Fan, S., Li, P., Gong, Z., Ren, W. & He, N. 2008. Promotion of pyrene degradation in rhizosphere of alfalfa (Medicago sativa L.). Chemosphere, 71, 1593–1598.

Fenlon, K.A., Jones, K.C. & Semple, K.T. 2007. Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. Journal of Environmental Monitoring, 9, 510.

Gao, Y. & Zhu, L. 2004. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere, 55, 1169–1178.

Günther, T., Dornberger, U. & Fritsche, W. 1996. Effects of ryegrass on

biodegradation of hydrocarbons in soil. Chemosphere, 33, 203–215.

Hamdi, H., Benzarti, S., Aoyama, I. & Jedidi, N. 2012. Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (Medicago sativa L.). International Biodeterioration and Biodegradation, 67, 40–47.

Henner, P., Schiavon, M., Druelle, V. & Lichtfouse, E. 1999. Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. Organic Geochemistry, 30, 963–969.

Joner, E.J., Corgié, S.C. & Leyval, C. 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biology and Biochemistry, 34, 859–864.

Joner, E.J., Johansen, A., Loibner, A.P., Cruz, M.A. Dela, Szolar, O.H.J., Portal, J.M. & Leyval, C. 2001. Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. Environmental Science & Technology, 35, 2773–2777.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, 165, 382–396.

Macleod, C.J.A. & Semple, K.T. 2002. The adaptation of two similar soils to pyrene catabolism. Environmental Pollution, 119, 357–364.

Maila, M.P. & Cloete, T.E. 2002. Germination of Lepidium sativum as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. International Biodeterioration & Biodegradation, 50, 107–113.

Maliszewska-Kordybach, B. & Smreczak, B. 2000. Ecotoxicological activity of soils polluted with polycyclic aromatic hydrocarbons (pahs) - effect on plants. Environmental Technology (United Kingdom), 21, 1099–1110.

Miya, R.K. & Firestone, M.K. 2001. Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environment Quality, 30, 1911–1918.

Moubasher, H.A., Hegazy, A.K., Mohamed, N.H., Moustafa, Y.M., Kabiel, H.F. & Hamad, A.A. 2015. Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. International Biodeterioration & Biodegradation, 98, 113–120.

Mueller, K.E. & Shann, J.R. 2007. Effects of tree root-derived substrates and inorganic nutrients on pyrene mineralization in rhizosphere and bulk soil. Journal of Environment Quality, 36, 120.

Muratova, A.Y., Dmitrieva, T. V., Panchenko, L. V. & Turkovskaya, O. V. 2008. Phytoremediation of oil-sludge-contaminated soil. International Journal of Phytoremediation, 10, 486–502.

Muratova, A., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A.,

Hirche, F., Merbach, W. & Turkovskaya, O. 2009. Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of Sorghum bicolor (L.) Moench. Environmental and Experimental Botany, 66, 514–521.

Muratova, A., Hübner, T., Tischer, S., Turkovskaya, O., Möder, M. & Kuschk, P. 2003. Plant–rhizosphere-microflora association during phytoremediation of PAH-ontaminated soil. International Journal of Phytoremediation, 5, 137–151.

Nichols, T.D., Wolf, D.C., Rogers, H.B., Beyrouty, C. a. & Reynolds, C.M. 1997. Rhizosphere microbial populations in contaminated soils. Water, Air, & Soil Pollution, 95, 165–178.

OECD. 2006. OECD Test Guideline 208: Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test. Guidelines for the Testing of Chemicals, Terrestrial Plant Test Seedling Emergence and Seedling Growth Test, 227, 1–21, (At: http://www.oecd-ilibrary.org/environment/test-no-208-terrestrial-plant-test-seedling-emergence-and-seedling-growth-test_9789264070066-en.).

Olson, P.E., Castro, A., Joern, M., Duteau, N.M., Pilon-Smits, E.A.H. & Reardon, K.F. 2007. Comparison of plant families in a greenhouse phytoremediation study on an aged polycyclic aromatic hydrocarbon-contaminated soil. International Journal of Environmental Quality, 36, 1461–1469.

Oyelami, A.O., Okere, U. V., Orwin, K.H., De Deyn, G.B., Jones, K.C. & Semple, K.T. 2013. Effects of plant species identity, diversity and soil fertility on biodegradation of phenanthrene in soil. Environmental Pollution, 173, 231–237.

Phillips, L.A., Greer, C.W., Farrell, R.E. & Germida, J.J. 2009. Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments. Applied Soil Ecology, 42, 9–17.

Phillips, L.A., Greer, C.W. & Germida, J.J. 2006. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biology and Biochemistry, 38, 2822–2833.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39.

Radwan, S.S., Al-Awadhi, H., Sorkhoh, N. a. & El-Nemr, I.M. 1998. Rhizospheric hydrocarbon-utilizing microorganisms as potential contributors to phytoremediation for the oil Kuwaiti desert. Microbiological Research, 153, 247–251.

Reilley, K.A., Banks, M.K. & Schwab, A.P. 1996. Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality, 25, 212–219.

Rentz, J.A., Alvarez, P.J.J. & Schnoor, J.L. 2005. Benzo[a]pyrene co-metabolism in the presence of plant root extracts and exudates: Implications for phytoremediation. Environmental Pollution, 136, 477–484.

Rezek, J., in der Wiesche, C., Mackova, M., Zadrazil, F. & Macek, T. 2008. The effect of ryegrass (Lolium perenne) on decrease of PAH content in long term

contaminated soil. Chemosphere, 70, 1603–1608.

Rorison, H. & Rorbinson, D. 1986. Mineral nutrition. In: Methods in Plant Ecology (eds. Moore, P.D. & Chapman, S.D.). Blackwell Scientific Publications, London, United Kingdom.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Alder, J. 2006. Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues. Environmental Pollution, 141, 519–525.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Saito, H. 2011. Study of PAH dissipation and phytoremediation in soils: Comparing freshly spiked with weathered soil from a former coking works. Journal of Hazardous Materials, 192, 1219–1225.

Sun, M., Fu, D., Teng, Y., Shen, Y., Luo, Y., Li, Z. & Christie, P. 2011. In situ phytoremediation of PAH-contaminated soil by intercropping alfalfa (Medicago sativa L.) with tall fescue (Festuca arundinacea Schreb.) and associated soil microbial activity. Journal of Soils and Sediments, 11, 980–989.

Thompson, O.A., Wolf, D.C., Mattice, J.D. & Thoma, G.J. 2008. Influence of nitrogen addition and plant root parameters on phytoremediation of pyrene-contaminated soil. Water, Air, and Soil Pollution, 189, 37–47.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Trapp, S. & Karlson, U. 2001. Aspects of phytoremediation of organic pollutants. Journal of Soils and Sediments, 1, 37–43.

U. S. Environmental Protection Agency. 2012. OCSPP 850.4100: Seedling Emergence and Seedling Growth. EPA Ecological Effects Test Guidelines.

Wenzel, W.W. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant and Soil, 321, 385–408.

Yoshitomi, K.. & Shann, J.. 2001. Corn (Zea mays L.) root exudates and their impact on 14C-pyrene mineralization. Soil Biology and Biochemistry, 33, 1769–1776.

5.8 Supplementary information

Table SI 1. Shoot and root biomass (g, dw) at the end of the phytotoxicity assay. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Treatment	*Lolium perenne*	*Sorghum bicolor*	*Medicago sativa*	*Trifolium repens*	*Glycine max*	*Achillea millefolium*
Shoot biomass						
	Species					
T1	^{ab}0.006 ± 0.001	^{ab}0.031 ± 0.004	^{a}0.009 ± 0.001	^{a}0.004 ± 0.000	^{a}0.482 ± 0.098	^{a}0.002 < 0.001
T2	^{b}0.008 ± 0.001	^{cd}0.044 ± 0.004	^{a}0.011 ± 0.001	^{bc}0.014 ± 0.001	^{a}0.467 ± 0.017	^{a}0.003 ± 0.001
T3	^{b}0.007 ± 0.001	^{abc}0.032 ± 0.002	^{a}0.010 ± 0.002	^{a}0.004 ± 0.001	^{a}0.453 ± 0.015	^{a}0.001 < 0.001
T4	^{b}0.007 ± 0.001	^{abcd}0.040 ± 0.002	^{a}0.015 ± 0.007	^{a}0.006 ± 0.001	^{a}0.505 ± 0.028	^{a}0.003 < 0.001
T5	^{ab}0.006 ± 0.000	^{abcd}0.035 ± 0.001	^{a}0.010 ± 0.001	^{a}0.006 ± 0.000	^{a}0.415 ± 0.028	^{a}0.002 < 0.001
T6	^{ab}0.005 ± 0.000	^{a}0.028 ± 0.003	^{a}0.012 ± 0.004	^{a}0.004 ± 0.000	^{a}0.454 ± 0.029	^{a}0.002 ± 0.001
T7	^{b}0.007 ± 0.000	^{abcd}0.038 ± 0.001	^{a}0.011 ± 0.002	^{b}0.012 ± 0.001	^{a}0.449 ± 0.040	^{a}0.002 ± 0.001
T8	^{b}0.007 ± 0.001	^{abcd}0.038 ± 0.003	^{a}0.013 ± 0.002	^{a}0.006 ± 0.001	^{a}0.481 ± 0.096	^{a}0.002 < 0.001
Cd	^{a}0.004 ± 0.000	^{abcd}0.034 ± 0.001	^{a}0.013 ± 0.005	^{a}0.004 ± 0.000	^{a}0.525 ± 0.049	^{a}0.003 ± 0.001
Cbc	^{ab}0.006 ± 0.001	^{d}0.045 ± 0.001	^{a}0.012 ± 0.002	^{bc}0.016 ± 0.001	^{a}0.514 ± 0.057	^{a}0.003 < 0.001
Cs	^{ab}0.005 ± 0.000	^{cd}0.043 ± 0.001	^{a}0.017 ± 0.003	^{d}0.025 ± 0.000	^{a}0.584 ± 0.078	^{a}0.003 ± 0.001
Cg	^{ab}0.006 ± 0.001	^{bcd}0.042 ± 0.003	^{a}0.009 ± 0.001	^{c}0.017 ± 0.002	^{a}0.555 ± 0.129	^{a}0.002 < 0.001
Root biomass						
T1	^{b}0.018 ± 0.000	^{ab}0.093 ± 0.027	^{a}0.006 ± 0.001	^{a}0.002 ± 0.000	^{a}0.110 ± 0.014	^{abc}0.010 ± 0.002
T2	^{a}0.011 ± 0.001	^{b}0.143 ± 0.022	^{a}0.006 ± 0.001	^{d}0.008 ± 0.001	^{a}0.168 ± 0.012	^{bc}0.016 ± 0.002
T3	^{a}0.011 ± 0.002	^{ab}0.102 ± 0.013	^{a}0.006 ± 0.001	^{a}0.003 ± 0.001	^{a}0.148 ± 0.003	^{ab}0.008 ± 0.002

T4	[a]0.010	± 0.001	[ab]0.082	± 0.009	[a]0.008	± 0.005	[ab]0.003	± 0.000	[a]0.113	± 0.014	[abc]0.010	± 0.002
T5	[a]0.008	± 0.000	[ab]0.095	± 0.020	[a]0.007	± 0.001	[abc]0.004	± 0.001	[a]0.092	± 0.010	[abc]0.010	± 0.001
T6	[a]0.008	± 0.001	[a]0.082	± 0.003	[a]0.008	± 0.002	[ab]0.004	± 0.000	[a]0.141	± 0.005	[a]0.004	± 0.002
T7	[a]0.008	± 0.001	[a]0.081	± 0.007	[a]0.005	± 0.001	[cd]0.008	± 0.001	[a]0.122	± 0.019	[abc]0.010	± 0.004
T8	[a]0.008	± 0.001	[ab]0.088	± 0.008	[a]0.006	± 0.002	[ab]0.004	± 0.000	[a]0.148	± 0.051	[abc]0.009	< 0.001
Cd	[a]0.009	± 0.002	[a]0.072	± 0.003	[a]0.009	± 0.003	[a]0.002	± 0.000	[a]0.131	± 0.010	[bc]0.013	± 0.001
Cbc	[a]0.008	± 0.001	[a]0.077	± 0.007	[a]0.007	± 0.002	[bcd]0.007	± 0.000	[a]0.153	± 0.023	[c]0.017	± 0.001
Cs	[a]0.009	± 0.001	[a]0.069	± 0.008	[a]0.011	± 0.002	[e]0.013	± 0.000	[a]0.156	± 0.018	[bc]0.015	± 0.001
Cg	[a]0.011	± 0.001	[a]0.079	± 0.003	[a]0.005	± 0.001	[abcd]0.006	± 0.002	[a]0.169	± 0.049	[bc]0.014	± 0.002

Treatments: T1 (100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel), T2 (100 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal), T3 (300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel), T4 (300 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal), T5 (100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), T6 (300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), T7 (100 mg kg^{-1} Σ PAH), T8 (300 mg kg^{-1} Σ PAH), C_d (Diesel control: 0.1% (w/w) diesel), C_{ac} (Activated charcoal control: 0.1 % (dry weight) activated charcoal), C_s (Solvent control: 10 ml kg^{-1} 1:1 toluene:acetone), C_g (Growth control: Clean soil)

Table SI 2. Root / Shoot ratio at the end of the phytotoxicity assay. Values represent the mean ± standard error of the mean (*n*=3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Treatment	Species											
	Lolium perenne		*Sorghum bicolor*		*Medicago sativa*		*Trifolium repens*		*Glycine max*		*Achillea millefolium*	
T1	^{c}3.00	± 0.21	^{ab}2.95	± 0.54	^{a}0.61	± 0.06	^{ab}0.67	± 0.11	^{a}0.23	± 0.02	^{a}0.82	± 0.12
T2	^{ab}1.44	± 0.14	^{b}3.22	± 0.16	^{a}0.52	± 0.10	^{ab}0.57	± 0.08	^{a}0.36	± 0.04	^{a}1.37	± 0.40
T3	^{ab}1.73	± 0.33	^{b}3.21	± 0.60	^{a}0.58	± 0.09	^{ab}0.73	± 0.10	^{a}0.33	± 0.01	^{a}1.09	± 0.11
T4	^{ab}1.50	± 0.19	^{ab}2.09	± 0.31	^{a}0.48	± 0.08	^{ab}0.50	± 0.06	^{a}0.22	± 0.02	^{a}0.58	± 0.16
T5	^{ab}1.38	± 0.05	^{ab}2.70	± 0.54	^{a}0.68	± 0.05	^{ab}0.58	± 0.12	^{a}0.22	± 0.01	^{a}0.95	± 0.09
T6	^{ab}1.47	± 0.17	^{ab}2.92	± 0.15	^{a}0.67	± 0.09	^{b}0.82	± 0.07	^{a}0.31	± 0.01	^{a}0.86	± 0.03
T7	^{a}1.21	± 0.11	^{ab}2.17	± 0.21	^{a}0.50	± 0.02	^{ab}0.66	± 0.03	^{a}0.27	± 0.04	^{a}0.83	± 0.08
T8	^{a}1.18	± 0.06	^{ab}2.30	± 0.25	^{a}0.45	± 0.07	^{ab}0.60	± 0.07	^{a}0.29	± 0.04	^{a}0.79	± 0.17
Cd	^{bc}2.44	± 0.44	^{ab}2.10	± 0.05	^{a}0.67	± 0.05	^{ab}0.57	± 0.07	^{a}0.25	± 0.03	^{a}0.76	± 0.23
Cbc	^{ab}1.45	± 0.21	^{ab}1.71	± 0.14	^{a}0.58	± 0.06	^{ab}0.43	± 0.05	^{a}0.30	± 0.02	^{a}1.18	± 0.12
Cs	^{ab}1.70	± 0.25	^{a}1.59	± 0.16	^{a}0.66	± 0.03	^{ab}0.49	± 0.02	^{a}0.27	± 0.04	^{a}1.00	± 0.21
Cg	^{ab}1.87	± 0.11	^{ab}1.92	± 0.17	^{a}0.55	± 0.07	^{a}0.34	± 0.07	^{a}0.31	± 0.08	^{a}0.99	± 0.25

Treatments: T1 (100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel), T2 (100 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal), T3 (300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel), T4 (300 mg kg^{-1} Σ PAH + 0.1 % (dry weight) activated charcoal), T5 (100 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), T6 (300 mg kg^{-1} Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), T7 (100 mg kg^{-1} Σ PAH), T8 (300 mg kg^{-1} Σ PAH), C_d (Diesel control: 0.1% (w/w) diesel), C_{ac} (Activated charcoal control: 0.1 % (dry weight) activated charcoal), C_s (Solvent control: 10 ml kg^{-1} 1:1 toluene:acetone), C_g (Growth control: Clean soil).

Table SI 3. Lag phases, maximum rates and total extents of ^{14}C-phenanthrene mineralisation after 14 days mineralisation assays, showing the different root amendments and soil treatments at 1, 14 and 28 days after soil spiking and amendment. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments (separated by lines) assessed by post hoc Tukey tests

Soil treatment	Root amendment	Lag phase (d)	Fastest rate of mineralisation (% d^{-1})	Total extent mineralised (%)
1 day contact time				
PAH	Non amended	^{b}4.85 ± 0.37	^{b}25.83 ± 9.67	^{c}59.55 ± 1.50
	L. perenne	^{a}4.12 ± 0.02	^{ab}16.78 ± 2.57	^{b}45.14 ± 3.30
	S. bicolor	^{b}5.07 ± 0.01	^{ab}18.95 ± 0.80	^{b}44.50 ± 1.10
	M. sativa	^{b}5.15 ± 0.11	^{a}5.35 ± 0.35	^{a}33.01 ± 0.56
	G. max	^{b}5.09 ± 0.01	^{ab}20.13 ± 1.55	^{b}43.85 ± 1.79
PAH+AC	Non amended	^{ab}5.14 ± 0.08	^{a}17.61 ± 2.22	^{ab}47.93 ± 1.19
	L. perenne	^{b}5.16 ± 0.02	^{a}10.39 ± 0.95	^{a}31.59 ± 0.32
	S. bicolor	^{c}7.28 ± 0.21	^{a}8.15 ± 2.55	^{a}34.46 ± 1.51
	M. sativa	^{c}6.71 ± 0.41	^{a}10.95 ± 3.42	^{b}40.29 ± 1.65
	G. max	^{a}4.27 ± 0.06	^{a}15.89 ± 3.27	^{b}44.36 ± 4.41
PAH+D	Non amended	^{b}5.49 ± 0.43	^{a}23.42 ± 0.49	^{a}61.31 ± 1.65
	L. perenne	^{a}4.09 ± 0.02	^{a}28.56 ± 0.64	^{a}60.32 ± 1.25
	S. bicolor	^{a}4.37 ± 0.03	^{a}31.73 ± 4.59	^{a}58.09 ± 5.83
	M. sativa	^{b}6.02 ± 0.02	^{a}18.19 ± 7.70	^{a}58.69 ± 5.72
	G. max	^{b}6.09 < 0.01	^{a}27.82 ± 0.65	^{a}56.15 ± 2.79
PAH+AC+D	Non amended	^{b}6.00 < 0.01	^{a}42.18 ± 10.7	^{b}73.34 ± 0.37
	L. perenne	^{b}6.12 ± 0.04	^{a}17.28 ± 5.26	^{a}42.74 ± 3.52
	S. bicolor	^{a}4.26 ± 0.03	^{a}16.98 ± 1.67	^{a}41.78 ± 5.59
	M. sativa	^{a}3.80 ± 0.57	^{a}15.69 ± 3.62	^{a}35.34 ± 1.91
	G. max	^{b}6.12 ± 0.07	^{a}12.53 ± 0.29	^{a}50.64 ± 1.99
PS	Non amended	^{a}7.33 ± 0.04	^{b}31.95 ± 1.16	^{b}66.62 ± 10.1
	L. perenne	^{a}4.56 ± 1.04	^{a}4.55 ± 0.38	^{a}32.65 ± 8.43
	S. bicolor	^{a}5.11 ± 0.01	^{ab}23.83 ± 1.18	^{ab}47.96 ± 0.84
	M. sativa	^{a}5.74 ± 0.28	^{b}28.87 ± 1.79	^{ab}52.20 ± 1.56
	G. max	^{a}4.93 ± 1.34	^{ab}12.46 ± 10.5	^{ab}35.27 ± 8.64
AC	Non amended	^{b}7.88 ± 0.45	^{a}11.76 ± 1.51	^{a}33.00 ± 0.67
	L. perenne	^{ab}5.10 ± 0.12	^{a}8.35 ± 3.35	^{a}28.73 ± 3.55
	S. bicolor	^{ab}5.92 ± 0.84	^{a}4.71 ± 1.25	^{a}27.60 ± 2.21
	M. sativa	^{ab}6.62 ± 0.32	^{a}5.35 ± 0.32	^{a}30.74 ± 1.44
	G. max	^{a}4.19 ± 0.09	^{a}12.31 ± 0.51	^{a}34.67 ± 2.68
D	Non amended	^{bc}5.58 ± 0.43	^{a}26.12 ± 5.97	^{a}61.74 ± 1.04
	L. perenne	^{a}5.10 ± 0.12	^{a}27.71 ± 4.69	^{a}54.95 ± 2.87
	S. bicolor	^{c}6.28 ± 0.01	^{a}13.89 ± 0.55	^{a}48.56 ± 4.18
	M. sativa	^{a}3.22 ± 0.01	^{a}17.95 ± 4.59	^{a}51.45 ± 5.53
	G. max	^{b}5.05 ± 0.01	^{a}28.66 ± 4.02	^{a}55.13 ± 5.07
AC+D	Non amended	^{c}6.25 ± 0.10	^{a}15.33 ± 6.24	^{a}55.24 ± 0.66
	L. perenne	^{b}5.14 ± 0.09	^{a}16.29 ± 3.57	^{a}56.51 ± 0.10
	S. bicolor	^{a}4.17 ± 0.03	^{a}25.32 ± 5.67	^{a}51.23 ± 2.77
	M. sativa	^{c}6.17 ± 0.10	^{a}12.87 ± 1.32	^{a}44.64 ± 2.95

	G. max	[c]6.08 ± 0.24	[a]11.39 ± 5.53	[a]50.37 ± 4.38
Solvent	Non amended	[c]6.10 ± 0.19	[a]25.60 ± 0.47	[a]49.61 ± 5.80
	L. perenne	[a]4.09 ± 0.01	[a]33.80 ± 1.44	[a]53.99 ± 1.22
	S. bicolor	[a]4.76 ± 0.35	[a]29.11 ± 3.79	[a]51.74 ± 1.02
	M. sativa	[ab]5.11 ± 0.03	[a]23.56 ± 8.86	[a]54.05 ± 3.09
	G. max	[ab]5.08 ± 0.02	[a]31.52 ± 3.06	[a]57.50 ± 1.95
14 days contact time				
PAH	Non amended	[a]1.35 ± 0.01	[a]9.56 ± 0.38	[a]38.68 ± 0.84
	L. perenne	[a]1.42 ± 0.07	[a]24.10 ± 2.58	[b]69.27 ± 0.57
	S. bicolor	[b]1.82 ± 0.01	[a]19.83 ± 4.41	[c]101.41 ± 0.69
	M. sativa	[a]1.51 ± 0.04	[a]21.11 ± 3.32	[c]101.85 ± 9.04
	G. max	[b]2.02 ± 0.05	[a]26.73 ± 3.86	[c]103.03 ± 3.49
PAH+AC	Non amended	[a]1.63 ± 0.06	[a]7.40 ± 0.46	[a]35.48 ± 1.69
	L. perenne	[b]2.12 ± 0.04	[ab]15.41 ± 1.39	[c]93.18 ± 0.63
	S. bicolor	[b]2.24 ± 0.12	[ab]14.11 ± 2.07	[c]89.48 ± 0.81
	M. sativa	[b]2.13 ± 0.07	[ab]18.61 ± 2.79	[b]68.42 ± 0.01
	G. max	[b]2.33 ± 0.10	[b]21.72 ± 3.02	[b]64.82 ± 3.07
PAH+D	Non amended	[a]1.30 ± 0.06	[a]9.79 ± 0.61	[a]39.50 ± 2.83
	L. perenne	[c]1.80 ± 0.01	[a]9.86 ± 1.18	[a]56.00 ± 8.17
	S. bicolor	[b]1.33 ± 0.04	[a]11.83 ± 1.79	[a]53.81 ± 4.21
	M. sativa	[b]1.36 ± 0.08	[a]13.50 ± 0.98	[a]52.95 ± 1.95
	G. max	[bc]1.62 ± 0.11	[a]11.55 ± 2.24	[a]43.71 ± 3.47
PAH+AC+D	Non amended	[a]1.65 ± 0.06	[a]5.28 ± 0.36	[a]40.18 ± 1.23
	L. perenne	[a]1.64 ± 0.05	[b]13.96 ± 0.45	[a]45.28 ± 2.12
	S. bicolor	[b]2.07 ± 0.03	[b]11.45 ± 0.16	[a]47.27 ± 1.52
	M. sativa	[b]2.09 ± 0.12	[b]10.63 ± 1.99	[a]41.16 ± 3.62
	G. max	[b]2.09 ± 0.03	[b]11.76 ± 0.50	[a]43.12 ± 2.01
PS	Non amended	[a]2.79 ± 0.13	[a]5.97 ± 0.77	[a]34.70 ± 3.32
	L. perenne	[c]5.19 ± 0.21	[c]21.98 ± 0.15	[a]44.45 ± 0.93
	S. bicolor	[bc]4.25 ± 0.08	[bc]14.80 ± 0.74	[a]41.75 ± 0.04
	M. sativa	[ab]3.39 ± 0.11	[ab]11.95 ± 1.53	[a]38.16 ± 0.99
	G. max	[ab]3.37 ± 0.09	[ab]11.53 ± 2.43	[a]40.22 ± 3.56
AC	Non amended	[ab]4.23 ± 0.13	[a]3.70 ± 0.43	[ab]25.13 ± 1.76
	L. perenne	[c]7.67 ± 0.06	[a]3.69 ± 0.92	[a]18.74 ± 1.14
	S. bicolor	[b]4.70 ± 0.08	[a]3.87 ± 0.10	[b]26.66 ± 0.77
	M. sativa	[a]4.14 ± 0.11	[a]3.02 ± 0.01	[ab]24.86 ± 0.76
	G. max	[ab]4.18 ± 0.09	[a]4.19 ± 0.02	[b]27.93 ± 1.75
D	Non amended	[a]2.91 ± 0.17	[a]7.60 ± 0.81	[a]38.58 ± 3.04
	L. perenne	[a]3.11 ± 0.07	[a]10.14 ± 1.44	[a]40.17 ± 2.07
	S. bicolor	[b]3.95 ± 0.07	[a]13.61 ± 3.51	[a]35.67 ± 5.05
	M. sativa	[c]4.54 ± 0.03	[a]9.84 ± 1.70	[a]37.31 ± 0.30
	G. max	[c]4.78 ± 0.13	[a]9.01 ± 1.26	[a]34.91 ± 0.82
AC+D	Non amended	[ab]5.46 ± 0.04	[a]5.55 ± 0.19	[a]32.46 ± 0.25
	L. perenne	[a]4.46 ± 0.10	[a]7.83 ± 0.59	[a]33.64 ± 1.65
	S. bicolor	[c]5.55 ± 0.12	[a]7.46 ± 2.29	[a]33.54 ± 5.92
	M. sativa	[ab]5.37 ± 0.24	[a]7.73 ± 1.08	[a]33.75 ± 1.18
	G. max	[ab]5.22 ± 0.35	[a]5.47 ± 0.14	[a]36.25 ± 1.62
Solvent	Non amended	[b]4.89 ± 0.14	[a]6.96 ± 1.42	[a]36.96 ± 4.65
	L. perenne	[c]5.74 ± 0.06	[a]14.10 ± 1.17	[a]42.74 ± 3.78
	S. bicolor	[b]4.92 ± 0.13	[a]12.55 ± 2.02	[a]42.02 ± 2.85

	M. sativa	[a]4.21 ± 0.11	[a]7.52 ± 0.54	[a]37.11 ± 2.55
	G. max	[ab]4.48 ± 0.06	[a]8.01 ± 1.52	[a]35.60 ± 2.75
28 days contact time				
PAH	Non amended	[b]1.28 < 0.01	[a]8.63 ± 0.22	[a]35.09 ± 3.08
	L. perenne	[a]1.06 ± 0.01	[bc]41.09 ± 0.92	[c]101.87 ± 2.77
	S. bicolor	[c]1.44 < 0.01	[bc]37.33 ± 0.66	[b]85.48 ± 4.85
	M. sativa	[bc]1.40 ± 0.03	[b]34.72 ± 2.20	[c]102.82 ± 2.31
	G. max	[a]1.11 ± 0.02	[c]45.69 ± 1.49	[bc]93.87 ± 1.33
PAH+AC	Non amended	[a]0.94 ± 0.03	[a]12.34 ± 0.64	[a]35.06 ± 1.59
	L. perenne	[a]0.77 ± 0.01	[c]42.74 ± 0.32	[c]92.79 ± 2.88
	S. bicolor	[a]0.91 ± 0.12	[b]32.05 ± 1.50	[bc]84.60 ± 5.67
	M. sativa	[a]0.76 ± 0.05	[ab]23.35 ± 3.06	[b]74.89 ± 3.24
	G. max	[a]0.69 ± 0.05	[b]24.51 ± 2.76	[bc]86.10 ± 1.81
PAH+D	Non amended	[b]1.27 ± 0.07	[a]10.81 ± 1.07	[a]39.53 ± 2.96
	L. perenne	[a]0.82 ± 0.06	[c]35.30 ± 4.61	[b]87.21 ± 4.78
	S. bicolor	[b]1.21 ± 0.02	[bc]30.55 ± 1.46	[b]77.41 ± 2.59
	M. sativa	[b]1.21 ± 0.03	[bc]28.94 ± 1.01	[b]73.11 ± 1.23
	G. max	[b]1.09 < 0.01	[b]23.41 ± 1.45	[b]68.61 ± 6.76
PAH+AC+D	Non amended	[b]1.14 ± 0.06	[a]9.96 ± 0.70	[a]37.77 ± 4.27
	L. perenne	[a]0.82 ± 0.13	[bc]26.11 ± 1.97	[b]58.29 ± 3.15
	S. bicolor	[a]0.73 ± 0.02	[b]21.22 ± 0.94	[b]59.88 ± 3.26
	M. sativa	[a]0.55 ± 0.03	[c]26.97 ± 0.89	[b]64.55 ± 1.85
	G. max	[a]0.61 ± 0.06	[bc]24.49 ± 1.25	[b]64.75 ± 3.38
PS	Non amended	[a]2.43 ± 0.07	[a]7.49 ± 0.46	[a]34.72 ± 1.34
	L. perenne	[c]4.45 ± 0.07	[b]24.27 ± 3.31	[b]69.06 ± 1.38
	S. bicolor	[ab]2.62 ± 0.04	[ab]13.52 ± 3.06	[b]54.95 ± 2.55
	M. sativa	[ab]2.48 ± 0.06	[ab]18.94 ± 4.33	[b]59.41 ± 3.53
	G. max	[b]2.78 ± 0.03	[ab]12.65 ± 0.39	[a]40.89 ± 1.00
AC	Non amended	[a]3.49 ± 0.14	[a]3.74 ± 0.34	[b]28.55 ± 2.03
	L. perenne	[c]6.73 ± 0.02	[a]4.11 ± 0.60	[a]20.70 ± 0.49
	S. bicolor	[b]4.03 ± 0.02	[a]4.45 ± 0.12	[b]28.05 ± 0.61
	M. sativa	[a]3.53 ± 0.08	[a]4.10 ± 0.52	[b]28.85 ± 0.81
	G. max	[a]3.49 ± 0.05	[a]5.29 ± 0.18	[b]32.99 ± 0.67
D	Non amended	[a]2.61 ± 0.06	[a]7.04 ± 0.47	[a]39.20 ± 1.36
	L. perenne	[ab]2.83 ± 0.07	[b]16.43 ± 2.92	[b]52.01 ± 3.52
	S. bicolor	[c]3.20 ± 0.09	[ab]12.70 ± 2.00	[ab]48.79 ± 3.32
	M. sativa	[bc]2.95 ± 0.03	[ab]10.14 ± 0.45	[a]39.98 ± 2.60
	G. max	[bc]3.03 ± 0.05	[ab]10.57 ± 1.56	[a]40.07 ± 0.30
AC+D	Non amended	[c]3.84 ± 0.01	[ab]7.29 ± 0.03	[a]36.50 ± 0.59
	L. perenne	[b]3.38 ± 0.01	[b]12.35 ± 0.46	[a]40.86 ± 0.27
	S. bicolor	[c]3.69 ± 0.11	[ab]7.26 ± 1.96	[a]37.37 ± 3.73
	M. sativa	[a]2.86 ± 0.06	[a]6.93 ± 0.73	[a]35.33 ± 1.89
	G. max	[b]3.27 ± 0.04	[ab]7.41 ± 0.70	[a]37.38 ± 2.57
Solvent	Non amended	[ab]3.68 ± 0.14	[a]7.02 ± 1.49	[a]36.85 ± 2.76
	L. perenne	[c]5.87 ± 0.16	[a]11.83 ± 0.55	[a]37.54 ± 1.08
	S. bicolor	[b]4.02 ± 0.16	[a]7.48 ± 1.10	[a]37.09 ± 1.57
	M. sativa	[a]3.19 ± 0.09	[a]7.37 ± 1.07	[a]33.64 ± 1.26
	G. max	[a]3.20 ± 0.13	[a]8.64 ± 0.78	[a]38.41 ± 0.62

Treatments: PAH (100 mg kg-1 Σ PAH), PAH + AC (100 mg kg-1 Σ PAH + 0.1 % (dry weight) activated charcoal), PAH + D (100 mg kg-1 Σ PAH + 0.1% (w/w) diesel), PAH + AC + D (100 mg kg-1 Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), PS (soil as collected from the field), AC (0.1 % (dry weight) activated charcoal), D (0.1% (w/w) diesel), AC + D (0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), Solvent (1:1 Toluene : Acetone solution).

Figure SI 4. .Mineralisation of ^{14}C-phenanthrene in soil (a-c) without ^{12}C PAHs, and amended with (d-f) 0.1% activated charcoal (dw), (g-i) 0.1% diesel (w/w), and (j-l) 0.1% activated charcoal (dw) + 0.1 % diesel (w/w). Root amendments: (●) *L. perenne*, (○) *S. bicolor*, (▼) *M. sativa*, (▽) *G. max*, and (■) without roots after 1, 14, and 28 days ageing respectively. Error bars represent the standard error of the mean (n = 3).

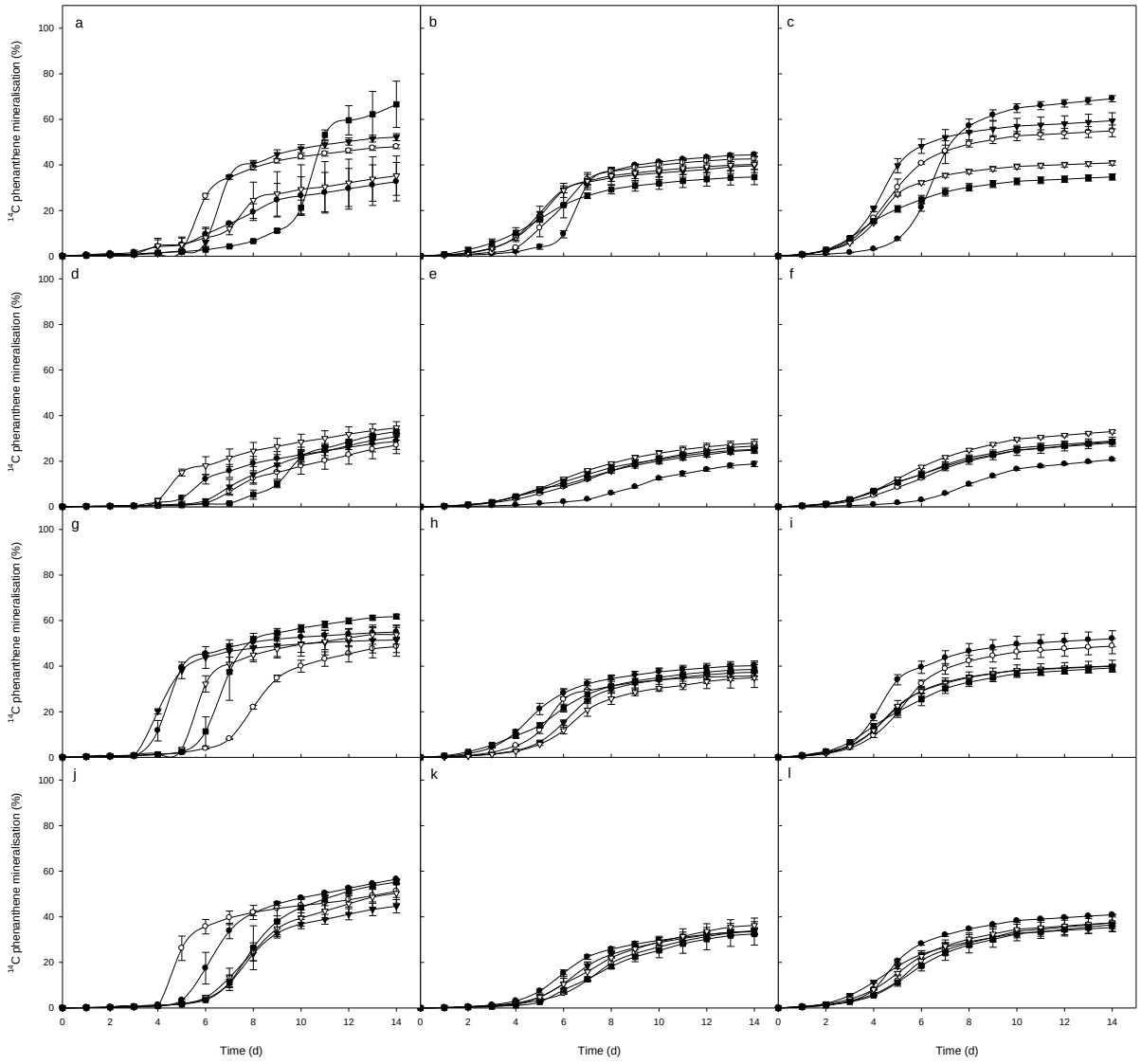

Table SI 5. Colony forming units (CFUs) of heterotrophs and phenanthrene degraders in soil before and after mineralisation of ^{14}C-phenanthrene at 1, 14 and 28 days after soil spiking and root amendment. Values represent the mean ± standard error of the mean (n = 3). Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments (separated by lines) assessed by post hoc Tukey tests. Data without a letter (*) was analysed through Kruskal-Wallis mean rank of the group's test without significant differences ($p > 0.05$)

		Before mineralisation		After mineralisation	
Treatment	Root amendment	Heterotrophs (CFU x 10^4 g^{-1})	Phenanthrene degraders (CFU x 10^4 g^{-1})	Heterotrophs (CFU x 10^4 g^{-1})	Phenanthrene degraders (CFU x 10^4 g^{-1})
1 day contact time					
PAH	Non amended	*9.60 ± 0.30	^{a}3.00 < 0.01	^{a}9.00 ± 2.10	^{a}12.60 ± 1.20
	L. perenne	*5.85 ± 0.15	^{cd}14.10 ± 0.60	^{b}18.60 ± 0.30	^{c}33.60 ± 0.60
	S. bicolor	*4.20 < 0.01	^{bc}6.45 ± 0.15	^{bc}26.40 ± 0.30	^{c}46.35 ± 0.45
	M. sativa	*24.45 ± 0.75	^{d}16.95 ± 0.15	^{c}75.90 ± 0.30	^{a}7.65 ± 0.75
	G. max	*8.55 ± 0.45	^{ab}7.20 < 0.01	^{a}29.85 ± 1.65	^{b}39.00 ± 0.90
PAH+AC	Non amended	*5.40 ± 0.30	^{a}3.60 < 0.01	^{a}11.40 ± 0.30	^{b}20.40 ± 2.70
	L. perenne	*6.60 ± 0.30	^{abc}11.85 ± 0.15	^{bc}20.10 ± 0.30	^{cd}34.20 ± 1.80
	S. bicolor	*4.05 ± 0.15	^{c}5.55 ± 0.45	^{b}19.35 ± 0.45	^{c}28.20 ± 1.50
	M. sativa	*9.60 ± 0.30	^{b}4.80 ± 0.30	^{c}32.10 ± 2.70	^{a}18.15 ± 0.75
	G. max	*37.05 ± 0.15	^{d}17.70 < 0.01	^{c}34.80 ± 0.30	^{d}49.35 ± 1.05
PAH+D	Non amended	*6.90 ± 0.30	^{a}4.20 ± 0.30	^{a}21.30 ± 0.60	^{b}30.60 < 0.01
	L. perenne	*5.40 ± 0.30	^{c}17.25 ± 0.15	^{a}25.05 ± 0.75	^{a}13.50 ± 0.30
	S. bicolor	*5.10 < 0.01	^{c}11.40 < 0.01	^{a}23.40 ± 5.10	^{a}15.15 ± 0.15
	M. sativa	*16.35 ± 0.75	^{b}6.60 ± 0.60	^{a}54.90 ± 0.30	^{a}11.85 ± 0.15
	G. max	*28.95 ± 0.75	^{c}19.95 ± 0.15	^{a}28.80 ± 0.30	^{ab}24.60 ± 0.60
PAH+AC+D	Non amended	*7.35 ± 0.45	^{b}7.95 ± 0.15	^{b}17.55 ± 0.15	^{b}28.50 ± 0.60

	L. perenne	*9.75 ± 0.15	c9.45 ± 1.05	a11.10 ± 1.20	a22.50 ± 1.20
	S. bicolor	*10.50 ± 0.00	a8.85 ± 0.15	c30.75 ± 2.25	c38.10 ± 5.70
	M. sativa	*6.75 ± 0.15	b6.00 ± 0.60	d40.20 ± 2.10	a22.05 ± 2.55
	G. max	*3.90 ± 0.00	cd7.65 ± 0.45	bc28.20 ± 0.30	ab24.75 ± 0.45
PS	Non amended	*7.50 ± 0.30	a7.05 ± 0.15	a21.30 ± 0.90	b25.65 ± 1.95
	L. perenne	*11.70 ± 0.90	a7.50 ± 0.30	c26.40 < 0.01	a12.60 ± 0.30
	S. bicolor	*13.35 ± 0.15	a7.50 < 0.01	b27.45 ± 1.05	b25.05 ± 0.75
	M. sativa	*15.90 ± 0.90	b22.65 ± 0.45	d85.05 ± 2.25	b26.55 ± 0.15
	G. max	*35.55 ± 1.35	ab7.65 ± 0.45	ab31.05 ± 1.95	bc40.50 ± 0.90
AC	Non amended	*10.05 ± 0.15	c7.65 ± 0.15	a13.65 ± 1.35	b21.00 ± 0.30
	L. perenne	*12.90 ± 0.30	b6.45 ± 0.15	a13.35 ± 0.75	c25.20 < 0.01
	S. bicolor	*7.05 ± 0.15	a5.70 < 0.01	b16.65 ± 1.05	d36.90 ± 0.60
	M. sativa	*19.35 ± 0.45	e24.30 ± 0.90	c32.10 ± 2.70	a18.75 ± 0.45
	G. max	*37.35 ± 0.75	d9.45 ± 1.05	ab14.70 ± 1.80	e40.80 ± 0.30
D	Non amended	*10.20 < 0.01	a11.25 ± 0.15	b31.35 ± 0.45	b31.20 ± 0.60
	L. perenne	*8.85 ± 0.45	ab7.95 ± 0.15	b31.65 ± 1.95	a17.25 ± 1.05
	S. bicolor	*25.20 ± 0.30	b12.00 < 0.01	a26.25 ± 0.15	b36.15 ± 1.05
	M. sativa	*24.15 ± 0.15	ab6.60 < 0.01	a28.35 ± 0.75	b27.75 ± 1.35
	G. max	*41.70 ± 1.20	a4.35 ± 0.15	a15.60 < 0.01	a23.55 ± 1.95
AC+D	Non amended	*5.40 ± 0.30	d11.70 < 0.01	a13.05 ± 1.05	bc23.85 ± 1.05
	L. perenne	*10.35 ± 0.45	c12.30 < 0.01	cd22.80 ± 0.60	a14.70 < 0.01
	S. bicolor	*11.25 ± 0.15	a3.30 < 0.01	ab14.70 ± 0.60	b20.70 ± 1.50
	M. sativa	*13.50 ± 0.30	bc13.20 ± 0.60	d60.00 ± 1.80	a10.65 ± 0.15
	G. max	*35.40 ± 0.30	b3.90 < 0.01	bc18.15 ± 1.65	c24.30 ± 1.50
Solvent	Non amended	*4.95 ± 0.15	a7.20 < 0.01	a12.90 ± 1.50	a23.25 ± 0.75
	L. perenne	*30.30 ± 0.01	ab4.10 ± 0.30	ab33.00 ± 1.20	a17.85 ± 0.45
	S. bicolor	*8.25 ± 0.45	b4.95 ± 0.15	b20.85 ± 0.75	ab33.00 < 0.01

	M. sativa	*12.90 ± 0.60	c9.90 < 0.01	c53.70 ± 0.60	a12.90 ± 0.60
	G. max	*9.75 ± 0.75	c5.70 ± 0.30	ab33.60 ± 0.60	b44.55 ± 0.15
		14 days contact time			
PAH	Non amended	*3.00 < 0.01	a3.30 < 0.01	c23.40 ± 0.60	a34.20 ± 0.30
	L. perenne	*7.80 ± 0.30	b11.40 ± 0.30	a14.85 ± 0.15	c50.55 ± 4.95
	S. bicolor	*14.40 ± 0.30	b13.20 ± 1.50	b18.75 ± 0.75	ab46.65 ± 0.45
	M. sativa	*7.20 ± 0.30	c45.90 < 0.01	c25.65 ± 0.75	ab42.45 ± 0.45
	G. max	*6.90 < 0.01	b13.35 ± 0.45	ab18.00 ± 0.90	a33.90 ± 1.20
PAH+AC	Non amended	*6.30 ± 0.30	a2.40 < 0.01	b13.80 ± 0.30	a24.90 ± 0.30
	L. perenne	*6.60 < 0.01	c11.85 ± 0.15	c18.60 ± 0.90	bc37.20 ± 3.00
	S. bicolor	*6.30 ± 0.30	b9.00 ± 0.30	ab11.25 ± 0.75	ab26.40 ± 0.30
	M. sativa	*4.20 < 0.01	e18.60 ± 0.30	bc14.85 ± 0.75	c37.95 ± 3.15
	G. max	*5.55 ± 0.15	d16.35 ± 0.15	a9.60 ± 0.60	abc32.40 ± 0.60
PAH+D	Non amended	*5.25 ± 0.15	a7.80 ± 0.30	c41.40 ± 0.30	c36.60 ± 0.30
	L. perenne	*15.15 ± 0.75	b9.30 < 0.01	b21.75 ± 0.15	c37.50 ± 0.30
	S. bicolor	*9.90 < 0.01	b9.90 < 0.01	ab19.20 ± 0.30	b26.85 ± 0.45
	M. sativa	*13.95 ± 0.15	d38.55 ± 0.45	a18.30 ± 0.90	b26.70 ± 0.60
	G. max	*9.90 ± 0.60	c22.95 ± 0.15	c41.85 ± 0.45	a6.45 ± 0.45
PAH+AC+D	Non amended	*5.25 ± 0.15	a7.35 ± 0.45	b18.00 < 0.01	a10.20 ± 1.50
	L. perenne	*7.50 < 0.01	a6.15 ± 0.45	ab14.10 ± 1.20	d30.90 ± 0.60
	S. bicolor	*7.65 ± 0.15	a5.25 ± 0.45	a13.65 ± 1.05	b18.60 ± 1.50
	M. sativa	*5.85 ± 0.15	b12.45 ± 0.75	d28.20 ± 0.60	cd25.50 ± 0.60
	G. max	*5.70 < 0.01	a5.40 < 0.01	c22.65 ± 0.15	bc21.00 ± 0.90
PS	Non amended	*7.20 ± 0.30	a3.90 < 0.01	c31.50 ± 1.80	a10.80 ± 2.40
	L. perenne	*4.65 ± 0.45	c12.90 ± 0.30	a19.35 ± 0.15	c43.35 ± 0.45
	S. bicolor	*6.30 < 0.01	b7.80 < 0.01	a15.00 ± 0.60	c37.05 ± 0.15

	M. sativa	*6.30 ± 0.30	d24.75 ± 0.45	b24.75 ± 0.15	b28.05 ± 0.75
	G. max	*4.80 ± 0.00	c12.90 ± 0.90	c30.30 ± 0.30	a13.50 ± 0.30
AC	Non amended	*6.75 ± 0.15	a11.10 < 0.01	bc17.70 < 0.01	a7.80 ± 0.60
	L. perenne	*8.40 ± 0.30	c24.90 < 0.01	ab15.60 < 0.01	d59.55 ± 1.35
	S. bicolor	*8.10 < 0.01	b16.20 ± 0.90	ab13.20 ± 1.20	b24.60 ± 2.10
	M. sativa	*7.05 ± 0.45	d35.25 ± 0.75	c21.15 ± 1.05	c39.00 ± 0.60
	G. max	*6.00 ± 0.30	a11.40 < 0.01	a12.90 ± 0.90	b21.90 ± 0.60
D	Non amended	*5.40 < 0.01	a8.25 ± 1.05	a13.95 ± 0.75	ab25.65 ± 1.35
	L. perenne	*11.70 < 0.01	a11.40 ± 0.30	b20.85 ± 1.35	a14.40 ± 0.60
	S. bicolor	*5.25 ± 0.15	c42.30 < 0.01	c26.25 ± 1.35	ab22.05 ± 0.45
	M. sativa	*9.45 ± 0.75	b37.35 ± 1.44	c27.15 ± 0.45	b29.25 ± 4.65
	G. max	*7.35 ± 0.75	a9.45 ± 0.75	c29.55 ± 0.15	b32.55 ± 2.55
AC+D	Non amended	*9.45 ± 0.45	c12.60 ± 0.30	a11.10 < 0.01	a13.50 ± 0.60
	L. perenne	*13.80 < 0.01	d26.55 ± 0.45	ab18.15 ± 1.35	bc31.05 ± 1.35
	S. bicolor	*14.10 ± 0.30	a3.75 ± 0.15	bc27.75 ± 0.75	d41.70 ± 0.60
	M. sativa	*7.05 ± 0.15	e35.55 ± 0.15	bc27.15 ± 0.75	cd34.05 ± 1.95
	G. max	*7.35 ± 0.75	b9.45 ± 0.75	c29.55 ± 0.15	b32.55 ± 2.55
Solvent	Non amended	*3.00 < 0.01	ab7.35 ± 0.15	b25.95 ± 2.25	ab21.15 ± 1.05
	L. perenne	*11.85 ± 0.06	c11.70 ± 0.90	b29.55 ± 0.45	b37.65 ± 0.15
	S. bicolor	*8.70 < 0.01	bc9.60 ± 0.30	b27.00 < 0.01	ab24.75 ± 0.15
	M. sativa	*9.15 ± 0.15	d17.40 ± 0.30	a16.95 ± 0.15	b39.00 ± 8.10
	G. max	*10.20 ± 0.00	a5.40 < 0.01	b28.20 ± 1.20	a15.45 ± 0.15
28 days contact time					
PAH	Non amended	a7.20 < 0.01	*10.95 ± 0.15	b16.80 ± 1.20	*9.45 ± 0.15
	L. perenne	a7.35 ± 0.15	*10.50 ± 0.30	a3.75 ± 0.15	*17.55 ± 1.05
	S. bicolor	a8.40 < 0.01	*7.35 ± 0.15	a7.95 ± 0.15	*26.40 ± 3.00

	M. sativa	[b]12.75 ± 0.15	*16.95 ± 0.15	[b]17.55 ± 0.45	*28.05 ± 0.75
	G. max	[b]12.75 ± 0.15	*15.60 ± 0.30	[c]34.20 ± 3.00	*29.85 ± 0.45
PAH+AC	Non amended	a7.05 ± 0.15	*7.80 ± 0.30	[a]8.40 < 0.01	*5.10 ± 0.30
	L. perenne	[b]12.45 ± 0.15	*20.10 < 0.01	[a]9.60 ± 0.30	*30.75 ± 1.35
	S. bicolor	[c]22.50 ± 0.90	*16.20 ± 0.90	[b]10.20 ± 0.30	*21.60 < 0.01
	M. sativa	[a]9.30 ± 0.30	*24.00 ± 0.60	[c]15.75 ± 0.15	*27.90 ± 0.60
	G. max	[ab]11.70 < 0.01	*12.60 ± 1.20	[c]17.70 ± 0.30	*16.80 ± 0.30
PAH+D	Non amended	[a]4.05 ± 0.15	*12.90 ± 0.30	[a]7.95 ± 1.95	*11.55 ± 0.15
	L. perenne	[b]7.65 ± 0.15	*15.90 < 0.01	[a]8.55 ± 0.15	*23.70 ± 1.20
	S. bicolor	[a]4.50 < 0.01	*16.50 < 0.01	[b]13.95 ± 0.15	*29.40 ± 3.00
	M. sativa	[c]16.50 < 0.01	*15.30 ± 0.90	[c]29.40 ± 1.50	*17.85 ± 0.15
	G. max	[b]7.35 ± 0.45	*12.00 ± 0.30	[bc]19.20 ± 1.20	*12.30 ± 0.60
PAH+AC+D	Non amended	[ab]9.30 ± 0.90	*11.55 ± 0.15	[bc]19.80 ± 0.30	*9.45 ± 0.15
	L. perenne	[a]7.50 < 0.01	*12.30 < 0.01	[ab]12.15 ± 0.15	*10.35 ± 0.75
	S. bicolor	[a]7.50 < 0.01	*12.45 ± 0.15	[a]8.70 ± 0.90	*12.30 ± 0.60
	M. sativa	[b]10.80 < 0.01	*19.50 < 0.01	[b]16.65 ± 0.15	*19.80 ± 1.20
	G. max	[ab]9.90 ± 0.30	*13.05 ± 0.15	[b]15.60 ± 2.10	*9.60 < 0.01
PS	Non amended	[a]5.85 ± 0.15	*8.70 < 0.01	[a]12.45 ± 0.45	*7.35 ± 0.75
	L. perenne	[b]11.10 ± 1.20	*13.80 ± 0.30	[c]27.75 ± 1.05	*21.30 ± 0.60
	S. bicolor	[ab]8.55 ± 0.15	*9.45 ± 0.15	[ab]14.10 ± 1.80	*11.85 ± 0.45
	M. sativa	[b]10.80 ± 0.60	*16.20 ± 0.30	[a]9.90 < 0.01	*17.70 ± 1.20
	G. max	[a]4.80 < 0.01	*9.00 < 0.01	[b]17.25 ± 0.45	*13.95 ± 0.75
AC	Non amended	[a]3.90 < 0.01	*11.25 ± 0.15	[a]12.45 ± 0.15	*7.35 ± 0.15
	L. perenne	[b]9.00 ± 0.60	*11.70 < 0.01	[a]12.45 ± 0.15	*25.80 ± 0.30
	S. bicolor	[b]9.30 ± 0.30	*11.10 ± 0.60	[ab]14.10 ± 0.30	*18.15 ± 0.15
	M. sativa	[b]10.65 ± 1.05	*16.20 < 0.01	[b]15.30 ± 0.30	*26.55 ± 1.65
	G. max	[ab]7.95 ± 0.15	*10.35 ± 0.15	[b]15.75 ± 1.05	*12.15 ± 0.15

D	Non amended	[a]2.40 ± 0.30	*8.25 ± 0.15	[a]11.10 < 0.01	*12.15 ± 1.65
	L. perenne	[d]10.80 ± 0.60	*4.11 ± 0.30	[b]19.95 ± 0.15	*30.90 ± 1.50
	S. bicolor	[b]6.00 < 0.01	*20.55 ± 0.15	[b]17.85 ± 2.55	*10.65 ± 0.45
	M. sativa	[a]4.20 < 0.01	*38.55 ± 0.75	[c]24.60 ± 1.20	*24.15 ± 1.05
	G. max	[c]8.40 ± 0.30	*17.10 ± 0.30	[c]27.45 ± 0.45	*10.95 ± 0.15
AC+D	Non amended	[a]6.30 ± 0.30	*7.80 < 0.01	[a]11.70 ± 0.30	*9.45 ± 0.15
	L. perenne	[b]8.55 ± 0.15	*36.60 ± 0.60	[c]26.70 ± 0.60	*27.75 ± 0.45
	S. bicolor	[ab]7.80 < 0.01	*22.20 ± 0.30	[a]10.80 ± 0.30	*11.25 ± 0.45
	M. sativa	[b]9.45 ± 0.15	*29.40 ± 2.40	[b]19.20 ± 2.40	*14.25 ± 0.45
	G. max	[a]5.55 ± 0.15	*9.60 ± 0.60	[a]10.35 ± 1.05	*6.45 ± 0.15
Solvent	Non amended	[a]5.70 < 0.01	*7.05 ± 0.15	[b]19.65 ± 2.85	*5.55 ± 0.15
	L. perenne	[a]5.10 ± 0.30	*17.55 ± 0.45	[c]24.60 ± 1.20	*14.10 ± 0.30
	S. bicolor	[a]4.80 ± 0.60	*9.30 ± 0.30	[c]25.95 ± 0.15	*15.15 ± 1.05
	M. sativa	[b]7.95 ± 0.15	*26.10 ± 0.90	[b]18.30 ± 3.00	*6.60 ± 0.30
	G. max	[b]7.35 ± 0.15	*12.00 ± 0.30	[a]14.55 ± 1.65	*6.15 ± 0.15

Treatments: PAH (100 mg kg-1 Σ PAH), PAH + AC (100 mg kg-1 Σ PAH + 0.1 % (dry weight) activated charcoal), PAH + D (100 mg kg-1 Σ PAH + 0.1% (w/w) diesel), PAH + AC + D (100 mg kg-1 Σ PAH + 0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), PS (soil as collected from the field), AC (0.1 % (dry weight) activated charcoal), D (0.1% (w/w) diesel), AC + D (0.1% (w/w) diesel + 0.1 % (dry weight) activated charcoal), Solvent (1:1 Toluene : Acetone solution).

Chapter 6

Bioaccessibility of ^{14}C-phenanthrene from root amended contaminated soil

Bioaccessibility of ^{14}C-phenanthrene from root amended contaminated soil

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6.1 Abstract

When plants are used to promote the biodegradation of these contaminants, it can be presumed that plants will increase its bioaccessible fraction, however, this is rarely evaluated. This gains importance when considering that the bioaccessibility of organic contaminants, such as hydrocarbons, is considered one of the main factors limiting the biodegradation processes. Therefore, this study aimed to assess the impact of plant roots on the bioaccessibility of ^{14}C-phenanthrene in spiked soil. To do this, changes in the hydroxypropyl-β-cyclodextrin (HPCD) extractable and biodegradable fractions of ^{14}C-phenanthrene were evaluated. After 1, 25, 50 and 100 days of PAH-soil contact time, $^{12/14}$C-phenanthrene spiked soil was amended with roots from two grasses and one legume. Mineralisation and HPCD extractability of ^{14}C-phenanthrene were quantified after 1, 21 and 42 days root-$^{12/14}$C-phenanthrene soil interaction time. Although the extractable fraction of ^{14}C-phenanthrene was not enhanced by the presence of roots, mineralisation was significantly promoted by the presence of the roots after at least 50 days of soil-PAH contact time. Overall results showed that a higher biodegradation of phenanthrene might be achieved by the incorporation of roots into the contaminated soil following a period of bacterial adaptation. Results from this study contribute to the understanding of the effect that rhizosphere conditions have towards the bioaccessibility of hydrocarbons in soil.

Keywords: Phenanthrene, diesel, hydrocarbons, plant roots, mineralisation.

6.2 Introduction

The fate and behaviour of organic contaminants in soil are controlled by a number of physical, chemical and biological factors and have been detailed by different authors (Riding *et al.*, 2013). As the contact time between the soil and the contaminant increases, there is also a decrease in the extractability and bioavailability, often described in terms of a biological measurement, such as biodegradation or ecotoxicity; this is the principle of ageing (Hatzinger & Alexander, 1995; Semple *et al.*, 2003). The ageing process has consistently been considered as an important limiting condition for the biodegradation of hydrophobic organic contaminants (HOCs) in soil (White *et al.*, 1997; Alexander, 2000) . Consequently, an accurate quantification of the magnitude by which the biologically available fraction of an organic contaminant has been affected will directly reflect on an adequate assessment and management of contaminated land (Riding *et al.*, 2013) . The extent by which an organic contaminant is susceptible to interact with soil biota can be evaluated through biological and chemical protocols including biodegradation, biological uptake, mild extractions and passive sampling (Reid *et al.*, 2001; Tang *et al.*, 2002; Song *et al.*, 2011; Bernhardt *et al.*, 2013; Riding *et al.*, 2013). These approaches have allowed assessing the bioaccessibility and potential risks of different HOCs within the environment including many priority polycyclic aromatic hydrocarbons (PAHs) (Riding *et al.*, 2013).

Biodegradation of PAHs in contaminated soil can be stimulated through different approaches. Among these, the presence of plants has been identified as having a beneficial effect upon this condition (Cunningham *et al.*, 1995). This is especially important when the soil is in close proximity to the roots where there is a modification of physical, chemical and biological properties that can promote the dissipation of contaminants (Anderson *et al.*, 1993; Joner *et al.*, 2002). Consequently, the microbial-enhanced ability of this region of the soil to break down contaminants, also known as the rhizosphere effect, has been the object of study for multiple authors (Joner & Leyval, 2003; Muratova *et al.*, 2003; Pilon-Smits, 2005; Agnello *et al.*, 2014) . Research is encouraged by the possibility of these desirable characteristics serving as a low-cost protocol for the enhancement of the biodegradation of hydrocarbons in soil, which may be translated to bioremediation. Although the mechanisms through which

the rhizosphere this process are complex, there are traits that have been identified as a contributing factor for the bioremediation of organic contaminants. Among these, the input of substances and organic matter due to root exudation, decay and turnover have been hypothesized to be important contributing factors (Anderson *et al.*, 1993; Pilon-Smits, 2005; Agnello *et al.*, 2014; Martin *et al.*, 2014). These processes represent a constant source of organic substrates that the microorganisms use as readily available sources of carbon and nutrients (Anderson *et al.*, 1993; Broeckling *et al.*, 2008; Martin *et al.*, 2014). It has been suggested that processes such as root decay and turnover might be able to promote the degradation of organic contaminants such as polycyclic aromatic hydrocarbons (Leigh *et al.*, 2002). When plant roots die and decompose, these have been observed to release substrates capable of providing multiple benefits such as enhancing soil nutrient and carbon cycling (Matamala, 2003), contribute to the accumulation of soil organic matter (SOM) (Rasse *et al.*, 2005) and promote microbial activity in the soil (Leigh *et al.*, 2002). Authors have observed the important role that specific root-released compounds such as phenolic substances (Fletcher & Hegde, 1995; Leigh *et al.*, 2002; Parrish *et al.*, 2005) or nutrients (Vogt *et al.*, 1998) might have on the biodegradation of organic contaminants.

It has been hypothesised that the use of root amendments should represent a realistic way to simulate natural root decay and release root-derived substrates (Miya & Firestone, 2001; Rentz *et al.*, 2004; Mueller & Shann, 2007). Additionally, an advantage of this approach would be the elimination of any possible competition for resources such as mineral nutrients between living plants and microorganisms (Chaineau *et al.*, 2000). However, there are few studies describing the influence of root amendments from different plant species on PAH mineralization (Miya & Firestone, 2001; Ite *et al.*, 2016), where results have showed that it is possible to enhance the biodegradation of phenanthrene (Miya & Firestone, 2001) and naphthalene (Miya & Firestone, 2001; Ite *et al.*, 2016) through this approach. The present study aims to contribute on the elucidation of to what extent a simulated rhizosphere of different plant species can influence the bioaccessibility of phenanthrene in contaminated soil while isolating any possible interference caused by the presence of the whole plants. Most importantly, results from this study will allow to accurately identify if *(1)* soil-contaminant contact time, *(2)* the approach used for

the introduction of the roots, and *(3)* the root-soil interaction time have a significant impact in the process.

6.3 Methodology

6.3.1 Soil preparation and spiking

Uncontaminated clay loam soil from the top layer (0 - 20 cm) was collected at an agricultural field in Myerscough Agricultural College, Preston, U.K. Soil was partially air-dried for 24 h and passed through a 2 mm sieve. Soil physical and chemical characteristics are described by Towell *et al.* (2011). Soil was rehydrated with dH_2O to 70% water holding capacity (Towell *et al.*, 2011a) and spiked following the stainless steel spoon approach proposed by Doick, Lee and Semple (2003). The spiking procedure was done by mixing the previously rehydrated soil with either one of two separate standards containing $^{12/14}$C-phenanthrene using acetone as the carrier solvent to deliver a final concentration of 100 mg kg^{-1} (dw) phenanthrene with an associated ^{14}C-activity of 83 Bq g^{-1} (dw). Additionally, one of the standards also included 0.1% (w/w) diesel. After spiking and venting, soil was placed in sealed sterilized amber jars and incubated in the dark at 21 ±1 ºC in a controlled environment room until needed. ^{14}C-activity in the soil was assessed at the beginning of every time point through sample oxidation as described by Rhodes *et al.* (2012).

6.3.2 Roots preparation

Seeds from *Lolium perenne* L., *Sorghum bicolor* L. (Poaceae) and *Medicago sativa* L. (Fabaceae) were sown in the same test soil without the presence of phenanthrene. Seeds were allowed to germinate and grown in a glasshouse at 25 ± 3 ºC with a 16/8 h photoperiod. Once the germination period was finished (> 50 % germinated seeds), seedlings were grown for three weeks before harvesting. Plants were removed from the soil and the roots separated from the above ground material and roots were then cleaned through three consecutive washes with sterilised dH_2O (Miya & Firestone, 2001). Each wash consisted on placing a beaker containing the roots and dH_2O onto an orbital shaker for 30 minutes at 70 rpm (Muratova *et al.*, 2003) in a controlled environment room at 21 ± 1 ºC. Washed roots were blotted with absorbent paper and used to prepare two different soil amendments from each species. Roots from each

species were either cut into 1-2 mm pieces or ground using mortar and pestle to form a homogeneous paste. Subsamples from each of the amendments were taken and oven dried at 80 ºC for 12 hours for the assessment of the individual species moisture content.

6.3.3 Microbial inoculum

An inoculum of bacteria isolated from hydrocarbon contaminated soil and identified as *Pseudomonas* sp. was prepared on a minimum basal salts (MBS) medium enriched with 0.1 g phenanthrene L^{-1} (Reid *et al.*, 2001; Towell *et al.*, 2011a). The solution was incubated onto an orbital shaker (100 rpm) at 21 ± 1 ºC for 4 days to reach bacteria exponential growth phase. After this time, the inoculum was harvested following the methodology described by Towell *et al.* (2011), delivering a final concentration of 10^6 cells ml^{-1}.

6.3.4 Experimental design

In order to assess the effects of soil-PAH contact time, $^{12/14}$C-phenanthene spiked soil was incubated over 1, 25, 50 and 100 days before root amendments were introduced into the soil. At each time point, the incubated soil was separated into equal parts and mixed with one of the root amendments (1 % dw). An additional portion of the soil with and without diesel was mixed but not amended and was used as the control. Once mixed, root amended soils and controls were placed into sterilized amber glass jars and incubated for up to 42 days. After 1, 21 and 42 days root-soil interaction time, subsamples of the root amended soil and controls were taken for the assessment of mineralisation and bioaccessibility of ^{14}C-phenanthene using complete randomized arrangements. Additional subsamples were taken from the control soils at each time point for the measurement of the initial ^{14}C-activity through sample oxidation (Model 307, Packard) as described by Rhodes *et al.* (2012).

6.3.5 Mineralisation assays

The assessment of the mineralisation of ^{14}C-pheanthrene followed the methodology described by Reid *et al.* (2001). At each time point, 10 g soil (dw) was placed into 250 ml modified Schott bottles (respirometer). Each respirometer also included 25 ml of sterilized minimal basal salts (MBS) medium (Fenlon *et al.*, 2007), and 5 ml of the

microbial inoculum (10^6 cells ml^{-1}) to give a final soil:liquid ratio of 1:3. Each respirometer was equipped a $^{14}CO_2$ trap consisting on a suspended 7 ml glass scintillation vial containing 1 ml 1M NaOH solution. Respirometers (n = 3) were placed onto an orbital shaker (100 rpm) in a controlled environment room at 21 ± 1 ºC in the dark for 14 days. Every 24 hours, the $^{14}CO_2$ trap was replaced with fresh NaOH solution, while the incubated one was mixed with liquid scintillation cocktail. Activity was assessed after 24 hours incubation in the dark (to reduce the effects of luminescence) by liquid scintillation counting (LSC) for 10 minutes using a Canberra Packard Tri-Carb 2250CA, U.K. equipment (Towell *et al.*, 2011a).

6.3.6 Assessment of bioaccessibility

Bioaccessibility of ^{14}C-phenanthrene was assessed through HPCD shake extractions following the methodology described by Vázquez-Cuevas and Semple (2016). At every time point, 1.25 g soil (dw) were subsampled from each treatment (n = 3) and mixed with 25 ml 50 mM HPCD solution in Teflon centrifuge tubes. Tubes were placed onto an orbital shaker (100 rpm) for 22 h in a controlled environment room at 21 ± 1 ºC) and centrifuged at 3000 *x g* for 1 h. An aliquot (5 ml) was then mixed with 15 ml liquid scintillation cocktail and assessed by LSC as previously described. Residual activity in the soil was assessed by sample oxidation as described by Rhodes *et al.* (2012).

6.3.7 Statistical analysis

Following blank correction, statistical analyses of the results were carried with IBM SPSS 21 software (95% confidence interval). Normal distribution of the data was tested with Shapiro-Wilk tests and transformations were used when normal distribution was not observed. Changes on the mineralisation across ageing times and the effect of diesel were evaluated by a two-way mixed repeated measures ANOVA (Bonferroni). Relationship and comparisons between bioavailable and mineralised ^{14}C-phenanthrene were assessed by linear regression modelling. For all cases, differences between the treatments at each individual time point were analysed using One-Way ANOVA (Tukey) or Kruskal-Wallis test for normal and non-normal distributed data respectively. Graphical representations of the results were done with the software SigmaPlot 2000.

6.4 Results

6.4.1 Mineralisation of ^{14}C-phenanthrene influenced by plant roots

6.4.1.1 Effects of soil-PAH contact time

The impact of roots on the mineralisation of ^{14}C-phenanthrene was assessed after 1, 25, 50 and 100 days of soil-PAH contact time, kinetics are presented in Tables SI 1 - 4. The findings showed that mineralisation of ^{14}C-phenanthrene was significantly reduced at longer soil-PAH contact times ($p < 0.001$), especially when compared against values observed after 1 day (Figures 1 and 2). The total extents of mineralisation at the beginning of soil-PAH contact times were reduced from 65.3 and 77.0 % after 1 day soil-PAH contact time to 20.0 and 13.9 % after 100 days, in the presence and absence of diesel, respectively.

Subsequent root-soil interaction times also presented similar patterns with reduced extents of mineralisation after the longest interaction times (42 days) compared to those measured at the beginning of the incubations (1 day). Similar to soil-PAH contact time, the effects of time when roots interacted with the soil were clearer at the initial stage of the experimentation (1 day soil-PAH contact time) than after 100 days (Figures 1 and 2).

These trends were observed for both the absence (Figure 1) and presence (Figure 2) of diesel. Furthermore, the introduction of diesel also produced significant changes within mineralisation kinetics ($p < 0.003$). This was generally reflected by longer lag phases longer lag phases ($p < 0.002$), as well as reduced maximum rates ($p < 0.003$) and total extents of mineralisation ($p < 0.001$) when diesel was present.

6.4.1.2 Impact of root biomass

The effects of the root amendments were assessed at each soil-PAH contact time (1, 25, 50 and 100 days) and followed for up to 42 days of root-soil interaction time. Overall results showed that although significant differences were observed among treatments ($p < 0.05$), this was not the case when treatments were compared against the controls ($p > 0.05$).

When soil was amended with roots from *L perenne*, the biomass did not have a significant effect on the mineralisation of ^{14}C-phenanthrene within the first 50 days of soil-PAH contact time ($p > 0.05$). After 50 d soil-PAH contact time, the introduction of either root pieces or paste from this species had significantly enhancing effects on the phenanthrene/diesel contaminated soil, especially after 42 days of root-soil contact time. This was reflected on shorter lag phases (2.6 and 2.4 d, respectively) compared to 3.66 d observed in the control soil. Similarly, maximum rates (2.2 and 2.6 % d^{-1}, respectively) and total extents (19.2 and 21.7 %, respectively) were significantly enhanced by either form or *L. perenne* roots when compared against the control.

As described for *L. perenne*, similar behaviour was observed when soil was amended with roots from *S. bicolor* in either form (paste or pieces). For this species, data was only significantly different ($p < 0.05$) in soil amended with the phenanthrene/diesel mixture after 50 days of soil-PAH contact time and mainly after 42 days of root-soil contact time. As with the other grass species, values from soil amended with root paste or pieces from *S. bicolor* reflected shorter lag phases (2.5 and 2.6 d, respectively), as well as fastest rates (2.4 and 2.2 % d^{-1}, respectively) and larger total extents (23.3 and 18.8 %, respectively) than the control soil (lag phase: 3.7 d, fastest rate: 1.7 % d^{-1}, total extent:15.2 %, respectively).

When soil was amended with roots from *M. sativa* (legume), the effects were also significant ($p < 0.05$) after 50 days soil-PAH contact time. Moreover, significant differences were observed in both soil treatments with and without diesel. Roots from this species as paste were capable of inducing greater total extents of mineralisation after 1 d (47.3 %) and 21 d (26.7 %) of root-soil contact time when compared to the control soil spiked with phenanthrene only (37.2 % and 23.0 %, respectively) after 50 days soil-PAH contact time. As observed with both grass species, when diesel was also present in the soil, significantly greater extents of mineralisation were mainly observed after 42 days of incubation of the roots in the soil for both root pieces and root paste. This led to shorter lag phases of 2.0 d, and fastest rates of 2.6 and 2.7 % d^{-1}) and greater extents of mineralisation of 22.0 and 22.5 %, compared to the control soil with a lag phase of 3.7 d, a fastest rate of 1.7 % d^{-1} and total extent of mineralisation of 15.2 %).

Overall, soil amended with any of the root treatments required an adaptation period before mineralisation was enhanced by the treatments. In addition, this process was observed to be longer in soil spiked with phenanthrene only. In this case, mineralisation was generally observed to be greater only after 100 d soil-PAH contact time and 1 d since the introduction of the roots. On the other hand, when diesel was also present in the soil, significantly greater mineralisation kinetics were observed after 50 d soil-PAH contact time and 42 d of root-soil incubation.

6.4.2 Bioaccessibility of ^{14}C-phenanthrene

6.4.2.1 Root induced changes to the bioaccessibility of ^{14}C-phenanthrene

The bioaccessibility of ^{14}C-phenanthrene, as measured by HPCD extractability, was not consistently affected throughout experiment (Table SI 5). General trends showed that the extractability of ^{14}C-phenanthrene decreased as soil-PAH contact time increased. ^{14}C-Phenanthrene extractability ranged from 88.62 % and 86.68 % after 1 d soil-PAH contact time to 16.37 % and 14.75 % after 100 d in the control soil containing ^{14}C-phenanthrene and ^{14}C-phenanthrene/diesel respectively.

The presence of the plant root biomass resulted in significantly greater levels of ^{14}C-phenanthrnene extractability after 21 days of root-soil incubation when diesel was not spiked. This effect was presented after 1 d (F = 3.127, p = 0.037), 25 d (F = 4.236, p = 0.012) and 50 d (p = 0.042) soil-PAH contact time. Despite these differences, no clear pattern was observed regarding plant species or the form of the root amendment. However, when diesel was also present in the soil, these differences where not observed. Overall data from this experiment showed that extractability of ^{14}C-phenanthrene was similar ($p > 0.05$) among treatments when compared to the control soil.

6.4.2.2 Relationship between HPCD extractability and mineralisation of ^{14}C-phenanthrene

The capacity of HPCD extractability to predict the mineralisable fraction of ^{14}C-phenanthrene was also investigated (Figure 3). Results showed that there was a statistically significant ($p \leq 0.001$) linear relationship between the ^{14}C-phenanthrene extracted (22 h) and mineralised (14 d). This correlation was higher in soil amended

with diesel (r^2 =0.775, n = 84, $p \leq 0.001$) than when it was absent (r^2 = 0.556, n = 84, $p \leq 0.001$). Data from soil containing the phenanthrene/diesel mixture also showed to have a closer 1:1 relationship reflected on slopes closer to 1 (Table SI 6).

In this same soil condition, data showed to have a stronger fit (r^2) in the control soil than when it was amended with the different root biomass treatments (Table SI 6). Furthermore, linear correlations of the two soil conditions (Figures 3 A-B) showed that the larger deviation from the 1:1 relationship (broken line) reflected an over-estimation of the mineralisation by the HPCD extraction on soils freshly amended with root biomass (i.e. 1 day of soil-PAH contact time).

6.5 Discussion

6.5.1 Influence of root amendment on the biodegradation of ^{14}C-phenanthrene in soil

Studies looking at the behaviour of organic contaminants in soil typically report the decline of the freely available fractions of these substances across time due to ageing processes (Hatzinger & Alexander, 1995; Reid *et al.*, 2000a; Macleod & Semple, 2002; Semple *et al.*, 2006). Results from the present investigation showed that, overall, this behaviour was not affected by the incorporation of plant root biomass into the spiked soil. Generally, findings showed a consistent reduction on the mineralisation of ^{14}C-phenanthrene with longer soil-PAH contact times despite the increment on the root-soil interaction time. In addition to this commonly observed pattern, the results indicated that plant-root biomass tended to reduce the mineralisation of ^{14}C-phenanthrene at the beginning of the incubation with the soil (1 d). Moreover, the present study highlighted the importance of the temporal factor for the biodegradation of ^{14}C-phenanthrene affected by plant roots. These results suggest that root biomass might have served as an additional sorption surface at this initial stage, which was then decomposed resulting in the release of the sorbed PAH alongside with root-derived substrates at longer root-soil incubation times (21 d and 42 d). Although the specific mechanism behind this behaviour was not assessed, a similar effect has been reported before for other PAHs (Schwab *et al.*, 1998), where the PAH was observed to be adsorbed onto root debris before being transformed or translocated. This hypothesis is also supported by the findings reported by other

authors, who also proposed the ability of plant roots to physically limit the biodegradation of PAHs (Miya & Firestone, 2000; Jiao *et al.*, 2007; Sun *et al.*, 2010).

In addition to the sorption processes previously discussed, the incorporation of plant root biomas can also be defined as an additional source of organic matter, which is expected to also promote the sequestration of organic contaminants (Richnow *et al.*, 1999; Boucard *et al.*, 2005). This same mechanism can can also derive on the modification within soil organic mater fractionation and its cycling (Makhadmeh, 2001), which is a behaviour normally observed thougout the rhizosphere. These particular processes are proposed as one of the main mechanisms leading to the generalised absence of significantly different bioaccessibility observed in this investigation, By this, ^{14}C-phenanthrene that under other circumstances might had become more accessible due to the presence of root exudates and root-derived substrates got sequestrated by one of these root-promoted fractions of organic matter that were originally less abundant.

On the other hand, the presence of diesel was also observed to have a significant impact on the mineralisation of ^{14}C-phenanthrene, producing a significant but less obvious effect of plant-root amendments towards mineralisation of the PAH. Althrough low molecular weight hydrocarbons typically found in in diesel have been reported tu promote greter mineralisation of larger molecular weight hydrocarbons (Lee, Doick and Semple, 2003), overall results from this experiment showed the opposite trend. These results suggest that the presence of diesel can inhibit the biodegradation of ^{14}C-phenanthrene in soil, agreeing with the reported by other authors such as Swindell and Reid (2006) and Vázquez-Cuevas, Stevens and Semple (2018). Among the different factors that might have produced this effect it is possible to identify *(i)* an initial toxicity of diesel towards microbial populations, and *(ii)* a reduction of the freely avaliable fraction of ^{14}C-phenanthene. First, as it has been previously obsreved, the microbial catabolic response can be retarded by the presence of diesel due to a toxic effect produced by different components found in diesel oil (Lee, Doick and Semple, 2003).

6.5.2. Relationship between HPCD extractability and mineralisation

It was observed that soil treatments, including diesel, resulted in a stronger 1:1 (HPCD:mineralised) relationship than when the oil was not amended into the soil. Given the generalised overestimation of the bioaccessible ^{14}C-phenanthrnene in the absence of diesel, it can be acknowledged that microbial communities were not able to mineralise the entire extent of this fraction within this soil condition. This behaviour can be attributed to the influence of diesel promoting the growth of more catabolically active bacteria (Siddiqui & Adams, 2002; Margesin *et al.*, 2007), as well as shift on the bacterial populations (Sutton *et al.*, 2013). By this, the more abundant and active bacterial community present in diesel-amended soil was able to mineralise a larger proportion of the bioaccessible ^{14}C-phenanthrene, leading to the observed closer 1:1 relationship between these two endpoints.

In addition, extractable^{14}C-phenanthrene was shown to have modest linear correlations against the extents of mineralisation. Regression values obtained in this study at early stages of the experiment agree to the results presented by Doick et al. (2006) for soil without ageing, where large concentrations of phenanthrene (up to 100 mg kg^{-1}) had a tendency to show a greater deviation from the 1:1 linear correlation. Furthermore, differences between mineralisable and HPCD extractable fractions of ^{14}C-phenanthrene might have been further induced by the promotion of sorption processes due to the presence of decaying root material and their associated substrates (Sun *et al.*, 2010). Results suggest that by this, decomposing plant-root material at different stages might be able to induce a change within the behaviour of ^{14}C-phenanthrene, therefore affecting its HPCD extractability.

6.6 Conclusions

Mineralisation of ^{14}C-phenanthrene can be enhanced by the amendment with plant root biomass from any spices or form, especially after a longer PAH-soil contact time. The time required for bacteria to significantly mineralise larger amounts of the PAH was observed to be shorter when soil was also spiked with diesel (50 days) than when this was absent (100 days). Similarly, the root-soil interaction time was also different depending on either the presence (42 days) or absence (1 day) of diesel. These results

show that after an adaptation period towards phenanthrene, the performance of catabolically active bacteria can be promoted by root biomass amendments and diesel. Furthermore, although diesel was observed to have a generalised inhibitory effect towards mineralisation, it was also capable to produce a more efficient utilisation of the bioaccessible fraction of ^{14}C-phenanthrene.

Bioaccessibility of ^{14}C-phenanthrene was assessed through its HPCD extractability, this was not significantly affected by root amendments. These results might have been the combination of the introduction of additional sorption surfaces (root biomass) and underlaying methodological discrepancies between the mineralisation and extraction approaches. Results from this study contribute to the understanding of the importance of the temporal factor towards the impact of plant root biomass decay for biodegradation of hydrocarbons in soil.

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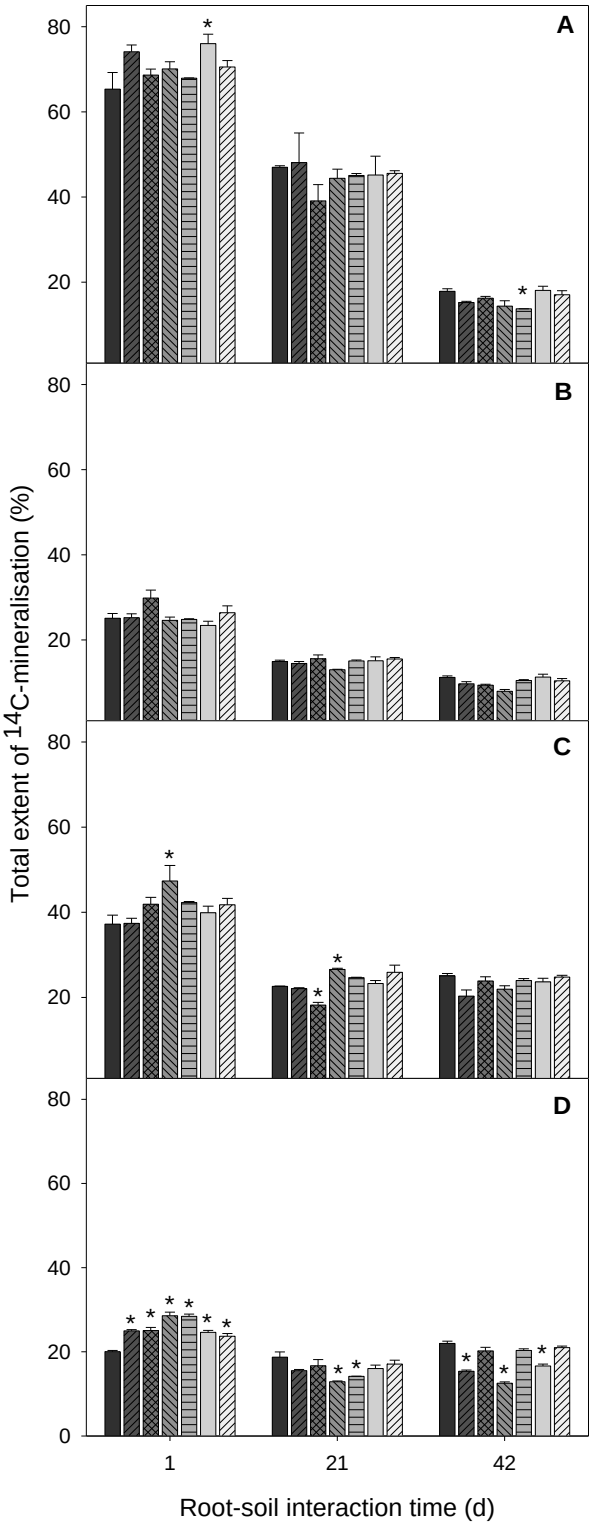

Figure 1. Total extents of mineralisation (%) after 1 (A), 25 (B), 50 (C) and 100 (D) soil-PAH contact times from phenanthrene spiked soil after 1, 21 and 42 d root-soil interaction time. Treatments are: Control, *L. perenne* paste, *L. perenne* pieces, *M. sativa* paste, *M. sativa* pieces, *S. bicolor* paste, *S. bicolor* pieces. Marked (*) cases represent significant differences ($p < 0.05$) of a treatment against the control. Error bars represent the standard error of the mean ($n = 3$).

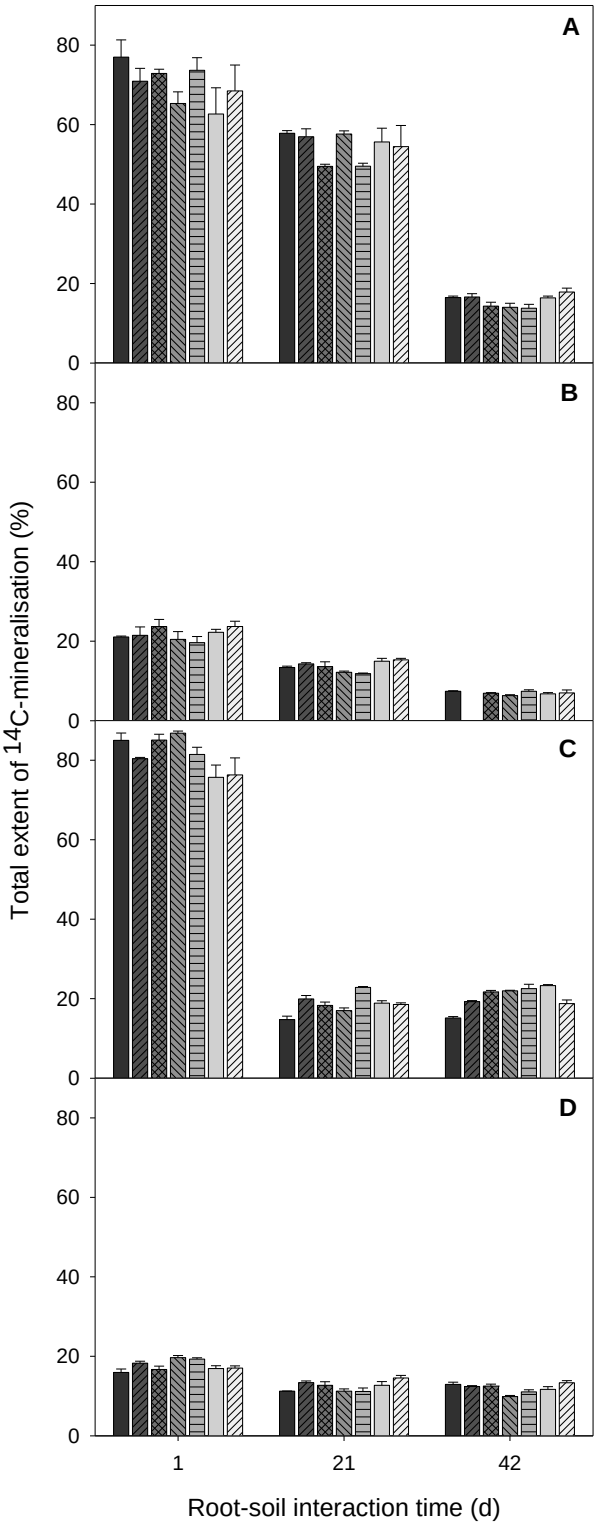

Figure 2. Total extents of mineralisation (%) after 1 (A), 25 (B), 50 (C) and 100 (D) soil-PAH contact times from phenanthrene/diesel spiked soil after 1, 21 and 42 d root-soil interaction time. Treatments are: Control, *L. perenne* paste, *L. perenne* pieces, *M. sativa* paste, *M. sativa* pieces, *S. bicolor* paste, *S. bicolor* pieces. Marked (*) cases represent significant differences ($p < 0.05$) of a treatment against the control. Error bars represent the standard error of the mean ($n = 3$).

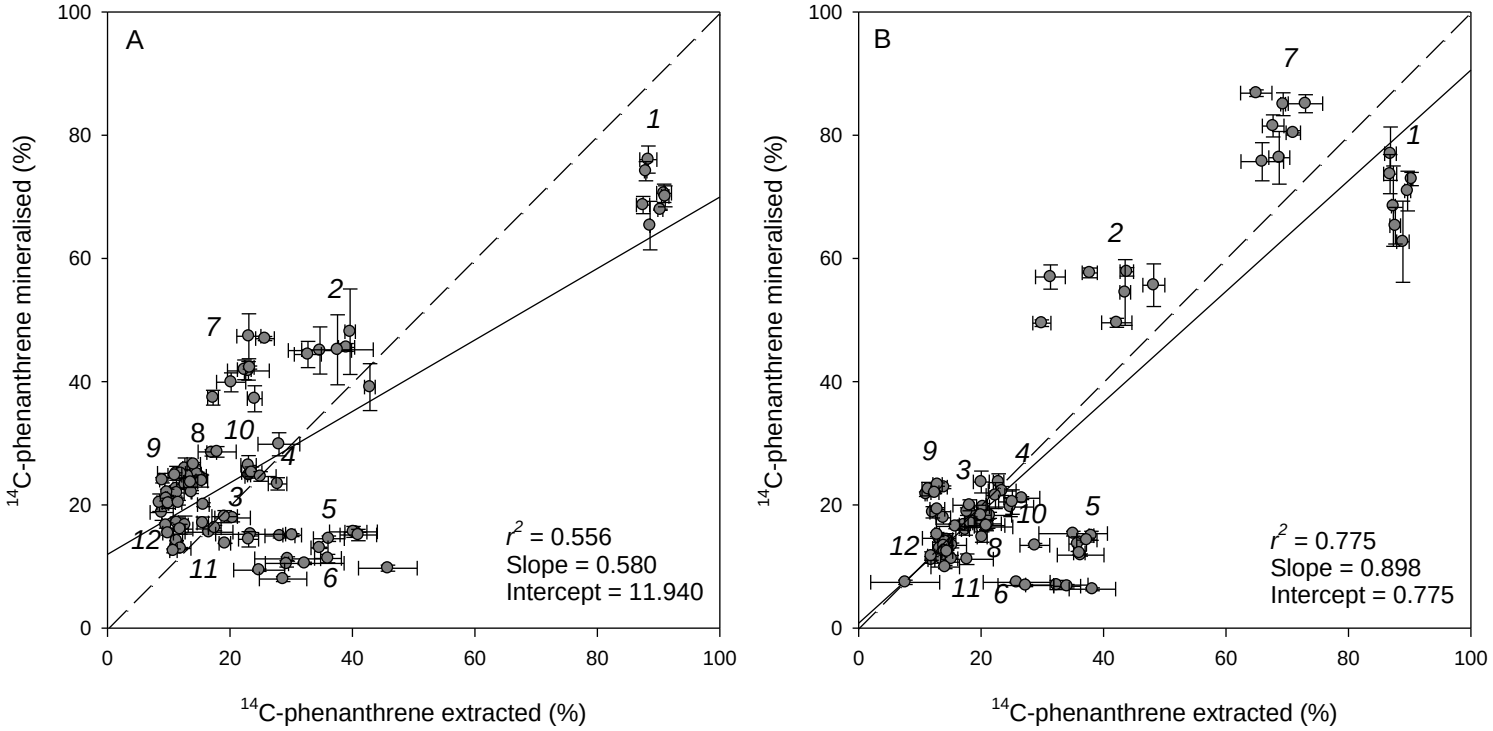

Figure 3. Linear regression between ^{14}C-phenanthrene extracted with HPCD after 22 h and the extent of ^{14}C-phenanthrene mineralised over 14 days from $^{12/14}$C-phenanthrene contaminated soil in the absence (A) and presence (B) of diesel (n = 84). Broken line represents the 1:1 fit. Error bars represent the standard error of the mean (n = 3). Italic numbers represent the specific time point of the series: one day soil-PAH contact time and 1(*1*), 21(*2*) and 42 (*3*) days root-soil contact time, 25 days soil-PAH contact time and 1(*4*), 21(*5*) and 42 (*6*) days root-soil contact time, 50 days soil-PAH contact time and 1(*7*), 21(*8*) and 42 (*9*) days root-soil contact time, and 100 days soil-PAH contact time and 1(*10*), 21(*11*) and 42 (*12*) days root-soil contact time.

6.7 References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D. & Esposito, G. 2014. Enhanced phytoremediation: A review of low molecular weight organic acids and surfactants used as amendments. Critical Reviews in Environmental Science and Technology, 44, 2531–2576.

Alexander, M. 2000. Aging, bioavaiability, and overestimation of risk from environmental pollutants. Environmental Science & Technology, 34, 4259–4265.

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science & Technology, 27, 2630–2636.

Bernhardt, C., Derz, K., Kördel, W. & Terytze, K. 2013. Applicability of non-exhaustive extraction procedures with Tenax and HPCD. Journal of Hazardous Materials, 261, 711–717.

Boucard, T.K., Bardgett, R.D., Jones, K.C. & Semple, K.T. 2005. Influence of plants on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil. Environmental Pollution, 133, 53–62.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. & Vivanco, J.M. 2008. Root exudates regulate soil fungal community composition and diversity. Applied and Environmental Microbiology, 74, 738–744.

Chaineau, C.H., Morel, J.L. & Oudot, J. 2000. Biodegradation of fuel oil hydrocarbons in the rhizosphere of maize. Journal of Environmental Quality, 29, 569–578.

Cunningham, S.D., Berti, W.R. & Huang, J.W. 1995. Phytoremediation of contaminated soils. Trends in Biotechnology, 13, 393–397.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Fenlon, K.A., Jones, K.C. & Semple, K.T. 2007. Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. Journal of Environmental Monitoring, 9, 510.

Fletcher, J.S. & Hegde, R.S. 1995. Release of phenols by perennial plant roots and their potential importance in bioremediation. Chemosphere, 31, 3009–3016.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

Ite, A.E., Adebisi, O.O., Hanney, N.F. & Semple, K. 2016. The Effect of Rhizosphere Soil and Root Tissues Amendment on Microbial Mineralisation of Target C – Hydrocarbons in

Contaminated Soil. International Journal of Environmental Bioremediation & Biodegradation, 4, 21–34.

Jiao, X.C., Xu, F.L., Dawson, R., Chen, S.H. & Tao, S. 2007. Adsorption and absorption of polycyclic aromatic hydrocarbons to rice roots. Environmental Pollution, 148, 230–235.

Joner, E.J., Corgié, S.C. & Leyval, C. 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biology and Biochemistry, 34, 859–864.

Joner, E.J. & Leyval, C. 2003. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environmental Science & Technology, 37, 2371–2375.

Lee, P.H., Doick, K.J. & Semple, K.T. 2003. The development of phenanthrene catabolism in soil amended with transformer oil. FEMS Microbiology Letters, 228, 217–223.

Leigh, M.B., Fletcher, J.S., Fu, X. & Schmitz, F.J. 2002. Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environmental Science & Technology, 36, 1579–1583.

Macleod, C.J.A. & Semple, K.T. 2002. The adaptation of two similar soils to pyrene catabolism. Environmental Pollution, 119, 357–364.

Makhadmeh, I.M. 2001. Ecophysiology of the Plant ± Rhizosphere System. Plant Production, 9, 1–9.

Margesin, R., Hämmerle, M. & Tscherko, D. 2007. Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: Effects of hydrocarbon concentration, fertilizers, and incubation time. Microbial Ecology, 53, 259–269.

Martin, B.C., George, S.J., Price, C.A., Ryan, M.H. & Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. Science of the Total Environment, 472, 642–653.

Matamala, R. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science, 302, 1385–1387.

Miya, R.K. & Firestone, M.K. 2000. Phenanthrene-degrader community dynamics in rhizosphere soil from a common annual grass. Journal of Environment Quality, 29, 584.

Miya, R.K. & Firestone, M.K. 2001. Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environment Quality, 30, 1911–1918.

Mueller, K.E. & Shann, J.R. 2007. Effects of tree root-derived substrates and inorganic nutrients on pyrene mineralization in rhizosphere and bulk soil. Journal of Environment Quality, 36, 120.

Muratova, A., Hübner, T., Tischer, S., Turkovskaya, O., Möder, M. & Kuschk, P. 2003. Plant–rhizosphere-microflora association during phytoremediation of PAH-ontaminated soil. International Journal of Phytoremediation, 5, 137–151.

Parrish, Z.D., Banks, M.K. & Schwab, A.P. 2005. Effect of root death and decay on dissipation of polycyclic aromatic hydrocarbons in the rhizosphere of yellow sweet clover and tall fescue. Journal of Environment Quality, 34, 207.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39.

Rasse, D.P., Rumpel, C. & Dignac, M.F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil, 269, 341–356.

Reid, B.J., Jones, K.C. & Semple, K.T. 2000. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environmental Pollution, 108, 103–112.

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D. & Semple, K.T. 2001. A simple 14C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. FEMS Microbiology Letters, 196, 141–146.

Rentz, J.A., Alvarez, P.J.J. & Schnoor, J.L. 2004. Repression of Pseudomonas putida phenanthrene-degrading activity by plant root extracts and exudates. Environmental Microbiology, 6, 574–583.

Rhodes, A.H., Riding, M.J., McAllister, L.E., Lee, K. & Semple, K.T. 2012. Influence of activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil. Environmental Science & Technology, 46, 12445–12451.

Richnow, H.H., Eschenbach, A., Mahro, B., Kästner, M., Annweiler, E., Seifert, R. & Michaelis, W. 1999. Formation of nonextractable soil residues: A stable isotope approach. Environmental Science & Technology, 33, 3761–3767.

Riding, M.J., Doick, K.J., Martin, F.L., Jones, K.C. & Semple, K.T. 2013. Chemical measures of bioavailability/bioaccessibility of PAHs in soil: Fundamentals to application. Journal of Hazardous Materials, 261, 687–700.

Schwab, a. P., Al-Assi, a. a. & Banks, M.K. 1998. Adsorption of Naphthalene onto Plant Roots. Journal of Environment Quality, 27, 220.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Siddiqui, S. & Adams, W.A. 2002. The fate of diesel hydrocarbons in soils and their effect on the germination of perennial ryegrass. Environmental Toxicology, 17, 49–62.

Song, Y., Wang, F., Yang, X., Liu, C., Kengara, F.O., Jin, X. & Jiang, X. 2011. Chemical extraction to assess the bioavailability of chlorobenzenes in soil with different aging periods. Journal of Soils and Sediments, 11, 1345–1354.

Sun, T.R., Cang, L., Wang, Q.Y., Zhou, D.M., Cheng, J.M. & Xu, H. 2010. Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. Journal of Hazardous Materials, 176, 919–925.

Sutton, N.B., Maphosa, F., Morillo, J.A., Al-Soud, W.A., Langenhoff, A.A.M., Grotenhuis, T., Rijnaarts, H.H.M. & Smidt, H. 2013. Impact of long-term diesel contamination on soil microbial community structure. Applied and Environmental Microbiology, 79, 619–630.

Swindell, A.L. & Reid, B.J. 2006. Influence of diesel concentration on the fate of phenanthrene in soil. Environmental Pollution, 140, 79–86.

Tang, J., Liste, H.H. & Alexander, M. 2002. Chemical assays of availability to earthworms of polycyclic aromatic hydrocarbons in soil. Chemosphere, 48, 35–42.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Vázquez-Cuevas, G.M. & Semple, K.T. 2016. Measurement of Hydrocarbon Bioavailability in Soil. In: Hydrocarbon and Lipid Microbiology Protocols - Springer Protocols Handbooks (eds. McGenity, T., Timmis, K. & Nogales, B.), pp. 231–246. Springer, Berlin, Heidelberg.

Vázquez-Cuevas, G.M., Stevens, C.J. & Semple, K.T. 2018. Enhancement of14C-phenanthrene mineralisation in the presence of plant-root biomass in PAH-NAPL amended soil. International Biodeterioration and Biodegradation, 126, 78–85, (At: http://dx.doi.org/10.1016/j.ibiod.2017.09.021.).

Vogt, K. a, Vogt, D.J. & Bloomfield, J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant and Soil, 200, 71–89.

White, J.C., Kelsey, J.W., Hatzinger, P.B. & Alexander, M. 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. Environmental Toxicology and Chemistry, 16, 2040–2045.

6.8 Supplementary information

Table SI 1. Mineralisation kinetics from phenanthrene and phenanthrene/diesel contaminated soil. Lag phases (d), maximum rates (% d^{-1}) and total extents (%) of ^{14}C-phenanthrene after 1 day soil-PAH contact time and 1, 21 and 42 days of root-soil contact time. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Root- soil contact time	Parameter	No roots	*Lolium perenne* paste	*Lolium perenne* pieces	*Medicago sativa* paste	*Medicago sativa* pieces	*Sorghum bicolor* paste	*Sorghum bicolor* pieces
				Phenanthrene				
1 day	Lag phase	0.2 < 0.1	0.1 < 0.1	0.2 < 0.1	0.2 < 0.1	0.2 < 0.1	0.1 < 0.1	0.2 < 0.1
	Maximum rate	25.0 ± 2.8	34.2 ± 5.1	30.4 ± 0.7	26.1 ± 1.2	26.6 ± 0.3	34.6 ± 6.1	30.3 ± 2.6
	Total extent	65.3 ± 3.9	74.1 ± 1.6	68.6 ± 1.4	70.1 ± 1.7	67.9 ± 0.1	*76.0 ± 2.2	70.5 ± 1.5
21 days	Lag phase	0.6 < 0.1	0.6 ± 0.1	0.8 ± 0.1	0.5 < 0.1	0.6 < 0.1	0.7 ± 0.1	0.6 < 0.1
	Maximum rate	8.4 ± 0.2	8.9 ± 1.9	7.1 ± 0.1	10.1 ± 1.0	8.3 ± 0.3	7.6 ± 0.7	8.1 ± 0.4
	Total extent	47.0 ± 0.3	48.1 ± 6.9	39.1 ± 3.8	44.4 ± 2.1	45.0 ± 0.5	45.2 ± 4.4	45.5 ± 0.6
42 days	Lag phase	2.0 < 0.1	2.0 ± 0.1	2.0 ± 0.3	2.1 ± 0.2	1.7 < 0.1	1.9 ± 0.2	1.8 ± 0.2
	Maximum rate	3.1 ± 0.2	3.0 ± 0.1	2.9 ± 0.1	*2.6 ± 0.2	3.5 < 0.1	3.1 ± 0.2	2.9 ± 0.3
	Total extent	17.8 ± 0.6	15.2 ± 0.3	16.2 ± 0.4	14.4 ± 1.2	*13.7 < 0.1	18.1 ± 0.1	17.0 ± 1.0
				Phenanthrene/diesel				
1 day	Lag phase	0.1 < 0.1	0.2 < 0.1	0.1 < 0.1	0.2 < 0.1	0.2 < 0.1	0.2 < 0.1	0.2 < 0.1
	Maximum rate	40.2 ± 5.3	21.5 ± 3.7	33.2 ± 2.4	27.7 ± 1.2	30.8 ± 4.2	*26.0 ± 2.9	29.7 ± 2.8
	Total extent	77.0 ± 4.3	70.9 ± 3.2	72.9 ± 1.1	65.3 ± 3.0	73.7 ± 3.2	62.7 ± 6.6	68.5 ± 6.5
21 days	Lag phase	0.4 < 0.1	0.4 < 0.1	0.5 < 0.1	0.4 < 0.1	0.5 < 0.1	0.5 < 0.1	0.6 ± 0.1
	Maximum rate	11.7 ± 1.2	11.5 ± 1.1	10.8 ± 0.2	11.8 ± 1.4	10.8 ± 0.2	11.2 ± 1.2	9.9 ± 2.1
	Total extent	57.8 ± 0.7	57.0 ± 2.0	49.5 ± 0.5	57.6 ± 0.8	49.5 ± 0.7	55.6 ± 3.5	54.4 ± 5.3
42 days	Lag phase	2.8 ± 0.2	2.2 < 0.1	2.7 ± 0.3	2.7 ± 0.2	2.2 ± 0.3	2.4 ± 0.2	2.1 ± 0.1
	Maximum rate	3.1 < 0.1	2.9 ± 0.3	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.3	2.5 < 0.1	3.1 ± 0.4
	Total extent	16.5 ± 0.4	16.6 ± 0.8	14.3 ± 1.0	14.0 ± 1.0	13.8 ± 1.0	16.4 ± 0.4	17.9 ± 1.0

Table SI 2. Mineralisation kinetics from phenanthrene and phenanthrene/diesel contaminated soil. Lag phases (d), maximum rates (% d^{-1}) and total extents (%) of ^{14}C-phenanthrene after 25 days soil-PAH contact time and 1, 21 and 42 days of root-soil contact time. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Root- soil contact time	Parameter	No roots	*Lolium perenne* paste	*Lolium perenne* pieces	*Medicago sativa* paste	*Medicago sativa* pieces	*Sorghum bicolor* paste	*Sorghum bicolor* pieces
				Phenanthrene				
1 day	Lag phase	1.6 ± 0.1	1.8 < 0.1	1.45 ± 0.1	1.7 ± 0.1	1.7 < 0.1	2.0 < 0.1	1.5 ± 0.1
	Maximum rate	3.4 ± 0.1	3.1 < 0.1	3.69 ± 0.3	3.3 ± 0.4	3.1 < 0.1	2.9 < 0.1	3.5 ± 0.3
	Total extent	25.1 ± 1.1	25.2 ± 0.9	29.83 ± 1.9	24.6 ± 0.8	24.8 ± 0.2	23.4 ± 1.0	26.4 ± 1.6
21 days	Lag phase	3.0 ± 0.2	3.0 ± 0.2	2.94 < 0.1	3.1 ± 0.3	2.8 < 0.1	2.6 ± 0.2	2.7 ± 0.2
	Maximum rate	1.8 ± 0.1	1.9 ± 0.2	2.01 < 0.1	2.4 ± 0.4	2.1 < 0.1	2.2 ± 0.1	2.1 ± 0.1
	Total extent	14.9 ± 0.3	14.5 ± 0.4	15.60 ± 0.9	13.0 ± 0.1	15.1 ± 0.2	15.1 ± 0.9	15.5 ± 0.3
42 days	Lag phase	4.6 ± 0.1	5.5 ± 0.1	4.90 ± 0.1	*6.2 ± 0.5	5.0 < 0.1	4.3 ± 0.5	5.0 ± 0.2
	Maximum rate	1.4 < 0.1	1.2 ± 0.1	1.44 < 0.1	1.1 < 0.1	1.4 < 0.1	1.6 ± 0.2	1.4 < 0.1
	Total extent	11.2 ± 0.4	9.7 ± 0.5	9.38 ± 0.2	7.9 ± 0.4	10.5 ± 0.2	11.3 ± 0.7	10.4 ± 0.5
				Phenanthrene/diesel				
1 day	Lag phase	2.3 ± 0.1	2.3 ± 0.3	2.1 ± 0.1	2.7 ± 0.4	2.9 ± 0.3	2.5 ± 0.1	1.8 ± 0.3
	Maximum rate	2.7 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	2.1 ± 0.3	2.6 ± 0.3	2.6 ± 0.2	3.3 ± 0.5
	Total extent	21.1 ± 0.2	21.5 ± 2.1	23.7 ± 1.8	20.4 ± 2.0	19.6 ± 1.5	22.2 ± 0.7	23.7 ± 1.3
21 days	Lag phase	4.0 ± 0.1	3.6 ± 0.1	3.6 ± 0.2	3.9 ± 0.2	4.3 ± 0.1	3.3 ± 0.3	3.2 ± 0.1
	Maximum rate	1.8 ± 0.1	1.9 < 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	2.1 ± 0.3	2.0 ± 0.1
	Total extent	13.4 ± 0.3	14.3 ± 0.3	13.6 ± 1.2	12.2 ± 0.3	11.8 ± 0.2	15.0 ± 0.7	15.3 ± 0.4
42 days	Lag phase	7.2 ± 0.3	ND	8.3 ± 0.5	9.9 ± 0.6	7.3 ± 0.6	8.8 ± 0.8	8.4 ± 1.6
	Maximum rate	1.1 < 0.1	ND	1.0 < 0.1	0.8 < 0.1	1.1 < 0.1	0.9 < 0.1	1.0 ± 0.1
	Total extent	7.4 ± 0.1	ND	6.9 ± 0.2	6.3 ± 0.2	7.4 ± 0.3	6.8 ± 0.3	7.0 ± 0.7

*ND: Not determined

Table SI 3. Mineralisation kinetics from phenanthrene and phenanthrene/diesel contaminated soil. Lag phases (d), maximum rates (% d^{-1}) and total extents (%) of ^{14}C-phenanthrene after 50 days soil-PAH contact time and 1, 21 and 42 days of root-soil contact time. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Root- soil contact time	Parameter	No roots (Control)	*Lolium perenne* paste	*Lolium perenne* pieces	*Medicago sativa* paste	*Medicago sativa* pieces	*Sorghum bicolor* paste	*Sorghum bicolor* pieces
				Phenanthrene				
1 day	Lag phase	0.5 < 0.1	0.6 ± 0.1	0.4 ± 0.01	0.3 < 0.1	0.4 < 0.1	0.5 ± 0.1	0.4 < 0.1
	Maximum rate	9.5 ± 0.8	8.9 ± 0.9	11.4 ± 0.37	*16.0 ± 2.8	11.1 ± 0.4	0.3 ± 1.3	11.9 ± 0.3
	Total extent	7.2 ± 2.1	37.4 ± 1.2	41.9 ± 1.61	*47.3 ± 3.6	42.3 ± 0.3	39.9 ± 1.5	41.7 ± 1.5
21 days	Lag phase	2.2 ± 0.2	2.7 < 0.1	2.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	2.4 ± 0.1	1.9 ± 0.3
	Maximum rate	2.8 ± 0.2	2.8 < 0.1	2.4 ± 0.2	2.9 ± 0.2	2.9 ± 0.2	2.7 ± 0.1	3.1 ± 0.4
	Total extent	22.6 ± 0.1	22.1 ± 0.2	*18.2 ± 0.6	*26.6 ± 0.3	24.6 ± 0.1	23.3 ± 0.7	25.9 ± 1.7
42 days	Lag phase	1.7 ± 0.1	2.4 ± 0.1	2.1 ± 0.3	*2.6 < 0.1	1.9 < 0.1	2.1 ± 0.1	2.0 < 0.1
	Maximum rate	3.2 < 0.1	2.3 ± < 0.1	3.1 ± 0.4	2.5 ± 0.1	3.0 ± 0.1	2.5 ± 0.1	2.8 ± 0.1
	Total extent	25.0 ± 0.6	*20.3 ± 1.4	23.9 ± 1.0	21.9 ± 0.8	24.0 ± 0.4	23.6 ± 0.8	24.8 ± 0.4
				Phenanthrene/diesel				
1 day	Lag phase	0.2 < 0.1	0.1 < 0.1	0.1 < 0.1	*0.1 < 0.1	0.2 < 0.1	0.2 < 0.1	0.1 < 0.1
	Maximum rate	26.3 ± 1.4	33.3 ± 2.3	35.3 ± 2.3	*47.4 ± 2.2	33.6 ± 5.4	30.4 ± 4.0	38.3 < 0.1
	Total extent	85.0 ± 1.8	80.4 ± 0.4	85.1 ± 1.4	86.8 ± 0.5	81.5 ± 1.8	75.7 ± 3.1	76.3 ± 4.3
21 days	Lag phase	3.5 ± 0.1	*2.9 < 0.1	*2.64 < 0.1	*2.8 ± 0.1	*2.6 ± 0.1	*2.6 < 0.1	*2.7 ± 0.1
	Maximum rate	2.0 ± 0.2	2.5 ± 0.4	2.15 < 0.1	2.6 ± 0.1	2.9 ± 0.2	2.2 < 0.1	2.2 ± 0.1
	Total extent	14.8 ± 0.8	*19.9 ± 0.9	*18.31± 0.8	17.0 ± 0.7	*22.8 ± 0.2	*18.9 ± 0.6	*18.5 ± 0.4
42 days	Lag phase	3.7 < 0.1	*2.6 < 0.1	*2.37 ± 0.1	*2.0 < 0.1	*2.0 ± 0.1	*2.5 < 0.1	*2.6 ± 0.1
	Maximum rate	1.7 < 0.1	*2.2 < 0.1	*2.58 ± 0.2	*2.6 ± 0.1	*2.7 < 0.1	*2.4 ± 0.1	*2.2 ± 0.1
	Total extent	15.2 ± 0.3	*19.2± 0.3	*21.72 ± 0.4	*22.0 ± 0.1	*22.5 ± 1.1	*23.3 ± 0.3	*18.8 ± 0.9

Table SI 4. Mineralisation kinetics from phenanthrene and phenanthrene/diesel contaminated soil. Lag phases (d), maximum rates (% d^{-1}) and total extents (%) of ^{14}C-phenanthrene after 100 days soil-PAH contact time and 1, 21 and 42 days of root-soil contact time. Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Root- soil contact time	Parameter	No roots (Control)	*Lolium perenne* paste	*Lolium perenne* pieces	*Medicago sativa* paste	*Medicago sativa* pieces	*Sorghum bicolor* paste	*Sorghum bicolor* pieces
				Phenanthrene				
1 day	Lag phase	1.8 ± 0.1	1.7 < 0.1	1.6 < 0.1	1.7 ± 0.1	*1.6 < 0.1	1.7 < 0.1	1.8 ± 0.1
	Maximum rate	2.9 ± 0.1	*3.5 < 0.1	*3.7 ± 0.1	*4.0 ± 0.1	*3.8 < 0.1	*3.4 < 0.1	*3.6 ± 0.1
	Total extent	20.0 ± 0.3	*25.0 ± 0.3	*25.0 ± 0.7	*28.6 ± 0.8	*28.4 ± 0.5	*24.6 ± 0.5	*23.7 ± 0.7
21 days	Lag phase	2.4 ± 0.1	1.8 ± 0.2	2.1 ± 0.3	2.9 < 0.1	2.2 < 0.1	2.11 ± 0.16	2.1 ± 0.1
	Maximum rate	2.8 ± 0.1	3.0 ± 0.1	2.6 ± 0.3	2.5 < 0.1	3.0 ± 0.1	2.67 ± 0.14	3.0 ± 0.2
	Total extent	18.7 ± 1.2	15.5 ± 0.3	16.7 ± 1.4	*12.9 ± 0.2	*14.2 < 0.1	16.04 ± 0.80	17.1 ± 1.0
42 days	Lag phase	2.1 ± 0.2	2.2 ± 0.2	1.7 ± 0.1	2.9 ± 0.3	2.3 < 0.1	2.13 ± 0.16	2.0 ± 0.1
	Maximum rate	3.2 ± 0.2	2.5 ± 0.1	3.0 ± 0.2	2.7 ± 0.2	*4.7 ± 0.3	2.92 ± 0.26	3.3 ± 0.3
	Total extent	22.0 ± 0.5	*15.4 ± 0.3	20.2 ± 0.8	*12.5 ± 0.3	20.3 ± 0.4	*16.64 ± 0.44	21.0 ± 0.4
				Phenanthrene/diesel				
1 day	Lag phase	3.8 ± 0.2	3.3 ± 0.2	3.6 ± 0.1	3.4 < 0.1	3.2 ± 0.1	3.7 ± 0.1	3.3 ± 0.1
	Maximum rate	2.2 ± 0.1	2.5 ± 0.1	1.8 ± 0.3	*2.8 < 0.1	2.7 ± 0.1	c2.3 ± 0.1	2.3 ± 0.1
	Total extent	16.0 ± 1.0	18.3 ± 0.5	16.7 ± 0.8	*19.7 ± 0.5	*19.3 ± 0.4	16.9 ± 0.7	17.0 ± 0.6
21 days	Lag phase	5.1 < 0.1	3.4 ± 0.1	3.9 ± 0.5	3.7 ± 0.4	4.3 ± 0.4	3.6 ± 0.3	3.4 ± 0.2
	Maximum rate	1.2 ± 0.1	1.7 ± 0.2	1.7 ± 0.3	*2.1 ± 0.2	1.6 ± 0.1	2.0 ± 0.1	*2.2 ± 0.2
	Total extent	11.2 ± 0.1	13.4 ± 0.4	12.7 ± 0.9	11.2 ± 0.6	11.2 ± 0.9	12.7 ± 0.9	*14.6 ± 0.6
42 days	Lag phase	3.8 ± 0.2	3.9 ± 0.1	3.9 ± 0.3	*4.6 ± 0.2	4.1 ± 0.2	3.9 ± 0.4	3.2 ± 0.3
	Maximum rate	1.7 ± 0.2	1.4 < 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	2.0 ± 0.1
	Total extent	b12.9 ± 0.6	12.4 ± 0.2	12.5 ± 0.5	*9.9 ± 0.3	11.1 ± 0.5	11.7 ± 0.7	13.3 ± 0.5

Table SI 5. Comparison between hydroxypropyl-β-cyclodextrin extractable (%, 22 h) and mineralisable (%, 14 d) fractions of ^{14}C-phenanthrene. Values are presented as the mean ± standard error of the mean (n = 3)

		1 day soil-root contact time		21 days soil-root contact time		42 days soil-root contact time	
		^{14}C-phe extracted (%)	^{14}C-phe mineralised (%)	^{14}C-phe extracted (%)	^{14}C-phe mineralised (%)	^{14}C-phe extracted (%)	^{14}C-phe mineralised (%)
1 day soil-PAH contact time							
Phenanthrene	No roots	88.62 ± 0.01	65.32 ± 3.93	25.72 ± 1.52	46.98 ± 0.34	20.46 ± 2.90	17.85 ± 0.61
	S. bicolor pieces	90.90 ± 1.19	70.55 ± 1.50	39.05 ± 1.33	45.54 ± 0.64	15.57 ± 0.93	17.04 ± 0.97
	L. perenne pieces	87.48 ± 1.09	68.65 ± 1.39	42.86 ± 0.88	39.10 ± 3.82	17.53 ± 1.04	16.23 ± 0.42
	M. sativa pieces	90.30 ± 0.42	67.89 ± 0.10	34.70 ± 5.16	45.04 ± 0.47	19.18 ± 0.94	13.74 ± 0.01
	S. bicolor paste	88.34 ± 1.40	76.03 ± 2.20	37.57 ± 5.83	45.16 ± 4.44	19.14 ± 2.07	18.07 ± 0.98
	L. perenne paste	87.91 ± 0.20	74.14 ± 1.56	39.63 ± 0.86	48.09 ± 6.94	23.39 ± 5.71	15.23 ± 0.28
	M. sativa paste	91.09 ± 0.61	70.06 ± 1.70	32.73 ± 2.19	44.40 ± 2.12	23.06 ± 1.64	14.39 ± 1.24
Phenanthrene + Diesel	No roots	86.88 ± 0.96	76.98 ± 4.33	43.81 ± 1.08	57.81 ± 0.68	15.81 ± 3.09	16.49 ± 0.37
	S. bicolor pieces	87.35 ± 0.32	68.47 ± 6.52	43.55 ± 0.87	54.45 ± 5.33	13.84 ± 1.20	17.87 ± 0.99
	L. perenne pieces	90.31 ± 0.22	72.87 ± 1.09	29.93 ± 1.49	49.48 ± 0.54	14.44 ± 0.95	14.34 ± 0.97
	M. sativa pieces	86.83 ± 1.07	73.67 ± 3.17	42.18 ± 2.47	49.54 ± 0.74	14.17 ± 0.95	13.81 ± 0.97
	S. bicolor paste	88.90 ± 1.00	62.70 ± 6.57	48.22 ± 1.80	55.65 ± 3.46	20.84 ± 4.35	16.40 ± 0.45
	L. perenne paste	89.68 ± 0.50	70.93 ± 3.22	31.33 ± 2.43	56.97 ± 1.97	20.89 ± 3.01	16.62 ± 0.84
	M. sativa paste	87.65 ± 0.87	65.30 ± 2.96	37.76 ± 1.24	57.64 ± 0.79	14.17± 0.46	14.02 ± 1.03
25 days soil-PAH contact time							
Phenanthrene	No roots	11.24 ± 3.07	25.10 ± 1.13	28.17 ± 2.43	14.94 ± 0.32	29.43 ± 5.38	11.21 ± 0.36
	S. bicolor pieces	23.04 ± 1.26	26.39 ± 1.61	40.57 ± 1.80	15.55 ± 0.33	29.26 ± 0.31	10.41 ± 0.53
	L. perenne pieces	28.00 ± 3.41	29.83 ± 1.87	40.18 ± 3.90	15.60 ± 0.89	24.79 ± 4.17	9.38 ± 0.20
	M. sativa pieces	23.12 ± 0.25	24.81 ± 0.17	30.18 ± 1.53	15.07 ± 0.24	32.22 ± 6.42	10.49 ± 0.19

	S. bicolor paste	27.78 ± 1.51	23.39 ± 1.00	40.97 ± 3.00	15.10 ± 0.92	36.02 ± 2.20	11.27 ± 0.67
	L. perenne paste	23.57 ± 1.66	25.23 ± 0.91	36.15 ± 2.53	14.49 ± 0.45	45.79 ± 4.78	9.70 ± 0.49
	M. sativa paste	24.99 ± 2.53	24.60 ± 0.77	34.64 ± 1.37	13.00 ± 0.11	28.68 ± 3.88	7.94 ± 0.41
Phenanthrene + Diesel	No roots	26.67 ± 2.94	21.08 ± 0.23	28.79 ± 2.42	13.40 ± 0.32	25.82 ± 5.45	7.41 ± 0.13
	S. bicolor pieces	22.87 ± 0.85	23.71 ± 1.33	35.05 ± 5.60	15.31 ± 0.38	32.37 ± 5.10	6.98 ± 0.75
	L. perenne pieces	20.05 ± 1.31	23.70 ± 1.77	35.80 ± 1.43	13.62 ± 1.20	27.32 ± 4.52	6.90 ± 0.18
	M. sativa pieces	24.81 ± 3.78	19.63 ± 1.55	36.27 ± 3.81	11.83 ± 0.17	7.62 ± 5.64	7.42 ± 0.34
	S. bicolor paste	23.46 ± 2.26	22.24 ± 0.73	38.02 ± 0.40	14.97 ± 0.73	34.08 ± 2.19	6.77 ± 0.28
	L. perenne paste	22.37 ± 1.51	21.49 ± 2.11	37.28 ± 1.65	14.28 ± 0.32	ND	ND
	M. sativa paste	25.14 ± 2.28	20.45 ± 1.98	36.07 ± 0.94	12.18 ± 0.29	38.19 ± 3.80	6.29 ± 0.23
	50 days soil-PAH contact time						
Phenanthrene	No roots	24.05 ± 1.21	37.21 ± 2.10	11.21 ± 0.49	22.59 ± 0.09	12.02 ± 0.72	25.05 ± 0.58
	S. bicolor pieces	23.02 ± 3.42	41.75 ± 1.50	12.64 ± 0.35	25.90 ± 1.69	11.02 ± 1.12	24.76 ± 0.43
	L. perenne pieces	22.36 ± 1.12	41.91 ± 1.61	72.44 ± 5.50	18.21 ± 0.62	15.41 ± 0.96	23.87 ± 0.99
	M. sativa pieces	23.27 ± 0.68	42.28 ± 0.26	13.14 ± 0.37	24.65 ± 0.08	8.96 ± 0.43	23.98 ± 0.44
	S. bicolor paste	20.22 ± 2.38	39.88 ± 1.54	12.70 ± 1.40	23.26 ± 0.70	13.59 ± 1.17	23.64 ± 0.84
	L. perenne paste	17.24 ± 0.87	37.38 ± 1.20	13.72 ± 0.07	22.06 ± 0.23	8.51 ± 0.43	20.32 ± 1.43
	M. sativa paste	23.11 ± 1.97	47.34 ± 3.65	14.09 ± 1.11	26.56 ± 0.27	11.35 ± 1.18	21.91 ± 0.83
Phenanthrene + Diesel	No roots	69.34 ± 0.40	85.01 ± 1.85	20.18 ± 0.81	14.76 ± 0.84	12.83 ± 0.38	15.17 ± 0.33
	S. bicolor pieces	68.71 ± 1.73	76.33 ± 4.27	18.40 ± 1.10	18.54 ± 0.41	12.12 ± 0.45	18.78 ± 0.91
	L. perenne pieces	72.99 ± 2.82	85.10 ± 1.45	19.99 ± 1.69	18.31 ± 0.84	11.04 ± 0.15	21.72 ± 0.36
	M. sativa pieces	67.71 ± 1.76	81.49 ± 1.79	13.95 ± 0.55	22.84 ± 0.21	11.40 ± 1.05	22.54 ± 1.09
	S. bicolor paste	65.92 ± 3.48	75.69 ± 3.09	17.73 ± 0.23	18.88 ± 0.62	12.91 ± 0.76	23.28 ± 0.27
	L. perenne paste	71.00 ± 1.17	80.37 ± 0.36	18.14 ± 2.72	19.92 ± 0.90	12.88 ± 1.22	19.25 ± 0.28
	M. sativa paste	64.96 ± 2.55	86.80 ± 0.55	18.32 ± 0.99	17.00 ± 0.70	12.48 ± 0.66	21.97 ± 0.15
	50 days soil-PAH contact time						

Phenanthrene	No roots	15.67 ± 0.95	20.02 ± 0.32	8.86 ± 1.88	18.73 ± 1.25	9.73 ± 0.39	22.01 ± 0.51
	S. bicolor pieces	12.81 ± 0.57	23.69 ± 0.66	11.35 ± 0.23	17.09 ± 0.93	9.65 ± 0.15	20.99 ± 0.36
	L. perenne pieces	14.21 ± 0.45	25.07 ± 0.74	12.68 ± 0.52	16.71 ± 1.45	9.95 ± 0.05	20.24 ± 0.84
	M. sativa pieces	17.03 ± 0.81	28.45 ± 0.51	11.28 ± 0.98	14.17 ± 0.02	11.61 ± 0.53	20.33 ± 0.37
	S. bicolor paste	15.03 ± 1.13	24.62 ± 0.47	11.90 ± 2.06	16.04 ± 0.80	9.58 ± 0.65	16.64 ± 0.44
	L. perenne paste	14.68 ± 0.64	24.97 ± 0.31	16.58 ± 3.90	15.54 ± 0.27	9.90 ± 0.88	15.37 ± 0.30
	M. sativa paste	17.93 ± 3.11	28.59 ± 0.85	11.95 ± 1.67	12.90 ± 0.16	10.78 ± 0.59	12.53 ± 0.33
Phenanthrene + Diesel	No roots	17.46 ± 0.65	15.95 ± 0.87	13.54 ± 0.33	11.22 ± 0.07	13.26 ± 1.16	12.92 ± 0.57
	S. bicolor pieces	19.81 ± 1.16	17.05 ± 0.56	13.16 ± 2.72	14.57 ± 0.64	13.94 ± 0.71	13.34 ± 0.55
	L. perenne pieces	17.18 ± 0.72	16.71 ± 0.83	14.20 ± 0.20	12.71 ± 0.89	14.02 ± 0.39	12.54 ± 0.48
	M. sativa pieces	18.16 ± 0.73	19.29 ± 0.37	17.72 ± 4.31	11.16 ± 0.86	11.90 ± 0.55	11.06 ± 0.54
	S. bicolor paste	21.15 ± 2.17	16.92 ± 0.74	14.64 ± 0.47	12.69 ± 0.94	11.88 ± 0.60	11.68 ± 0.68
	L. perenne paste	21.41 ± 3.58	18.28 ± 0.48	15.26 ± 2.39	13.40 ± 0.41	14.45 ± 0.93	12.41 ± 0.21
	M. sativa paste	20.34 ± 1.04	19.67 ± 0.55	15.19 ± 1.02	11.23 ± 0.56	14.16 ± 2.34	9.90 ± 0.27

*ND = Not determined

Table SI 6. Linear regression between ^{14}C-phenanthrene extracted with HPCD after 22 h and the extent of ^{14}C-phenanthrene mineralised over 14 days for individual root amendments (n = 36)

	Root amendment	r^2	Intercept	Slope	F	p-value
Phenanthrene	No roots	0.534	14.970	0.517	38.967	≤ 0.001
	S. bicolor pieces	0.575	13.559	0.559	40.059	≤ 0.001
	L. perenne pieces	0.430	11.071	0.489	26.673	≤ 0.001
	M. sativa pieces	0.559	12.880	0.560	43.012	≤ 0.001
	S. bicolor paste	0.567	10.585	0.560	43.012	≤ 0.001
	L. perenne paste	0.469	11.043	0.564	30.080	≤ 0.001
	M. sativa paste	0.518	10.624	0.606	36.485	≤ 0.001
Phenanthrene + Diesel	No roots	0.847	-3.834	1.053	187.527	≤ 0.001
	S. bicolor pieces	0.726	2.261	0.835	89.923	≤ 0.001
	L. perenne pieces	0.741	1.381	0.887	97.437	≤ 0.001
	M. sativa pieces	0.792	1.417	0.913	129.280	≤ 0.001
	S. bicolor paste	0.669	2.059	0.787	68.717	≤ 0.001
	L. perenne paste	0.737	4.533	0.844	86.891	≤ 0.001
	M. sativa paste	0.607	0.557	0.873	52.467	≤ 0.001

Chapter 7

Mineralisation of ^{14}C-phenanthrene in complex PAH-diesel oil mixtures contaminated soil in the presence of *Sorghum bicolor* and *Medicago sativa* mono- and mixed cultures

Mineralisation of ^{14}C-phenanthrene in complex PAH-diesel oil mixtures contaminated soil in the presence of *Sorghum bicolor* and *Medicago sativa* mono- and mixed cultures

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7.1 Abstract

The aim of this study was to assess ^{14}C-phenanthrene mineralisation profiles in mixtures of PAH-diesel contaminated soil in the presence of *Sorghum bicolor* and *Medicago sativa.* Monocultures of both plant species tolerated the complex mixtures based on growth and survival, and increased rates and extents of ^{14}C-phenanthrene mineralisation in soil. The influence of PAH concentration on ^{14}C-phenanthrene mineralisation profiles varied in planted and unplanted treatments. The rates and extents of ^{14}C-phenanthrene mineralisation tended to decrease in diesel amended soil, especially at low PAH concentrations. To the best of the authors' knowledge, this is the first report of ^{14}C-phenanthrene mineralisation patterns in complex PAH-diesel oil mixtures contaminated soil especially with respect to the specified plant species. The findings offer new insights on mono- and multi-species phytotoxicity as well as plant-assisted biodegradation of PAH mixtures in soil which may be useful in the risk assessment and remediation of contaminated sites.

Keywords: PAH mixtures; diesel oil amendment; Phytotoxicity; *Sorghum bicolor*; *Medicago sativa*; ^{14}C-phenanthrene mineralisation.

7.2. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic contaminants with two or more benzene rings fused together. Generally, these compounds are of concern to human and environmental health due to their carcinogenicity, toxicity, and persistence in the environment (Juhasz & Naidu, 2000). The USEPA has classified 16 PAHs as priority pollutants as they are classified as either probable or possible human carcinogens, including phenanthrene (Phe), benzo[a]anthracene (BaA), and benzo[a]pyrene (BaP) (Yan *et al.*, 2004; Couling *et al.*, 2010; US Environmental Protection Agency, 2013). PAHs differ in their physico-chemical properties; typically as the molecular size increases, water solubility, volatility and biodegradability decrease, while K_{ow}, K_d, K_{oc} and half-life increase (Stroud *et al.*, 2007a; Semple *et al.*, 2013). Although PAHs are released into the environment from natural combustion of organic matter, anthropogenic activities constitute the most important sources (Wilson & Jones, 1993). For example, burning of fossil fuel, coal, and wood, vehicular emissions, heating, and accidental spills of crude oil and other petroleum products among others are well known sources of PAH release into the environment (Baldrian *et al.*, 2000; Wong *et al.*, 2005; Cachada *et al.*, 2014). As a result of these emissions and their mobility in the environment, PAHs are found in almost all environments including in locations some distance from their primary sources (Halsall *et al.*, 2001).

Soil is considered a major sink for PAHs in the environment (Wild & Jones, 1995; Semple *et al.*, 2001). PAHs can be found as complex mixtures in soil, where they associate with other chemicals such as phenols, aliphatic hydrocarbons and metals (Allan *et al.*, 2007; Thavamani *et al.*, 2012). PAHs also exist in co-contamination with non-aqueous phase liquids (NAPLs) such as transformer oil from electrical cables and diesel oil from deliberate and accidental oil spillage around petroleum hydrocarbon contaminated sites (Wilson & Jones, 1993; Luthy *et al.*, 1994; Molina-Barahona *et al.*, 2004). The implication is that co-contamination is likely to change the fate and behaviour of PAHs in soil (Lee *et al.*, 2003; Couling *et al.*, 2010; Semple *et al.*, 2013). Swindell & Reid (2006) examined the influence of diesel on phenanthrene loss in soil and found that diesel concentrations within 0 and 2,000 mg kg^{-1} resulted in increased phenanthrene dissipation, while at 20,000 mg kg^{-1}, phenanthrene loss was retarded. Another study reported that although microbial respiration increased with increasing

cable oil concentration, mineralisation of ^{14}C-phenyldodecane decreased (Towell *et al.*, 2011b).

Various studies have revealed the potential of plant-assisted biodegradation of PAHs in soil (Aprill & Sims, 1990; Banks *et al.*, 2003; Huang *et al.*, 2004; White *et al.*, 2006; Meng *et al.*, 2011; Chen *et al.*, 2016; Deng & Zeng, 2017). For example, Aprill & Sims (1990) reported greater losses of 4 and 5 ringed-PAHs in planted sandy-loam soils, when compared to unplanted controls. In another study, ryegrass planted in soil contaminated soil with a mixture of hydrocarbons (HCs) decreased the initial HC concentration by up to 97 %, whereas 82 % was lost from the unplanted soil (Günther *et al.*, 1996). In another study, 30 to 40 % less degradation was measured in unplanted soils containing a mixture of pyrene and anthracene than in soils planted with grasses and a leguminous plant species (Reilley *et al.*, 1996). In another study, Xu *et al.* (2006) reported a decline in the concentration of pyrene and phenanthrene from 52.52 mg kg^{-1} and 58.19 mg kg^{-1} down to 4.12 mg kg^{-1} and 6.77 mg kg^{-1} respectively after 60 d in a soil planted with maize. Similarly, Meng *et al.* (2011) reported greater PAH losses in soil planted with ryegrass, white clover, and celery as mono- and mixed cultures when compared to non-planted controls. Up to 99 % of PAH loss was attributed to plant-enhanced biodegradation while only 2 % was due to direct plant uptake, concluding that PAH losses in planted soils are mostly driven by plant-microbe interactions rather than plant uptake (Meng *et al.*, 2011).

Plant species differ in their morphology and physiology such as in root and shoot properties (Mueller & Shann, 2006; Zhu *et al.*, 2016; Panchenko *et al.*, 2017). The root exudates secreted by plants can serve as biosurfactants or readily available carbon sources to support contaminant biodegradation within the rhizosphere (Fan *et al.*, 2008; Wenzel, 2009; Gao *et al.*, 2015b, 2017). In addition, mixed cultures of two or more plant species have been reported to enhance rates and extents of biodegradation (Chen *et al.*, 2016), potentially due to nutrient- and metabolites-richer rhizosphere, when compared to their corresponding monocultures (Wenzel, 2009). Since the effectiveness of plant-assisted biodegradation may differ with plant species (D'Orazio *et al.*, 2013), finding appropriate plant species mix may represent a confounding factor for phytoremediation (Panchenko *et al.*, 2017; Thijs *et al.*, 2017). Biodegradation enhancement of PAHs in soil using leguminous and grass crops such as monocultures

of *Medicago sativa* L. (Fabaceae) (Liu *et al.*, 2004; Sun *et al.*, 2011) and *Sorghum bicolor* (L.) Moench (Poaceae) (Banks *et al.*, 2003; Muratova *et al.*, 2009a; b) respectively have been reported previously. Owing to the desirable properties of *M. sativa* and *S. bicolor* (Hutchinson *et al.*, 2001; Ibragimova *et al.*, 2006; Dubrovskaya *et al.*, 2017), and the possibility for synergistic root interactions and co-secretion of root exudates, mixed culture of both plant species may better enhance mineralisation of ^{14}C-phenanthrene in PAH mixtures contaminated soils, in comparison to their monocultures.

For this present study, it was hypothesised that (i) both plants species would show tolerance in PAH-diesel oil mixture contaminated soil, regardless of PAH concentration or diesel amendment. (ii) Increases in PAH mixture concentration, and diesel amendment, would decrease rates and extents of ^{14}C-phenanthrene mineralisation in soil. (iii) Rates and extents of ^{14}C-phenanthrene mineralisation would be greater in planted treatments (monocultures or mixed cultures), and (iv) rates and extents of ^{14}C-phenanthrene mineralisation in treatments associated with mixed cultures would be greater than those of monocultures. To address these hypotheses, the following objectives were (i) to assess the tolerance (growth and survival) of *M. sativa* and *S. bicolor* in PAH-diesel oil mixture contaminated soil; (ii) to assess microbial mineralisation of ^{14}C-phenanthrene in soil spiked with a mixture of three PAHs and amended with diesel oil, and (iii) to evaluate and compare microbial mineralisation of ^{14}C-phenanthrene in PAH-diesel oil mixture in planted and unplanted treatments. To the best of the authors' knowledge, this is the first report of ^{14}C-phenanthrene mineralisation patterns in complex PAH-diesel oil mixtures contaminated soil with respect to the specified plant species.

7.3 Materials and methods

7.3.1 Chemicals and other materials

Non-labelled phenanthrene (>98 %), benzo[a]pyrene (>97 %), benzo[a]anthracene (>95%), sodium hydroxide (reagent grade), plate count agar (Fluka analytical), and toluene were purchased from Sigma-Aldrich, UK. [9-^{14}C] phenanthrene (3.7 MBq ml^{-1}) was obtained from American Radiolabelled chemicals, Inc., USA, Goldstar liquid scintillation cocktail (LSC) from Meridian, UK, general purpose agar (agar-agar),

general purpose grade Ringer's solution tablets, acetone (HPLC grade), as well as the chemicals used for preparing minimum basal salts (MBS) solution were acquired from Fisher Scientific, UK. Seeds of *M. sativa* and *S. bicolor* were purchased from Moles Seeds Ltd., UK and Chiltern Seeds, UK respectively. Commercial diesel was obtained from a local UK petrol station.

7.3.2 Soil preparation

A pristine clay-loam soil collected from Myerscough Agricultural College, Preston, Lancashire, PR3 0RY, U.K. Soil was partially air-dried and then passed through a 2 mm sieve. Thereafter, the sieved soil was stored in the dark at 4 ºC until needed. Soil properties have been previously determined (Couling *et al.*, 2010) and are presented in Table SI 1. Air-dried soil was spiked with ^{12}C-PAH standard (ΣPAH = Phe + BaP + BaA) at 100 mg kg^{-1} and 300 mg kg^{-1}, as well as diesel (0.1 % w/w) when applicable. Spiking was done using an inoculum approach following the protocol described by Doick & Semple (2003). Briefly, the soil was rehydrated to approximately 35 % moisture content with deionised water, after which three batches of 250 g soil were placed in a mixing bowl and spiked with ^{12}C-PAH standard in acetone:toluene (1:1 v/v) carrier solvent mixture. Solvent was allowed to vent in a fume cupboard. Soil was then thoroughly homogenised.

7.3.3 Plant-assisted biodegradation test

7.3.3.1 Assessment of seedling emergence and phytotoxicity

Seedling emergence and growth test of both plant species was conducted following relevant OECD and USEPA guidelines (OECD, 2006; U. S. Environmental Protection Agency, 2012) with slight modifications. Prior to the growth test, a seed viability test was done for both plant species. Seeds (n = 10) of each species were placed on a moistened filter paper in a petri dish. The petri dish was covered and placed in a controlled temperature room (21 ± 1 ºC) and assessed daily for germination. For the pot experiment, 90 mm plastic pots were filled with 50 g soil according to the treatments (Table 1) with a disc of filter paper fitted at the bottom to avoid soil loss. In addition, individual pot trays were fitted under each pot in order to control any leachate and avoid cross contamination. Thereafter, seeds (n = 10) were sown into the

pots in the case of monocultures and 5 seeds each for the mixed cultures (i.e. *M. sativa* + *S. bicolor*). The growth experiment was carried out in a glasshouse under standard growing conditions (OECD, 2006; U. S. Environmental Protection Agency, 2012), with the pots arranged using a completely randomized design (n = 3). The plants were grown and observed for 21 d. To maintain validity of the seedling emergence and growth test, percentage seedling emergence was 70 % in the uncontaminated control soil (OECD, 2006; U. S. Environmental Protection Agency, 2012). Plants in the control pots should not exhibit visible phytotoxic effects, and plant's survival was 90 % (OECD, 2006; U. S. Environmental Protection Agency, 2012). Germination, survival and general visual detrimental effects were assessed daily, while percentage seedling emergence and growth was determined after 21 d (OECD, 2006). Further, weekly measurements of plant heights were made while other visual toxic effects were also observed. At the end of the growth assay, planted treatments were destructively sampled in order to determine plant biomass. The shoots were harvested from the soil surface while the roots were carefully harvested after inverting the pots on a clean polythene sheet. Afterwards, the roots were gently rinsed to detach soil from the surface and then dried with a clean paper towel. The fresh weights of the shoots and roots were measured, after which they were oven-dried at 60 ºC for 24 h and their dry weights assessed. The root to shoot (R:S) ratios were then calculated.

7.3.3.2 ^{14}C-Respirometry assay

To assess plant-assisted biodegradation, evolution of ^{14}C-phenanthrene mineralisation in planted (after 0 and 21 d) and unplanted soils (after 0, 21, and 42 d) was monitored in 250 ml modified Schott bottles (n = 3) at 20 ± 1 ºC, following the methods described by Reid *et al.* (2001). Overall, each respirometer consisted of a bottle with a Teflon®-lined screw cap and a $^{14}CO_2$ trap, i.e. a 7 ml glass scintillation vial containing 1 M NaOH (1 ml). Soil (10 ± 0.2 g) was placed into each respirometer with 30 ml sterile MBS (to form a 1:3 soil slurry); and 5 µl ^{14}C-phenanthrene to deliver an activity of 398 Bq. The slurry system was used to ensure complete distribution of ^{14}C-phenanthrene in the soil samples (Doick & Semple, 2003). An analytical blank (unspiked) was also prepared to monitor levels of background ^{14}C-activity. The respirometers were then placed onto an orbital shaker (Janke and Kunkel, IKA® - Labortechnik KS 501 Digital) and shaken at 100 rpm to ensure adequate mixing of the

slurry throughout a 14 d incubation period in a controlled temperature room (20 ± 1 ^{o}C). Sampling, which involves the replacement of the $^{14}CO_2$ trap with a fresh one, was carried out every 24 h during the incubation period. To determine the amount of $^{14}CO_2$ trapped, 5 ml liquid scintillation cocktail was added to the removed trap, after which each sample was kept in the dark for 24 h to reduce the effect of chemolumniscence. Quantification of trapped ^{14}C-activity was carried out using a Canberra Packard Tri-Carb 2300TR scintillation analyser, following standard counting protocols and automatic quench correction techniques. The biodegradation parameters assessed in this study are; lag phase (defined as the time taken to reach 5% mineralisation), fastest rate (% $^{14}CO_2$ d^{-1}), and cumulative extent of mineralisation expressed as a percentage of the initial ^{14}C-phenanthrene activity which has been mineralised to $^{14}CO_2$ during each sampling time (Reid *et al.*, 2001).

7.3.3.3 Enumeration of microbial cell numbers

The number of indigenous microbial colonies (total heterotrophs and PAH degraders) assessed as colony forming units per grams soil dry weight (CFUs g^{-1}) was estimated after 0 and 21 d (planted treatments), and 0, 21, and 42 d (unplanted treatments) following standard aseptic plate counting techniques (Alef, 1995). Briefly, 1 ± 0.2 g soil (n = 3) was extracted with 10 ml sterile Ringer's solution and serially diluted. Each diluted extract was inoculated on plate count agar for total heterotrophic bacteria, and agar-agar plates pre-amended with 0.1 % ΣPAH as sole carbon source for the corresponding degrader organisms. The inoculated plates were then incubated for 10 d, at 25 ± 1 ^{o}C (Incubator Shaker G25, New Brunswick Scientific Co. Inc., USA), and microbial colonies enumerated after 4, 7 and 10 d. The ratio of degraders to total heterotrophs was also determined.

7.3.4 Statistical analysis

Data analysis was carried out using Sigmastat 13.0 (Systat Software, Inc.), and graphs were presented using SigmaPlot for Windows 13.0 (Systat Software, Inc.). Levene's test was used to determine normality of data. The statistical significance of results, i.e. effects of increase in concentration, diesel amendment, plant species, growing pattern on plant heights and plant biomass, and biodegradation parameters such as lag phases, rates and cumulative extents of ^{14}C-phenanthrene mineralisation, and microbial

numbers, were assessed at the 95 % confidence level using ANOVA (following Tukey's post hoc method) as well as Student t-test where applicable. Repeated measures ANOVA using Bonferroni's method was used to perform multiple pairwise comparisons.

7.4 Results and discussion

7.4.1 Effects of PAHs and diesel on seedling emergence and growth

After 21 d incubation following sowing, no significant differences were observed regarding the percentage of emergence ($p > 0.05$), even though values measured in soil spiked with 300 mg kg^{-1} ΣPAH were consistently greater than in soil amended with 100 mg kg^{-1} ΣPAH (Table SI 2). Plant heights also followed this trend ($p > 0.05$) in both mono- and mixed-cultures when compared to the control (Figures 1 and 2). Plant tolerance in PAH contaminated soils has been previously reported (Banks *et al.*, 2003; Cheema *et al.*, 2010; Hamdi *et al.*, 2012). For instance, the heights of *M. sativa*, *Brassica napus,* and *Lolium* sp. grown in pyrene amended soil were statistically similar to the uncontaminated controls which may imply species tolerance in the contaminated soil used (Ghanem *et al.*, 2010). However, PAHs in soil are generally not acutely toxic to plants (Chouychai *et al.*, 2007; Sverdrup *et al.*, 2007; Khan *et al.*, 2012b; D'Orazio *et al.*, 2013; Marchand *et al.*, 2016). PAHs may be unavailable to interact with plants due to sorption in soil, a phenomenon which increases with increasing PAH hydrophobicity as well as soil organic matter content (Luthy *et al.*, 1997), and may thereby minimise PAH toxicity to plants in soil (Sverdrup *et al.*, 2007). Also, Muratova *et al.* (2009b) reported that a phenanthrene concentration of 10 and 100 mg kg^{-1} did not inhibit growth and survival of *S. bicolor*, although plant biomass was affected at 100 mg kg^{-1}. Some studies however reported that diesel oil affected the germination and seedling emergence of plants and this effect has been attributed to volatile constituents of diesel fuel (Adam & Duncan, 1999, 2002; Bamgbose & Anderson, 2015). However, these effects are reduced significantly with ageing (Bona *et al.*, 2011; Wei *et al.*, 2017). In the present study, both *S. bicolor* and *M. sativa* tolerated the complex PAH-diesel oil mixtures contaminated soil as regards seedling emergence and plant growth under the prevalent assay conditions and no apparent signs of stress were observed. Such tolerance might be attributed to a

combination of plant morphological and physiological characteristics, and soil-PAH interactions (Cunningham & Ow, 1996; Salt *et al.*, 1998; White *et al.*, 2006; Wenzel, 2009; Hamdi *et al.*, 2012; de Boer & Wagelmans, 2016).

7.4.2 Effects of PAHs and diesel on plant biomass

A change in plant root biomass is also an important parameter that can be monitored during plant-enhanced biodegradation (Cheema *et al.*, 2010). High plant root biomass may favour microbial activity in soil through enrichment of rhizosphere (Banks *et al.*, 2003; Fan *et al.*, 2008; Wenzel, 2009). With respect to varying PAH concentrations and diesel amendment, changes in plant biomass among treatments were consistently observed in this study (Table 2). Root biomass (dry weight) of *S. bicolor* across all treatments was greater than in the control. The greatest biomass value of *S. bicolor* was recorded in soil spiked with 100 mg kg^{-1} ΣPAH and amended with diesel, as it exceeded the control by approximately 2 fold ($p < 0.05$). However, *M. sativa* exhibited a significantly greater ($p < 0.05$) biomass only in soil spiked with 100 mg kg^{-1} ΣPAH and amended with diesel, compared to the control. Observation of treatments with the same ΣPAH concentration (i.e. either 100 mg kg^{-1} or 300 mg kg^{-1}) in this study revealed that root biomass and R:S ratio were generally greater in the diesel amended than unamended soils for both mono- and mixed-cultures (Table 2). For example, root biomass of *S. bicolor* and *M. sativa* monocultures in soil spiked with 100 mg kg^{-1} ΣPAH and amended with diesel was greater by 33 % and 31 % respectively, compared to similar treatments without diesel amendment. In the same regard, R:S ratios was greater by 40 % and 18 % in the 100 mg kg^{-1} treatments with diesel for *S. bicolor* and *M. sativa* respectively, as well as by 28% and 45% in the 300 mg kg^{-1} treatments with diesel. Generally, diesel amendment appeared to inhibit the adverse effects of PAHs on plant biomass and R:S ratios; this was one of the key observations in this study. It is suggested that diesel in diesel-amended treatments may have promoted PAH partitioning into the diesel phase (Boyd & Sun, 1990), especially at low PAH concentrations in soil. This is such that closely-associating roots in the diesel-amended soil show minimal effects on biomass production compared to diesel-unamended treatments.

Overall, soil spiked with 100 mg kg^{-1} and amended with diesel showed the greatest root biomass and R:S ratios for both species within all treatments and growing

patterns. With increase in ΣPAH concentration from 100 mg kg^{-1} to 300 mg kg^{-1}, root biomass and R:S ratios generally decreased, especially for *M. sativa*; however, the differences were not statistically significant ($p > 0.05$). These results are similar to those presented by Cheema *et al.* (2010)Cheema et al. (2010), where the authors reported that after 65 d of plant growth, root biomass and R:S ratio of *M. sativa* were mostly affected in soil amended with a mixture of 200 mg kg^{-1} phenanthrene and 199.3 mg kg^{-1} pyrene, when compared to rape seed exposed to the same treatment. These trends have also been observed for maize, ryegrass and white clover, exhibiting decreased biomass values with increasing concentrations of phenanthrene and pyrene mixtures in loam soil, but the differences were not statistically significant (Xu et al., 2006). These trends may have resulted from inherent non-acute toxicity of PAHs especially at higher concentrations in spiked soils (Wei *et al.*, 2017). In addition, PAH-contaminated soils may inhibit the flow of water and nutrients to plants, thereby affecting plant's ability to increase biomass especially at higher PAH concentrations (Reilley *et al.*, 1996).

The relationships between root and shoot biomass, especially R:S ratios, are important indicators of plant health (Cairns *et al.*, 1997), although interpretation of such relationships is not always clear-cut (Mokany *et al.*, 2006). Plant root systems absorb water and mineral nutrients from soil, and transports them to plant shoots, while shoot systems fix CO_2 needed for photosynthesis and transports food to other non-photosynthetic parts of the plant (Cairns *et al.*, 1997). It is thought that a reduced R:S ratio is unfavourable for plants as it indicates shoot proliferation at the expense of root, however, reduced R:S ratio especially at higher concentrations (300 mg kg^{-1} ΣPAH) does not still exclude plant tolerance within the growth assay conditions (Harris, 1992; Cheema *et al.*, 2010). One reason for the reduced R:S ratios, especially at higher concentrations may have been due to increased root proliferation to allow increased transport of water and nutrients aboveground thereby increasing shoot biomass at the expense of root biomass, and hence a reduced R:S ratio (Harris, 1992). For instance, percentage decreases in shoot biomass from 100 mg kg^{-1} to 300 mg kg^{-1} ΣPAH were approximately 4 % and 22 % in *S. bicolor* and *M. sativa*, respectively; whereas, the root biomass similarly decreased by approximately 18 % and 25 %. This finding therefore implies that the rate at which root biomass proliferate may have been less compared to shoot biomass, which may have resulted in the reduced R:S ratios

observed at 300 mg kg^{-1} ΣPAH compared to 100 mg kg^{-1} ΣPAH. Similarly, roots are likely to be more susceptible to damage from soil contamination as they are in direct contact with soil, thereby adversely affecting water and mineral transport functions (Cheema *et al.*, 2010). As a result, greater energy may be expended on translocating carbohydrates produced above-ground to below-ground biomass resulting in an increased R:S ratio (Harris, 1992; Reilley *et al.*, 1996). However, an evaluation of the moisture content of roots and shoots after harvesting both plant species did not present any significant difference ($p > 0.05$) within each of the treatments, nor between each treatment and control (Figure SI 1). Hence, root functioning in terms of water transport may not have been significantly impaired due to PAH-diesel oil contamination in soil during the growth duration. These findings revealed reduced plant biomass and R:S ratios for both plant species in PAH-diesel oil mixture contaminated soils especially in the 300 mg kg^{-1} ΣPAH treatment, however potential toxicity or stress signs were not apparent throughout the growth period, which may support the notion of both plant species being tolerant of PAH-diesel oil contaminated soil.

7.4.3 ^{14}C-phenathrene mineralisation in unplanted and planted treatments

The microbial catabolic activities across unplanted and planted treatments are shown in Table 3. The presence of a lag phase is indicative of the time needed to allow microbial adaptation in soil, and it has been suggested previously that a decreasing lag phase prior to mineralisation could be attributable to microbial adaptation processes (Macleod & Semple, 2002). Varying lag phases were observed in the unplanted soils, which significantly shortened ($p < 0.05$) with in soil-contaminant contact time (Table 3). This was more pronounced in the planted soils (Table 3). Results revealed that the indigenous microorganisms in the unplanted control were catabolically active. However, microbial activities were much slower as revealed by longer lag phases, compared to the unplanted treatments (Table 3, Figure 3A). The indigenous microorganisms in soil may have access to various carbon sources, including ubiquitously-distributed PAHs, although background PAH concentrations were considered to be negligible (Igunnugbemi, 2013).

Across all unplanted treatments, the soil spiked with 100 mg kg^{-1} ΣPAH generally exhibited shorter lag phases than those spiked with 300 mg kg^{-1} ΣPAH with and

without diesel amendment at 0 d. Overall, lag phases were not significantly different within and across all unplanted treatments and these ranged from 3.84 ± 0.50 d up to 5.34 ± 0.58 d at 0 d. Only treatments with 100 mg kg^{-1} ΣPAH with and without diesel amendment presented lag phases significantly shorter ($p < 0.05$ and $p < 0.02$ respectively) when compared to untreated control soil. After 21 and 42 d, reduced lag phases, greater maximum rates and cumulative extents of mineralisation were observed in all treatments, compared to 0 d (Figures 3 - 4). Lag phases generally shortened to less than 1 d in both planted and unplanted treatments (Table 3). Rhodes *et al.* (2008) also reported statistically shorter ($p < 0.05$) lag phases after 42 d and 84 d soil-phenanthrene contact time in natural and artificial soils compared to those observed after 1 d contact time. An increase in indigenous microbial activities was observed in the planted (C3) compared to the unplanted (C4) controls (Table 3) as shown by significantly longer lag phases ($p < 0.05$) and cumulative extents of ^{14}C-phenanthrene mineralisation ($p < 0.001$). This shows the influence of both plant species at increasing indigenous microbial activities in soil, which may have implications for contaminant biodegradation. This was further reflected by the greater CFUs of total heterotrophs and PAH degraders in the planted controls than in the unplanted control, especially for *M. sativa* (Table 3).

Plant roots release root exudates containing mineralisable oxygen, water, enzymes, and a diverse array of low molecular weight carbon-containing compounds such as amino acids, sugars, organic acids, and phenols (Bais *et al.*, 2006; Muratova *et al.*, 2009c). These root exudates may enrich the rhizosphere and serve as readily-mineralisable carbon sources for microorganisms involved in symbiotic root-microbe interactions (Bais *et al.*, 2006; Wenzel, 2009; Gao *et al.*, 2017). Continuous mineralisation and incorporation of these carbon sources increases microbial biomass, thereby supporting microbial growth, activity, and contaminant biodegradation (Reilley *et al.*, 1996; Molina-Barahona *et al.*, 2004; Gao *et al.*, 2015b, 2017). Such symbiotic root-microbe interactions in soil have been previously reported for *M. sativa* (Fan *et al.*, 2008) and *S. bicolor* (Banks *et al.*, 2003; Muratova *et al.*, 2009a). Specifically, enzymatic metabolites via cationic peroxidases from *M. sativa* and *S. bicolor* are key mechanisms for PAH biodegradation in soil in the presence of the plant species (Dubrovskaya *et al.*, 2017).

Mineralisation was observed to vary throughout the experiment. At 0 d, fastest rates (0.98 ± 0.37 % $^{14}CO_2$ d^{-1}) and greatest cumulative extents of ^{14}C-phenanthrene mineralisation (59.27 ± 6.09 %) were observed only in the unplanted treatment with 100 mg kg^{-1} ΣPAH and amended with diesel ($p < 0.05$). The corresponding 300 mg kg^{-1} ΣPAH treatment exhibited the slowest rates (0.20 ± 0.002 % $^{14}CO_2$ d^{-1}) as well as the lowest cumulative extents (24.68 ± 3.48 %) of mineralisation. This trend was further reflected by a greater ratio of degraders to total heterotrophs in soil with 100 mg kg^{-1} ΣPAH, compared to soil with 300 mg kg^{-1} ΣPAH as shown in Figure SI 2A. However, microbial numbers (PAH degraders and total heterotrophs) within and across corresponding treatments were not significantly different ($p \geq 0.05$) (Table 3). In the unplanted treatments at 21 d (Table 3), rates of mineralisation were fastest ($p < 0.001$) in soil spiked with 300 mg kg^{-1} ΣPAH especially in the diesel unamended treatment; whereas, cumulative extents of mineralisation were greater in soil containing 100 mg kg^{-1} ΣPAH without diesel. The maximum rates of mineralisation within the planted treatments in comparison to their corresponding unplanted controls were statistically similar ($p > 0.05$). This observation is consistent with previous findings where microbial respiration was not affected by plant species identity (Groffman *et al.*, 1996; Oyelami *et al.*, 2013), and have been suggested to be due to spatial limitations between indigenous microorganisms and plants in soil (Oyelami *et al.*, 2013).

Considering biodegradation parameters such as lag phases, fastest rates and cumulative extents of ^{14}C-phenanthrene mineralisation, observations at 0 d appeared to depict mineralisation patterns which may have been largely influenced by the availability of freshly spiked ΣPAH in soil. It is well known that freshly spiked PAHs are more mobile and bioavailable in soil than aged PAHs (Alexander, 1999, 2000, Semple *et al.*, 2003, 2007), due to minimal influence of soil-contaminant sequestration processes (Luthy *et al.*, 1997; Northcott & Jones, 2001; National Academies, 2003). Sorption forces are usually more apparent at lower concentrations (Pignatello & Xing, 1996), hence, soil with higher concentrations of freshly spiked PAHs may be subject to greater contaminant bioavailability compared to soil with lower concentrations (Hwang & Cutright, 2004a; b). Since PAHs are potentially toxic, adverse effects on soil enzymatic, as well as microbial numbers and catabolic activities are likely to be observed (Kanaly & Harayama, 2000; John *et al.*, 2012). In this present study, PAH

inherent toxicity to indigenous microorganisms, especially in soils spiked with 300 mg kg^{-1} ΣPAH, may have resulted in the pattern observed of biodegradation parameters in unplanted soil at 0 d soil-PAH contact time. This result is consistent with those of Couling *et al.* (2010) who reported greater biodegradation parameters in soil spiked with lower concentrations of individual PAHs, and/or a mixture of naphthalene, phenanthrene and pyrene, with single or multiple dosing of each concentrations. However, the differences between biodegradation parameters at low and high PAH concentrations were usually statistically similar ($p > 0.05$) (Couling *et al.*, 2010). In addition, while Oyelami *et al.* (2013) observed that unplanted soils amended with different concentrations of PAH mixtures showed corresponding responses in degrader numbers and activities which may have resulted in consequent ^{14}C-phenanthrene mineralisation, observations from the present study did not generally show such trends (Table 3, Figures SI 2 - SI 3).

The rates of PAH mineralisation in planted and unplanted treatments were generally statistically similar; however, cumulative extents of mineralisation also need to be considered to evaluate plant-assisted biodegradation. Cumulative extents of mineralisation at 21 d were significantly greater in soils spiked with 300 mg kg^{-1} ΣPAH with diesel for both *S. bicolor* ($p < 0.001$) and *M. sativa* ($p = 0.003$) monocultures, compared to corresponding unplanted treatments. However, a contrasting trend was generally observed ($p < 0.05$) in soils spiked with 100 mg kg^{-1} and 300 mg kg^{-1} ΣPAH without diesel, which implied that plant-assisted biodegradation in these diesel-unamended treatments was not evident in these soils. Similar findings have also been reported previously (Mueller & Shann, 2006; Smith *et al.*, 2011; Cennerazzo *et al.*, 2017). For instance, Cennerazzo *et al.* (2017) reported that biodegradation in soil spiked with 300 mg kg^{-1} phenanthrene within a 21 d *Lolium perenne* monoculture was not significantly different from the unplanted treatment. In contrast, cumulative extents of mineralisation in soil spiked with 100 mg kg^{-1} ΣPAH with diesel from only *M. sativa* monoculture were significantly greater ($p = 0.013$) than that in corresponding unplanted treatment. Cumulative extents of mineralisation were generally statistically similar ($p > 0.05$) within planted treatments (mono- and mixed cultures). The only exception was in *S. bicolor* planted soil spiked with 300 mg kg^{-1} ΣPAH and without diesel, which showed a significantly greater ($p = 0.003$) cumulative extents of mineralisation compared to corresponding *M. sativa* treatment.

Further, cumulative extents of mineralisation within treatments were statistically similar ($p > 0.05$) at 42 d, except for soil spiked with 100 mg kg^{-1} ΣPAH without diesel where cumulative extents of mineralisation were significantly greater ($p < 0.05$) than corresponding treatment with diesel.

In this study, diesel amendment generally inhibited the rates and cumulative extents of ^{14}C-phenanthrene mineralisation in soils at 21 and 42 d; however, the trend was not consistent, as had been previously documented for other NAPLs (Lee *et al.*, 2003). Diesel, itself being a hydrophobic non-aqueous phase liquid (Adam & Duncan, 1999), contains the greatest amount of PAHs and aromatics when compared to other medium distillate fuel oils (Wang *et al.*, 1990). It is therefore suggested that due to its hydrophobic nature, diesel may further increase PAH partitioning processes (Boyd & Sun, 1990; Rutherford *et al.*, 1997; Ghosh *et al.*, 2000), especially in soils with low concentrations of PAHs. Hence, decreased PAH mobility, bioavailability, toxicity, and biodegradation may occur (Boyd & Sun, 1990; Sun & Boyd, 1991; Zwiernik *et al.*, 1999), as also evident from the results of plant biomass and R:S ratios previously discussed. Therefore, soil with greater PAH concentrations and amended with diesel may show greater rates and extents of mineralisation compared to one with lower PAH concentrations, especially in the presence of relevant plant species. In addition, rates and extents of mineralisation are likely to be greater in diesel unamended treatments and especially at lower PAH concentrations since an additional sorbent phase (diesel) is absent. The modifying effects of diesel amendment on rates and extents of PAH mineralisation in spiked soil may be dependent on concentration of diesel amended (Hernández-Ortega *et al.*, 2014), and these effects are likely to be greater in highly weathered field-contaminated soils (Smith *et al.*, 2011; Bona *et al.*, 2011; Wei *et al.*, 2017). For intance, phenanthrene degradation was reported to have increased in a pasture soil with diesel concentration of 0 - 2,000 mg kg^{-1}, but then decreased when diesel concentration was increased to 20,000 mg kg^{-1} (Swindell & Reid, 2006a). Towell *et al.* (2011) also investigated the effect of cable oil concentration on biodegradation of ^{14}C-phenyldodecane in an agricultural soil and reported that even though microbial respiration increased with increasing oil concentration (0.001 - 10 %, w/w dry weight of soil), mineralisation of ^{14}C-phenyldodecane decreased. In this present study, greater rates and cumulative extents of mineralisation at 21 and 42 d were mostly observed in diesel unamended treatments

with similar ΣPAH concentrations (100 mg kg^{-1} or 300 mg kg^{-1}). The nature of NAPLs and associated concentration are factors to be considered in PAH biodegradation. Key questions to answer in future investigations are; at what concentration and soil-contaminant contact time does diesel oil increase or decrease PAH biodegradation, as well as identifying the mechanisms controlling the influence of diesel oil on PAH bioavailability in aged soil? Such investigations may have implications for biodegradation of complex PAH-diesel oil mixtures, especially in historically contaminated soils.

Based on previous studies (Xu *et al.*, 2006; Meng *et al.*, 2011), it was expected that a mixed culture of both plant species used in this study would co-enhance rates and extents of ^{14}C-phenanthrene mineralisation in soil compared to their individual monocultures, rather, the mixed culture associated treatments did not significantly enhance rates and extents of ^{14}C-phenanthrene mineralisation (Table 3). Either of the monocultures generally exhibited significantly greater ($p < 0.05$) extents of mineralisation compared to the mixed culture. Oyelami *et al.* (2013) also reported that plant species richness had no significant effects on phenanthrene biodegradation in long-term aged soil. To the best of our knowledge, there have been no published studies evaluating the plant-assisted biodegradation potential of *M. sativa* and *S. bicolor* mixed cultures in PAH-diesel oil contaminated soil. Belowground interactions between many plant roots are yet to be understood and fully investigated (Bais *et al.*, 2006). Although based on daily visual assessment, plant growth aboveground in the controls did not appear limited, however, plant biomass and R:S ratios were generally more reduced in mixed cultures than individual monocultures both within control and PAH-diesel oil amended treatments. An antagonistic interaction between the roots of both plant species in this study may not be totally excluded (Hedge & Miller, 1990; Muratova *et al.*, 2009a). In this regard, it is speculated that greater energy may have been expended by both plant roots towards surviving competition and associated adverse effects, rather than supporting microbial activity in the mixed culture as generally shown in Table 3. Similarly, associated microorganisms within the rhizosphere may also expend energy competing for preferable rhizospheric microhabitats rather than co-enhance biodegradation (van Veen *et al.*, 1997). Such counter-productive survival interactions within the rhizosphere may affect the combined potential of both plant roots as well as associated microorganisms to better

enhance rates and extents of ^{14}C-phenanthrene mineralisation in the PAH-diesel oil co-contaminated soil.

7.5 Conclusions

S. bicolor and *M. sativa* mono- and mixed- cultures were tolerant of the PAH-diesel oil amended soil given the observed data relating to plant health characteristics such as percentage plant emergence, growth and survival, and plant biomass through the 21 d growth period in this study. Generally, PAH concentration, diesel amendment, and plant species differences influenced plant health characteristics, microbial numbers and activity which in turn may have influenced microbial mineralisation and plant-assisted biodegradation. Overall, increase in PAH concentration reduced plant biomass and R:S ratios, as well as adversely affected lag phases, and rates and extents of ^{14}C-phenanthrene mineralisation especially at initial stages of soil-contaminant interactions. In contrast, maximum rates and cumulative extents of ^{14}C-phenanthrene mineralisation were greater at advanced stages of soil-contaminant contact time especially in the more concentrated PAH-contaminated soils with monocultures of the plant species used. The contrasting rates and extents of ^{14}C-phenanthrene at 0, 21, and 42 d may have resulted from the combined effects of microbial adaptation, reduced effects of sorption at higher PAH concentrations, and plant-assisted biodegradation. Diesel amendment supported plant biomass production as well as increase in R:S ratios, however, appeared to inhibit rates and extents of ^{14}C-phenanthrene mineralisation in soil. Such diesel-oil associated influences may be dependent on both the properties and concentration of PAHs and diesel oil amended. The mechanisms through which diesel oil (and other NAPLs) controls the fate and behaviour of complex PAH mixtures in soil should be further investigated.

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Table 1. Experimental treatments

Treatment	Characteristics
C1	Control = un-spiked soil + carrier solvent + diesel + plants
C2	Control = un-spiked soil + carrier solvent + plants
C3	Control = untreated soil + plants
C4	Control = un-spiked soil + no plants
T1	100 mg kg^{-1} ∑PAH + carrier solvent + diesel + plants
T2	300 mg kg^{-1} ∑PAH + carrier solvent + diesel + plants
T3	100 mg kg^{-1} ∑PAH + carrier solvent + plants
T4	300 mg kg^{-1} ∑PAH + carrier solvent + plants
C5	Control = 100 mg kg-1 ∑PAH + carrier solvent + diesel + no plants
C6	Control = 300 mg kg-1 ∑PAH + carrier solvent + diesel + no plants
C7	Control = 100 mg kg-1 ∑PAH + carrier solvent + no plants
C8	Control = 300 mg kg-1 ∑PAH + carrier solvent + no plants

Table 2**.** Plant biomass in monocultures and mixed cultures: dry weight (g) of shoots and roots, and Root:Shoot ratio (R:S). Values represent the mean ± 1 standard error of the mean (SEM) (n = 3)

Treatment	Shoot weight (g)	Root weight (g)	Root:Shoot
		S. bicolor	
C3	0.376 ± 0.029	0.710 ± 0.025	1.915 ± 0.174
T1	0.313 ± 0.021	1.162 ± 0.284	3.647 ± 0.765
T2	0.301 ± 0.022	0.956 ± 0.161	3.210 ± 0.602
T3	0.362 ± 0.013	0.782 ± 0.062	2.170 ± 0.212
T4	0.373 ± 0.030	0.846 ± 0.050	2.303 ± 0.249
		M. sativa	
C3	0.086 ± 0.009	0.046 ± 0.003	0.548 ± 0.074
T1	0.085 ± 0.007	0.052 ± 0.002	0.614 ± 0.057
T2	0.066 ± 0.012	0.039 ± 0.010	0.576 ± 0.094
T3	0.072 ± 0.019	0.036 ± 0.003	0.501 ± 0.020
T4	0.134 ± 0.034	0.034 ± 0.014	0.318 ± 0.139
		M. sativa + *S. bicolor*	
C3	0.042 ± 0.008[a] 0.251 ± 0.012[b]	0.475 ± 0.036[c]	1.620 ± 0.100[d]
T1	0.028 ± 0.004 0.226 ± 0.022	0.742 ± 0.075	2.961 ± 0.388
T2	0.030 ± 0.005 0.145 ± 0.010	0.433 ± 0.047	2.453 ± 0.183
T3	0.039 ± 0.001 0.273 ± 0.021	0.532 ± 0.032	1.722 ± 0.194
T4	0.290 ± 0.095 0.287 ± 0.000	0.430 ± 0.038	0.823 ± 0.238

C3: Control = untreated soil + plants. T1: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants. T2: 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants. T3: 100 mg kg^{-1} ΣPAH + carrier solvent + plants. T4: 300 mg kg^{-1} ΣPAH + carrier solvent + plants "a" and "b": Shoot weights (g) of *M. sativa* and *S. bicolor* respectively. "c": sum of root weights for both *M. sativa* and *S. bicolor*. d: c / (a + b).

Table 3. ^{14}C-Phenanthrene mineralisation and indigenous microbial numbers (CFU g-1soil) profiles. Values represent the mean ± 1 standard error of the mean (SEM) (n = 3)

Soil treatment	Plant species	Lag phase (d)	Maximum rates (% $^{14}CO_2$ d^{-1})	Cumulative extents (%)	Total heterotrophs (CFU x 10^5 g^{-1})	PAH degraders (CFU x 10^5 g^{-1})
			0 days			
C4		7.50 ± 0.84	0.88 ± 0.09	45.42 ± 3.17	7.97 ± 3.48	0.69 ± 0.17
C5		4.41 ± 0.20	0.98 ± 0.37	59.27 ± 6.09	8.73 ± 3.05	1.50 ± 0.10
C6	NA	5.34 ± 0.58	0.20 < 0.01	24.68 ± 3.48	19.00 ± 2.04	1.90 ± 0.20
C7		3.84 ± 0.50	0.39 ± 0.15	39.97 ± 2.02	11.70 ± 1.62	1.83 ± 0.29
C8		5.24 ± 0.90	0.34 ± 0.20	37.96 ± 0.49	7.97 ± 3.13	0.80 ± 0.31
			21 days			
C3	*S. bicolor*	2.44 ± 0.34	1.13 ± 0.33	52.61 ± 3.32	1.93 ± 1.04	0.70 ± 0.13
	M. sativa	2.74 ± 0.31	1.26 ± 0.11	60.72 ± 1.21	10.37 ± 4.88	0.95 ± 0.62
	Mixed	2.12 ± 0.03	1.36 ± 0.20	58.82 ± 5.43	16.37 ± 9.38	0.46 ± 0.10
C4	Unplanted	3.15 ± 0.52	0.32 ± 0.16	27.81 ± 8.80	2.63 ± 0.91	0.69 ± 0.17
T1	*S. bicolor*	< 1	1.16 ± 0.25	51.40 ± 3.63	7.43 ± 3.13	1.04 ± 0.19
	M. sativa	< 1	1.61 ± 0.07	55.28 ± 1.67	32.33 ± 11.3	1.88 ± 0.81
	Mixed	< 1	1.03 ± 0.03	55.45 ± 5.02	7.10 ± 0.72	1.52 ± 0.30
C5	Unplanted	< 1	1.19 ± 0.16	48.15 ± 6.30	17.50 ± 4.65	1.50 ± 0.10
T2	*S. bicolor*	< 1	1.81 ± 0.10	68.36 ± 1.25	11.20 ± 5.96	1.77 ± 0.34
	M. sativa	< 1	1.93 ± 0.08	62.33 ± 6.34	14.17 ± 5.53	1.95 ± 0.23
	Mixed	< 1	1.56 ± 0.09	63.75 ± 3.66	6.07 ± 1.96	1.63 ± 0.49
C6	Unplanted	< 1	1.77 ± 0.12	54.17 ± 4.13	5.17 ± 1.57	1.90 ± 0.20
T3	*S. bicolor*	< 1	1.36 ± 0.18	62.52 ± 1.08	5.33 ± 0.56	1.37 ± 0.15
	M. sativa	< 1	1.38 ± 0.24	61.57 ± 4.29	3.97 ± 0.62	1.86 ± 0.41
	Mixed	< 1	1.43 ± 0.11	63.23 ± 3.49	6.90 ± 2.76	1.48 ± 0.24
C7	Unplanted	< 1	1.67 ± 0.03	71.58 ± 4.56	11.97 ± 5.09	1.83 ± 0.29
T4	*S. bicolor*	< 1	1.80 ± 0.28	61.72 ± 1.70	6.70 ± 0.76	1.63 ± 0.75

	M. sativa	< 1	1.57 ± 0.08	53.48 ± 3.21	12.23 ± 1.71	2.59 ± 0.45
	Mixed	< 1	1.67 ± 0.02	59.42 ± 2.05	8.43 ± 3.29	2.08 ± 0.36
C8	Unplanted	< 1	1.77 ± 0.11	66.70 ± 5.82	8.03 ± 1.14	0.80 ± 0.31
			42 days			
C4		3.95 ± 0.38	0.53 ± 0.19	43.72 ± 0.87	5.07 ± 0.03	1.46 ± 0.52
C5		< 1	0.91 ± 0.15	52.73 ± 3.79	13.10 ± 0.76	0.79 ± 0.25
C6	NA	< 1	1.24 ± 0.15	63.12 ± 3.75	12.00 ± 1.92	1.87 ± 0.09
C7		< 1	0.56 ± 0.21	67.13 ± 3.25	22.43 ± 6.76	2.04 ± 0.37
C8		< 1	1.67 ± 0.08	65.46 ± 0.66	10.47 ± 2.09	1.68 ± 0.25

C3: Control = untreated soil + plants. C4: Control = untreated soil + no plants. C5: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants. C6: 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants. C7: 100 mg kg^{-1} ΣPAH + carrier solvent + no plants. C8: 300 mg kg^{-1} ΣPAH + carrier solvent + no plants. T1: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants. T2: 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants. T3: 100 mg kg^{-1} ΣPAH + carrier solvent + plants. T4: 300 mg kg^{-1} ΣPAH + carrier solvent + plants. N/A = Not Applicable. Mixed = mixed culture of *S. bicolor* and *M. sativa*.

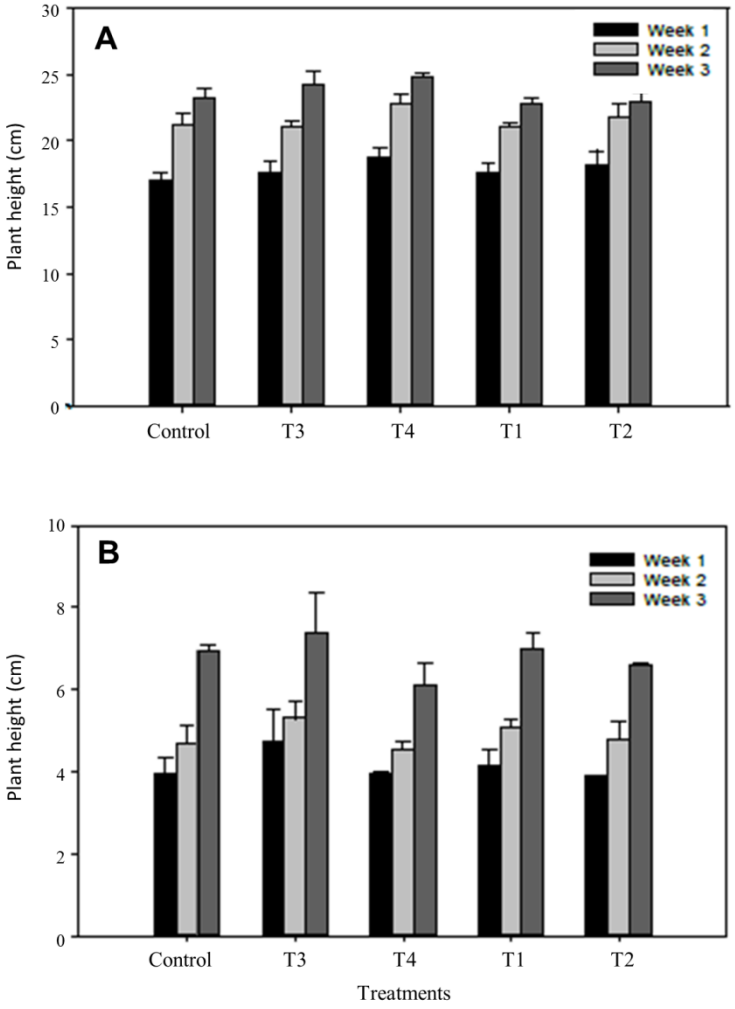

Figure 1. Weekly plant heights of Sorghum bicolor (A) and Medicago sativa (B) from monocultures across treatments ($p > 0.05$). Control = untreated soil + plants or C3, 100-D = 100 mg kg^{-1} ΣPAH + carrier solvent + plants or T3, 300-D = 300 mg kg^{-1} ΣPAH + carrier solvent + plants or T4, 100+D = 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants or T1, and 300+D = 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants or T2. Note the different scale on y-axis. Data represents the mean ± 1 standard error of the mean (SEM) ($n = 3$).

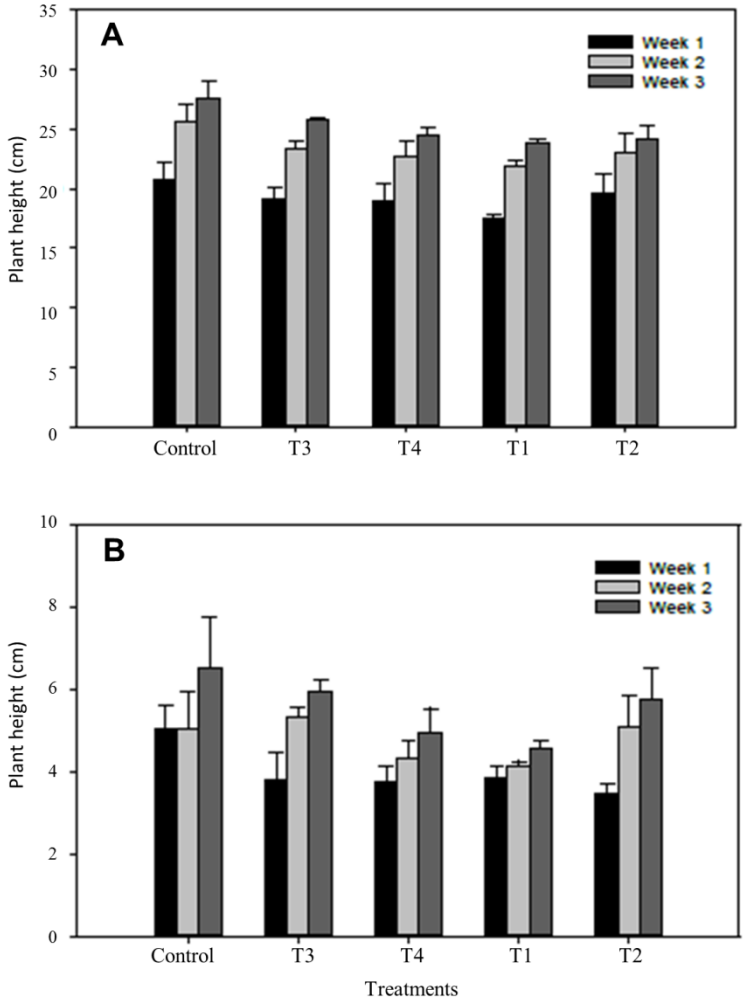

Figure 2. Weekly plant heights of Sorghum bicolor (A) and Medicago sativa (B) from mixed cultures across treatments ($p > 0.05$). Control = untreated soil, T3 = 100 mg kg^{-1} ΣPAH + carrier solvent, T4= 300 mg kg^{-1} ΣPAH + carrier solvent, T1 = 100 mg kg^{-1} ΣPAH + carrier solvent + diesel, and T2 = 300 mg kg^{-1} ΣPAH + carrier solvent + diesel. Note the different scale on y-axis. Data represents the mean ± 1 standard error of the mean (SEM) ($n = 3$).

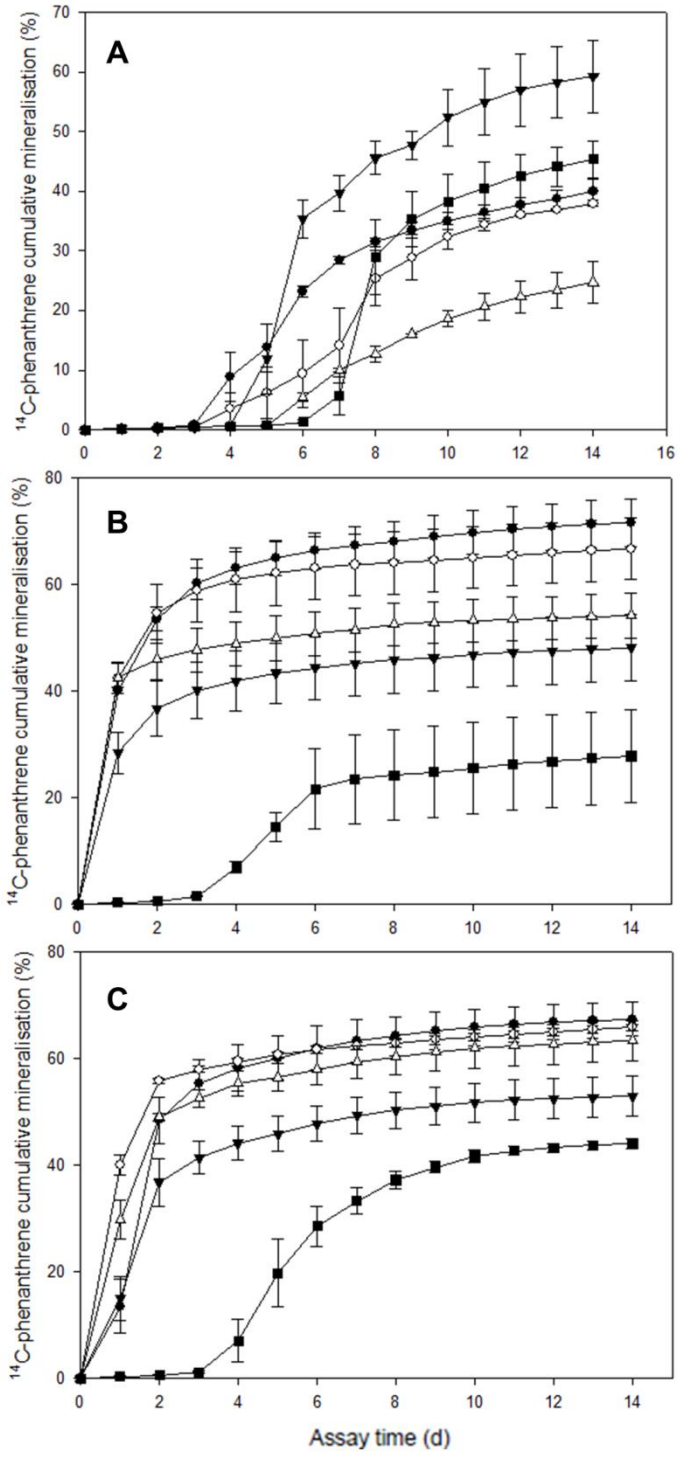

Figure 3. Development of ^{14}C-phenanthrene mineralisation at 0 d (A), 21 d (B) and 42 d (C) respectively. Control = Untreated soil + no plants, C4 (■). Soil amended with: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants, C5 (▼); and 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants, C6 (Δ); 100 mg kg^{-1} ΣPAH + carrier solvent + no plants, i.e. C7 (●); 300 mg kg^{-1} ΣPAH + carrier solvent + no plants, C8 (○). Note the different scale on y-axis. Data represents the mean ± 1 standard error of the mean (SEM) (n = 3).

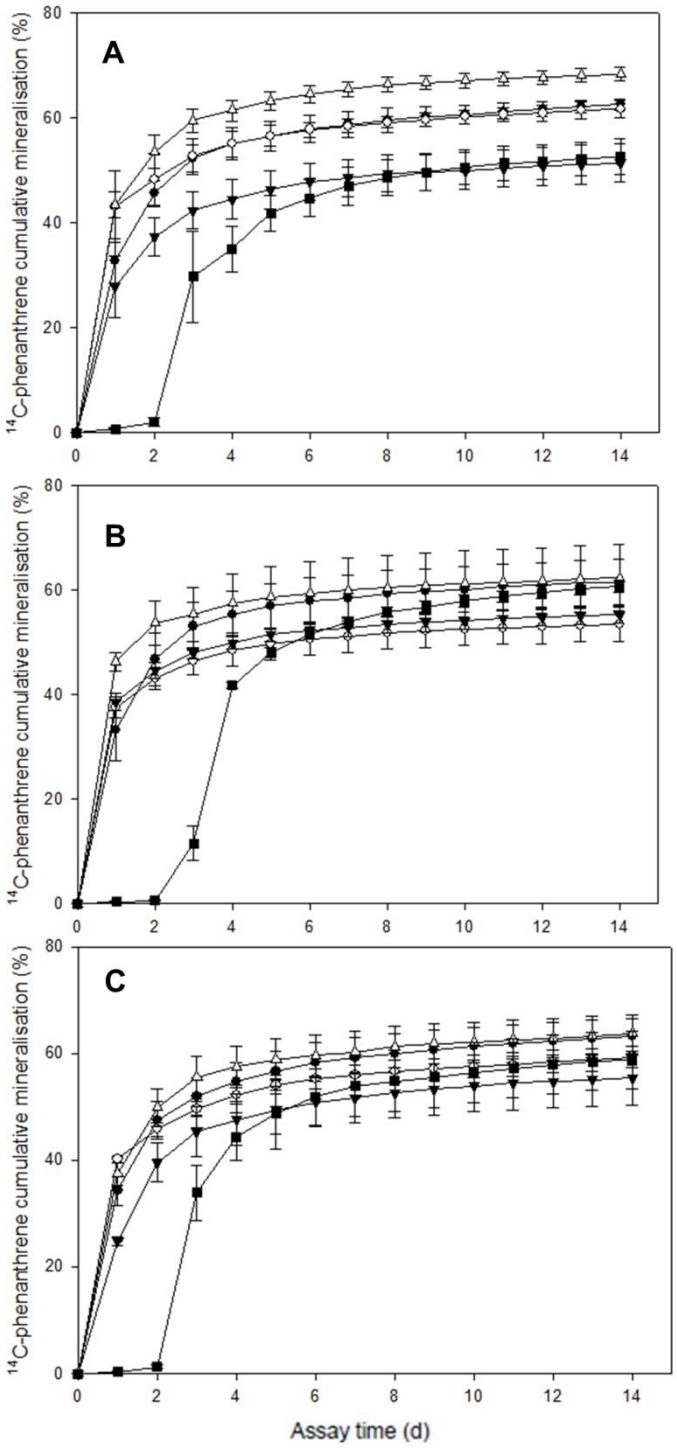

Figure 4. Development of ^{14}C-phenanthrene mineralisation at 21 d in monocultures of S. bicolor (A) and M. sativa (B), and mixed culture (C) respectively. Control = untreated soil + no plants, C3 (■). Soil amended with: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants, T1 (▼); and 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants, T2 (Δ); 100 mg kg^{-1} ΣPAH + carrier solvent + plants, T3 (●); 300 mg kg^{-1} ΣPAH + carrier solvent + plants, T4 (○).Data represents the mean ± 1 standard error of the mean (SEM) (n = 3).

7.6 References

Adam, G. & Duncan, H. 1999. Effect of diesel fuel on growth of selected plant species. Environmental Geochemistry and Health, 21, 353–357.

Adam, G. & Duncan, H. 2002. Influence of diesel fuel on seed germination. Environmental Pollution, 120, 363–370.

Alef, K. 1995. Enrichment, isolation and counting of soil microorganisms. In: Methods in Applied Soil Microbiology and Biochemistry (eds. Alef, K. & Nannipieri, P.), pp. 123–191. 1st ed. Academic Press.

Alexander, M. 1999. Biodegradation and bioremediation. Academic Press.

Alexander, M. 2000. Aging, bioavaiability, and overestimation of risk from environmental pollutants. Environmental Science & Technology, 34, 4259–4265.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2007. Cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and phenols in contaminated soil slurries. Environmental Science & Technology, 41, 5498–5504.

Aprill, W. & Sims, R.C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere, 20, 253–265.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. & Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, 57, 233–266.

Baldrian, P., in der Wiesche, C., Gabriel, J., Nerud, F. & Zadrazil, F. 2000. Influence of cadmium and mercury on activities of lignolytic enzimes and degradation of polycyclic aromatic hydrocarbons by Pleurotus ostreatus in soil. Applied and Environmental Microbiology, 66, 2471–2478.

Bamgbose, I. & Anderson, T.A. 2015. Phytotoxicity of three plant-based biodiesels, unmodified castor oil, and Diesel fuel to alfalfa (Medicago sativa L.), lettuce (Lactuca sativa L.), radish (Raphanus sativus), and wheatgrass (Triticum aestivum). Ecotoxicology and Environmental Safety, 122, 268–274.

Banks, M.K., Kulakow, P., Schwab, a P., Chen, Z. & Rathbone, K. 2003. Degradation of crude oil in the rhizosphere of Sorghum bicolor. International Journal of Phytoremediation, 5, 225–34.

de Boer, J. & Wagelmans, M. 2016. Polycyclic aromatic hydrocarbons in soil – Practical options for remediation. Clean - Soil, Air, Water, 44, 648–653.

Bona, C., Rezende, I.M. de, Santos, G. de O. & Souza, L.A. de. 2011. Effect of soil contaminated by diesel oil on the germination of seeds and the growth of Schinus terebinthifolius Raddi (Anacardiaceae) seedlings. Brazilian Archives of Biology and Technology, 54, 1379–1387.

Boyd, S.A. & Sun, S. 1990. Residual petroleum and polychlorobiphenyl oils as sorptive phases for organic contaminants in soils. Environmental Science &

Technology, 24, 142–144.

Cachada, A., Pereira, R., da Silva, E.F. & Duarte, A.C. 2014. The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges. Science of the Total Environment, 472, 463–480.

Cairns, M.A., Brown, S., Helmer, E.H. & Baumgardner, G.A. 1997. Root biomass allocation in the world's upland forests. Oecologia, 111, 1–11.

Cennerazzo, J., de Junet, A., Audinot, J.N. & Leyval, C. 2017. Dynamics of PAHs and derived organic compounds in a soil-plant mesocosm spiked with13C-phenanthrene. Chemosphere, 168, 1619–1627.

Cheema, S.A., Imran Khan, M., Shen, C., Tang, X., Farooq, M., Chen, L., Zhang, C. & Chen, Y. 2010. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. Journal of Hazardous Materials, 177, 384–389.

Chen, F., Tan, M., Ma, J., Zhang, S., Li, G. & Qu, J. 2016. Efficient remediation of PAH-metal co-contaminated soil using microbial-plant combination: A greenhouse study. Journal of Hazardous Materials, 302, 250–261.

Chouychai, W., Thongkukiatkul, A., Upatham, S., Lee, H., Pokethitiyook, P. & Kruatrachue, M. 2007. Phytotoxicity assay of crop plants to phenanthrene and pyrene contaminants in acidic soil. Environmental Toxicology, 22, 597–604.

Couling, N.R., Towell, M.G. & Semple, K.T. 2010. Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. Environmental Pollution, 158, 3411–3420.

Cunningham, S.D. & Ow, D.W. 1996. Promises and Prospects of Phytoremediation. Plant Physiology, 110, 715–719.

D'Orazio, V., Ghanem, A. & Senesi, N. 2013. Phytoremediation of pyrene contaminated soils by different plant species. Clean - Soil, Air, Water, 41, 377–382.

Deng, S. & Zeng, D. 2017. Removal of phenanthrene in contaminated soil by combination of alfalfa, white-rot fungus, and earthworms. Environmental Science and Pollution Research, 24, 7565–7571.

Doick, K.J. & Semple, K.T. 2003. The effect of soil:water ratios on the mineralisation of phenanthrene: LNAPL mixtures in soil. FEMS Microbiology Letters, 220, 29–33.

Dubrovskaya, E., Pozdnyakova, N., Golubev, S., Muratova, A., Grinev, V., Bondarenkova, A. & Turkovskaya, O. 2017. Peroxidases from root exudates of Medicago sativa and Sorghum bicolor: Catalytic properties and involvement in PAH degradation. Chemosphere, 169, 224–232.

Fan, S., Li, P., Gong, Z., Ren, W. & He, N. 2008. Promotion of pyrene degradation in rhizosphere of alfalfa (Medicago sativa L.). Chemosphere, 71, 1593–1598.

Gao, Y., Hu, X., Zhou, Z., Zhang, W., Wang, Y. & Sun, B. 2017. Phytoavailability and mechanism of bound PAH residues in filed contaminated soils. Environmental

Pollution, 222, 465–476.

Gao, Y., Yuan, X., Lin, X., Sun, B. & Zhao, Z. 2015. Low-molecular-weight organic acids enhance the release of bound PAH residues in soils. Soil and Tillage Research, 145, 103–110.

Ghanem, A., Orazio, V.D. & Senesi, N. 2010. Phytotoxicity assay of selected plants to Pyrene contaminated soil. World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia., 74–77.

Ghosh, U., Weber, A.S., Jensen, J.N. & Smith, J.R. 2000. Relationship between PCB desorption equilibrium, kinetics, and availability during land biotreatment. Environmental Science & Technology, 34, 2542–2548.

Groffman, P.M., Eagan, P., Sullivan, W.M. & Lemunyon, J.L. 1996. Grass species and soil type effects on microbial biomass and activity. Plant and soil, 183, 61–67.

Günther, T., Dornberger, U. & Fritsche, W. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere, 33, 203–215.

Halsall, C.J., Sweetman, A.J., Barrie, L.A. & Jones, K.C. 2001. Modelling the behaviour of PAHs during atmospheric transport from the UK to the Arctic. Atmospheric Environment, 35, 255–267.

Hamdi, H., Benzarti, S., Aoyama, I. & Jedidi, N. 2012. Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (Medicago sativa L.). International Biodeterioration and Biodegradation, 67, 40–47.

Harris, R.W. 1992. Root-shoot ratios. Journal of Arboriculture, 18, 39–42.

Hedge, R.S. & Miller, D.A. 1990. Allelopathy and autotoxicity in alfalfa: Characterization and effects of preceding crops and residue incorporation. Crop Sci, 30, 1255–1259.

Hernández-Ortega, H.A., Quintanar-Isaías, P.A., Jaramillo-Pérez, A.T., Alarcón, A., Ferrera-Cerrato, R. & Lazzarini-Lechuga, R. 2014. Diesel effects on root hydraulic conductivity and morphological changes of the vascular cylinder in Medicago sativa. Environmental and Experimental Botany, 105, 1–9.

Huang, X.D., El-Alawi, Y., Penrose, D.M., Glick, B.R. & Greenberg, B.M. 2004. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution, 130, 465–476.

Hutchinson, S.L., Schwab, A.P. & Banks, M.K. 2001. Phytoremediation of aged petroleum sludge. Journal of Environment Quality, 30, 1516.

Hwang, S. & Cutright, T.J. 2004a. Preliminary exploration of the relationships between soil characteristics and PAH desorption and biodegradation. Environment International, 29, 887–894.

Hwang, S.C. & Cutright, T.J. 2004b. Evidence of underestimation in PAH sorption/desorption due to system nonequilibrium and interaction with soil

constituents. Journal of Environmental Science and Health Part a-Toxic/hazardous Substances & Environmental Engineering, 39, 1147–1162.

Ibragimova, M., Rumyantseva, M., Onishchuk, O., Belova, V., Kurchak, O., Andronov, E., Dzyubenko, N. & Simarov, B. 2006. Symbiosis between the root-nodule bacterium Sinorhizobium meliloti and alfalfa (Medicago sativa) under salinization conditions. Microbiology, 75, 77–81.

Igunnugbemi, O.O. 2013. Biodegradation of aliphatic and polycyclic aromatic hydrocarbons in petroleum oil-contaminated soils. Lancaster University. (At: https://onesearch.lancaster-university.uk/primo-explore/fulldisplay?docid=44LAN_ALMA_DS2189824420001221&context=L&vid=LUL_VU1&lang=en_US&search_scope=LSCOP_44LAN_LANUL_LIB&adaptor=Local Search Engine&tab=books&query=any,contains,Biodegradation of . Accessed: 7/2/2018).

John, R.C., Essien, J.P., Akpan, S.B. & Okpokwasili, G.C. 2012. Polycyclic aromatic hydrocarbon-degrading bacteria from aviation fuel spill site at Ibeno, Nigeria. Bulletin of Environmental Contamination and Toxicology, 88, 1014–1019.

Juhasz, A.L. & Naidu, R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. International Biodeterioration & Biodegradation, 45, 57–88.

Kanaly, R. a & Harayama, S. 2000. Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria MINIREVIEW Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria. Journal of Bacteriology, 182, 2059–2067.

Khan, M.I., Cheema, S.A., Tang, X., Shen, C., Sahi, S.T., Jabbar, A., Park, J. & Chen, Y. 2012. Biotoxicity assessment of pyrene in soil using a battery of biological assays. Archives of Environmental Contamination and Toxicology, 63, 503–512.

Lee, P.H., Doick, K.J. & Semple, K.T. 2003. The development of phenanthrene catabolism in soil amended with transformer oil. FEMS Microbiology Letters, 228, 217–223.

Liu, S.L., Luo, Y.M., Cao, Z.H., Wu, L.H., Ding, K.Q. & Christie, P. 2004. Degradation of benzo[a]pyrene in soil with arbuscular mycorrhizal alfalfa. Environmental Geochemistry and Health, 26, 285–293.

Luthy, G.R., Ainken, R.G., Brusseau, L.M., Cunningham, D.S., Gschwend, M.P., Pignatello, J.J., Reinhard, M., Traina, J.S., Jr, Weber, J.W. & Westall, C.J. 1997. Critical review sequestration of hydrophobic organic contaminants by geosorbents. American Chemical Society, 31, 3341–3347.

Luthy, R.G., Dzombak, D.A., Peters, C.A., Roy, S.B., Ramaswami, A., Nakles, D. V. & Nott, B.R. 1994. Remediating tar-contaminated soils at manufactured gas plant sites. Environmental Science & Technology, 28.

Macleod, C.J.A. & Semple, K.T. 2002. The adaptation of two similar soils to pyrene catabolism. Environmental Pollution, 119, 357–364.

Marchand, L., Sabaris, C.Q., Desjardins, D., Oustrière, N., Pesme, E., Butin, D., Wicart, G. & Mench, M. 2016. Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons. Environmental Science and Pollution Research, 23, 3120–3135.

Meng, L., Qiao, M. & Arp, H.P.H. 2011. Phytoremediation efficiency of a PAH-contaminated industrial soil using ryegrass, white clover, and celery as mono- and mixed cultures. Journal of Soils and Sediments, 11, 482–490.

Mokany, K., Raison, R.J. & Prokushkin, A.S. 2006. Critical analysis of root: Shoot ratios in terrestrial biomes. Global Change Biology, 12, 84–96.

Molina-Barahona, L., Rodríguez-Vázquez, R., Hernández-Velasco, M., Vega-Jarquín, C., Zapata-Pérez, O., Mendoza-Cantú, A. & Albores, A. 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Applied Soil Ecology, 27, 165–175.

Mueller, K.E. & Shann, J.R. 2006. PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees. Chemosphere, 64, 1006–1014.

Muratova, A.Y., Golubev, S.N., Merbach, W. & Turkovskaya, O. V. 2009a. Biochemical and physiological peculiarities of the interactions between Sinorhizobium meliloti and Sorghum bicolor in the presence of phenanthrene. Microbiology, 78, 308–314.

Muratova, A., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A., Hirche, F., Merbach, W. & Turkovskaya, O. 2009b. Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of Sorghum bicolor (L.) Moench. Environmental and Experimental Botany, 66, 514–521.

Muratova, A., Pozdnyakova, N., Golubev, S., Wittenmayer, L., Makarov, O., Merbach, W. & Turkovskaya, O. 2009c. Oxidoreductase activity of sorghum root exudates in a phenanthrene-contaminated environment. Chemosphere, 74, 1031–1036.

National Academies. 2003. Bioavailability of contaminants in soils and sediments: Processes, tools, and applications.

Northcott, G.L. & Jones, K.C. 2001. Partitioning, extractability, and formation of non extractable PAH residues in soil.2. Effects on compund dissolution bahaviour. Environmental Science & Technology, 35, 1111–1117.

OECD. 2006. OECD Test Guideline 208: Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test. Guidelines for the Testing of Chemicals, Terrestrial Plant Test Seedling Emergence and Seedling Growth Test, 227, 1–21, (At: http://www.oecd-ilibrary.org/environment/test-no-208-terrestrial-plant-test-seedling-emergence-and-seedling-growth-test_9789264070066-en.).

Oyelami, A.O., Okere, U. V., Orwin, K.H., De Deyn, G.B., Jones, K.C. & Semple, K.T. 2013. Effects of plant species identity, diversity and soil fertility on biodegradation of phenanthrene in soil. Environmental Pollution, 173, 231–237.

Panchenko, L., Muratova, A. & Turkovskaya, O. 2017. Comparison of the

phytoremediation potentials of Medicago falcata L. And Medicago sativa L. in aged oil-sludge-contaminated soil. Environmental Science and Pollution Research, 24, 3117–3130.

Pignatello, J.J. & Xing, B. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environmental Science & Technology, 30, 1–11.

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D. & Semple, K.T. 2001. A simple 14C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. FEMS Microbiology Letters, 196, 141–146.

Reilley, K.A., Banks, M.K. & Schwab, A.P. 1996. Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality, 25, 212–219.

Rhodes, A.H., Hofman, J. & Semple, K.T. 2008. Development of phenanthrene catabolism in natural and artificial soils. Environmental Pollution, 152, 424–430.

Rutherford, P.M., Gray, M.R. & Dudas, M.J. 1997. Desorption of [14C]naphthalene from bioremediated and nonbioremediated soils contaminated with creosote compounds. Environmental Science and Technology, 31, 2515–2519.

Salt, D.E., Smith, R.D. & Raskin, I. 1998. Phytoremediation. Annual Review of Plant Biology, 49, 643–668.

Semple, K.T., Doick, K.J., Wick, L.Y. & Harms, H. 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. Environmental Pollution, 150, 166–176.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Semple, K.T., Reid, B.J. & Fermor, T.R. 2001. Impact of composting strategies on the treatment of soil contaminated with diesel fuel.pdf. Environmental Pollution, 112, 269–283.

Semple, K.T., Riding, M.J., McAllister, L.E., Sopena-Vazquez, F. & Bending, G.D. 2013. Impact of black carbon on the bioaccessibility of organic contaminants in soil. Journal of Hazardous Materials, 261, 808–816.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Saito, H. 2011. Study of PAH dissipation and phytoremediation in soils: Comparing freshly spiked with weathered soil from a former coking works. Journal of Hazardous Materials, 192, 1219–1225.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2007. Importance of chemical structure on the development of hydrocarbon catabolism in soil. FEMS Microbiology Letters, 272, 120–126.

Sun, S. & Boyd, S.A. 1991. Sorption of polychlorobiphenyl (PCB) congeners by residual PCB-Oil phases in soils. Journal of Environment Quality, 20, 557.

Sun, M., Fu, D., Teng, Y., Shen, Y., Luo, Y., Li, Z. & Christie, P. 2011. In situ phytoremediation of PAH-contaminated soil by intercropping alfalfa (Medicago sativa L.) with tall fescue (Festuca arundinacea Schreb.) and associated soil microbial activity. Journal of Soils and Sediments, 11, 980–989.

Sverdrup, L.E., Hagen, S.B., Krogh, P.H. & van Gestel, C.A.M. 2007. Benzo(a)pyrene shows low toxicity to three species of terrestrial plants, two soil invertebrates, and soil-nitrifying bacteria. Ecotoxicology and Environmental Safety, 66, 362–368.

Swindell, A.L. & Reid, B.J. 2006. Influence of diesel concentration on the fate of phenanthrene in soil. Environmental Pollution, 140, 79–86.

Thavamani, P., Megharaj, M. & Naidu, R. 2012. Multivariate analysis of mixed contaminants (PAHs and heavy metals) at manufactured gas plant site soils. Environmental Monitoring and Assessment, 184, 3875–3885.

Thijs, S., Sillen, W., Weyens, N. & Vangronsveld, J. 2017. Phytoremediation: State-of-the-art and a key role for the plant microbiome in future trends and research prospects. International Journal of Phytoremediation, 19, 23–38.

Towell, M.G., Paton, G.I. & Semple, K.T. 2011. The biodegradation of cable oil components: Impact of oil concentration, nutrient addition and bioaugmentation. Environmental Pollution, 159, 3777–3783.

U. S. Environmental Protection Agency. 2012. OCSPP 850.4100: Seedling Emergence and Seedling Growth. EPA Ecological Effects Test Guidelines.

US Environmental Protection Agency. 2013. Priority Pollutant List. (At: https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf. Accessed: 6/2/2018).

van Veen, J.A., van Overbeek, L.S. & van Elsas, J.D. 1997. Fate and activity of microorganisms introduced into soil. Microbiology and molecular biology reviews : MMBR, 61, 121–35.

Wang, X., Yu, X. & Bartha, R. 1990. Effect of bioremediation on Polycyclic Aromatic Hydrocarbon residues in soil. Environmental Science & Technology, 24, 1086–1089.

Wei, R., Ni, J., Li, X., Chen, W. & Yang, Y. 2017. Dissipation and phytoremediation of polycyclic aromatic hydrocarbons in freshly spiked and long-term field-contaminated soils. Environmental Science and Pollution Research, 24, 7994–8003.

Wenzel, W.W. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant and Soil, 321, 385–408.

White, P.M., Wolf, D.C., Thoma, G.J. & Reynolds, C.M. 2006. Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. Water, Air, and Soil Pollution, 169, 207–220.

Wild, S.R. & Jones, K.C. 1995. Polynuclear aromatic hydrocarbons in the United

Kingdom environment: A preliminary source inventtory and budget. Environmental Pollution, 88, 91–108.

Wilson, S.C. & Jones, K.C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environmental pollution (Barking, Essex : 1987), 81, 229–249.

Wong, K.W., Toh, B.A., Ting, Y.P. & Obbard, J.P. 2005. Biodegradation of phenanthrene by the indigenous microbial biomass in a zinc amended soil. Letters in Applied Microbiology, 40, 50–55.

Xu, S.Y., Chen, Y.X., Wu, W.X., Wang, K.X., Lin, Q. & Liang, X.Q. 2006. Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. Science of the Total Environment, 363, 206–215.

Yan, J., Wang, L., Fu, P.P. & Yu, H. 2004. Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 557, 99–108.

Zhu, X., Jin, L., Sun, K., Li, S., Li, X. & Ling, W. 2016. Phenanthrene and pyrene modify the composition and structure of the cultivable endophytic bacterial community in ryegrass (Lolium multiflorum lam). International Journal of Environmental Research and Public Health, 13.

Zwiernik, M.J., Quensen, J.F. & Boyd, S.A. 1999. Residual petroleum in sediments reduces the bioavailability and rate of reductive dechlorination of aroclor 1242. Environmental Science & Technology, 33, 3574–3578.

7.7 Supplementary information

Table SI 1. Properties of soil (Adapted from Couling et al., 2010). Values represent the mean ± 1 standard error of the mean (SEM) (n = 3)

Soil properties	Value
pH (in dH_2O)	6.50 ± 0.08
Moisture content (%)	21.07 ± 2.78
Elemental analysis (%)	
Total extractable carbon	1.80 ± 0.03
Total extractable nitrogen	0.14 ± 0.01
Total extractable organic carbon	1.60 ± 0.07
Soil organic matter	2.70 ± 0.04
Soil particle size (%)	
Clay	19.50 ± 0.70
Silt	20.00 ± 0.87
Sand	60.40 ± 1.20
Coarse	0.12 ± 0.01
Medium	6.90 ± 0.10
Fine	53.30 ± 0.60
Texture	Clay loam

Table SI 2. Plant emergence (%) in soil after 21 days

Plant species	Emergence per treatment (%)					Growing pattern
	Control	TI	T2	T3	T4	
S. bicolor	100	100	93	97	97	Mono culture
	93	100	87	83	100	Mixed culture
M. sativa	83	87	73	70	60	Mono culture
	93	93	93	100	87	Mixed culture

Control = untreated soil; T1: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel. T2: 300 mg kg^{-1} ΣPAH + carrier solvent + diesel. T3: 100 mg kg^{-1} ΣPAH + carrier solvent. T4: 300 mg kg^{-1} ΣPAH + carrier solvent. Differences between planted treatments and control were not statistically significant ($p > 0.05$).

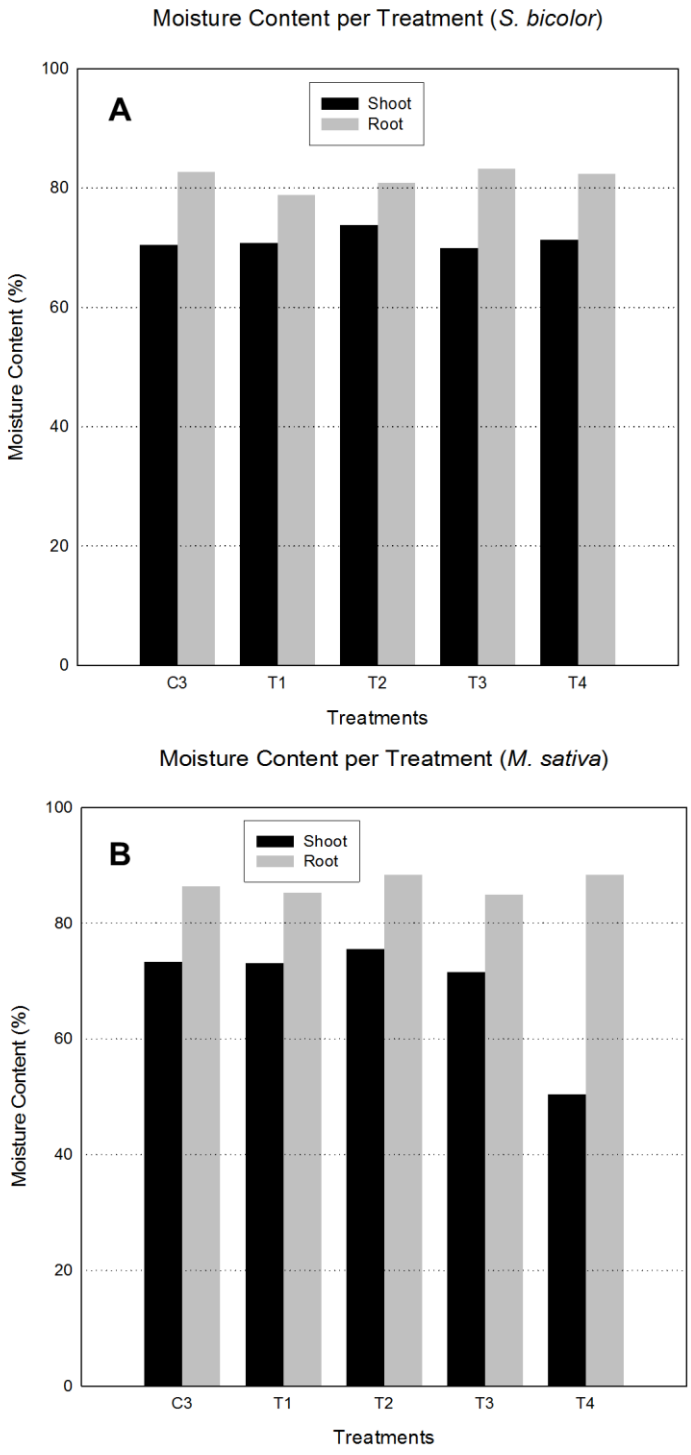

Figure SI 3. Moisture content in root and shoot per treatment after 21 d in monocultures of *S. bicolor* (A) and *M. sativa* (B) respectively. C3 = control untreated soil, T1: 100 mg kg^{-1} ΣPAH + carrier solvent + diesel. T2: 300 mg kg^{-1} ΣPAH + carrier solvent + diesel. T3: 100 mg kg^{-1} ΣPAH + carrier solvent. T4: 300 mg kg^{-1} ΣPAH + carrier solvent.

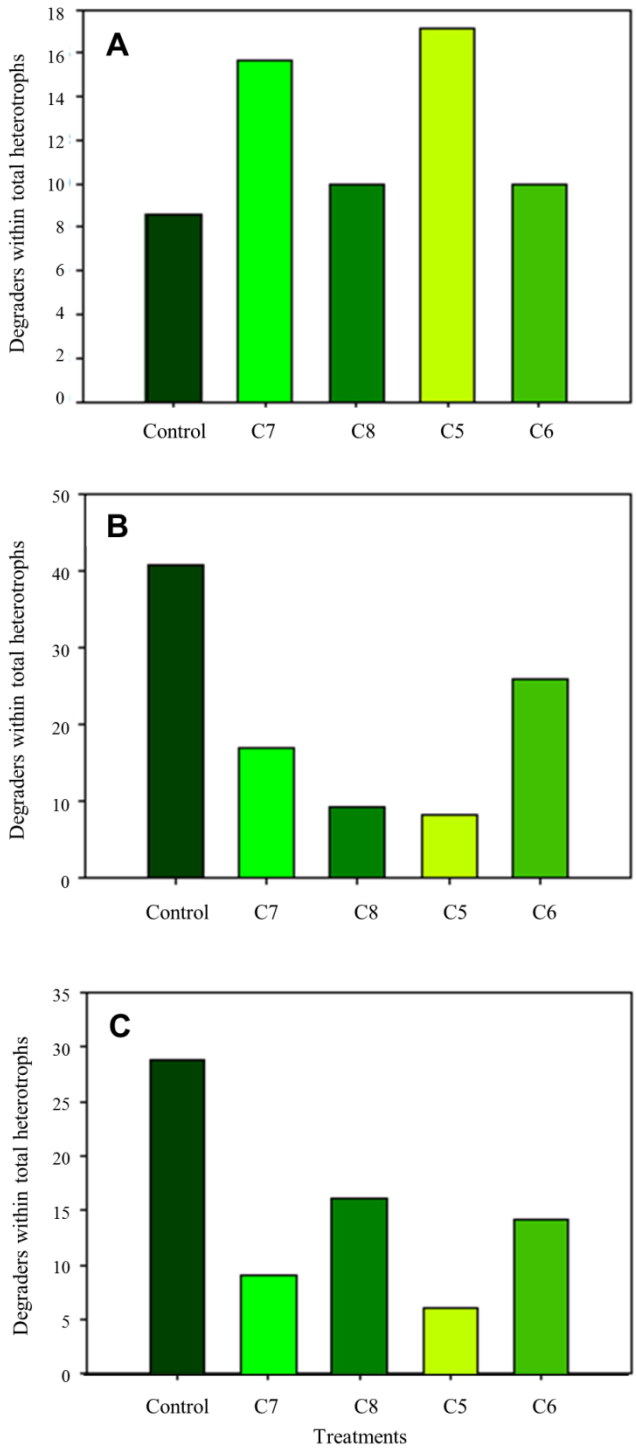

Figure SI 4. Ratio of PAH degraders to total heterotrophs in each treatment at 0 d (A), 21 d (B) and 42 d (C) respectively. C7 = 100 mg kg^{-1} ΣPAH + carrier solvent + no plants, C8 = 300 mg kg^{-1} ΣPAH + carrier solvent + no plants, C5 = 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants, and C6 = 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants.

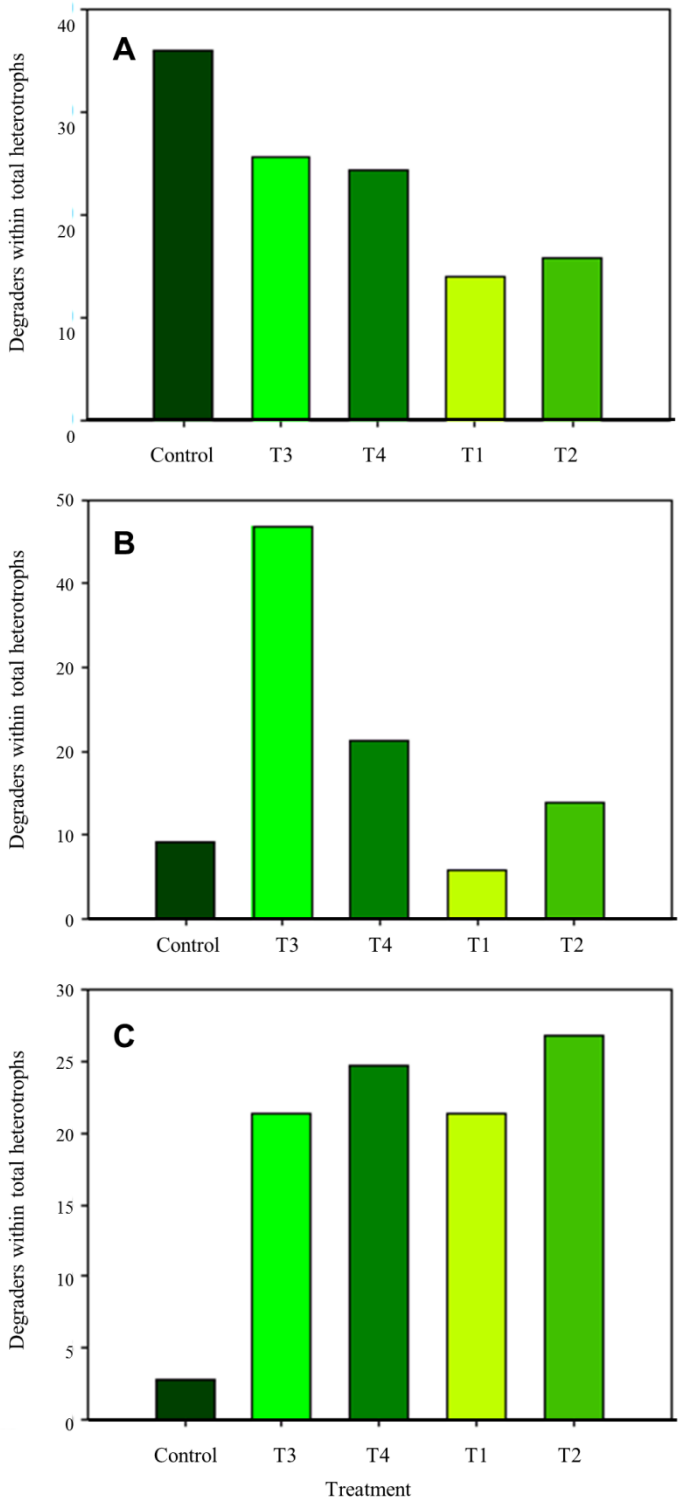

Figure SI 5. Ratio of degraders to total heterotrophs in each treatment at 21 d in monocultures of S. bicolor (A) and M. sativa (B), and mixed culture (C) respectively. Control = untreated planted soil, T3 = 100 mg kg^{-1} ΣPAH + carrier solvent, T4 = 300 mg kg^{-1} ΣPAH + carrier solvent, T1 = 100 mg kg^{-1} ΣPAH + carrier solvent + diesel, and T2 = 300 mg kg^{-1} ΣPAH + carrier solvent + diesel.

Chapter 8

The effect of organic acids on the fate of ^{14}C-phenanthrene in contaminated soil

The effect of organic acids on the fate of ^{14}C-phenanthrene in contaminated soil

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8.1 Abstract

The interaction between root exudates and soil microbes has been hypothesised as the primary mechanism for the biodegradation of organic pollutants in the rhizosphere. However, the mechanisms governing this loss process are not completely understood. This study aimed to investigate the effect of two important compounds within root exudates (citric and malic acid) on ^{14}C-phenanthrene desorption and bioaccessibility in soil. Overall results showed that the presence of both citric and malic acid enhanced desorption of ^{14}C-phenanthrene; this appeared to be concentration dependant. Increases in extractability were not reflected in a higher bioaccessibility. Despite enhancing the desorption of ^{14}C-phenanthrene in soil, there is no direct evidence indicating that citric or malic acids have the ability to promote the biodegradation of ^{14}C-phenanthrene from soil. Results from this study provide a novel understanding of the role that substrates, typically found within the rhizosphere due to root exudation, play in the bioaccessibility and biodegradation of hydrocarbons in contaminated soil.

Keywords: Phenanthrene, organic acids, root exudates, desorption, bioavailability, ageing

8.2 Introduction

The rhizosphere is defined as the soil in closest proximity to plant roots and has been hypothesised to enhance the biodegradation of organic contaminants such as aliphatic and aromatic hydrocarbons through different mechanisms (Anderson *et al.*, 1993; Pilon-Smits, 2005). These include the promotion of (1) larger microbial populations (Anderson *et al.*, 1993) and shifts on their community composition (Joner *et al.*, 2002), (2) source of biologically important substrates including nutrients and readily available sources of carbon (Reilley *et al.*, 1996; Dakora & Phillips, 2002; Martin *et al.*, 2014), and (3) increasing the bioavailability of the contaminants due to root exudates, decay and turnover (Siciliano & Germida, 1998; Martin *et al.*, 2014). The amount and type of substances released by roots is highly dependent on a series of factors including plant species and age, as well as particular soil and environmental conditions (Jones, 1998; Shukla *et al.*, 2011; Agnello *et al.*, 2014; Martin *et al.*, 2014). However, a number of low molecular weight compounds, such as amino acids, sugars and organic acids, have been identified as common constituents of root exudates (Jones *et al.*, 2003; van Hees *et al.*, 2005), where organic acids including citric, malic and oxalic are considered the most abundant (Jones & Brassington, 1998).

The use of root exudates for the dissipation of organic contaminants in soil has been reported (Miya & Firestone, 2001; Yoshitomi & Shann, 2001; Joner *et al.*, 2002). These investigations have used simulated rhizosphere conditions by the introduction of artificial or natural root exudates in order to approach the subject in a more controlled manner (Miya & Firestone, 2001; Joner *et al.*, 2002). From these studies, research has been developed to consider the effect of these substances on shifts of the microbial populations and/or communities (Joner *et al.*, 2002; Shukla *et al.*, 2011), overall dissipation of contaminants (Joner *et al.*, 2002), and their effect on soil physical and chemical properties (Shukla *et al.*, 2011). Authors such as Sun *et al.* (2013), Martin *et al.* (2014) and Gao *et al.* (2015) have pointed out that although efforts have been directed towards investigating the effect of root exudates on the biodegradation of hydrocarbons in contaminated soil, information regarding the role of single compounds from this solution is scarce. Within these few studies, authors have reported that organic acids commonly found in root exudates can promote the desorption of phenanthrene from soil (Gao *et al.*, 2010b, 2015b; Ling *et al.*, 2015).

Changes in the extractability of polycyclic aromatic hydrocarbons (PAHs) might act as a predictor of the microbial biodegradability of different species of PAHs, where the rates of desorption can be used as predictors of biodegradation (Cornelissen *et al.*, 1998a). As fractions of hydrocarbons are transferred from soil to solution through the desorption process; these can also become more bioaccessible and susceptible to be metabolised by the soil microbial community (Semple *et al.*, 2003). Therefore, the possibility of enhancing the desorption of PAHs by using organic acids to promote or enhance biodegradation in soil has been identified as a promising strategy, but remains poorly explored (Martin *et al.*, 2014). In addition, the extent into which these organic acids affect the biodegradation of the desorbed hydrocarbon has not been considered. Therefore, the aim of this study was to investigate the effect of two low molecular weight organic acids (LOAs) commonly found within root exudates in the extractability and bioaccessibility of ^{14}C-phenanthrene contaminated soil. Phenanthrene was selected as a model PAH given its widespread distribution, biodegradability and persistent properties in soil. For this, mineralisation, hydroxypropyl-β-cyclodextrin (HPCD) extractability and desorption kinetics of ^{14}C-phenanthrene were assessed in the presence of organic acids at a range of concentrations. Results from this experiment provide a novel perspective of the effect of organic acids on the fate of ^{14}C-phenanthrene soil by investigating (1) desorbing capacity of citric and malic acid and (2) the extent by which these can promote a higher bioavailability and mineralisation.

8.3 Methodology

8.3.1 Soil preparation and spiking

An uncontaminated clay loam soil (top 20 cm, 2.7 % organic matter) was collected from Myerscough Agricultural College, Preston, U.K. Partially air-dried soil (24 h) was passed through a 2 mm sieve and stored in the dark at 4 ± 1 ºC until needed. Main soil physical and chemical characteristics have been described by Towell *et al.* (2011). Sieved soil was rehydrated (50% water holding capacity (whc)) and spiked following the procedure proposed by Doick *et al.* (2003) using a soil inoculum and mixing with a stainless steel spoon. Standards used for spiking contained $^{12/14}$C phenanthrene dissolved in acetone to deliver a final concentration of 100 mg kg^{-1} (dw) phenanthrene

with an associated ^{14}C-activity of 83 Bq g^{-1} (dw). Spiked soil was placed in sealed sterilized amber jars and incubated in the dark at 21 ± 1 ºC in a controlled environment room for up to 15 weeks. Determination of the total ^{14}C-phenanthrene associated activity in the soil was assessed at every time point by sample oxidation following the methodology described by Rhodes *et al.* (2012).

8.3.2 Influence of organic acids on the mineralisation of ^{14}C-phenanthrene

Mineralisation of ^{14}C-phenanthrene was evaluated following the methodology developed and tested by Reid *et al.* (2001) and Semple *et al.* (2006). Soil (10 g dw) aged over 14 and 50 days was placed into 250 modified bottles fitted with a 1 M NaOH $^{14}CO_2$ trap (n = 3). To assess the effect of organic acids towards the mineralisation process, citric, malic, oxalic and succinic acids were selected as representative LOAs often observed in the rhizosphere (van Hees *et al.*, 2005). Solutions containing individual organic acids within its naturally appearing range of concentration in rhizosphere soil solution (0.1 and 0.5 mmol l^{-1}) (van Hees *et al.*, 2005) were used as the base for the preparation of a minimal basal salts (MBS) medium used for the mineralisation assay. Soil was mixed with the modified MBS containing the organic acids (25 ml) and 5 ml of a bacterial inoculum of *Mycobacterium gilvum* (10^5 cells ml^{-1}) suspended in MBS without organic acids to achieve a final 3:1 liquid:soil ratio (Vázquez-Cuevas & Semple, 2016). Bottles were placed onto an orbital shaker at 100 rpm in a controlled environment room at 21 ± 1 ºC in the dark. $^{14}CO_2$ evolution was assessed by periodically (up to every 24 h) replacing the trap, mixing with 5 ml liquid scintillation cocktail and assessed by liquid scintillation counting (LSC) (10 min - Canberra Packard Tri-Carb 2300, U.K.).

8.3.3 Influence of organic acids on the extractability of ^{14}C-phenanthrene

8.3.3.1 Preliminary tests

A series of preliminary tests were carried in order to optimize the general experimental parameters and design of the extraction assays. Solutions of deionised water containing citric, malic, oxalic and succinic acids solutions of individual organic acids were prepared at 0.1 and 0.5 mmol l^{-1}. Desorption kinetics of ^{14}C-phenanthrene with these solutions (n = 3) were assessed from spiked soil following the methodology described below.

The temporal effect of organic acids on the bioaccessibility of ^{14}C-phenanthrene was also assessed (n = 3). Soil was saturated with malic acid solution (100 % whc) at two concentrations (0.5 and 500 mmol l^{-1}) and incubated in a controlled environment room 21 ± 1 ºC for 1, 3, 6, 8 or 24 h. Each experimental unit was also fitted with a 1 M NaOH $^{14}CO_2$ trap for the assessment of any possible dissipation of ^{14}C-phenanthrene by microbial respiration during the incubation time. Soil was extracted with 50 mM HPCD solutions after each incubation time following the methodology described below. $^{14}CO_2$ traps were assessed by adding 5 ml of liquid scintillation cocktail and assessed by LSC as previously described.

Based on the results from the preliminary tests, citric and malic acid were selected for further investigation. Solutions with these two organic acids were prepared at 0.5, 100, 250, 500 and 1000 mmol l^{-1} using deionized water; these were used for the HPCD extractability and desorption assays. Selected concentrations ranged from naturally appearing LOAs concentrations (van Hees *et al.*, 2005) up to maximum tested concentrations within experiments with similar aims (Gao *et al.*, 2015a; Ling *et al.*, 2015).

8.3.3.2 HPCD extraction of ^{14}C-phenanthrene from soil

Changes in the bioaccessibility of ^{14}C-phenanthrene were measured by HPCD extractions from soil aged over 1 and 15 weeks. At each time point, 1.25 g soil (dw) were placed into Teflon centrifuge tubes (n = 5); soil was saturated (100 % whc) with citric and malic acid solution (0.5, 100, 250, 500 and 1000 mmol l^{-1}). The tubes where then sealed and stored in a controlled environment room (21 ± 1 ºC) for 8 h. After this time, 25 ml of 50 mM HPCD solution was added. Tubes were placed onto an orbital shaker (100 rpm) for 22 h at 21 ±1 ºC. Afterwards, samples were centrifuged (3000 *x g* for 1 h) and 5 ml of the supernatant was placed in a glass scintillation vial and mixed with 15 ml liquid scintillation cocktail. Samples were assessed through LSC as described previously. Remaining ^{14}C-associated activity in the soil was assessed by sample oxidation (Rhodes *et al.*, 2012).

8.3.3.3 Desorption of ^{14}C-phenanthrene by organic acids

Tests were performed following a randomized design (n = 5) and blind sampling. Desorption kinetics were assessed after 1 and 15 weeks soil-contaminant contact time;

at each time point, 4 g soil (dw) was placed into Teflon centrifuge tubes and mixed with 25 ml of organic acid solution at a given concentration (0.5, 100, 250, 500 and 1000 mmol l^{-1}). Tubes were placed onto an orbital shaker (100 rpm) in a controlled environment room at 21 ±1 ºC. Soil samples were sequentially extracted after 1, 4, 6, 12, 24, 45, 90, 180 and 360 h by centrifuging at 3000 *x g* for 1 h. Aliquots (5 ml) were mixed with 15 ml liquid scintillation cocktail in a glass scintillation vial and assessed by LSC. Residual activity in the soil after the last extraction was assessed by sample oxidation as described by Rhodes *et al.* (2012).

Desorption of ^{14}C-phenanthrene was examined by two- (Equation 1) and three-compartment (Equation 2) first-order kinetics (Cornelissen *et al.*, 1998b; Rhodes *et al.*, 2010b):

Equation 1:

$$S_t / S_0 = [F_{rap} \bullet \exp(-k_{rap} \bullet t)] + [F_{slow} \bullet \exp(-k_{slow} \bullet t)]$$

Equation 2:

$$S_t / S_0 = [F_{rap} \bullet \exp(-k_{rap} \bullet t)] + [F_{slow} \bullet \exp(-k_{slow} \bullet t)] + [F_{very\ slow} \bullet \exp(-k_{very\ slow} \bullet t)]$$

where S_t represents the amount of ^{14}C-phenanthrene sorbed to the soil at the desorption time t (h) and S_0 is the initial total amount of ^{14}C-phenanthrene at the beginning of the assay (time 0). F_{rap}, F_{slow} and $F_{very\ slow}$ (%) are the rapid, slow and very slow desorbing fractions and k_{rap}, k_{slow} and $k_{very\ slow}$ (h^{-1}) are the rate constants for the rapid, slow and very slow desorption, respectively. The model assumes that $k_{very\ slow} \leq k_{slow} \leq k_{rap}$ (Rhodes *et al.*, 2010b; Clegg *et al.*, 2014), and that the addition of the desorbing fractions equals 100 % (Clegg *et al.*, 2014). The values of F_{rap}, F_{slow}, $F_{very\ slow}$, k_{rap}, k_{slow} and $k_{very\ slow}$ were obtained by exponential curve fitting using Excel Solver add-in, using a non-linear least squares method.

8.3.4 Statistical analysis

Statistical analyses were carried using the SPSS 21 (95 % confidence interval). Normality of the data was verified by Shapiro-Wilk tests, transformations were applied in cases where a normal distribution was not observed. Analyses of the

differences across time were carried by Student's t-test and Wilcoxon test for normal and not normally distributed data respectively. Differences between the treatments at each time point were analysed using One-Way ANOVA (Tukey) or Kruskal-Wallis test for normal and not-normal distributed data respectively. Graphical representations of the results were done with the software Sigma Plot 2000.

8.4 Results

8.4.1 Short-term impact of organic acids on the mineralisation of ^{14}C-phenanthrene in soil

The impact of citric, malic, oxalic and succinic acids within a naturally occurring range of concentrations was tested on the mineralisation of ^{14}C-phenanthrene. Organic acids were only observed to produce significant differences on the mineralisation of ^{14}C-phenanthrene, after 14 days soil-PAH contact time, while remaining unaffected after 50 days of soil-PAH contact time. The data showed that after a short soil-PAH contact time (14 d), the presence of citric acid (0.1 mmol l^{-1}) resulted in a significantly faster rate of mineralisation (29.52 % d^{-1}) than the control (20.99 % d^{-1}) (F = 2.795, p = 0.016) (Table 1). At this same time point, although not significant (p = 0.077), the lag phase of the control soil was longer (18.28 h) than in soil incubated with organic acids (4.11 – 5.42 h).

8.4.2 Preliminary tests for the selection of organic acids and soil-organic acid contact time for the assessment of ^{14}C-phenanthre bioaccessibility in soil

Citric, malic, oxalic and succinic acids did not impact significantly on the desorption of ^{14}C-phenanthrene from the soil (Table SI-1) when compared against the control (p > 0.05). Despite of this, soil extracted with citric and malic acid at 0.5 mmol l^{-1} were the only two treatments presenting higher rapidly desorbing fractions (F_{rap}), with (44.16 %) and (49.76 %) respectively, than the control (40.94 %). Based on this, these two organic acids were selected for further investigation at a wider range of concentrations (0.5, 100, 250, 500 and 1000 mmol l^{-1}) in order to assess the full potential of these compounds to impact the desorption of ^{14}C-phenanthrene in soil.

As significant differences were not observed within the different organic acids used in the preliminary assay looking at their impact in ^{14}C-phenanthrene desorption kinetics;

malic acid was selected as a representative organic acid for the optimisation of the methodology for the assessment of bioaccessibility. Results from the test looking at the temporal impact of malic acid in HPCD-extractable ^{14}C-phenanthrene fraction showed that soil-organic acid contact time did not have a significant effect on the bioaccessible fraction of this hydrocarbon ($p > 0.05$). However, data showed that the largest extractable proportion of ^{14}C-phenanthrene was obtained after 8 h of soil-organic acid incubation (control soil, 8.15 %), compared to the lowest value presented after 48 hours (control soil, 1.10 %). Therefore, 8 h soil-organic acid incubation was considered to be the most suitable contact time and consequently selected for further investigation. Furthermore, mineralisation from the HPCD extractable experimental units within the incubation time was observed to be negligible.

8.4.3 Bioaccessibility of ^{14}C-phenanthrene in soil

Changes on the bioaccessibility of ^{14}C-phenanthrene in soil was assessed by HPCD extractions and were observed to be significantly different over time (Table 2, $t = 66.682$, $p < 0.001$). After one week of soil-PAH contact time, saturation of soil with organic acids (100 % whc, 8 h) did not have significant effects on the bioaccessibility of ^{14}C-phenanthrene ($F = 1.981$, $p = 0.059$). In the case of soil incubated for 15 weeks, the addition of 500 mmol l^{-1} citric acid significantly enhanced the bioaccessible fraction of ^{14}C-phenanthrene (14.92 %) compared to the control (6.72 %) ($F = 4.513$, $p = 0.003$).

8.4.4 Desorption of ^{14}C-phenanthrene with organic acids

The amount of ^{14}C-phenanthrene that was desorbed from soil was significantly affected by the presence of organic acids ($p < 0.001$) (Table 3). Citric acid at the highest concentration (1000 mmol l^{-1}) consistently produced a significantly higher desorption than any other treatment after 1 week (39.27 %) and 15 weeks (47.86 %) soil-PAH contact time ($p < 0.001$). Furthermore, this was the only treatment capable of enhancing the desorption of ^{14}C-phenanthrene after 1 week soil-PAH contact time. The presence of citric acid (0.5 - 250 mmol l^{-1}) and malic acid (0.5 - 500 mmol l^{-1}) significantly reduced the total desorbable fraction of ^{14}C-phenanthrene soil after one week soil-PAH contact time ($p < 0.001$). In contrast, only the lowest concentrations (0.5 mmol l^{-1}) of citric acid (13.04 %) and malic acid (12.51 %) produced significantly

lower levels of desorption of ^{14}C-phenanthrene than the control (18.96 %) after 15 weeks soil-PAH contact time. This was in contrast to the desorption behaviour observed at concentrations above 100 mmol l^{-1} citric acid and 250 mmol l^{-1} malic acid, where desorbed ^{14}C-phenanthrene was significantly higher ($p < 0.001$).

8.4.4.1 Impact of organic acids on ^{14}C-phenanthrene desorption kinetics

Desorbing fractions (F_{rap} and F_{slow}; F_{rap}, F_{slow} and $F_{very\ slow}$) and rate constants (k_{rap} and k_{slow}; k_{rap}, k_{slow} and $k_{very\ slow}$) from the two- and three-compartment model fitting, respectively, are presented on Tables 4 and 5. Squared deviations data showed a better fit by the three-compartment fitting (Table SI-3, $p < 0.001$); therefore, further analysis was focused on the values estimated by this desorption model. Desorbing fractions (%) and rate constants (h^{-1}) obtained by the three-compartment model (Figures SI 4-9) showed significant differences for all cases ($p < 0.001$). After one week soil-PAH contact time, significantly higher fractions of ^{14}C-phenanthrene were rapidly desorbed by 1000 mmol l^{-1} citric (19.22 %) and malic acid (20.20%) than in the control soil (12.08 %). In contrast, lower concentrations of malic acid (100 and 250 mmol l^{-1}) and citric acid (100 mmol l^{-1}) significantly reduced the rapidly desorbing fractions. Rapidly desorbing rate constants were not affected by the majority of the treatments with the exception of the effect produced by citric acid at 100 mmol l^{-1}. Slowly desorbing fractions were significantly reduced by all treatments apart from citric acid (1000 mmol l^{-1}), which was found to be similar to the control. Furthermore, rate constants of this fraction (k_{slow}) were significantly enhanced in most of the treatments (except 0.5 and 1000 mmol l^{-1} citric acid), with a longest slowly desorbing phase produced in the present of malic acid (0.139 – 0.146 h^{-1}) when compared against the control (0.013 h^{-1}). Very slowly desorbing fractions accounted for the largest phase in all of the treatments. Moreover, organic acids significantly increased this fraction in all treatments (except 1000 mmol l^{-1} citric acid), ranging from 77.1 to 88.09 % against the 72.12 % when dH_2O was used as extractant. Very slowly desorbing rate constants were also significantly higher in the presence of citric (≥ 500 mmol l^{-1}) and malic acid at all tested concentrations.

After 15 weeks incubation, high concentrations of citric (500 – 1000 mmol l^{-1}) and malic (500 mmol l^{-1}) acid were found to significantly enhance the rapidly desorbing fraction of ^{14}C-phenanthrene, representing up to 25.12 % compared to the control

(13.11 %). Moreover, low concentrations of both organic acids (0.5 mmol l^{-1}) had the opposite effect, significantly reducing the fraction of ^{14}C-phenanthrene desorbed to 3.38 and 5.50 % respectively. Similarly, rapidly desorbing rate constants were also significantly larger when soil was extracted with citric (0.5 – 1000 mmol l^{-1}) and malic acid (1000 mmol l^{-1}). Slowly desorbing fractions (F_{slow}) were significantly reduced by all tested concentrations of citric acid and 0.5, 100 and 1000 mmol l^{-1} malic acid, while the corresponding desorption rate constants displayed the opposite behaviour. Fractions desorbed in the very slow phase were significantly increased by all treatments (except 500 mmol l^{-1}malic acid) going from 3.23 % in the control up to 92.50% when soil was treated with 0.5 mmol l^{-1}malic acid. Very slowly desorbing rate constants were similar to the control with the exception of 100 mmol l^{-1}malic acid where significantly higher values were observed ($p < 0.001$).

8.5 Discussion

8.5.1 Effect of organic acids in the bioaccessibility of ^{14}C-phenanthrene in soil

The general absence of effects by organic acids on the mineralisation of ^{14}C-phenanthrene reported in this study has also been observed by Cébron *et al.* (2011) and Louvel *et al.* (2011), both of whom worked with root exudates containing mixtures of organic acids. Despite this trend, both authors were able to observe an initial acceleration of the mineralisation process (Cébron *et al.*, 2011; Louvel *et al.*, 2011), as was the case of citric acid (100 mmol l^{-1}) in this present study. Cébron *et al.* (2011) further discussed that this general absence of effects might be the consequence of an enhanced binding of phenanthrene to SOM and other soil inorganic fractions such as mineral clays produced by the organic acids and that ultimately reflected on a reduced availability of phenanthrene for microbial degradation.

Similar trends were also observed when the bioaccessibility of ^{14}C-phenanthrene was measured through its HPCD extractability. Bioaccessibility was only significantly higher in one of the treatments (500 mmol l^{-1} citric and malic acid after 15 weeks soil-PAH contact time). These findings contrast with that reported by other authors where PAH availability can be significantly promoted by different LOAs assessed through *n*-butanol extractions (Ling *et al.*, 2009; Sun *et al.*, 2012a, 2013a; Kong *et al.*, 2013; Gao *et al.*, 2015b). Disagreement between these two trends is suggested to be due to

differences in the methodologies used for this purpose, where *n*-butanol extracted PAH not only extracting the freely and potentially freely available (bioaccessible) fraction of the hydrocarbon but also soil bound PAH residues as pointed by Ling *et al.* (2009). Although *n*-butanol has been proposed to act as a predictor for the bioavailability of PAHs in soil (Kelsey *et al.*, 1997; Liste & Alexander, 2002), this extractant has also been observed to exhibit greater extraction efficiencies when compared against HPCD extractability (Swindell & Reid, 2006b). Furthermore, *n*-butanol has also been demonstrated to act as a more exhaustive extractant than HPCD (Reid *et al.*, 2000b; Swindell & Reid, 2006b) leading to an overestimation of the bioaccessible fraction of PAHs in soil. Further, *n*-butanol has been shown to extract similar quantities of PAHs as DCM, which is often used to determine the total concentrations of contaminants in soil (Reid *et al.*, 2000b).

8.5.2 Impact of LOAs on the desorption of ^{14}C-phenanthrene in soil

The total desorbable fraction of ^{14}C-phenanthrene did not decrease as a function of time over the course of the incubation when soil was extracted with organic acids ($\geq$ 100 mmol l^{-1}). Despite the general acknowledgement of the negative correlation between the extractability of organic contaminants and contact time (Hatzinger & Alexander, 1995; Semple *et al.*, 2003), this behaviour was only observed in the control and the lowest tested concentration of organic acids after 15 weeks of soil-PAH contact time. This trend suggests that high amounts of organic acids could potentially restrict the reduction of bioaccessibility of ^{14}C-phenanthrene, therefore limiting the ageing process. Although not observed before, this behaviour could be the reflection of a dual effect of organic acids on phenanthrene sorption reported by Ouvrard *et al.* (2006) who described the impact of LOAs as a combined process characterised by an initial short term enhanced sorption of phenanthrene by SOM, followed by an increased mass transfer of the hydrocarbon due to the destabilisation of this soil fraction. Similarly, data from the present study showed a general reduction of the extractable ^{14}C-phenanthrene after a short period of ageing while organic acids were consistently observed to promote a larger desorption after 15 weeks of soil-PAH contact time when compared against the control. This increase in the extractability of PAHs by LOAs has also been reported by other authors (Ling *et al.*, 2009, 2015, Gao *et al.*, 2010a; b, 2015b; Kong *et al.*, 2013), but rarely considered the impact of soil-

PAH ageing included in the present study. Although not common, the reduction of ^{14}C-phenanthrene desorption in the presence of organic acids observed after a short soil-PAH contact time in the present study has been reported before (Ouvrard *et al.*, 2006; Zhu *et al.*, 2009; Gao *et al.*, 2015b). This initial behaviour has been associated with the capacity of small amounts of oxalate, citrate and malate to promote the sorption of anions to the soil (Jones & Brassington, 1998; Jones *et al.*, 2003). In a similar way, phenanthrene has been hypothesised to be also sorbed through the development of new sorption sites by these sorbed organic acids (Ouvrard *et al.*, 2006; Gao *et al.*, 2015a).

LOAs have been acknowledged to significantly influence the physical, chemical and biological properties of soil (Jones & Darrah, 1994; Jones, 1998). As such, the main mechanism behind the enhancement of the desorption of PAHs in soil impacted by organic acids has been proposed to be the solubilisation of soil organic matter (SOM) with a subsequent release SOM-associated hydrocarbons (Ouvrard *et al.*, 2006; Agnello *et al.*, 2014). This hypothesis is supported by findings from different authors who have reported consistently higher amounts of dissolved organic matter and certain minerals when artificial root exudates (Gao *et al.*, 2010a) and single LOAs (Ling *et al.*, 2009, 2015, Gao *et al.*, 2010b, 2015a; Sun *et al.*, 2012a; Kong *et al.*, 2013) were used to extract PAHs from contaminated soil. In a similar way, previously immobilised aromatic compounds have been observed to be released from soil to pore water after the introduction of organic acids solution (White *et al.*, 2003; Gao *et al.*, 2015b; Keiluweit *et al.*, 2015).

8.5.2.1 Desorption kinetics

^{14}C-Phenanthrene desorption kinetics in the presence of the organic acids displayed 3-compartment desorption behaviour. Although desorption of organic contaminants has been widely observed to behave following a rapid desorption followed by a slower phase (Cornelissen *et al.*, 1998b), this rapid/slow/very slow release from the soil has also been observed (Rhodes *et al.*, 2010b). Unlike other studies where desorption kinetics were performed using an extractant previously known (or suspected) to positively correlate with the biodegradable fraction of the contaminant under study (Cornelissen *et al.*, 2001; Rhodes *et al.*, 2010b); the present research was focused on the assessment of the desorbing potential that organic acids might be able to provide.

Bearing this in mind, the proportion of ^{14}C-phenanthrene desorbed at each of these phases should be considered as a measure of the behaviour of this PAH under the influence of organic acids rather than an indication of its bioaccessibility.

The different fractions described by the desorption kinetics can be interpreted as the biodegradable (F_{rap}) and less accessible F_{slow} and/or $F_{very\ slow}$ fractions of the organic contaminant (Cornelissen *et al.*, 1998b; Rhodes *et al.*, 2010b). Results from this investigation showed that the majority of the treatments had a tendency to enhance the very slowly desorbing fraction ($F_{very\ slow}$).These results could be interpreted as that the presence of organic acids might be able to promote the mobilisation of a significant proportion of the bioaccessible fraction of ^{14}C-phenanthrene (F_{rap}) towards a less accessible form (F_{slow} and $F_{very\ slow}$), therefore limiting the biological degradation of the contaminant (Pignatello & Xing, 1996; Clegg *et al.*, 2014). Moreover, similar behaviour has been observed to occur during the mineralisation of organic acids, where these compounds have been observed to induce shifts of $^{14}CO_2$ production from a rapid to a slower phase (Oburger *et al.*, 2009).

8.6 Conclusions

The total extractable fraction of ^{14}C-phenanthrene can be significantly enhanced by citric and malic acid. This effect is most likely to be observed at a longer soil-PAH contact time, where organic acids showed to restrict the ageing effect. Despite these enhancing effects, desorption kinetics showed that a large proportion of the desorbable ^{14}C-phenanthrene was being mobilised towards the least desorbable fraction (F_{slow} and $F_{very\ slow}$). Results from the assessment of accessibility and mineralisation of ^{14}C-phenanthrene showed that despite the enhancement of the hydrocarbon extractable fraction by citric and malic acid; there is no clear evidence suggesting that this condition can promote the microbial utilisation of ^{14}C-phenanthrene. This study contributes to the understanding of the role of root exudation within the rhizosphere towards the bioaccessibility and biodegradation of hydrocarbons in contaminated soil.

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Table 1. Mineralisation kinetics of ^{14}C-phenanthrene from soil affected by organic acids after 14 and 50 days ageing. Values of the lag phases (h), maximum rates (% d^{-1}) and total extents (%) represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments at each time point assessed by post hoc Tukey tests.

		14 days ageing					
Treatment	Concentration (mmol l^{-1})	Lag phase*		Fastest rate		Total extent	
Control	0	18.288	± 0.358	[a]20.991	± 0.564	[ab]57.483	± 0.617
Citric acid	0.1	4.104	± 0.417	[b]29.520	± 2.316	[ab]65.325	± 3.590
	0.5	4.728	± 0.382	[ab]25.519	± 1.439	[ab]63.863	± 2.559
Malic acid	0.1	4.440	± 0.347	[ab]27.114	± 1.268	[ab]63.741	± 1.430
	0.5	4.320	± 0.364	[ab]27.902	± 1.050	[b]68.370	± 0.101
Oxalic acid	0.1	5.424	± 0.564	[ab]22.562	± 2.125	[a]54.085	± 3.386
	0.5	4.632	± 0.397	[ab]26.093	± 1.576	[ab]58.915	± 1.694
Succinic acid	0.1	4.656	± 0.260	[ab]25.806	± 0.664	[ab]63.543	± 2.409
	0.5	4.800	± 0.445	[ab]25.270	± 1.938	[ab]60.683	± 3.183
		50 days ageing					
Treatment	Concentration (mmol l^{-1})	Lag phase		Fastest rate		Total extent	
Control	0	[a]84.350	± 2.400	[a]1.613	± 0.257	[a]11.581	± 1.321
Citric acid	0.1	[a]81.128	± 1.945	[a]1.607	± 0.128	[a]11.419	± 0.560
	0.5	[a]48.488	± 1.400	[a]1.651	± 0.020	[a]11.688	± 0.332
Malic acid	0.1	[a]69.532	± 2.020	[a]1.592	± 0.108	[a]12.535	± 0.474
	0.5	[a]85.519	± 2.319	[a]1.345	± 0.089	[a]10.590	± 0.667
Oxalic acid	0.1	[a]69.922	± 0.894	[a]1.553	± 0.026	[a]12.387	± 0.410
	0.5	[a]72.237	± 1.494	[a]1.670	± 0.128	[a]12.959	± 0.935
Succinic acid	0.1	[a]78.770	± 1.819	[a]1.661	± 0.140	[a]11.093	± 0.591
	0.5	[a]56.177	± 2.119	[a]1.959	± 0.154	[a]13.641	± 0.159

*Not normally distributed data analysed by Kruskal-Wallis non-parametric test (p = 0.077)

Table 2. HPCD extractable fraction of ^{14}C-phenanthrene from soil after 1 and 15 weeks ageing following saturation with organic acids solution (100 % whc, 8 h). Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments at each time point (Tukey)

Treatment	Concentration (mmol l^{-1})	Bioaccessible ^{14}C-pheannthrene (%)	
		1 week*	15 weeks
Control	0	^{a}79.841 ± 0.717	^{ab}6.721 ± 0.970
Citric acid	0.5	^{a}78.964 ± 2.070	^{a}5.033 ± 0.955
	100	^{a}78.769 ± 1.346	^{ab}6.492 ± 1.646
	250	^{a}79.239 ± 1.243	^{abc}9.804 ± 1.033
	500	^{a}79.852 ± 0.633	^{c}14.929 ± 0.582
	1000	^{a}76.745 ± 0.164	^{bc}11.223 ± 1.524
Malic acid	0.5	^{a}81.138 ± 0.611	^{ab}7.992 ± 0.690
	100	^{a}80.517 ± 0.366	^{abc}8.938 ± 1.509
	250	^{a}78.540 ± 0.793	^{ab}8.764 ± 2.035
	500	^{a}77.937 ± 0.579	^{abc}10.726 ± 0.871
	1000	^{a}76.737 ± 1.003	^{ab}8.594 ± 1.284

Table 3. Total ^{14}C-phenanthrene desorbed from soil after 1 and 15 weeks ageing. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments at each time point (Tukey).

Treatment	Concentration (mmol l^{-1})	Desorbed ^{14}C-pheannthrene (%)	
		1 week	15 weeks
Control	0	^{de}27.980 ± 1.636	^{b}18.958 ± 0.931
Citric acid	0.5	^{ab}20.755 ± 0.432	^{a}13.038 ± 1.010
	100	^{a}17.221 ± 0.211	^{cd}26.462 ± 0.431
	250	^{ab}19.709 ± 0.081	^{de}31.264 ± 1.851
	500	^{cd}26.579 ± 0.795	^{f}40.006 ± 0.655
	1000	^{f}39.274 ± 1.921	^{g}47.856 ± 1.060
Malic acid	0.5	^{ab}20.068 ± 0.376	^{a}12.507 ± 0.176
	100	^{a}16.552 ± 0.119	^{bc}21.986 ± 1.363
	250	^{ab}18.820 ± 0.256	^{cd}25.923 ± 0.983
	500	^{bc}22.558 ± 0.266	^{e}32.955 ± 1.134
	1000	^{e}31.720 ± 1.249	^{ef}36.184 ± 2.054

Table 4. Desorbing fractions (F_{rap} and F_{slow}) and constant rates (k_{rap} and k_{slow}) calculated by a two-compartment model. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})	
1 week ageing									
Control	0	^{cd}16.571	± 0.442	^{a}0.128	± 0.004	^{cd}83.428	± 0.442	^{c}0.001	<0.001
Citric acid	0.5	^{abc}14.393	± 0.381	^{ab}0.142	± 0.004	^{def}85.606	± 0.381	^{ab}<0.001	<0.001
	100	^{a}12.313	± 0.160	^{b}0.145	± 0.001	^{f}87.687	± 0.160	^{a}<0.001	<0.001
	250	^{abc}14.423	± 0.108	^{ab}0.134	± 0.001	^{def}85.576	± 0.108	^{a}<0.001	<0.001
	500	^{bcd}19.090	± 0.706	^{ab}0.142	± 0.006	^{c}80.909	± 0.706	^{b}0.001	<0.001
	1000	^{e}28.381	± 1.587	^{ab}0.140	± 0.005	^{a}71.618	± 1.587	^{d}0.001	<0.001
Malic acid	0.5	^{abc}15.123	± 0.104	^{b}0.139	± 0.002	^{def}84.876	± 0.104	^{a}<0.001	<0.001
	100	^{a}11.901	± 0.143	^{ab}0.142	± 0.002	^{f}88.098	± 0.143	^{a}<0.001	<0.001
	250	^{ab}13.447	± 0.119	^{ab}0.146	± 0.002	^{ef}86.553	± 0.119	^{a}<0.001	<0.001
	500	^{d}16.122	± 0.272	^{ab}0.141	± 0.003	^{cde}83.877	± 0.272	^{ab}<0.001	<0.001
	1000	^{f}22.899	± 0.936	^{ab}0.140	± 0.003	^{b}77.100	± 0.936	^{c}0.001	<0.001
15 weeks ageing									
Control	0	^{b}14.683	± 3.591	^{ab}0.195	± 0.009	^{f}85.317	± 3.591	^{a}0.001	<0.001
Citric acid	0.5	^{a}6.789	± 0.247	^{ab}0.170	± 0.012	^{g}93.211	± 0.247	^{a}<0.001	<0.001
	100	^{bc}14.566	± 0.436	^{bc}0.217	± 0.020	^{ef}85.434	± 0.436	^{b}0.001	<0.001
	250	^{b}19.534	± 0.493	^{b}0.186	± 0.014	^{bc}80.466	± 0.493	^{bc}0.001	<0.001
	500	^{f}25.150	± 0.876	^{bc}0.213	± 0.009	^{c}74.850	± 0.876	^{cd}0.001	<0.001
	1000	^{g}30.907	± 0.377	^{c}0.262	± 0.006	^{a}69.093	± 0.377	^{d}0.002	<0.001
Malic acid	0.5	^{a}7.533	± 0.333	^{bc}0.206	± 0.023	^{g}92.467	± 0.333	^{a}<0.001	<0.001
	100	^{bcd}15.906	± 0.770	^{a}0.108	± 0.021	^{def}84.094	± 0.770	^{a}<0.001	<0.001
	250	^{cde}17.146	± 0.621	^{ab}0.156	± 0.021	^{cde}82.854	± 0.621	^{ab}0.001	<0.001

500	[e]20.412	± 0.598	[bc]0.209	± 0.007	[a]79.588	± 0.598	[bc]0.001	<0.001
1000	[f]24.585	± 1.845	[ab]0.156	± 0.009	[b]75.415	± 1.845	[bc]0.001	<0.001

Table 5. Desorbing fractions (F_{rap}, F_{slow} and $F_{very\ slow}$) and constant rates (k_{rap}, k_{slow} and $k_{very\ slow}$) calculated by a three-compartment model. Values represent the mean ± standard error of the mean (n = 5). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests.

Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})		$F_{very\ slow}$ (%)		$k_{very\ slow}$ (h^{-1})	
1 week ageing													
Control	0	^{de}12.078	± 0.418	^{ab}0.210	± 0.008	^{e}15.801	± 0.850	^{a}0.013	± 0.001	^{b}72.122	± 1.251	<^{a}0.001	<0.001
Citric acid	0.5	^{bcd}10.164	± 0.564	^{b}0.226	± 0.014	^{cd}9.162	± 0.224	^{ab}0.021	± 0.004	^{d}80.674	± 0.708	<^{ab}0.001	<0.001
	100	^{a}7.099	± 0.457	^{c}0.319	± 0.037	^{b}7.136	± 0.418	^{c}0.042	± 0.003	^{efg}85.765	± 0.224	<^{abc}0.001	<0.001
	250	^{b}8.984	± 0.423	^{b}0.242	± 0.013	^{bc}7.992	± 0.219	^{bc}0.035	± 0.003	^{de}83.025	± 0.254	<^{abc}0.001	<0.001
	500	^{cde}11.822	± 0.656	^{bc}0.271	± 0.029	^{d}10.589	± 0.536	^{c}0.036	± 0.004	^{c}77.589	± 0.563	<^{cde}0.001	<0.001
	1000	^{f}19.220	± 0.344	^{b}0.234	± 0.007	^{e}16.563	± 0.614	^{abc}0.027	± 0.005	^{a}64.217	± 0.657	<^{cde}0.001	<0.001
Malic acid	0.5	^{cde}11.379	± 0.524	^{a}0.139	± 0.002	^{a}3.686	± 0.473	^{d}0.139	± 0.002	^{ef}84.935	± 0.086	<^{de}0.001	<0.001
	100	^{bc}9.614	± 0.136	^{a}0.142	± 0.002	^{a}2.288	± 0.008	^{d}0.142	± 0.002	^{g}88.098	± 0.143	<^{cde}0.001	<0.001
	250	^{bcd}11.074	± 0.115	^{a}0.146	± 0.002	^{a}2.373	± 0.005	^{d}0.146	± 0.002	^{fg}86.553	± 0.119	<^{de}0.001	<0.001
	500	^{e}13.632	± 0.260	^{a}0.141	± 0.003	^{a}2.491	± 0.012	^{d}0.141	± 0.003	^{ef}83.877	± 0.272	<^{e}0.001	<0.001
	1000	^{f}20.196	± 0.914	^{a}0.140	± 0.003	^{a}2.705	± 0.022	^{d}0.140	± 0.003	^{c}77.100	± 0.936	<^{f}0.001	<0.001
15 weeks ageing													
Control	0	^{bcd}13.115	± 0.712	^{b}0.172	± 0.010	^{d}83.646	± 3.300	<^{a}0.001	<0.001	^{a}3.239	± 3.234	<^{a}0.001	<0.001
Citric acid	0.5	^{a}6.389	± 0.180	^{bc}0.180	± 0.019	^{c}14.050	± 4.178	^{bc}0.005	± 0.001	^{b}79.561	± 4.036	<^{ab}0.001	<0.001
	100	^{bc}12.466	± 0.512	^{cd}0.298	± 0.023	^{c}16.386	± 0.491	^{cd}0.010	± 0.001	^{b}71.149	± 0.609	<^{b}0.001	<0.001
	250	^{bc}11.753	± 1.008	^{ef}0.511	± 0.053	^{c}18.569	± 1.781	^{de}0.027	± 0.007	^{b}69.678	± 2.345	<^{ab}0.001	<0.001
	500	^{ef}17.595	± 1.768	^{de}0.521	± 0.126	^{c}19.782	± 1.110	^{d}0.020	± 0.007	^{b}62.623	± 2.536	<^{ab}0.001	<0.001
	1000	^{g}25.120	± 1.153	^{de}0.398	± 0.046	^{c}15.909	± 2.951	^{de}0.026	± 0.005	^{b}58.971	± 2.787	^{ab}0.001	<0.001
Malic acid	0.5	^{a}5.504	± 0.526	^{bc}0.190	± 0.021	^{b}1.994	± 0.305	^{f}0.190	± 0.021	^{b}92.502	± 0.324	<^{a}0.001	<0.001
	100	^{cde}14.762	± 0.759	^{a}0.090	± 0.012	^{a}1.057	± 0.006	^{f}0.090	± 0.012	^{b}84.181	± 0.765	<^{a}0.001	<0.001
	250	^{def}16.916	± 0.492	^{b}0.150	± 0.010	^{d}58.727	± 1.719	^{a}0.001	± 0.000	^{b}24.357	± 1.776	<^{a}0.001	<0.001

500	[f]20.465	± 0.659	[bc]0.210	± 0.007	[d]58.849	± 1.998	[ab]0.001	<0.001	[ab]20.686	± 1.732	[a]0.001	<0.001
1000	[ab]9.066	± 0.771	[f]0.830	± 0.032	[c]18.118	± 1.007	[ef]0.061	± 0.002	[ef]72.816	± 1.353	[a]0.001	<0.001

8.7 References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D. & Esposito, G. 2014. Enhanced phytoremediation: A review of low molecular weight organic acids and surfactants used as amendments. Critical Reviews in Environmental Science and Technology, 44, 2531–2576.

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science & Technology, 27, 2630–2636.

Cébron, A., Louvel, B., Faure, P., France-Lanord, C., Chen, Y., Murrell, J.C. & Leyval, C. 2011. Root exudates modify bacterial diversity of phenanthrene degraders in PAH-polluted soil but not phenanthrene degradation rates. Environmental Microbiology, 13, 722–736.

Clegg, H., Riding, M.J., Oliver, R., Jones, K.C. & Semple, K.T. 2014. The impact of soil organic matter and soil sterilisation on the bioaccessibility of 14C-azoxystrobin determined by desorption kinetics. Journal of Hazardous Materials, 278, 336–342.

Cornelissen, G., Van Noort, P.C.M. & Covers, H.A.J. 1998a. Mechanism of slow desorption of organic compounds from sediments: A study using model sorbents. Environmental Science & Technology, 32, 3124–3131.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M.A. & Van Noort, P.C.M. 1998b. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environmental Science & Technology, 32, 966–970.

Cornelissen, G., Rigterink, H., ten Hulscher, D.E.M., Vrind, B.A. & van Noort, P.C.M. 2001. A simple tenax® extraction method to determine the availability of sediment-sorbed organic compounds. Environmental Toxicology and Chemistry, 20, 706–711.

Dakora, F.D. & Phillips, D.A. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil, 245, 35–47.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Gao, Y., Ren, L., Ling, W., Gong, S., Sun, B. & Zhang, Y. 2010a. Desorption of phenanthrene and pyrene in soils by root exudates. Bioresource Technology, 101, 1159–1165.

Gao, Y., Ren, L., Ling, W., Kang, F., Zhu, X. & Sun, B. 2010b. Effects of low-molecular-weight organic acids on sorption–desorption of phenanthrene in soils. Soil Science Society of America Journal, 74, 51.

Gao, Y., Wang, N., Li, H., Hu, X. & Goikavi, C. 2015a. Low-molecular-weight organic acids influence the sorption of phenanthrene by different soil particle size

fractions. Journal of Environment Quality, 44, 219.

Gao, Y., Yuan, X., Lin, X., Sun, B. & Zhao, Z. 2015b. Low-molecular-weight organic acids enhance the release of bound PAH residues in soils. Soil and Tillage Research, 145, 103–110.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L. & Lundström, U.S. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry, 37, 1–13.

Joner, E.J., Corgié, S.C. & Leyval, C. 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biology and Biochemistry, 34, 859–864.

Jones, D. 1998. Organic acids in the rhizosphere - a critical review. Plant and Soil, 205, 25–44.

Jones, D.L. & Brassington, D.S. 1998. Sorption of organic acids in acid soils and its implications in the rhizosphere. European Journal of Soil Science, 49, 447–455.

Jones, D.L. & Darrah, P.R. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil, 166, 247–257.

Jones, D.L.L., Dennis, P.G.G., Owen, a. G.G. & van Hees, P. a. W. a W. 2003. Organic acid behavior in soils - misconceptions and knowledge gaps. Plant and Soil, 248, 31–41.

Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K. & Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change, 5, 588–595.

Kelsey, J.W., Kottler, B.D. & Alexander, M. 1997. Selective chemiscal extractants to predict bioavaiability of soil-aged organic chemicals. Environmental Science & Technology, 31, 214–217.

Kong, H., Sun, R., Gao, Y. & Sun, B. 2013. Elution of polycyclic aromatic hydrocarbons in soil columns using low-molecular-weight organic acids. Soil Science Society of America Journal, 77, 72–82.

Ling, W., Ren, L., Gao, Y., Zhu, X. & Sun, B. 2009. Impact of low-molecular-weight organic acids on the availability of phenanthrene and pyrene in soil. Soil Biology and Biochemistry, 41, 2187–2195.

Ling, W., Sun, R., Gao, X., Xu, R. & Li, H. 2015. Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil. European Journal of Soil Science, 66, 339–347.

Liste, H.H. & Alexander, M. 2002. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere, 46, 1011–1017.

Louvel, B., Cébron, A. & Leyval, C. 2011. Root exudates affect phenanthrene biodegradation, bacterial community and functional gene expression in sand microcosms. International Biodeterioration and Biodegradation, 65, 947–953.

Martin, B.C., George, S.J., Price, C.A., Ryan, M.H. & Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. Science of the Total Environment, 472, 642–653.

Miya, R.K. & Firestone, M.K. 2001. Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environment Quality, 30, 1911–1918.

Oburger, E., Kirk, G.J.D., Wenzel, W.W., Puschenreiter, M. & Jones, D.L. 2009. Interactive effects of organic acids in the rhizosphere. Soil Biology and Biochemistry, 41, 449–457.

Ouvrard, S., Lapole, D. & Morel, J.L. 2006. Root exudates impact on phenanthrene availability. Water, Air, and Soil Pollution: Focus, 6, 343–352.

Pignatello, J.J. & Xing, B. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environmental Science & Technology, 30, 1–11.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39.

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D. & Semple, K.T. 2001. A simple 14C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. FEMS Microbiology Letters, 196, 141–146.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science & Technology, 34, 3174–3179.

Reilley, K.A., Banks, M.K. & Schwab, A.P. 1996. Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality, 25, 212–219.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Rhodes, A.H., Riding, M.J., McAllister, L.E., Lee, K. & Semple, K.T. 2012. Influence of activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil. Environmental Science & Technology, 46, 12445–12451.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic

organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Shukla, K.P., Sharma, S., Singh, N.K., Singh, V. & Tiwari, K. 2011. Nature and role of root exudates : Efficacy in bioremediation. African Journal of Biotechnology, 10, 9717–9724.

Siciliano, S.D. & Germida, J.J. 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environmental Reviews, 6, 65–79.

Sun, B., Gao, Y., Liu, J. & Sun, Y. 2012. The impact of different root exudate components on phenanthrene availability in soil. Soil Science Society of America Journal Abstract - Soil Chemistry, 76, 2041 2050.

Sun, B., Ling, W. & Wang, Y. 2013. Can root exudate components influence the availability of pyrene in soil? Journal of Soils and Sediments, 13, 1161–1169.

Swindell, A.L. & Reid, B.J. 2006. Comparison of selected non-exhaustive extraction techniques to assess PAH availability in dissimilar soils. Chemosphere, 62, 1126–1134.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Vázquez-Cuevas, G.M. & Semple, K.T. 2016. Measurement of Hydrocarbon Bioavailability in Soil. In: Hydrocarbon and Lipid Microbiology Protocols - Springer Protocols Handbooks (eds. McGenity, T., Timmis, K. & Nogales, B.), pp. 231–246. Springer, Berlin, Heidelberg.

White, J.C., Mattina, M.I., Lee, W.Y., Eitzer, B.D. & Iannucci-Berger, W. 2003. Role of organic acids in enhancing the desorption and uptake of weathered p ,p -DDE by Cucurbita pepo. Environmental Pollution, 124, 71–80.

Yoshitomi, K.. & Shann, J.. 2001. Corn (Zea mays L.) root exudates and their impact on 14C-pyrene mineralization. Soil Biology and Biochemistry, 33, 1769–1776.

Zhu, Y., Zhang, S., Huang, H. & Wen, B. 2009. Effects of maize root exudates and organic acids on the desorption of phenanthrene from soils. Journal of Environmental Sciences, 21, 920–926.

8.8 Supplementary information

Table SI 1. Desorption kinetics of ^{14}C-phenanthrene from mildly aged soil (50 d). Values represent the mean ± standard error of the mean (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests

Two-compartment fitting													
Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})					
Control	0	^{ab}40.939	± 3.861	^{a}0.090	± 0.019	^{ab}59.061	± 3.861	^{a}0.001	< 0.001				
Citric acid	0.1	^{a}25.905	± 6.193	^{a}0.133	± 0.020	^{b}74.095	± 6.193	^{a}0.001	< 0.001				
	0.5	^{ab}44.164	± 7.875	^{a}0.119	± 0.010	^{ab}55.836	± 7.875	^{a}0.001	< 0.001				
Malic acid	0.1	^{a}25.356	± 3.052	^{a}0.179	± 0.007	^{b}74.644	± 3.052	^{a}0.001	< 0.001				
	0.5	^{b}49.763	± 3.847	^{a}0.110	± 0.015	^{a}50.237	± 3.847	^{a}0.001	< 0.001				
Oxalic acid	0.1	^{ab}37.096	± 5.009	^{a}0.107	± 0.017	^{ab}62.904	± 5.009	^{a}0.001	< 0.001				
	0.5	^{ab}34.857	± 1.603	^{a}0.151	± 0.027	^{ab}65.143	± 1.603	^{a}0.001	± 0.001				
Succinic acid	0.1	^{ab}30.451	± 2.869	^{a}0.115	± 0.036	^{ab}69.549	± 2.869	^{a}0.000	< 0.001				
	0.5	^{ab}28.999	± 1.983	^{a}0.123	± 0.007	^{ab}71.001	± 1.983	^{a}0.001	< 0.001				
Three-compartment fitting													
Treatment	Concentration (mmol l^{-1})	F_{rap} (%)		k_{rap} (h^{-1})		F_{slow} (%)		k_{slow} (h^{-1})		$F_{very\ slow}$ (%)		$k_{very\ slow}$ (h^{-1})	
Control	0	^{ab}36.112	± 5.067	^{ab}0.138	± 0.023	^{a}56.511	± 2.855	^{ab}0.003	< 0.001	^{a}7.377	± 2.458	^{a}0.003	< 0.001
Citric acid	0.1	^{a}22.920	± 6.498	^{ab}0.168	± 0.035	^{a}51.399	± 18.98	^{ab}0.003	± 0.001	^{a}25.682	± 13.80	^{a}0.002	± 0.001
	0.5	^{b}56.929	± 9.031	^{a}0.130	± 0.083	^{a}30.697	± 10.45	^{ab}0.007	± 0.005	^{a}12.374	± 12.37	^{a}0.001	± 0.001
Malic acid	0.1	^{a}23.902	± 2.529	^{ab}0.289	± 0.045	^{a}61.384	± 9.804	^{ab}0.002	± 0.001	^{a}14.714	± 8.010	^{a}0.001	± 0.001
	0.5	^{ab}43.770	± 2.514	^{ab}0.193	± 0.035	^{a}46.991	± 8.865	^{ab}0.005	± 0.003	^{a}9.240	± 6.792	^{a}0.002	± 0.001
Oxalic acid	0.1	^{ab}31.645	± 7.967	^{ab}0.321	± 0.179	^{a}44.376	± 9.240	^{ab}0.007	± 0.005	^{a}23.979	± 15.78	^{a}0.001	< 0.001

	0.5	[ab]29.134	± 1.809	[ab]0.375	± 0.082	[a]38.480	± 9.898	[ab]0.007	± 0.003	[a]32.386	± 10.93	[a]0.001	± 0.001
Succinic acid	0.1	[ab]29.880	± 2.748	[a]0.147	± 0.058	[a]64.678	± 5.535	[a]0.001	< 0.001	[a]5.443	± 4.019	[a]0.001	± 0.001
	0.5	[a]19.530	± 0.664	[b]0.577	± 0.106	[a]24.270	± 5.003	[b]0.016	± 0.006	[a]56.200	± 5.500	[a]< 0.001	< 0.001

Table SI 2. Proportion of ^{14}C-phenanthrene (1) extracted with 50 mM HPCD solution, (2) mineralisation rate (%, h^{-1}) within the assessed contact time. Values represent the mean ± standard error of the mean (n = 3)

Contact time (h)	Treatment	Extracted (%)[1]		Mineralised (%)[2]	
1	Control	7.269	± 0.993	1.454	± 0.199
	0.5 mmol l^{-1}	5.777	± 0.767	1.155	± 0.153
	500 mmol l^{-1}	7.854	± 0.257	1.571	± 0.051
3	Control	6.062	± 0.943	0.505	± 0.236
	0.5 mmol l^{-1}	6.101	± 0.358	0.508	± 0.089
	500 mmol l^{-1}	7.268	± 1.315	0.605	± 0.329
6	Control	5.843	± 1.035	0.243	± 0.259
	0.5 mmol l^{-1}	6.264	± 0.954	0.261	± 0.238
	500 mmol l^{-1}	5.528	± 0.818	0.230	± 0.205
8	Control	8.146	± 1.876	0.254	± 0.469
	0.5 mmol l^{-1}	5.978	± 1.104	0.186	± 0.276
	500 mmol l^{-1}	8.495	± 1.182	0.265	± 0.296
24	Control	5.250	± 3.213	0.054	± 0.803
	0.5 mmol l^{-1}	3.805	± 1.050	0.039	± 0.263
	500 mmol l^{-1}	6.282	± 1.327	0.065	± 0.332
48	Control	1.102	± 0.108	0.091	± 0.432
	0.5 mmol l^{-1}	0.929	± 0.169	0.077	± 0.676
	500 mmol l^{-1}	1.257	± 0.157	0.104	± 0.628

Table SI 3. Sums of squared deviations of desorbed ^{14}C-phenanthrene fitted to a two- and three-compartment model. Values represent the mean ± standard error of the mean (n = 5)

Treatment	Concentration (mmol l^{-1})	Sum of squared difference 2 compartment fitting		Sum of squared difference 3-compartment fitting	
		1 week ageing			
Control	0	9.05E-04	± 1.0E-04	2.24E-04	± 2.8E-05
Citric acid	0.5	4.79E-04	± 3.6E-05	2.53E-04	± 7.8E-05
	100	3.97E-04	± 2.6E-05	1.39E-04	± 1.3E-05
	250	4.19E-04	± 2.4E-05	1.28E-04	± 1.1E-05
	500	8.97E-04	± 1.0E-04	2.73E-04	± 5.1E-05
	1000	2.40E-03	± 2.8E-04	7.09E-04	± 9.4E-05
Malic acid	0.5	5.44E-04	± 4.0E-05	5.44E-04	± 4.0E-05
	100	3.13E-04	± 1.3E-05	3.13E-04	± 1.3E-05
	250	4.98E-04	± 2.0E-05	4.98E-04	± 2.0E-05
	500	6.71E-04	± 1.4E-05	6.71E-04	± 1.4E-05
	1000	1.49E-03	± 1.7E-04	1.49E-03	± 1.7E-04
		15 weeks ageing			
Control	0	4.50E-04	± 6.0E-05	4.60E-04	± 6.0E-05
Citric acid	0.5	9.59E-05	± 4.6E-05	6.00E-05	± 4.0E-05
	100	1.17E-03	± 2.0E-04	2.20E-04	± 9.3E-05
	250	2.50E-03	± 3.9E-04	1.48E-04	± 5.0E-05
	500	3.59E-03	± 4.5E-04	9.20E-04	± 1.9E-04
	1000	2.99E-03	± 7.8E-04	3.70E-04	± 4.8E-05
Malic acid	0.5	3.92E-04	± 6.4E-05	4.20E-04	± 6.6E-05
	100	4.63E-04	± 1.3E-04	4.63E-04	± 1.3E-04
	250	1.98E-03	± 1.2E-04	1.28E-03	± 4.1E-04
	500	1.75E-03	± 1.0E-04	1.58E-03	± 2.3E-04
	1000	3.70E-03	± 4.7E-04	2.80E-04	± 6.8E-05

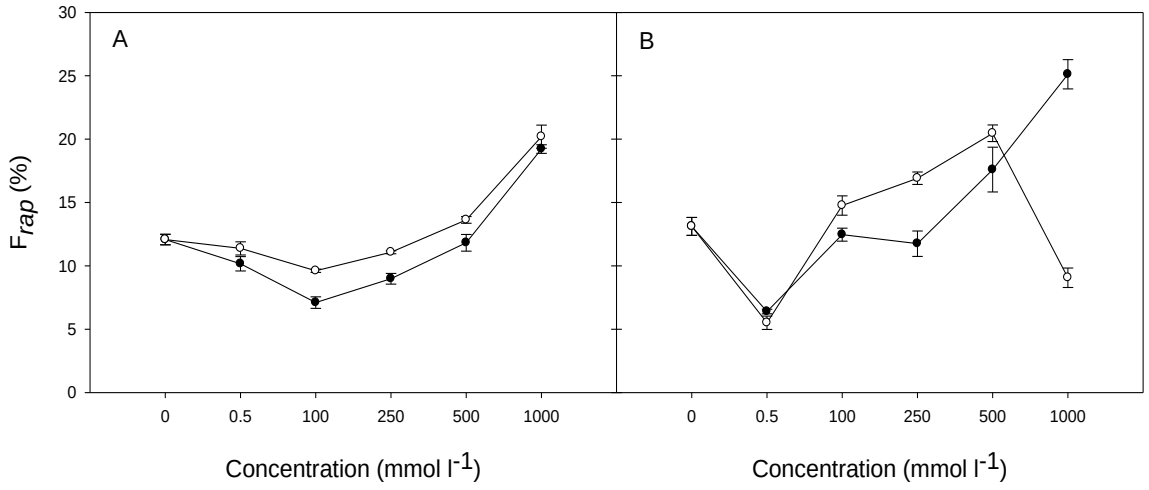

Figure SI 4. Rapid desorbing fractions (F_{rap}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

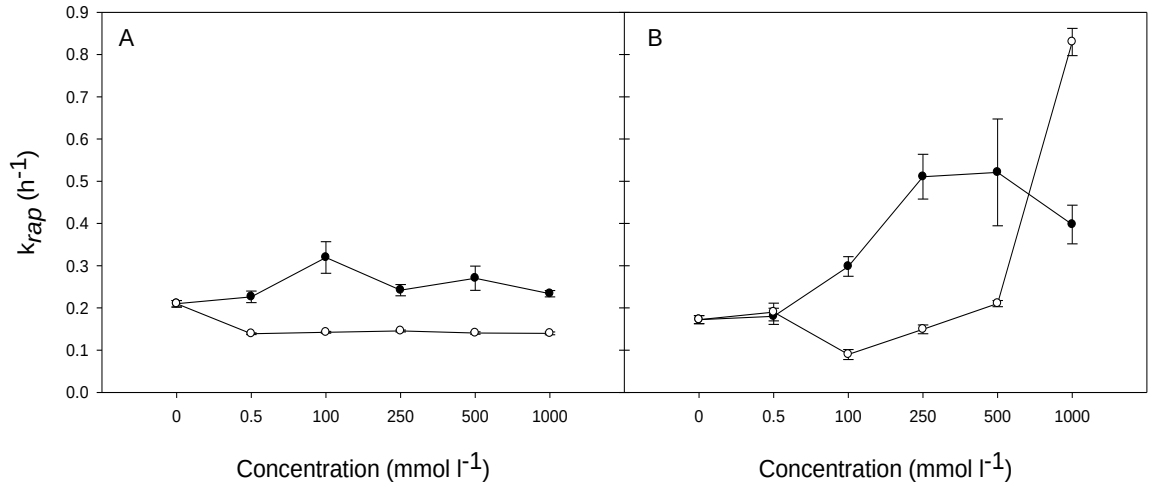

Figure SI 5. Rate constants of the rapid desorbing fractions (k_{rap}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

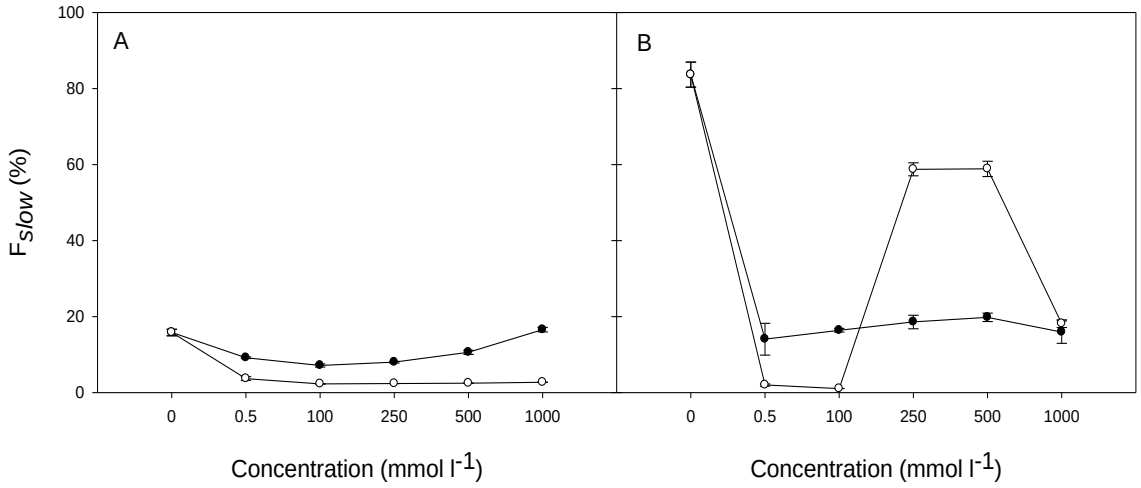

Figure SI 6. Slow desorbing fractions (F_{slow}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

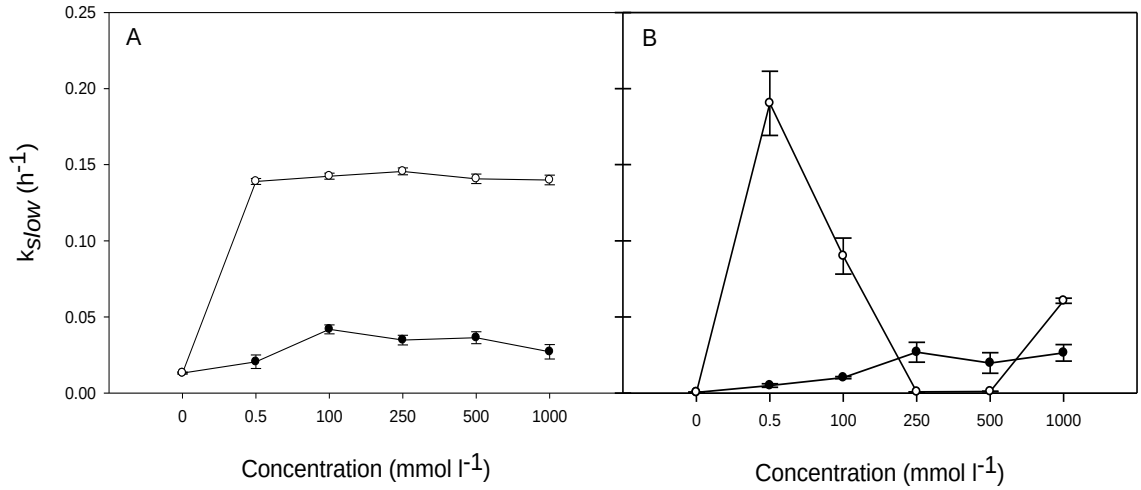

Figure SI 7. Rate constants of the slow desorbing fractions (k_{slow}) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

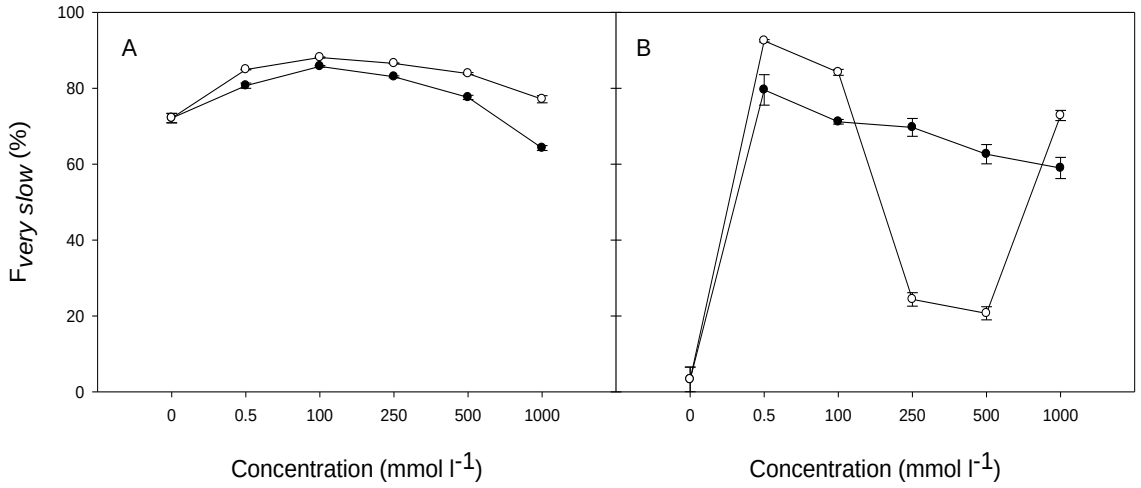

Figure SI 8. Very slow desorbing fractions ($F_{very\ slow}$) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

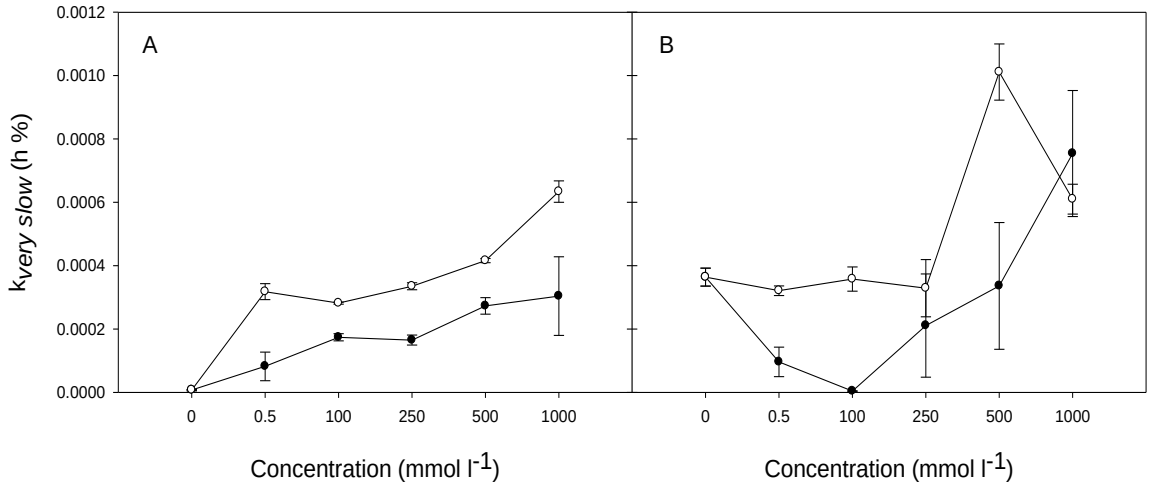

Figure SI 9. Rate constants of the very slow desorbing fractions ($k_{very\ slow}$) of ^{14}C-phenanthrene from soil aged for 1 (A) and 15 (B) weeks extracted with citric (●) and malic (○) acid. Values were obtained using a three-compartment model fitting. Error bars represent the standard error of the mean (n = 5).

Chapter 9

General discussion and conclusions

9.1 General discussion and conclusions

The study of the mechanisms driving the biodegradation of HOCs can be considered as a fundamental approach for the development of new remediation technologies for the management and treatment of contaminated soil. As such, the bioaccessibility of these hydrophobic contaminants is deemed to be a defining element within the study of the biodegradation, ecotoxicology and risk assessment of contaminated land (Semple *et al.*, 2004; Ortega-Calvo *et al.*, 2015). As it has been mentioned throughout this thesis, the extent by which HOCs are degraded can be significantly enhanced by the presence of plant-root biomass. Results showed that both indigenous catabolic response (Chapters 5 and 7) and biodegradation (Chapter 6) can be promoted by the presence of plant-root material in the hydrocarbon contaminated soil. This behaviour can be attributed to different mechanisms, among which the *(i)* promotion of larger and/or better adapted microbial populations (Siciliano & Germida, 1998; Pilon-Smits, 2005; Mueller & Shann, 2007), and *(ii)* increased bioaccessibility of the hydrocarbons (Siciliano & Germida, 1998; Martin *et al.*, 2014), are consistently considered among the main drivers for plant-enhanced biodegradation of hydrocarbons in soil.

Data presented throughout this thesis indicated that although mineralisation of ^{14}C-phenanthrene was enhanced by different plant-root treatments, these did not show to have a direct positive correlation with the number of colony forming units. This suggests that despite the general acknowledgment of larger microbial populations present in soil under the influence of plant roots (Anderson *et al.*, 1993; Salt *et al.*, 1998; Thijs *et al.*, 2017), this was not necessary in order to increase the catabolic response and biodegradation of ^{14}C-phenanthrene. On the contrary, fewer colony forming units where observed at the end of the experiment, which was also when a larger proportion of the hydrocarbon was mineralised. This temporal factor can be interpreted as the result of a microbial adaptation period towards both the hydrocarbons and the plant-roots (Macleod & Semple, 2002; Mueller & Shann, 2007). Moreover, the enhanced mineralisation could have been further promoted by the introduction of readily available nutrients and carbon from the decomposing root biomass (Miya & Firestone, 2000; Leigh *et al.*, 2002) after longer root-soil incubation times.

As mentioned above, in addition to the enhanced capability of the microbial populations to mineralise ^{14}C-phenanthrene, these can also impact this process through the promotion of a

larger bioaccessible fraction of the hydrocarbon. Results from the study on how root biomass impacts on the bioaccessibility of ^{14}C-phenanthrene performed in Chapter 6 did not confirm this as the mechanism enabling the observed significantly higher biodegradation of the hydrocarbon. As data from the hydroxypropyl-β-cyclodextrin (HPCD) extractions showed, plant-root amendments did not affect the bioaccessibility of ^{14}C-phenanthrene regardless the significantly larger biodegradation presented after 21 and/or 42 days of root-soil incubation. It would be expected a direct positive association between mineralisation and bioaccessibility, therefore these results suggest that plant-root biomass could be promoting a larger mineralisation through a different mechanism. Given that enhanced biodegradation of hydrocarbons through the action of plant roots is normally attributed to root exudation (Khan *et al.*, 2013), results support this hypothesis by the enhancement of the biodegradation of ^{14}C-phenanthrene only after the release of root-derived substrates through root-decomposition processes. It is also possible that plant-root biomass could be physically limiting the bioaccessibility of what otherwise would be freely available ^{14}C-phrenanthrene, this through the promotion of sorption processes (Jones & Darrah, 1992; Pignatello, 1998; Miya & Firestone, 2000; Jiao *et al.*, 2007; Sun *et al.*, 2010).

Through the analysis of the combined results, it is possible to conclude that plant-root biomass can enhance the biodegradation of hydrocarbons through two main mechanisms. First, by buffering the initial toxic effects of the hydrocarbons by the promotion of new sorption surfaces and physically limit the freely available fraction of the contaminant. Secondly, the sorbed fraction of the hydrocarbon will become bioavailable through the root decay and turnover, which will not only release the previously sorbed HOC, but will also introduce root-derived substrates and provide readily available nutrients and carbon. This last phase becomes an important part of the process considering that one of the well-defined limitations of the microbial degradation of HOCs is the depletion of nutrients due to the initial highly active microbial activity (Bamforth & Singleton, 2005; Barnier *et al.*, 2014; Martin *et al.*, 2014).

As pointed before, results indicated that the presence of plant-root biomass might have acted as a sorbent and consequently shadowed the full potential of root-derived substrates towards the biodegradation of HOCs in soil. As a result, the impact of some root-derived substrates towards biodegradation, bioaccessibility and desorption of ^{14}C-phenanthrene was studied as detailed in Chapter 8. In this case, the presence of organic acids within the naturally

occurring concentrations were observed to have no significant impact towards bioaccessibility and biodegradation of ^{14}C-phenanthrene. In contrast, higher concentrations (above the naturally appearing range) were capable to promote the total desorbable fraction, as well as the bioaccessibility of the hydrocarbon, although biodegradation was still unaffected. These results contrast with the observed when plant-root biomass was amended into the soil (Chapters 5 and 6), where even though bioaccessibility was not enhanced, mineralisation of ^{14}C-phenanthrene was consistently promoted by the treatments. Even though organic acids have been proposed as the type of root-derived substrates responsible for the increased biodegradation of hydrocarbons within the rhizosphere (Agnello *et al.*, 2014; Martin *et al.*, 2014; Rohrbacher & St-Arnaud, 2016), this is not confirmed by the present results. Despite this, overall results indicate that high concentrations of organic acids might have the ability to produce a larger bioaccessibility and desorption, which has been hypothesised as one of the main mechanisms promoting the biodegradation of HOCs (Rhodes *et al.*, 2010b; Bernhardt *et al.*, 2013). More importantly, trends showed that only high concentrations of organic acids produced this effect, while small amounts of the substrates had the opposite effect and enhanced the sorption processes. This contrasting behaviour is attributed to different mechanisms, although all inherent to the modification of soil physical and chemical characteristics. Although with different direction and magnitude, the impact of the organic acids on the total desorbable fraction of ^{14}C-phenanthrene can been attributed to the modification of the soil mineral structure (Siciliano & Germida, 1998) and soil organic matter (Martin *et al.*, 2014). Additionally, these substrates also had different effects depending on the soil-PAH contact times and organic acid concentration. Although organic acids were observed to enhance the total desorbable fractions at higher concentrations, desorbed ^{14}C-phenanthrene was also observed to be extracted within slowly and very slowly desorbing fractions, often interpreted as non-biodegradable (Cornelissen *et al.*, 1997; Rhodes *et al.*, 2010b). The use of organic acids towards extractability of PAHs from soil has been previously reported as a promising aid for the biodegradation of hydrocarbons from soil (White *et al.*, 2003; An *et al.*, 2010; Gao *et al.*, 2010a; b; Agnello *et al.*, 2014). However, data from the present thesis (Chapter 8) represents the first investigation studying the impact of these substrates on the desorption kinetics of a PAH in soil. As mentioned above, despite promoting a larger desorbable fraction of ^{14}C-phenanthrene, a considerable fraction of the hydrocarbon was desorbed within the non-biodegradable phase. These results, combined with the absence of impact on the mineralisation of ^{14}C-phenanthrene, indicate that organic acids

alone do not represent the substrate by which biodegradation is promoted in the rhizosphere. This effect is likely to occur due to the important role that roots play within the exudation process, regulating the rate and concentration at which organic acids and other substrates are released into the soil (Sun *et al.*, 2010; Keiluweit *et al.*, 2015).

Overall results showed that plant-root biomass (Chapters 5 and 6) and root derived substrates (Chapter 8) have the ability to significantly impact the behaviour of ^{14}C-phenanthrene in soil. This included the microbial catabolic response (Chapter 5), biodegradation (Chapter 6), bioaccessibility (Chapter 6), and desorption kinetics (Chapter 8). Plant-root biomass was observed to significantly promote the biodegradation of ^{14}C-phenanthrene, while single substrates typically found in the rhizosphere (organic acids) did not. Data from this thesis showed that the biodegradable fraction of ^{14}C-phenanthrene can be enhanced by plant-root biomass, especially after longer root-soil incubation times, and therefore reducing recalcitrant/non-biodegradable fraction, confirming the proposed root-induced changes (Introduction – Figure 3). Root enhanced biodegradation of hydrocarbons is proposed to be a feasible approach for the treatment of contaminated soil. More importantly, it consistently showed to be an effective methodology to promote faster and larger mineralisation of ^{14}C-phenanthrene while keeping a constant bioaccessibility of the contaminant, often associated with the toxic and harmful fractions (Ortega-Calvo *et al.*, 2015).

Given these results, it is possible to suggest that the reduction of the concentration of hydrocarbons normally reported when a phytoremediation approach is used are mainly the result of the additional sorption processes promoted by the roots and not due to the biodegradation of the contaminant. Furthermore, the enhanced biodegradation of hydrocarbons through the action plant-root biomass is more likely to be observed with longer plant-soil-contaminant contact times. This promoted by the decomposition of the plant-root biomass which will release into the soil not only the root-derived substrates but the sorbed hydrocarbon as well. Considering this, in can be concluded that the application of plant-root biomass into the soil represents a viable and low-cost approach for (i) the control of the bioaccessibility of the contaminant when the contamination event is still recent, and (ii) the enhancement of the biodegradation processes when the non-biodegradable fraction has been considerably reduced due to ageing.

Extrapolating these results into the phytoremediation of hydrocarbons from contaminated soil, it is possible to enhance the biodegradation of hydrocarbons and other HOCs through the action of plants. Nonetheless, it is important to mention that in contrast with a regular phytoremediation approach, the use of plant-root biomass eliminates any competition for resources between the plants and soil microbial communities, which has been previously identified as a limitation for phytoremediation protocols (Salt *et al.*, 1998; Pilon-Smits, 2005). Consequently, it is recommended further research regarding the impact of not only plant-root biomass but also in combination with the whole plant system towards the biodegradation of hydrocarbons from soil. It is hypothesised that by following this protocol it would be possible to overcome some of the widely known limitations of phytoremediation. This includes the control of phytotoxic effects by the initial sorption processes promoted by plant-root biomass, and the incorporation of additional sources of root-derived substrates at the beginning of the phytoremediation protocol, reducing its depletion due to plant-microbe competition.

9.2 References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D. & Esposito, G. 2014. Enhanced phytoremediation: A review of low molecular weight organic acids and surfactants used as amendments. Critical Reviews in Environmental Science and Technology, 44, 2531–2576.

An, C., Huang, G., Yu, H., Wei, J., Chen, W. & Li, G. 2010. Effect of short-chain organic acids and pH on the behaviors of pyrene in soil-water system. Chemosphere, 81, 1423–1429.

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science & Technology, 27, 2630–2636.

Bamforth, S.M. & Singleton, I. 2005. Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. Journal of Chemical Technology and Biotechnology, 80, 723–736.

Barnier, C., Ouvrard, S., Robin, C. & Morel, J.L. 2014. Desorption kinetics of PAHs from aged industrial soils for availability assessment. Science of the Total Environment, 470–471, 639–645.

Bernhardt, C., Derz, K., Kördel, W. & Terytze, K. 2013. Applicability of non-exhaustive extraction procedures with Tenax and HPCD. Journal of Hazardous Materials, 261, 711–717.

Cornelissen, G., vanNoort, P.C.M. & Govers, H. a J. 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with Tenax(R) and effects of contact time and solute hydrophobicity. Environmental Toxicology And Chemistry, 16, 1351–1357.

Gao, Y., Ren, L., Ling, W., Gong, S., Sun, B. & Zhang, Y. 2010a. Desorption of phenanthrene and pyrene in soils by root exudates. Bioresource Technology, 101, 1159–1165.

Gao, Y., Ren, L., Ling, W., Kang, F., Zhu, X. & Sun, B. 2010b. Effects of low-molecular-weight organic acids on sorption–desorption of phenanthrene in soils. Soil Science Society of America Journal, 74, 51.

Jiao, X.C., Xu, F.L., Dawson, R., Chen, S.H. & Tao, S. 2007. Adsorption and absorption of polycyclic aromatic hydrocarbons to rice roots. Environmental Pollution, 148, 230–235.

Jones, D.L. & Darrah, P.R. 1992. Re-sorption of organic components by roots of Zea mays L. and its consequences in the rhizosphere - I. Re-sorption of 14C labelled glucose, mannose and citric acid. Plant and Soil, 143, 259–266.

Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K. & Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change, 5, 588–595.

Khan, S., Afzal, M., Iqbal, S. & Khan, Q.M. 2013. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere, 90, 1317–1332.

Leigh, M.B., Fletcher, J.S., Fu, X. & Schmitz, F.J. 2002. Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environmental Science & Technology, 36, 1579–1583.

Macleod, C.J.A. & Semple, K.T. 2002. The adaptation of two similar soils to pyrene catabolism. Environmental Pollution, 119, 357–364.

Martin, B.C., George, S.J., Price, C.A., Ryan, M.H. & Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. Science of the Total Environment, 472, 642–653.

Miya, R.K. & Firestone, M.K. 2000. Phenanthrene-degrader community dynamics in rhizosphere soil from a common annual grass. Journal of Environment Quality, 29, 584.

Mueller, K.E. & Shann, J.R. 2007. Effects of tree root-derived substrates and inorganic nutrients on pyrene mineralization in rhizosphere and bulk soil. Journal of Environment Quality, 36, 120.

Ortega-Calvo, J.J., Harmsen, J., Parsons, J.R., Semple, K.T., Aitken, M.D., Ajao, C., Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R., Peijnenburg, W.J.G.M., Römbke, J., Streck, G. & Versonnen, B. 2015. From bioavailability science to regulation of organic chemicals. Environmental Science & Technology, 49, 10255–10264.

Pignatello, J.J. 1998. Soil organic matter as a nanoporous sorbent of organic pollutants. Advances in Colloid and Interface Science, 76–77, 445–467.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Rohrbacher, F. & St-Arnaud, M. 2016. Root exudation: The ecological driver of hydrocarbon rhizoremediation. Agronomy, 6, 19.

Salt, D.E., Smith, R.D. & Raskin, I. 1998. Phytoremediation. Annual Review of Plant Biology, 49, 643–668.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science & Technology, 38, 228A–231A.

Siciliano, S.D. & Germida, J.J. 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environmental Reviews, 6, 65–79.

Sun, T.R., Cang, L., Wang, Q.Y., Zhou, D.M., Cheng, J.M. & Xu, H. 2010. Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. Journal of Hazardous Materials, 176, 919–925.

Thijs, S., Sillen, W., Weyens, N. & Vangronsveld, J. 2017. Phytoremediation: State-of-the-art and a key role for the plant microbiome in future trends and research prospects. International

Journal of Phytoremediation, 19, 23–38.

White, J.C., Mattina, M.I., Lee, W.Y., Eitzer, B.D. & Iannucci-Berger, W. 2003. Role of organic acids in enhancing the desorption and uptake of weathered p ,p -DDE by Cucurbita pepo. Environmental Pollution, 124, 71–80.

Appendix

Measurement of hydrocarbon bioavailability in soil

Measurement of hydrocarbon bioavailability in soil

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1. Abstract

Bioavailability is recognised as being important in the study of biodegradation and ecotoxicity of organic contaminants in soils and sediments. The bioavailability of organic contaminants is controlled by biological, chemical and physical interactions and, as a result, will differ between soil types and biota. Over the last 30 years, numerous investigations have been carried out to quantify chemical bioavailability in soil. Much of this research has focussed on using microbial degradation as a measure of bioavailability as well as chemical assessments, with numerous methods published. Chemical methods described in this chapter rely on the extraction of hydrocarbons using aqueous solutions of cyclodextrin, which have been shown to provide a robust and reproducible measurement of the amount of hydrocarbon that is biodegradable when compared with biological approaches also described in this chapter. Clearly, a simple aqueous extraction that predicts the microbial degradable fraction of hydrocarbons will prove to be useful in the assessment of contaminated land by offering a predictive measure of amount of a contaminant(s) that may be removed before embarking on full-scale bioremediation.

Keywords: Bioavailability, mineralisation, HPCD, bioremediation, hydrocarbons, PAHs

2. Introduction

Currently, the risk assessment and management of contaminated soil is based upon total extractable concentrations, with only negligible consideration of the bioavailability of target contaminants. However, in the recent SETAC Europe 10th Special Science Symposium in October 2014, entitled ‘Bioavailability of organic chemicals: Linking science to risk assessment and regulation’, there was much discussion within the academic and end-user communities to consider how regulatory bodies might consider including bioavailability within risk assessment and management frameworks. Two components were discussed which

are of relevance here: (i) what is meant by bioavailability and (ii) methods for measuring bioavailability. There are many definitions of bioavailability, which have been reviewed by Semple *et al.* (2007) and Riding *et al.* (2013). In a recent report by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the definition proposed by Semple *et al.* (2004, 2007)was adopted (European Centre for Ecotoxicology and Toxicology of Chemicals, 2013), namely bioavailability and bioaccessibility. The *bioavailable compound* was defined as "that which is freely available to cross an organism's cellular membrane from the medium – at a given time". While the *bioaccessible compound* was defined as "that which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical. However, the chemical may be either physically removed from the organism or only bioavailable after a period of time" (Semple *et al.*, 2004). In a practical context, this means that bioaccessibility defines not only what is bioavailable, but also what is potentially bioavailable. In terms of measurement, bioaccessibility is more relevant than bioavailability in that chemical extractions will measure the latter due to the physico-chemical interactions between the extracting solution and the soil. However, for the purposes of this chapter, *bioavailability* will be used as the collective term of both concepts.

The bioavailability of hydrocarbons is affected by a wide range of factors including the physico-chemical characteristics of the contaminants, such as aqueous solubility and partitioning coefficients (K_{ow}, K_d and K_{oc}) (Hatzinger & Alexander, 1995); concentration and form of soil organic matter (Alexander, 2000; Reid *et al.*, 2000a); physical interactions (e.g. sorption/desorption processes) (Semple *et al.*, 2003); environmental factors such as temperature and precipitation (Reid *et al.*, 2000b), and hydrocarbon-soil contact time (Hatzinger & Alexander, 1995; Alexander, 2000; Reid *et al.*, 2000a). For the purposes of this chapter, a variety of microbiological *(A)*, and chemical *(B)* approaches are described, as these two approaches have been used extensively in the assessment of the bioavailability of hydrocarbons in soil and sediments (Reid *et al.*, 2000a).

A. Microbiological Methods: Biological approaches rely on the limitations of the biodegradation process, e.g. numbers, populations and activity of the degrading microorganisms present in the soil, physico-chemical properties of the contaminant, and soil and sediment properties.

1. Mineralisation of ^{14}C-hydrocarbons to $^{14}CO_2$.

In this method, a ^{14}C-hydrocarbon at a known amount of ^{14}C-activity (Bq g^{-1}) and a known concentration of its ^{12}C-homologue (mg kg^{-1}) is amended into soil and the extent to which it is catabolized to $^{14}CO_2$ is measured. This can be as a result of indigenous soil microflora (Rhodes *et al.*, 2010a; Oyelami *et al.*, 2013; Obuekwe & Semple, 2013b) or following the addition of a microbial inoculum (Rhodes *et al.*, 2008a; Towell *et al.*, 2011a), with the amount of $^{14}CO_2$ used as a measure of bioavailability for a wide range of aromatic (Semple *et al.*, 2006) and aliphatic (Stroud *et al.*, 2007b) hydrocarbons in soil.

2. Biodegradation assay.

   The measurement of the biodegradation of hydrocarbons can be used to approximate bioavailability, and relies on the measurement of ^{12}C-contaminant concentration using traditional analytical techniques. This approach has been reported in several studies investigating the bioavailability of chemicals added to soil as well as field-contaminated soil (Doick *et al.*, 2005b; Stokes *et al.*, 2005). Following solvent extraction and analysis by gas chromatography or high performance liquid chromatography of the target compound (initial concentration), the soil is incubated over time (e.g. 6 weeks) in soil: solution ratio of 1:3. At the end of the incubation, the soil is again extracted using a solvent and the contaminants measured. The difference between the initial and final concentration of the target hydrocarbons is considered the fraction that has been degraded by the soil microflora and hence the bioavailable fraction.

B. Chemical Methods: Chemical extractions involving organic solvents have been typically used for the assessment of total concentrations of contaminants in the environment. It is well established that the extraction of contaminants using organic solvents overestimates the total amount of hydrocarbons available to soil biota. In recent years, less aggressive extractions have been used for the prediction of bioavailability of selected organic contaminants in soil and sediment. The major limitation of these newer approaches is that they have been mostly used for the study of polycyclic aromatic hydrocarbons, and to a lesser extent, aliphatic hydrocarbons, so its application to other contaminants has not been fully understood yet (Semple *et al.*, 2004).

1. Organic solvent extractions.

This is the traditional chemical approach to measure hydrocarbon concentrations in soil; a typical example of this is the exhaustive Soxhlet extraction (Semple *et al.*, 2003). However, these methods have been proved to have little correlation to the actual bioavailable fraction of organic contaminants (Hatzinger & Alexander, 1995; Reid *et al.*, 2000a; Semple *et al.*, 2003), tending to overestimate the amount of bioavailable chemical in soil (Alexander, 2000). Less aggressive extractions using more polar solvents seem to be more representative in the assessment of bioavailability, but the extraction concentrations tend to be variable (Cui *et al.*, 2013). These solvent extraction methods are considered to be faster than other approaches, such as passive samplers (Gomez-Eyles *et al.*, 2012).

2. Solid phase micro-extraction (SPME).

   This is a passive sampling technique which uses polydimethylsiloxane coated solid phase micro-extraction (SPME) fibres. This method has been extensively used and modified over the years (Harwood *et al.*, 2012). Overall, results from this method have been found to be useful to predict bioavailability under certain conditions, being especially useful for sediments.

3. Polyoxymethylene-solid phase extraction (POM-SPE).

   This is a passive sampling technique frequently used for the assessment of bioavailable hydrophobic organic contaminants in soil and sediment (Stokes *et al.*, 2005). This method relies on a solid phase extraction (SPE) of the target hydrocarbon onto plastic polyoxymethylene (POM).

4. Tenax extraction.

   This is a desorption extraction, which measures the rapidly desorbable and aqueously soluble concentrations of organic contaminants in soil and sediments. Tenax beads are used to extract hydrocarbons from soil and sediments. This procedure is usually done by mixing Tenax beads with sediment or soil followed by a recovery step in which the contaminants are extracted from the beads using an organic solvent (Cui *et al.*, 2013). Results from Tenax extractions have shown to successfully predict the biodegradable fraction of hydrocarbons in soil and sediments (Cornelissen *et al.*, 2001).

5. Hydroxypropyl-β-cyclodextrin (HPCD) extraction.

   Like the Tenax method, the HPCD technique is a desorption extraction. In this case, cyclodextrin molecules form water-soluble inclusion complexes with organic contaminants thanks to the molecular structure and the presence of a hydrophobic

cavity. This aqueous extraction has been shown to predict the microbial availability of PAHs (Reid *et al.*, 1998; Rhodes *et al.*, 2008b) and aliphatic hydrocarbons (Stroud *et al.*, 2007b, 2008) in soil and sediments.

Organic solvents, Tenax and HPCD can also be used to characterise the desorption kinetics of hydrocarbons in soil. Typically, these hydrophobic contaminants display either rapid and slow desorbing behaviour (two compartment) or rapid, slow and very slow desorbing behaviour (three compartment) (Riding *et al.*, 2013). Several studies have shown the rapidly desorbing fraction, as measured by Tenax and HPCD, describe the microbially degradable fraction (Cornelissen *et al.*, 1998b; Cuypers *et al.*, 2002; Rhodes *et al.*, 2010b).

This chapter describes the HPCD extractions (Figure 1), and mineralisation and biodegradation assays (Figures 2 and 3, respectively) for the chemical and microbiological methods for the determination of the bioaccessibility of hydrocarbons in soil as these three approaches have proven to provide robust, accurate and reproducible predictions of bioaccessibility of hydrocarbons in soil. The methods are described below.

3. Materials

3.1 HPCD extraction

1. Soil sample (~1.25g wet weight)

2. HPCD solution: 12 g hydroxypropyl-β -cyclodextrin, 97% (Acros Organics – Thermo Fisher Scientific) dissolved in 150 ml deionised water

3. Teflon centrifuge tubes (30 ml)

4. Liquid scintillation cocktail (only if ^{14}C- approach is used)

5. Liquid scintillation counter (only if ^{14}C- approach is used)

6. Glass scintillation vials (20 ml)

7. HPLC grade dichloromethane (only if ^{12}C- approach is used)

3.2 Mineralisation

1. Soil sample (10 g wet weight)

2. Modified Teflon lined screw cap-Schott bottle (250 ml; "respirometer") [Note 1]

3. Minimal basal salts (MBS; 30 ml) solution (g l^{-1}): to 1 l deionised water add 0.3 g NaCl, 0.6 g $(NH_4)_2SO_4$, 0.6 g KNO_3, 0.25 g KH_2PO_4, 0.75 g K_2HPO_4, 0.15 g $MgSO_4 \cdot 7H_2O$, additionally 1 ml l^{-1} trace element solution should be added. [Note 2]

4. Trace element solution: to 250 ml deionised water add 5 µg $LiCl(LiBO_2)$, 20 µg $CaSO_4 \cdot 5H_2O$, 25 µg $ZnSO_4 \cdot 7H_2O$, 25 µg $Al_2(SO_4)_3 \cdot 16H_2O$, 25 µg $NiCl \cdot 6H_2O$, 25 µg $CoSO_4 \cdot 7H_2O(CoNO_3)$, 7.5 µg KBr, 7.5 µg KI, 150 µg $MnCl_2 \cdot 4H_2O$, 10 µg $SnCl_2 \cdot 2H_2O$, 75 µg $FeSO_4 \cdot 7H_2O$

5. ^{14}C-Labelled analogue (also known as stable isotopically labelled analogue) to give ~83 Becquerels (Bq) g^{-1} activity (American Radiolabelled Chemicals, Inc., USA)

6. Liquid scintillation cocktail (Goldstar liquid scintillation cocktail – Meridian Biotechnologies Ltd, UK)

7. Liquid scintillation counter

8. Glass scintillation vials (7 ml)

8. NaOH solution (1 M)

3.3 Biodegradation

1. Soil sample (5 g wet weight)

2. Conical flask (150 ml)

3. Minimal basal salts (MBS; 15 ml) solution (g l^{-1}): to 1 l deionised water add 0.3 g NaCl, 0.6 g $(NH_4)_2SO_4$, 0.6 g KNO_3, 0.25 g KH_2PO_4, 0.75 g K_2HPO_4, 0.15 g $MgSO_4 \cdot 7H_2O$, additionally 1 ml l^{-1} trace element solution should be added. [Note 2]

4. Trace element solution: to 250 ml deionised water add 5 µg $LiCl(LiBO_2)$, 20 µg $CaSO_4 \cdot 5H_2O$, 25 µg $ZnSO_4 \cdot 7H_2O$, 25 µg $Al_2(SO_4)_3 \cdot 16H_2O$, 25 µg $NiCl \cdot H_2O$, 25 µg

$CoSO_4 \cdot 7H_2O(CoNO_3)$, 7.5 µg KBr, 7.5 µg KI, 150 µg $MnCl_2 \cdot 4H_2O$, 10 µg $SnCl_2 \cdot 2H_2O$, 75 µg $FeSO_4 \cdot 7H_2O$

4. Methodology

4.1 Soil preparation and spiking

1. Soil is normally sieved through a 2 mm mesh without air-drying or partially air-dried, and then rehydrated to the required moisture content (up to water holding capacity) using deionized water.

2. Depending on the objectives of the experiment, the contaminant can be introduced into pristine soil (for further information regarding the approach for spiking of hydrocarbons into the soil please refer to Note 3) or collected from a contaminated site. Soil (e.g. 250 g if 1 kg is to be spiked) is placed in a glass bowl and the standard is added to the soil and mixed constantly with the use of a stainless-steel spoon for about 3 minutes, after which, the remaining part of the soil is gradually added until all the soil has been incorporated.

3. Once the soil has been spiked, a venting period is needed to allow remaining solvent to volatilise. This is dependent on the solvent used; for example, 30 - 60 minutes with periodic mixing is recommended for acetone. The type of solvent will depend on the solubility of the hydrocarbon(s) used for the preparation of the standard; acetone and ethanol are the most common ones. Soil sub-samples can be used to confirm hydrocarbon concentration or ^{14}C activity through standard chromatographic methods or sample oxidation respectively (Doick *et al.*, 2003).

4. After venting, soil moisture may need to be readjusted to the original moisture content using deionized water.

4.2 HPCD extraction

4.2.1 Setting up

1. Soil (~1.25 g wet weight) is placed in triplicate in a 35 ml capacity Teflon centrifuge tube [Note 4] and 25 ml of 50 mM HPCD solution is added. This soil:solution ratio has been assessed as the optimum for this procedure (Reid *et al.*, 2000b).

2. Tubes are placed horizontally onto a rotatory or a flatbed shaker (100 rpm) for 22 h at 20 ± 2 ºC.

3. After this, the tubes are centrifuged at 3000 *x g* rpm for 1 h.

4. Supernatant and soil are retained for analysis.

4.2.2 Analysis [Note 5]

1. *If ^{12}C-hydrocarbons are being measured*: supernatant is analysed for bioavailable hydrocarbons. This analysis can be carried out using different types of chromatographic instrumentation (e.g. HPLC, GC-MS (Stokes *et al.*, 2005)).

2. *If ^{14}C-hydrocarbons are being measured:* supernatant (5 ml) is placed into 20 ml glass liquid scintillation vials and mixed with 15 ml liquid scintillation cocktail. The vials are kept in the dark for 12 h and then ^{14}C- activity is measured using a liquid scintillation counter (LSC).

4.2.3 Desorption kinetics

The study of the desorption kinetics can be especially helpful when the aims of the experiment include the quantification of the influence of contact time on the partitioning and bioaccessibility of the contaminant (Semple *et al.*, 2003), allowing the quantification of rapidly and slowly desorbing fractions of a contaminant in soil and sediments. Tenax (Cornelissen *et al.*, 2001) or HPCD (Rhodes *et al.*, 2010b) extractions can be carried out after 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 26, 32, 56, 80 and 104 h shaking (Cornelissen *et al.*, 2001; Rhodes *et al.*, 2010b) to assess the desorption kinetics or organic compounds. The analysis of desorption data is achieved by using a *(1)* first-order two-compartment *(2)* model as follows (Rhodes *et al.*, 2010b).

(1) $S_t/S = F_{\text{rap}} \cdot \exp(-k_{\text{rap}} \cdot t) + F_{\text{slow}} \cdot \exp(-k_{\text{slow}} \cdot t)$

(2) $S_t/S = F_{\text{rap}} \cdot \exp(-k_{\text{rap}} \cdot t) + F_{\text{slow}} \cdot \exp(-k_{\text{slow}} \cdot t) + F_{\text{very slow}} \cdot \exp(-k_{\text{very slow}} \cdot t)$

where S_t is the amount of the compound sorbed into the soil at desorption time t (h), S is the initial amount of the compound before desorption. F_{rap}, F_{slow}, $F_{\text{very slow}}$ (%) are the rapidly,

slowly and very slowly desorbing fractions, respectively. k_{rap}, k_{slow}, $k_{\mathrm{very\,slow}}$ (h^{-1}) represent rate constants of rapid, slow and very slow desorption. It is important to understand that the model assumes that k_{slow} is less than k_{rap}. The values can be determined by the use of exponential curve fitting using a non-linear least squares method (Rhodes *et al.*, 2010b).

4.3 Mineralisation of ^{14}C-contaminants

The mineralisation assay is commonly maintained over 14 days, as this is normally the maximum amount of time required to reach the plateau phase. Under normal circumstances, this period of time will be long enough to observe the full mineralisation potential of the microcosms contained in the "respirometer".

4.3.1 Setting up and sampling of evolved $^{14}CO_2$

1. Pre-cleaned [Note 4] respirometers are set up (in triplicate); each one containing 10 ± 0.2 g soil (wet weight).

2. A soil:solution ratio of 1:3 is recommended in order to produce a rapid and reproducible determination of the biodegradable fraction of ^{14}C-hydrocarbons. Therefore, 30 ml MBS solution is added into each respirometer [Note 6]. Additionally, a set of 3 respirometers containing clean/control soil needs to be included in order to assess the background $^{14}CO_2$ production (the data from these needs to be subtracted from the treatments for blank correction).

3. The $^{14}CO_2$ trap consists of a 7 ml vial containing 1 ml 1N NaOH solution is placed into each respirometer.

4. Respirometers are then incubated (20 ± 2 ºC) onto an orbital shaker (100 rpm) for 14 days.

5. Every 24 h, the $^{14}CO_2$ trap is replaced with fresh NaOH solution. The replacement of the $^{14}CO_2$ traps needs to be done as fast as possible in order to minimize losses of $^{14}CO_2$ during sampling of the respirometers.

6. After the replacement of the $^{14}CO_2$ trap, 5 ml liquid scintillation cocktail is dispensed into each $^{14}CO_2$ trap and activity is assessed with the use of a LSC after 12 h stabilization period in the dark.

Results from LSC are typically expressed in disintegrations per minute (DPM). Knowing the total ^{14}C- activity spiked into the soil [Note 6], a direct cumulative curve of the percent of evolved $^{14}CO_2$ can be obtained.

4.4 Biodegradation

4.4.1 Setting up

1. Sterile microcosms (autoclaved conical flasks) are set up (in triplicate); each one containing 5 ± 0.1 g soil (wet weight). When spiked soil is used, a blank is recommended for the quantification of background contaminants.

2. A soil:solution ratio of 1:3 is recommended. Therefore 15 ml MS solution is added into each conical flask [Note 7]. Additionally, a set of flasks just with MBS is used as analytical blank.

3. The conical flasks are sealed with a non-absorbent cotton wool bung covered with aluminium foil.

4. Microcosms are then incubated (20 ± 2 ºC) onto an orbital shaker (100 rpm) for a defined time (e.g. 6 weeks). Other incubation temperatures may be used according to the aims of the experiment, e.g. when comparing the biodegradation process among different climates.

5. Additionally, a sample of the soil must be kept for subsequent extraction. The hydrocarbon concentration of this sample will be used as the initial concentration.

4.4.2 Preparation of the sample for analytical analysis

1. After the incubation period, the content of each flask is qualitatively transferred into a centrifuge tube and centrifuged at 3000 *x g* for 30 min.

2. The supernatant is discarded and the pellet is mixed (by hand) with 25 ml dH_2O and centrifuged again under the same conditions.

3. A fraction of the pellet is then analysed to know the final hydrocarbon concentration following standard analytical methods (e.g. HPLC, GC-MS (Stokes *et al.*, 2005)). There is a wide range of sample cleaning and extraction methodologies that can be used based on instrumental availability and/or personal preferences.

5. Data analysis

Results from the mineralisation assay can be presented as the cumulative evolution of $^{14}CO_2$ across time. Also, there are three parameters that are recommended to use for statistical analysis: (1) lag phase (time taken for mineralisation to reach 5% of the totality of the contaminant), (2) fastest rate of mineralisation, and (3) total extent of mineralisation.

In the case of biodegradation assays, results from this assessment can simply be presented as the change of concentration of the targeted hydrocarbon (difference between the initial and final concentration/activity). Additionally, if the evolution of the biodegradation process is considered of interest, in might be useful to include additional replications of the different soil conditions/treatments and sequentially sacrifice a set of flasks in order to monitor these differences across time (Figure 4). Following blank correction, statistical significance of treatments are analysed using general linear models (ANOVA) and a post hoc test (e.g. Tukey).

For the extractions using HPCD, these results can be considered as a direct measure of the bioaccessible fraction of the targeted hydrocarbon. To achieve this, it is recommended to test the chemical extraction against the biological endpoint to establish the relationship to the target contaminant(s). After blank correction, comparisons between HPCD extractability and a biological assay or other extraction methods are performed using Student *t*-tests and linear regression modelling.

For comparisons between a chemical and a biological approach, the use of a regression model allows to define the reliability of the linear relationship between the *x* and *y* values (r^2). As shown in Figure 5, parameters from the modelling present a close 1:1 correlation between the ^{14}C-phenanthrene extracted using HPCD solution and the amount of ^{14}C-phenanthrene mineralised after 10 days, showing the feasibility of using the chemical extraction as a predictor of the biodegradable fraction of phenanthrene.

For the study of the desorption kinetics, the goodness of fit to a certain model is recommended. Figure 6 shows the fitting of data of ^{14}C-phenanthrene extracted with HPCD solutions using a two-compartment model across time (after 0, 25, 50 and 100 d ageing) in four different soils. By doing this, it is possible to observe how the different desorbing fractions change because of the ageing processes.

In addition of the HPCD extractions, desorption kinetics can also be assessed through different chemical approaches, allowing to define which one can reproduce in a more reliable way the fraction of the contaminant that is available for biodegradation. Figure 7 shows the use of SPE, Triton X-100 and HPCD extractions for the estimation of the biodegradable fraction of PAHs of genuine field-contaminated sediment compared to the results from a 21 d biodegradation assay. It can be observed that SPE and HPCD extractions presented similar results when compared to the residual concentration of PAHs after the biodegradation period, especially for the extraction of 3 and 4-ring PAHs.

6. Notes

Note 1: An important factor of the respirometer configuration is the lid, which holds the $^{14}CO_2$ trap. To ensure a proper seal (minimizing $^{14}CO_2$ losses) a secured washer and nut must be included. Additionally, it has been observed that over time these components tend to get loose, therefore proper periodical maintenance is recommended.

Note 2: For all chemicals purity must be ≥ 98% unless stated otherwise.

Note 3: Specifically for standards including ^{14}C- analogues; there are two approaches for their introduction of the soil. When *(1)* ageing processes are not part of the objectives, the ^{14}C-standard is introduced directly into the respirometer with an expected activity of ~500 – 830 Bq per respirometer using a minimum amount of solvent (usually 5 µl) and a marginal concentration of the respective ^{12}C- (~10 mg kg^{-1}). On the other hand, if ageing processes are considered as a part of the assessment, *(2)* the ^{14}C-analogue spiking is carried out with the use of a standard containing both ^{12}C- hydrocarbon and ^{14}C- analogue as detailed in 3.1. If ^{14}C-analogues are not going to be used, contaminant standards are prepared by dissolving the desired amount of hydrocarbon using a minimum amount of solvent (typically acetone). For both cases, the moisture content of the soil has to be determined by drying (in triplicate) ~2 g soil at 105ºC for 24 h in porcelain crucibles. This data is necessary in order to assess the amount of contaminant to be added into the standard in order to deliver the desired amount of contaminant to the soil.

Note 4: Special attention should be given to the cleaning procedure of all glassware and centrifuge tubes. First, material needs to be thoroughly cleaned with soapy warm water and soaked in a 10 % Decon 90 solution overnight (1 h for Teflon centrifuge tubes). After this,

the material needs to be rinsed again and soaked in a deionized water bath for 1 h. Finally, once dry the material needs to be soaked in an acetone bath for a few seconds and allow venting before use.

Note 5: If a mass balance is considered of interest after HPCD extractions then the soil pellet must be retained for further analysis. *If only ^{12}C- has been used*; soil samples need to be extracted, cleaned and prepared for gas chromatograph analysis (GC-MS) following standard analytical methodologies (Stokes *et al.*, 2005). *If ^{14}C- analogue has been used,* the remaining activity in the soil is assessed through sample oxidation.

Note 6: Effectiveness of specific bacterial populations or communities can also be assessed through the mineralisation method. In this case, and to keep the 1:3 soil:solution ratio; 5 ml of the sub-cultured microbial inoculum suspended of MBS (each ml of inoculum should contain approximately 10^{7-8} cells) are added to each respirometer and the amount of MBS is reduced from 30 down to 25 ml.

Note 7: As in the case of the mineralisation assay, effectiveness of specific bacterial populations or communities can be assessed through the addition of active bacterial inoculum as long as the 1:3 soil:solution ratio is used . In this case, 12.5 ml MBS solution and 2.5 ml of inoculum is used as solution instead of 15 ml MBS solution.

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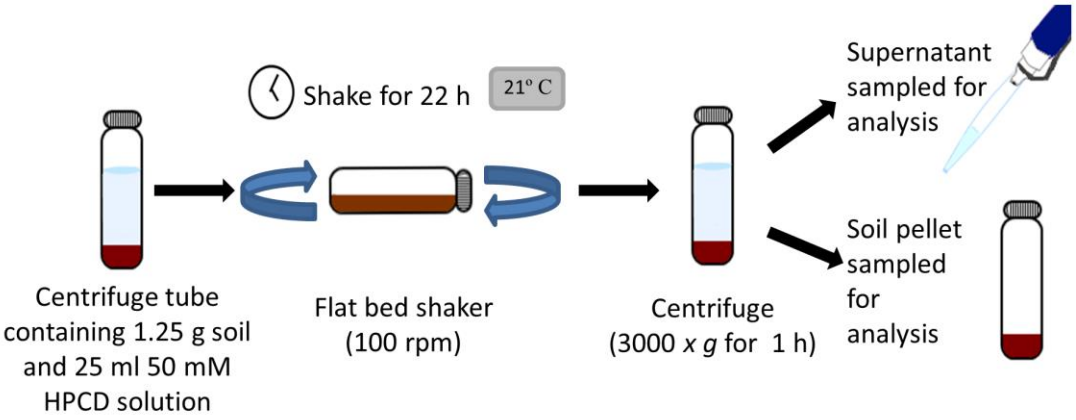

Figure 1. Diagram of HPCD shake extraction method. Overall, soil is mixed with cyclodextrin solution and shaken over 22 h. At the end, soil pellet is separated from the solution through centrifugation and analysed.

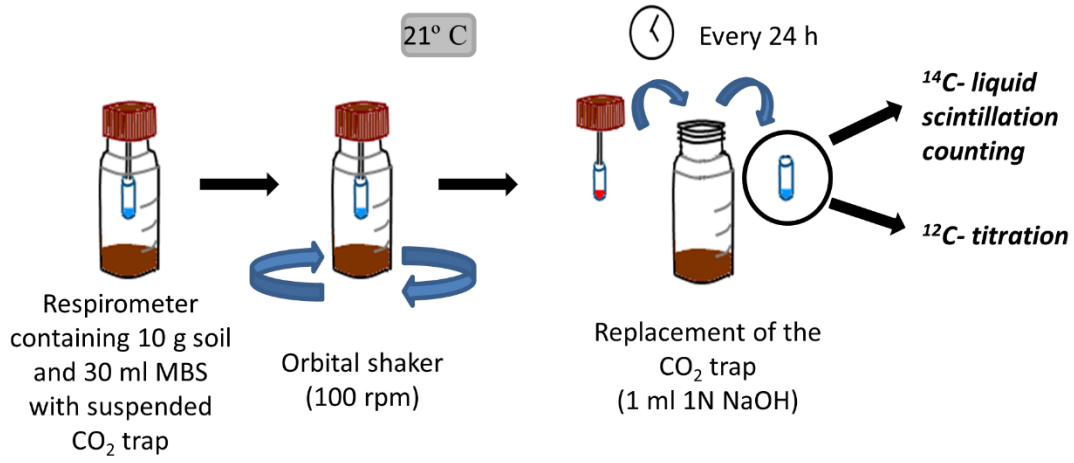

Figure 2. Diagram of mineralisation assay. Overall, soil slurry is placed into a "respirometer" with an integrated CO_2 trap [Note 1] and placed onto an orbital shaker. The CO_2 trap is typically replaced and analysed every 24 hours.

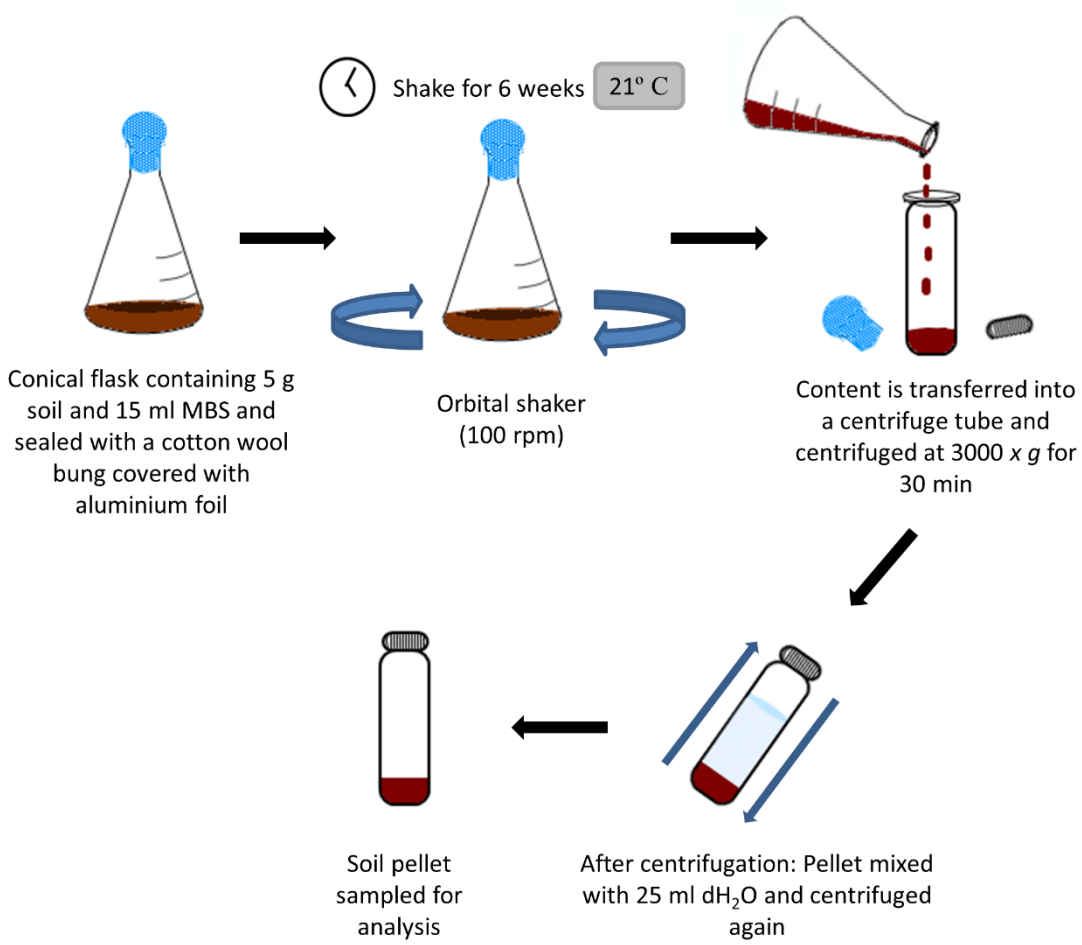

Figure 3. Diagram of biodegradation assay. Overall, soil slurry is placed into a conical flask and kept on an orbital shaker. Soil pellet and liquid are separated and analysed after certain amount of time (commonly 6 weeks). Initial and final concentrations of the analyte are used to estimate the fraction of the hydrocarbon that has been degraded.

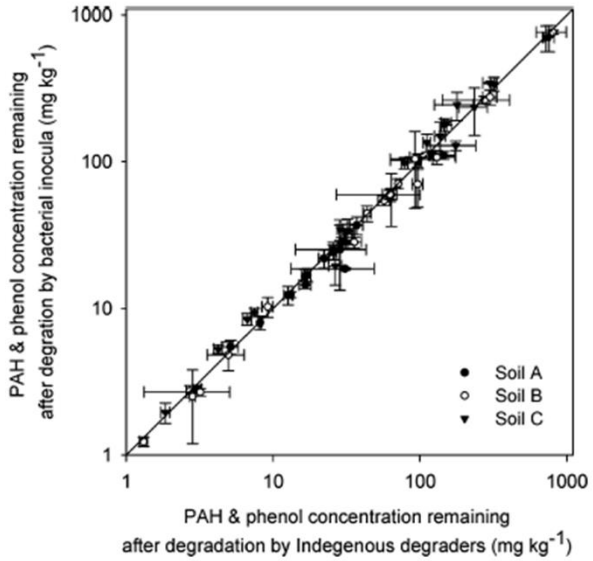

Figure 4. Example of correlation between concentration of PAH and phenol remaining in soil after a biodegradation assay using indigenous degraders or active bacterial inoculum (mixed culture of *p*-cresol, phenanthrene, pyrene and benzo[a]pyrene degraders) in three different types of soil. The 1:1 relationship is represented by the solid line. Reproduced from Allan *et al.* (2007) with permission from © 2007 American Chemical Association.

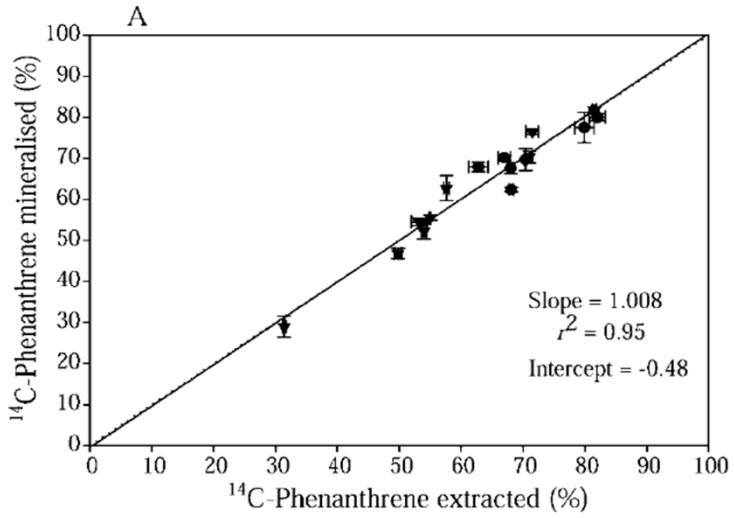

Figure 5. Example of linear correlations between ^{14}C-phenanthrene extracted with HPCD and ^{14}C-phenanthrene mineralised, in this case using multiple inoculations with *Pseudomona* sp., the dotted line represents the 1:1 fit while the solid is the result from the linear regression modelling. Reproduced from Rhodes *et al.* (2008b) with permission from © 2008 SETAC.

Soil type	F_{rap}	F_{slow}
A		
0	78.02 ± 2.55	21.03 ± 1.86
25	72.11 ± 2.97	26.97 ± 1.66
50	76.73 ± 2.40	22.28 ± 1.38
100	68.20 ± 2.90	30.06 ± 2.03
B		
0	83.99 ± 1.75	15.83 ± 1.25
25	82.67 ± 2.12	16.93 ± 0.96
50	79.96 ± 2.41	19.38 ± 1.16
100	79.16 ± 2.27	19.93 ± 1.12
C		
0	82.61 ± 1.99	16.75 ± 1.89
25	78.54 ± 1.74	20.33 ± 1.15
50	73.94 ± 2.44	24.18 ± 1.54
100	73.81 ± 2.44	23.45 ± 1.61
D		
0	79.18 ± 1.49	20.21 ± 1.23
25	71.79 ± 1.97	27.40 ± 1.23
50	69.45 ± 2.66	28.65 ± 1.63
100	36.51 ± 0.38	63.33 ± 0.26

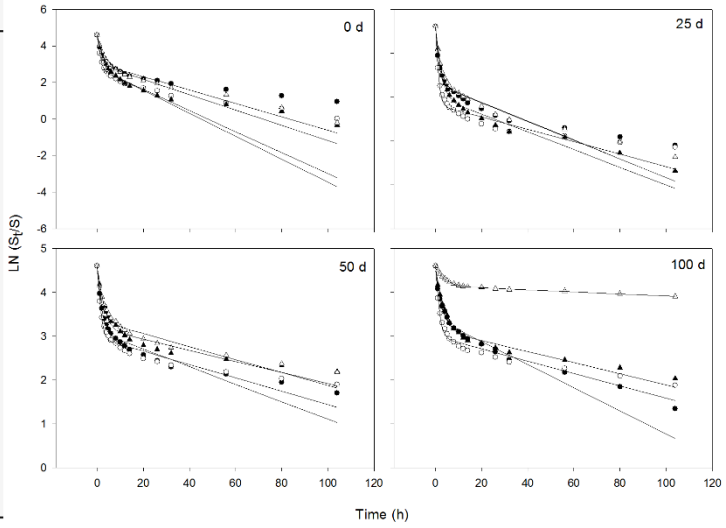

Figure 6. Example of the use and comparison of HPCD extractions and mineralisation of hydrocarbons for the study of desorption kinetics. The figure presents the desorption curves and values of phenanthrene from four different soils [A (●), B (○), C (▲) and D (Δ)] after 0, 25, 50 and 100 d ageing with a two-compartment exponential curve fitting. Reproduced (adapted) from Rhodes *et al.* (2010b) with permission from © 2010 Elsevier Ltd.

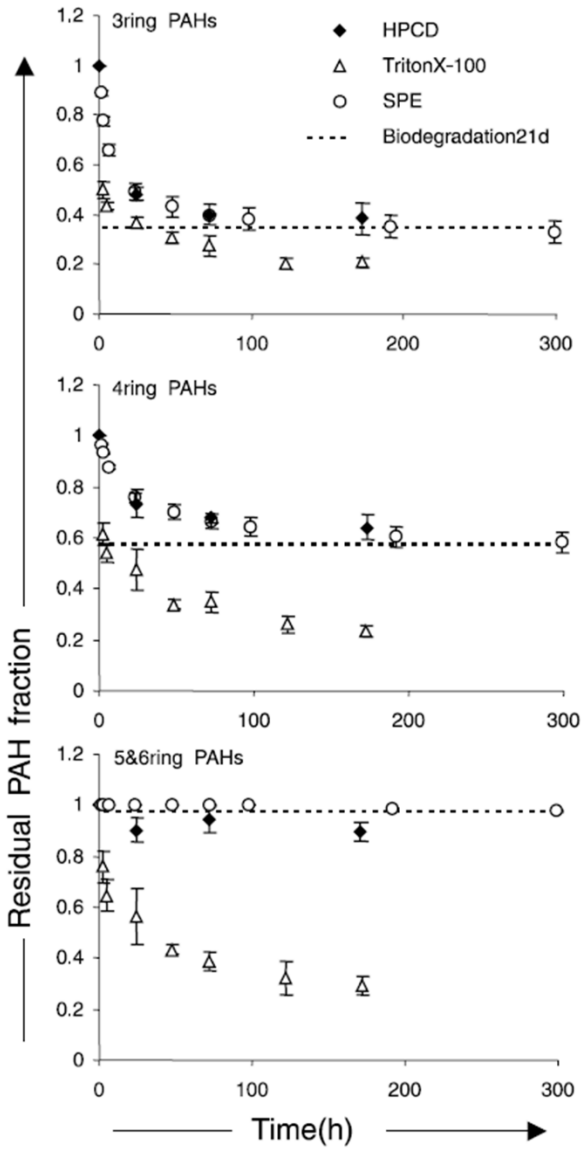

Figure 7. PAH concentrations obtained through SPE, HPCD, and Triton X-100 extractions from genuine field-contaminated sediments. The dotted line indicates the residual PAH concentration after a 21d biodegradation assay. Reproduced from Cuypers *et al.* (2002) with permission from © 2002 Elsevier B.V.

7. References

Alexander, M. 2000. Aging, bioavaiability, and overestimation of risk from environmental pollutants. Environmental Science & Technology, 34, 4259–4265.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2007. Cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and phenols in contaminated soil slurries. Environmental Science & Technology, 41, 5498–5504.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M.A. & Van Noort, P.C.M. 1998. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environmental Science & Technology, 32, 966–970.

Cornelissen, G., Rigterink, H., ten Hulscher, D.E.M., Vrind, B.A. & van Noort, P.C.M. 2001. A simple tenax® extraction method to determine the availability of sediment-sorbed organic compounds. Environmental Toxicology and Chemistry, 20, 706–711.

Cui, X., Mayer, P. & Gan, J. 2013. Methods to assess bioavailability of hydrophobic organic contaminants: Principles, operations, and limitations. Environmental Pollution, 172, 223–234.

Cuypers, C., Pancras, T., Grotenhuis, T. & Rulkens, W. 2002. The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere, 46, 1235–1245.

Doick, K.J., Dew, N.M. & Semple, K.T. 2005. Linking catabolism to cyclodextrin extractability: Determination of the microbial availability of PAHs in soil. Environmental Science & Technology, 39, 8858–8864.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

European Centre for Ecotoxicology and Toxicology of Chemicals. 2013. ECETOC Technical Report No. 117 - Understanding the Relationship between Extraction Technique and Bioavailability.

Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E. & Collins, C.D. 2012. Passive samplers provide a better prediction of PAH bioaccumulation in earthworms and plant roots than exhaustive, mild solvent, and cyclodextrin extractions. Environmental Science & Technology, 46, 962–969.

Harwood, A.D., Landrum, P.F. & Lydy, M.J. 2012. A comparison of exposure methods for SPME-based bioavailability estimates. Chemosphere, 86, 506–511.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

Obuekwe, I.S. & Semple, K.T. 2013. Impact of Zn and Cu on the development of phenanthrene catabolism in soil. Environmental Monitoring and Assessment, 185, 10039–10047.

Oyelami, A.O., Okere, U. V., Orwin, K.H., De Deyn, G.B., Jones, K.C. & Semple, K.T. 2013. Effects of plant species identity, diversity and soil fertility on biodegradation of phenanthrene in soil. Environmental Pollution, 173, 231–237.

Reid, B.J., Jones, K.C. & Semple, K.T. 2000a. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environmental Pollution, 108, 103–112.

Reid, B.J., Semple, K.T. & Jones, K.C. 1998. Prediction of bioavailability of persistent organic pollutants by a novel extraction technique. Contaminated Soil '98, Proceedings of the International FZK/TNO Conference on Contaminated Soil, 6th, Edinburgh, May 17-21, 1998, 2, 889–890.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000b. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science & Technology, 34, 3174–3179.

Rhodes, A.H., Carlin, A. & Semple, K.T. 2008a. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science & Technology, 42, 740–745.

Rhodes, A.H., Dew, N.M. & Semple, K.T. 2008b. Relationship between cyclodextrin extraction and biodegradation of phenanthrene in soil. Environmental toxicology and chemistry / SETAC, 27, 1488–95.

Rhodes, A.H., McAllister, L.E., Chen, R. & Semple, K.T. 2010a. Impact of activated charcoal on the mineralisation of 14C-phenanthrene in soils. Chemosphere, 79, 463–469.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010b. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Riding, M.J., Doick, K.J., Martin, F.L., Jones, K.C. & Semple, K.T. 2013. Chemical measures of bioavailability/bioaccessibility of PAHs in soil: Fundamentals to application. Journal of Hazardous Materials, 261, 687–700.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science & Technology, 38, 228A–231A.

Semple, K.T., Doick, K.J., Wick, L.Y. & Harms, H. 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. Environmental Pollution, 150, 166–176.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C. & Semple, K.T. 2005. Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl-β-cyclodextrin extraction technique. Environmental Toxicology and Chemistry, 24, 1325.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2007. Microbe-aliphatic hydrocarbon interactions in soil: Implications for biodegradation and bioremediation. Journal of Applied Microbiology, 102, 1239–1253.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2008. Linking chemical extraction to microbial degradation of 14C-hexadecane in soil. Environmental Pollution, 156, 474–481.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Chapter 10

Consolidated reference list

10. Consolidated reference list

Acar, Y.B. & Alshawabkeh, A.N. 1993. Principles of electrokinetic remediation. Environmental Science & Technology, 27, 2638–2647.

Adam, G. & Duncan, H. 1999. Effect of diesel fuel on growth of selected plant species. Environmental Geochemistry and Health, 21, 353–357.

Adam, G. & Duncan, H. 2002. Influence of diesel fuel on seed germination. Environmental Pollution, 120, 363–370.

Adams, R.H., Guzmán-Osorio, F.J. & Domínguez-Rodríguez, V.I. 2014. Field-scale evaluation of the chemical-biological stabilization process for the remediation of hydrocarbon-contaminated soil. International Journal of Environmental Science and Technology, 11, 1343–1352.

Adams, R.H., Guzman Osorio, F.J. & Cruz, J.Z. 2008. Water repellency in oil contaminated sandy and clayey soils. International Journal of Environmental Science and Technology, 5, 445–454.

Adams Schroeder, R.H., Dominguez Rodríguez, V.I. & Vinalay Carrillo, L. 2002. Evaluation of microbial respiration and ecotoxicity in contaminated soils representative of the pretroleum-producing region of southeastern Mexico. Terra Latinoamericana, 60, 253–265.

Adenipekun, C.O. & Fasidi, I.O. 2005. Bioremediation of oil-polluted soil by Lentinus subnudus, a Nigerian white-rot fungus. African Journal of Biotechnology, 4, 796–798.

Adesodun, J.K. & Mbagwu, J.S.C. 2008. Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresource Technology, 99, 5659–5665.

Admassu, W. & Korus, R.A. 1996. Engineering of bioremediation processes: needs and limitations. In: Bioremediation (eds. Crawford, R.L. & Crawford, D.L.), pp. 13–34. Cambridge University Press, Cambridge.

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D. & Esposito, G. 2014. Enhanced phytoremediation: A review of low molecular weight organic acids and surfactants used as amendments. Critical Reviews in Environmental Science and Technology, 44, 2531–2576.

Akpor, O.B., Igbinosa, O.E. & Igbinosa, O.O. 2007. Studies on the effect of petroleum hydrocarbon on the microbial and physico-chemicals characteristics of soil. African Journal of Biotechnology, 6, 1939–1943.

Alba, G.I., Cuevas, M.C. & Bustos, E. 2013. Comparing the electroremediation of gleysol soil contaminated with hydrocarbons with triton X-114 washing and bioremediation with solid cultures employing agroindustrial residues. International Journal of Electrochemical Science, 8, 6365–6376.

Alef, K. 1995. Enrichment, isolation and counting of soil microorganisms. In: Methods in Applied Soil Microbiology and Biochemistry (eds. Alef, K. & Nannipieri, P.), pp. 123–191. 1st ed. Academic Press.

Alexander, M. 1999. Biodegradation and bioremediation. Academic Press.

Alexander, M. 2000. Aging, bioavaiability, and overestimation of risk from environmental pollutants. Environmental Science & Technology, 34, 4259–4265.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2006. Prediction of mono- and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction. Environmental Pollution, 144, 562–571.

Allan, I.J., Semple, K.T., Hare, R. & Reid, B.J. 2007. Cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and phenols in contaminated soil slurries. Environmental Science & Technology, 41, 5498–5504.

Alvarez, V.M., Santos, S.C.C.D.S., Casella, R.D.C., Vital, R.L., Sebastin, G.V. & Seldin, L. 2008. Bioremediation potential of a tropical soil contaminated with a mixture of crude oil and production water. Journal of Microbiology and Biotechnology, 18, 1966–1974.

Amezcua-Allieri, M.A., Lead, J.R., Meléndez-Estrada, J. & Rodríguez-Vázquez, R. 2003. Phenanthrene Removal in a Selected Mexican Soil by the Fungus Penicillium frequentans: Role of C:N Ratio and Water Content. Soil and Sediment Contamination, 12, 387–399.

An, C., Huang, G., Yu, H., Wei, J., Chen, W. & Li, G. 2010. Effect of short-chain organic acids and pH on the behaviors of pyrene in soil-water system. Chemosphere, 81, 1423–1429.

Anderson, T.A., Guthrie, E.A. & Walton, B.T. 1993. Bioremediation in the Rhizosphere: Plant roots and associated microbes clean contaminated soil. Environmental Science & Technology, 27, 2630–2636.

Aprill, W. & Sims, R.C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere, 20, 253–265.

Arbeli, Z. & Fuentes, C.L. 2010. Microbial degradation of pesticides in tropical soils. In: (ed. Dion, P.), pp. 251–274.

Atlas, R.M.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological reviews, 45, 180–209.

Ayotamuno, J.M., Kogbara, R.B. & Agoro, O.S. 2009a. Biostimulation supplemented with phytoremediation in the reclamation of a petroleum contaminated soil. World Journal of Microbiology and Biotechnology, 25, 1567–1572.

Ayotamuno, M.J., Kogbara, R.B., Ogaji, S.O.T. & Probert, S.D. 2006. Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt, Nigeria. Applied Energy, 83, 1249–1257.

Ayotamuno, J.M., Okparanma, R.N. & Araka, P.P. 2009b. Bioaugmentation and composting of oil-field drill-cuttings containing polycyclic aromatic hydrocarbons (PAHs). Journal of Food, Agriculture and Environment, 7, 658–664.

Badr, T., Hanna, K. & De Brauer, C. 2004. Enhanced solubilization and removal of naphthalene and phenanthrene by cyclodextrins from two contaminated soils. Journal of Hazardous Materials, 112, 215–223.

Baek, K.-H., Kim, H.-S., Oh, H.-M., Yoon, B.-D., Kim, J. & Lee, I.-S. 2004. Effects of crude oil, oil components, and bioremediation on plant growth. Journal of Environmental Science and Health, Part A- Toxic/Hazardous Substances & Environmental Engineering, 39, 2465–2472.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. & Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, 57, 233–266.

Baldrian, P., in der Wiesche, C., Gabriel, J., Nerud, F. & Zadrazil, F. 2000. Influence of cadmium and mercury on activities of lignolytic enzimes and degradation of polycyclic aromatic hydrocarbons by Pleurotus ostreatus in soil. Applied and Environmental Microbiology, 66, 2471–2478.

Bamforth, S.M. & Singleton, I. 2005. Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. Journal of Chemical Technology and Biotechnology, 80, 723–736.

Bamgbose, I. & Anderson, T.A. 2015. Phytotoxicity of three plant-based biodiesels, unmodified castor oil, and Diesel fuel to alfalfa (Medicago sativa L.), lettuce (Lactuca sativa L.), radish (Raphanus sativus), and wheatgrass (Triticum aestivum). Ecotoxicology and Environmental Safety, 122, 268–274.

Bandala, E.R. & Torres, L.G. 2012. Remediation of soils and aquifers in tropical and subtropical regions. Physics and Chemistry of the Earth, 37–39, 1.

Banks, M.K., Kulakow, P., Schwab, a P., Chen, Z. & Rathbone, K. 2003. Degradation of crude oil in the rhizosphere of Sorghum bicolor. International Journal of Phytoremediation, 5, 225–34.

Bardi, L., Mattei, A. & Steffan, S. 2000. Hydrocarbon degradation by a soil microbial population with ??-cyclodextrin as surfactant to enhance bioavailability. Enzyme and Microbial Technology, 27, 709–713.

Barnier, C., Ouvrard, S., Robin, C. & Morel, J.L. 2014. Desorption kinetics of PAHs from aged industrial soils for availability assessment. Science of the Total Environment, 470–471, 639–645.

Barrow, P.C., Olivier, P. & Marzin, D. 1995. The reproductive and developmental toxicity profile of beta-cyclodextrin in rodents. Reproductive Toxicology, 9, 389–398.

Baudin, C., Pean, C., Perly, B. & Gosselin, P. 2000. Inclusion of organic pollutants in cyclodextrins and derivatives. International Journal of Environmental Analytical Chemistry, 77, 233–242.

Bekers, O., Beijnen, J.H., Tank, M.J.T.K., Bult, A. & Underberg, W.J.M. 1991. Effect of cyclodextrins on the chemical stability of mitomycins in alkaline solution. Journal of Pharmaceutical and Biomedical Analysis, 9, 1055–1060.

Beraldo De Morais, E. & Tauk-Tornisielo, S.M. 2009. Biodegradation of oil fefinery residues using mixed- culture of microorganisms isolated from a landfarming. Brazilian Archives of Biology and Technology, 52652, 1571–1578.

Bernhardt, C., Derz, K., Kördel, W. & Terytze, K. 2013. Applicability of non-exhaustive extraction procedures with Tenax and HPCD. Journal of Hazardous Materials, 261, 711–717.

Bhardwaj, R., Dorr, R.T. & Blanchard, J. 2000. Approaches to reducing toxicity of parenteral anticancer drug formulations using cyclodextrins. PDA Journal of Pharmaceutical Science and Technology, 54, 233–239.

Binet, P., Portal, J.M. & Leyval, C. 2000. Dissipation of 3 ± 6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biology and Biochemistry, 32, 2011–2017.

Biwer, A., Antranikian, G. & Heinzle, E. 2002. Enzymatic production of cyclodextrins. Applied Microbiology and Biotechnology, 59, 609–617.

de Boer, J. & Wagelmans, M. 2016. Polycyclic aromatic hydrocarbons in soil – Practical options for remediation. Clean - Soil, Air, Water, 44, 648–653.

Bollag, J.-M. & Bollag, W.B. 1995. Soil Contamination and the Feasibility of Biological Remediation. In: Bioremediation: Science and Applications (eds. Skipper, H.D. & Turco, R.F.), pp. 1–12. Soil Science Society of America, American Society of Agronomy, and Crop Science Society of America.

Bona, C., Rezende, I.M. de, Santos, G. de O. & Souza, L.A. de. 2011. Effect of soil contaminated by diesel oil on the germination of seeds and the growth of Schinus terebinthifolius Raddi (Anacardiaceae) seedlings. Brazilian Archives of Biology and Technology, 54, 1379–1387.

Bosma, T.N.P., Middeldorp, P.J.M., Schraa, G. & Zehnder, A.J.B. 1997. Mass transfer limitation of beiotransformation: Quantifying bioavailability. Environmental Science & Technology, 31, 248–252.

Boucard, T.K., Bardgett, R.D., Jones, K.C. & Semple, K.T. 2005. Influence of plants on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil. Environmental Pollution, 133, 53–62.

Boving, T.B., Wang, X. & Brusseau, M.L. 1999. Cyclodextrin-enhanced solubilization and removal of residual-phase chlorinated solvents from porous media. Environmental Science & Technology, 33, 764–770.

Boyd, S.A. & Sun, S. 1990. Residual petroleum and polychlorobiphenyl oils as sorptive phases for organic contaminants in soils. Environmental Science & Technology, 24, 142–144.

Brand, E., Peijnenburg, W., Goenenberg, B., Vink, J., Lijzen, J., ten Hulscher, D., Jonker, C., Romkens, P. & Roex, E. 2009. Towards implementation of bioavailability measurements in the Dutch regulatory framework. National Institute for Public Health and the Environment.

Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C. & Broll, G. 2006. Potential of vetiver (Vetiveria zizanioides (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. International Journal of Phytoremediation, 8, 273–284.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. & Vivanco, J.M. 2008. Root exudates regulate soil fungal community composition and diversity. Applied and Environmental Microbiology, 74, 738–744.

Brooijmans, R.J.W., Pastink, M.I. & Siezen, R.J. 2009. Hydrocarbon-degrading bacteria: The oil-spill clean-up crew. Microbial Biotechnology, 2, 587–594.

Brusseau, M.L., Wang, X. & Hu, Q. 1994. Enhanced transport of low-polarity organic compounds through soil by cyclodextrin. Environmental Science & Technology, 28, 952–956.

Brusseau, M.L., Wang, X. & Wang, W.-Z. 1997. Simultaneous elution of heavy metals and organic compounds from soil by cyclodextrin. Environmental Science & Technology, 31, 1087–1092.

Burgess, R.M. & Lohmann, R. 2004. Role of black carbon in the partitioning and bioavailability of organic pollutants. Environmental Toxicology and Chemistry, 23, 2531–2533.

Cachada, A., Pereira, R., da Silva, E.F. & Duarte, A.C. 2014. The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges. Science of the Total Environment, 472, 463–480.

Cairns, M.A., Brown, S., Helmer, E.H. & Baumgardner, G.A. 1997. Root biomass allocation in the world's upland forests. Oecologia, 111, 1–11.

Cal, K. & Centkowska, K. 2008. Use of cyclodextrins in topical formulations: Practical aspects. European Journal of Pharmaceutics and Biopharmaceutics, 68, 467–478.

Carmichael, L.M., Christman, R.F. & Pfaender, F.K. 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. Environmental Science & Technology, 31, 126–132.

Castro-Silva, C., Ruiz-Valdiviezo, V.M., Rivas-Rivera, S.G., Sosa-Trinidad, A.R., Luna-Guido, M., Delgado-Balbuena, L., Marsch, R. & Dendooven, L. 2015. Bioavailability and dissipation of anthracene from soil with different alkalinity and salinity. Journal of Environmental Biology, 36, 229–234.

Cébron, A., Louvel, B., Faure, P., France-Lanord, C., Chen, Y., Murrell, J.C. & Leyval, C. 2011. Root exudates modify bacterial diversity of phenanthrene degraders in PAH-polluted soil but not phenanthrene degradation rates. Environmental Microbiology, 13, 722–736.

Cennerazzo, J., de Junet, A., Audinot, J.N. & Leyval, C. 2017. Dynamics of PAHs and derived organic compounds in a soil-plant mesocosm spiked with13C-phenanthrene. Chemosphere, 168, 1619–1627.

Cerniglia, C.E. 1993. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3, 351–368.

Chagas-Spinelli, A.C.O., Kato, M.T., de Lima, E.S. & Gavazza, S. 2012. Bioremediation of a tropical clay soil contaminated with diesel oil. Journal of Environmental Management, 113, 510–516.

Chaillan, F., Le Flèche, A., Bury, E., Phantavong, Y.H., Grimont, P., Saliot, A. & Oudot, J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Research in Microbiology, 155, 587–595.

Chaineau, C.H., Morel, J.L. & Oudot, J. 2000. Biodegradation of fuel oil hydrocarbons in the rhizosphere of maize. Journal of Environmental Quality, 29, 569–578.

Chan-Quijano, J.G., Ochoa-Gaona, S., Pérez-hernández, I., Gutiérrez-Aguirre, M.A. & Saragos-Méndez, J. 2012. Germinación y sobrevivencia de especies arbóreas que crecen en suelos contaminados por hidrocarburos Germination and survival of tree species growing in soils contaminated by hydrocarbons. Teoría y Praxis, 12, 102–119.

Chatain, V., Hanna, K., De Brauer, C., Bayard, R. & Germain, P. 2004. Enhanced solubilization of arsenic and 2,3,4,6 tetrachlorophenol from soils by a cyclodextrin derivative. Chemosphere, 57, 197–206.

Chaudhary, P., Sahay, H., Sharma, R., Pandey, A.K., Singh, S.B., Saxena, A.K. & Nain, L. 2015. Identification and analysis of polyaromatic hydrocarbons (PAHs)—biodegrading bacterial strains from refinery soil of India. Environmental Monitoring and Assessment, 187.

Chávez-Gómez, B., Quintero, R., Esparza-García, F., Mesta-Howard, A.M., Zavala Díaz De La Serna, F.J., Hernández-Rodríguez, C.H., Gillén, T., Poggi-Varaldo, H.M., Barrera-Cortés, J. & Rodríguez-Vázquez, R. 2003. Removal of phenanthrene from soil by co-cultures of bacteria and fungi pregrown on sugarcane bagasse pith. Bioresource Technology, 89, 177–183.

Cheema, S.A., Imran Khan, M., Shen, C., Tang, X., Farooq, M., Chen, L., Zhang, C. & Chen, Y. 2010. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. Journal of Hazardous Materials, 177, 384–389.

Chen, Y.C., Banks, M.K. & Schwab, A.P. 2003. Pyrene Degradation in the Rhizosphere of Tall Fescue (Festuca arundinacea) and Switchgrass (Panicum virgatum L.). Environmental Science & Technology, 37, 5778–5782.

Chen, F., Tan, M., Ma, J., Zhang, S., Li, G. & Qu, J. 2016. Efficient remediation of PAH-metal co-contaminated soil using microbial-plant combination: A greenhouse study. Journal of Hazardous Materials, 302, 250–261.

Chen, Y., Tang, X., Cheema, S.A., Liu, W. & Shen, C. 2010. β-cyclodextrin enhanced phytoremediation of aged PCBs-contaminated soil from e-waste recycling area. Journal of Environmental Monitoring, 12, 1482.

Chen, B., Yuan, M. & Qian, L. 2012. Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. Journal of Soils and Sediments, 12, 1350–1359.

Chiapusio, G., Pujol, S., Toussaint, M.L., Badot, P.M. & Binet, P. 2007. Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (Lolium perenne L. and Trifolium pratense L.) in three spiked soils. Plant and Soil, 294, 103–112.

Chikere, C.B., Okpokwasili, G.C. & Chikere, B.O. 2009. Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. African Journal of Biotechnology, 8, 2535–2540, (At: http://www.academicjournals.org/AJB.).

Chiou, C.T., Malcolm, R.L., Brinton, T.I. & Kile, D.E. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environmental Science & Technology, 20, 502–508.

Cho, Y.-M., Smithenry, D.W., Ghosh, U., Kennedy, A.J., Millward, R.N., Bridges, T.S. & Luthy, R.G. 2007. Field methods for amending marine sediment with activated carbon and assessing treatment effectiveness. Marine Environmental Research, 64, 541–55.

Chouychai, W., Thongkukiatkul, A., Upatham, S., Lee, H., Pokethitiyook, P. & Kruatrachue, M. 2007. Phytotoxicity assay of crop plants to phenanthrene and pyrene contaminants in acidic soil. Environmental Toxicology, 22, 597–604.

Chun, Y., Sheng, G., Chiou, G.T. & Xing, B. 2004. Compositions and sorptive properties of crop residue-derived chars. Environmental Science & Technology, 38, 4649–4655.

Chung, N. & Alexander, M. 2002. Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil. Chemosphere, 48, 109–115.

Clegg, H., Riding, M.J., Oliver, R., Jones, K.C. & Semple, K.T. 2014. The impact of soil organic matter and soil sterilisation on the bioaccessibility of 14C-azoxystrobin determined by desorption kinetics. Journal of Hazardous Materials, 278, 336–342.

Clemente, A.R. & Durrant, L.R. 2005. Biodegradation of PAHs in soil by two deuteromycete fungi. Contaminated Soils, Sediments and Water, 9, 13–20.

Di Corcia, A., Caracciolo, A.B., Crescenzi, C., Giuliano, G., Murtas, S. & Samperi, R. 1999. Subcritical water extraction followed by liquid chromatography mass spectmmetry for determining terbuthylazine and its metabolites in aged and incubated soils. Environmental Science & Technology, 33, 3271–3277.

Corgié, S.C., Joner, E.J. & Leyval, C. 2003. Rhizospheric degradation of phenanthrene is a function of proximity to roots. Plant and Soil, 257, 143–150.

Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A. & Van Noort, P.C.M. 2005. Extensive sorption of organic compounds to black carbon, coal, and

kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environmental Science & Technology, 39, 6881–6895.

Cornelissen, G., Van Noort, P.C.M. & Covers, H.A.J. 1998a. Mechanism of slow desorption of organic compounds from sediments: A study using model sorbents. Environmental Science & Technology, 32, 3124–3131.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M.A. & Van Noort, P.C.M. 1998b. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. Environmental Science & Technology, 32, 966–970.

Cornelissen, G., Rigterink, H., ten Hulscher, D.E.M., Vrind, B.A. & van Noort, P.C.M. 2001. A simple tenax® extraction method to determine the availability of sediment-sorbed organic compounds. Environmental Toxicology and Chemistry, 20, 706–711.

Cornelissen, G., Rigterink, H., Van Noort, P.C.M. & Govers, H.A.J. 2000. Slowly and very slowly desorbing organic compounds in sediments exhibit Langmuir-type sorption. Environmental Toxicology and Chemistry, 19, 1532–1539.

Cornelissen, G., vanNoort, P.C.M. & Govers, H. a J. 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with Tenax(R) and effects of contact time and solute hydrophobicity. Environmental Toxicology And Chemistry, 16, 1351–1357.

Couling, N.R., Towell, M.G. & Semple, K.T. 2010. Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. Environmental Pollution, 158, 3411–3420.

Crampon, M., Bodilis, J., Le Derf, F. & Portet-Koltalo, F. 2016. Alternative techniques to HPCD to evaluate the bioaccessible fraction of soil-associated PAHs and correlation to biodegradation efficiency. Journal of Hazardous Materials, 314, 220–229.

Crampon, M., Bureau, F., Akpa-Vinceslas, M., Bodilis, J., Machour, N., Le Derf, F. & Portet-Koltalo, F. 2014. Correlations between PAH bioavailability, degrading bacteria, and soil characteristics during PAH biodegradation in five diffusely contaminated dissimilar soils. Environmental Science and Pollution Research, 21, 8133–8145.

Craswell, E.T. & Lefroy, R.D.B. 2001. The role and function of organic matter in tropical soils. Nutrient Cycling in Agroecosystems, 61, 7–18.

Crescenzi, C., Di Corcia, A., Nazzari, M. & Samperi, R. 2000. Hot phosphate-buffered water extraction coupled on-line with liquid chromatography/mass spectrometry for analyzing contaminants in soil. Analytical Chemistry, 72, 3050–5.

Crini, G. 2005. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Progress in Polymer Science (Oxford), 30, 38–70.

Crini, G. & Morcellet, M. 2002. Synthesis and applications of adsorbents containing cyclodextrins. Journal of Separation Science, 25, 789–813.

Cui, X., Mayer, P. & Gan, J. 2013. Methods to assess bioavailability of hydrophobic organic contaminants: Principles, operations, and limitations. Environmental Pollution, 172, 223–234.

Cunningham, S.D., Anderson, T.A., Schwab, A.P. & Hsu, F.C. 1996. Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy, 56, 55–114.

Cunningham, S.D., Berti, W.R. & Huang, J.W. 1995. Phytoremediation of contaminated soils. Trends in Biotechnology, 13, 393–397.

Cunningham, S.D. & Ow, D.W. 1996. Promises and Prospects of Phytoremediation. Plant Physiology, 110, 715–719.

Cunningham, S, D., Berti & W, R. 1993. Phytoremediation of contaminated soils: progress and promise. Symposium on Boremediation and Bioprocessing, 13, 265–268.

Cuypers, C., Clemens, R., Grotenhuis, T. & Rulkens, W. 2001. Prediction of petroleum hydrocarbon bioavailability in contaminated soils and sediments. Soil and Sediment Contamination, 10, 459–482.

Cuypers, H., Grotenhuis, T., Joziasse, J. & Rulkens, W. 2000. Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments. Environmental Science and Technology, 34, 2057–2063.

Cuypers, C., Pancras, T., Grotenhuis, T. & Rulkens, W. 2002. The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere, 46, 1235–1245.

D'Orazio, V., Ghanem, A. & Senesi, N. 2013. Phytoremediation of pyrene contaminated soils by different plant species. Clean - Soil, Air, Water, 41, 377–382.

Dadrasnia, A., Usman, M.M., Wei, K.S.C., Velappan, R.D., Jamali, H., Mohebali, N. & Ismail, S. 2016. Native soil bacterial isolate in Malaysia exhibit promising supplements on degrading organic pollutants. Process Safety and Environmental Protection, 100, 264–271.

Dakora, F.D. & Phillips, D.A. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil, 245, 35–47.

Dandie, C.E., Weber, J., Aleer, S., Adetutu, E.M., Ball, A.S. & Juhasz, A.L. 2010. Assessment of five bioaccessibility assays for predicting the efficacy of petroleum hydrocarbon biodegradation in aged contaminated soils. Chemosphere, 81, 1061–1068.

Deng, S. & Zeng, D. 2017. Removal of phenanthrene in contaminated soil by combination of alfalfa, white-rot fungus, and earthworms. Environmental Science and Pollution Research, 24, 7565–7571.

Dennis, P.G., Miller, A.J. & Hirsch, P.R. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiology Ecology, 72, 313–327.

Dew, N.M., Paton, G.I. & Semple, K.T. 2005. Prediction of [3-14C]phenyldodecane biodegradation in cable insulating oil-spiked soil using selected extraction techniques. Environmental Pollution, 138, 316–323.

Díaz-Ramírez, I., Escalante-Espinosa, E., Adams Schroeder, R., Fócill-Monterrubio, R. & Ramírez-Saad, H. 2013. Hydrocarbon Biodegradation Potential of Native and Exogenous Microbial Inocula in Mexican Tropical Soils. In: Biodegradation of Hazardous and Special Products (eds. Chamy, R. & Rosenkrans, F.). InTech.

Díaz-Ramírez, I.J., Escalante-Espinosa, E., Favela-Torres, E., Gutiérrez-Rojas, M. & Ramírez-Saad, H. 2008. Design of bacterial defined mixed cultures for biodegradation of specific crude oil fractions, using population dynamics analysis by DGGE. International Biodeterioration and Biodegradation, 62, 21–30.

Dibble, J.T. & Bartha, R. 1979. Effect of environmental parameters on the biodegradation of oil sludge. Applied and Environmental Microbiology, 37, 729–739.

Doick, K.J., Burauel, P., Jones, K.C. & Semple, K.T. 2005a. Effect of cyclodextrin and transformer oil amendments on the chemical extractability of aged [14C]polychlorinated biphenyl and [14C]polycyclic aromatic hydrocarbon residues in soil. Environmental Toxicology and Chemistry, 24, 2138–2144.

Doick, K.J., Clasper, P.J., Urmann, K. & Semple, K.T. 2006. Further validation of the HPCD-technique for the evaluation of PAH microbial availability in soil. Environmental Pollution, 144, 345–354.

Doick, K.J., Dew, N.M. & Semple, K.T. 2005b. Linking catabolism to cyclodextrin extractability: Determination of the microbial availability of PAHs in soil. Environmental Science & Technology, 39, 8858–8864.

Doick, K.J., Lee, P.H. & Semple, K.T. 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environmental Pollution, 126, 399–406.

Doick, K.J. & Semple, K.T. 2003. The effect of soil:water ratios on the mineralisation of phenanthrene: LNAPL mixtures in soil. FEMS Microbiology Letters, 220, 29–33.

Dou, J., Li, S., Cheng, L., Ding, A., Liu, X. & Yun, Y. 2011. The enhancement of naphthalene degradation in soil by hydroxypropyl-β- cyclodextrin. In: Procedia Environmental Sciences, pp. 26–31.

Duan, L., Naidu, R., Liu, Y., Palanisami, T., Dong, Z., Mallavarapu, M. & Semple, K.T. 2015. Effect of ageing on benzo[a]pyrene extractability in contrasting soils. Journal of Hazardous Materials, 296, 175–184.

Dubrovskaya, E., Pozdnyakova, N., Golubev, S., Muratova, A., Grinev, V., Bondarenkova, A. & Turkovskaya, O. 2017. Peroxidases from root exudates of Medicago sativa and Sorghum bicolor: Catalytic properties and involvement in PAH degradation. Chemosphere, 169, 224–232.

Eastburn, S.D. & Tao, B.Y. 1994. Applications of modified cyclodextrins. Biotechnology Advances, 12, 325–339.

European Centre for Ecotoxicology and Toxicology of Chemicals. 2013. ECETOC Technical Report No. 117 - Understanding the Relationship between Extraction Technique and Bioavailability.

Evans, F.F., Rosado, A.S., Sebastián, G. V., Casella, R., Machado, P.L.O.A., Holmström, C., Kjelleberg, S., Van Elsas, J.D. & Seldin, L. 2004. Impact of oil contamination and biostimulation on the diversity of indigenous bacterial communities in soil microcosms. FEMS Microbiology Ecology, 49, 295–305.

Fan, S., Li, P., Gong, Z., Ren, W. & He, N. 2008. Promotion of pyrene degradation in rhizosphere of alfalfa (Medicago sativa L.). Chemosphere, 71, 1593–1598.

De Farias, V., Maranho, L.T., De Vasconcelos, E.C., Da Silva Carvalho Filho, M.A., Lacerda, L.G., Azevedo, J.A.M., Pandey, A. & Soccol, C.R. 2009. Phytodegradation potential of Erythrina crista-galli L., Fabaceae, in petroleum-contaminated soil. Applied Biochemistry and Biotechnology, 157, 10–22.

Fava, F., Bertin, L., Fedi, S. & Zannoni, D. 2003. Methyl-β-cyclodextrin-enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils. Biotechnology and Bioengineering, 81, 381–390.

Fava, F. & Ciccotosto, V.F. 2002. Effects of randomly methylated-β-cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with a transformer oil. Applied Microbiology and Biotechnology, 58, 393–399.

Fava, F., Di Gioia, D. & Marchetti, L. 1998. Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Biotechnology and Bioengineering, 58, 345–355.

Fenlon, K.A., Jones, K.C. & Semple, K.T. 2007. Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. Journal of Environmental Monitoring, 9, 510.

Fenyvesi, É., Csabai, K., Molnár, M., Leitgi, L. & Gruiz, K. 2010. Cyclodextrins for the enhancement of soil remediation technologies. In: Construction for a Sustainable Environment - Proceedings of the International Conference of Construction for a Sustainable Environment.

Fenyvesi, E., Gruiz, K., Verstichel, S., De Wilde, B., Leitgib, L., Csabai, K. & Szaniszlo, N. 2005. Biodegradation of cyclodextrins in soil. Chemosphere, 60, 1001–1008.

Ferreira Do Nascimento, T.C., Santos Oliveira, F.J. & Pessoa De França, F. 2013. Biorremediación de un suelo tropical contaminado con residuos aceitosos intemperizados. Revista Internacional de Contaminacion Ambiental, 29, 21–28.

Fletcher, J.S. & Hegde, R.S. 1995. Release of phenols by perennial plant roots and their potential importance in bioremediation. Chemosphere, 31, 3009–3016.

Frick, C.M., Germida, J.J., Farrell, R.E. & Germida, J.J. 1999. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Technical Seminar on Chemical Spills, 105a–124a.

Gallegos Martínez, M., Gómez Santos, A., González Cruz, L., Montes De Oca García, M.A., Yúñez Trujillo, L., Zermeño Eguña Lis, J.A. & Gutiérrez-Rojas, M. 2000. Diagnostic and resulting approaches to restore petroleum-contaminated soil in a Mexican tropical swamp. Water Science and Technology, 42, 377–384.

Gao, Y., Hu, X., Zhou, Z., Zhang, W., Wang, Y. & Sun, B. 2017. Phytoavailability and mechanism of bound PAH residues in filed contaminated soils. Environmental Pollution, 222, 465–476.

Gao, H., Ma, J., Xu, L. & Jia, L. 2014. Hydroxypropyl-β-cyclodextrin extractability and bioavailability of phenanthrene in humin and humic acid fractions from different soils and sediments. Environmental Science and Pollution Research, 21, 8620–8630.

Gao, Y., Ren, L., Ling, W., Gong, S., Sun, B. & Zhang, Y. 2010a. Desorption of phenanthrene and pyrene in soils by root exudates. Bioresource Technology, 101, 1159–1165.

Gao, Y., Ren, L., Ling, W., Kang, F., Zhu, X. & Sun, B. 2010b. Effects of low-molecular-weight organic acids on sorption–desorption of phenanthrene in soils. Soil Science Society of America Journal, 74, 51.

Gao, Y., Wang, N., Li, H., Hu, X. & Goikavi, C. 2015a. Low-molecular-weight organic acids influence the sorption of phenanthrene by different soil particle size fractions. Journal of Environment Quality, 44, 219.

Gao, H., Xu, L., Cao, Y., Ma, J. & Jia, L. 2013. Effects of hydroxypropyl-β-cyclodextrin and β-cyclodextrin on the distribution and biodegradation of phenanthrene in NAPL-water system. International Biodeterioration and Biodegradation, 83, 105–111.

Gao, Y., Yuan, X., Lin, X., Sun, B. & Zhao, Z. 2015b. Low-molecular-weight organic acids enhance the release of bound PAH residues in soils. Soil and Tillage Research, 145, 103–110.

Gao, Y. & Zhu, L. 2004. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere, 55, 1169–1178.

Garon, D., Sage, L., Wouessidjewe, D. & Seigle-Murandi, F. 2004. Enhanced degradation of fluorene in soil slurry by Absidia cylindrospora and maltosyl-cyclodextrin. Chemosphere, 56, 159–166.

Gerhardt, K.E., Huang, X.D., Glick, B.R. & Greenberg, B.M. 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Science, 176, 20–30.

Germida, J.J., Frick, C.M. & Farrell, R.E. 2002. Phytoremediation of oil-contaminated soils. Developments in Soil Science, 28, 169–186.

Ghanem, A., Orazio, V.D. & Senesi, N. 2010. Phytotoxicity assay of selected plants to Pyrene contaminated soil. World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia., 74–77.

Ghosh, U., Weber, A.S., Jensen, J.N. & Smith, J.R. 2000. Relationship between PCB desorption equilibrium, kinetics, and availability during land biotreatment. Environmental Science & Technology, 34, 2542–2548.

Gomes, H.I., Dias-Ferreira, C. & Ribeiro, A.B. 2012. Electrokinetic remediation of organochlorines in soil: Enhancement techniques and integration with other remediation technologies. Chemosphere, 87, 1077–1090.

Gomez-Eyles, J.L., Collins, C.D. & Hodson, M.E. 2010. Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability. Environmental Pollution, 158, 278–284.

Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E. & Collins, C.D. 2012. Passive samplers provide a better prediction of PAH bioaccumulation in earthworms and plant roots than exhaustive, mild solvent, and cyclodextrin extractions. Environmental Science & Technology, 46, 962–969.

Gomez-Eyles, J.L., Sizmur, T., Collins, C.D. & Hodson, M.E. 2011. Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environmental Pollution, 159, 616–622.

Gong, X., Xu, X., Gong, Z., Li, X., Jia, C., Guo, M. & Li, H. 2015. Remediation of PAH-contaminated soil at a gas manufacturing plant by a combined two-phase partition system washing and microbial degradation process. Environmental Science and Pollution Research, 22, 12001–12010.

Greenland, D.J., Wild, A. & Adams, D. 1992. Organic matter dynamics in soils of the tropics - from myth to complex reality. Myths and science of soils in the tropics, 17–33.

Groffman, P.M., Eagan, P., Sullivan, W.M. & Lemunyon, J.L. 1996. Grass species and soil type effects on microbial biomass and activity. Plant and soil, 183, 61–67.

Grybos, M., Davranche, M., Gruau, G., Petitjean, P. & Pédrot, M. 2009. Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. Geoderma, 154, 13–19.

Guha, S., Jaffé, P.R. & Peters, C.A. 1998. Solubilization of PAH mixtures by a nonionic surfactant. Environmental Science & Technology, 32, 930–935.

Günther, T., Dornberger, U. & Fritsche, W. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere, 33, 203–215.

Gupta, S.K., Kumari, R., Prakash, O. & Lal, R. 2008. Pseudomonas panipatensis sp. nov., isolated from an oil-contaminated site. International Journal of Systematic and Evolutionary Microbiology, 58, 1339–1345.

De Haan, H., De Boer, T., Kramer, H.A. & Voerman, J. 1982. Applicability of light absorbance as a measure of organic carbon in humic lake water. Water Research, 16, 1047–1050.

Hahladakis, J.N., Calmano, W. & Gidarakos, E. 2013. Use and comparison of the non-ionic surfactants Poloxamer 407 and Nonidet P40 with HP-β-CD cyclodextrin, for the enhanced electroremediation of real contaminated sediments from PAHs. Separation and Purification Technology, 113, 104–113.

Halsall, C.J., Sweetman, A.J., Barrie, L.A. & Jones, K.C. 2001. Modelling the behaviour of PAHs during atmospheric transport from the UK to the Arctic. Atmospheric Environment, 35, 255–267.

Hamdi, H., Benzarti, S., Aoyama, I. & Jedidi, N. 2012. Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (Medicago sativa L.). International Biodeterioration and Biodegradation, 67, 40–47.

Hanna, K., Chiron, S. & Oturan, M.A. 2005. Coupling enhanced water solubilization with cyclodextrin to indirect electrochemical treatment for pentachlorophenol contaminated soil remediation. Water Research, 39, 2763–2773.

Harris, R.W. 1992. Root-shoot ratios. Journal of Arboriculture, 18, 39–42.

Hartnik, T., Jensen, J. & Hermens, J.L.M. 2008. Nonexhaustive β-cyclodextrin extraction as a chemical tool to estimate bioavailability of hydrophobic pesticides for earthworms. Environmental Science & Technology, 42, 8419–8425.

Harwood, A.D., Landrum, P.F. & Lydy, M.J. 2012. A comparison of exposure methods for SPME-based bioavailability estimates. Chemosphere, 86, 506–511.

Hatzinger, P.B. & Alexander, M. 1995. Effect of ageing of chemicals in soil on their biodegradability and extractability. Environmental Science & Technology, 29, 537–545.

Hawari, J., Paquet, L., Zhou, E., Halasz, A. & Zilber, B. 1996. Enhanced recovery of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from soil: Cyclodextrin versus anionic surfactants. Chemosphere, 32, 1929–1936.

Hawthorne, S.B. & Grabanski, C.B. 2000. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. Environmental Science & Technology, 34, 4103–4110.

Hawthorne, S.B., Grabanski, C.B., Martin, E. & Miller, D.J. 2000. Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: Recovery, selectivity and effects on sample matrix. Journal of Chromatography A, 892, 421–433.

Hawthorne, S.B., Poppendieck, D.G., Grabanski, C.B. & Loehr, R.C. 2002. 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. Environmental Science & Technology, 36, 4795–4803.

Hedge, R.S. & Miller, D.A. 1990. Allelopathy and autotoxicity in alfalfa: Characterization and effects of preceding crops and residue incorporation. Crop Sci, 30, 1255–1259.

Hedges, A.R. 1998. Industrial applications of cyclodextrins. Chemical Reviews, 98, 2035–2044.

van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L. & Lundström, U.S. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry, 37, 1–13.

Henner, P., Schiavon, M., Druelle, V. & Lichtfouse, E. 1999. Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. Organic Geochemistry, 30, 963–969.

Hernández-Espriú, A., Sánchez-León, E., Martínez-Santos, P. & Torres, L.G. 2013. Remediation of a diesel-contaminated soil from a pipeline accidental spill: Enhanced biodegradation and soil washing processes using natural gums and surfactants. Journal of Soils and Sediments, 13, 152–165.

Hernández-Ortega, H.A., Quintanar-Isaías, P.A., Jaramillo-Pérez, A.T., Alarcón, A., Ferrera-Cerrato, R. & Lazzarini-Lechuga, R. 2014. Diesel effects on root hydraulic conductivity and morphological changes of the vascular cylinder in Medicago sativa. Environmental and Experimental Botany, 105, 1–9.

Hernández-Valencia, I. & Mager, D. 2003. Uso de Panicum maximum y Brachiaria brizantha para fitorremediar suelos contaminados con un crudo de petróleo liviano. Bioagro, 15, 149–155.

Hickman, Z.A. & Reid, B.J. 2005. Towards a more appropriate water based extraction for the assessment of organic contaminant availability. Environmental Pollution, 138, 299–306.

Hickman, Z.A., Swindell, A.L., Allan, I.J., Rhodes, A.H., Hare, R., Semple, K.T. & Reid, B.J. 2008. Assessing biodegradation potential of PAHs in complex multi-contaminant matrices. Environmental Pollution, 156, 1041–1045.

Huang, X.D., El-Alawi, Y., Penrose, D.M., Glick, B.R. & Greenberg, B.M. 2004. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution, 130, 465–476.

Huesemann, M.H., Hausmann, T.S. & Fortman, T.J. 2003. Assessment of bioavailability limitations during slurry biodegradation of petroleum hydrocarbons in aged soils. Environmental Toxicology and Chemistry / SETAC, 22, 2853–2860.

Hutchinson, S.L., Schwab, A.P. & Banks, M.K. 2001. Phytoremediation of aged petroleum sludge. Journal of Environment Quality, 30, 1516.

Hütsch, B.W., Augustin, J. & Merbach, W. 2002. Plant rhizodeposition-an important source for carbon turnover in soils. Journal of Plant Nutrition and Soil Science, 165, 397–407.

Hwang, S. & Cutright, T.J. 2002. Biodegradability of aged pyrene and phenanthrene in a natural soil. Chemosphere, 47, 891–899.

Hwang, S. & Cutright, T.J. 2004a. Preliminary exploration of the relationships between soil characteristics and PAH desorption and biodegradation. Environment International, 29, 887–894.

Hwang, S.C. & Cutright, T.J. 2004b. Evidence of underestimation in PAH sorption/desorption due to system nonequilibrium and interaction with soil constituents. Journal of Environmental Science and Health Part a-Toxic/hazardous Substances & Environmental Engineering, 39, 1147–1162.

Hwang, S., Ramirez, N. & Cutright, T.J. 2003. The role of soil properties in pyrene sorption and desorption. 65–80.

Ibragimova, M., Rumyantseva, M., Onishchuk, O., Belova, V., Kurchak, O., Andronov, E., Dzyubenko, N. & Simarov, B. 2006. Symbiosis between the root-nodule bacterium Sinorhizobium meliloti and alfalfa (Medicago sativa) under salinization conditions. Microbiology, 75, 77–81.

Idris, M., Abdullah, S.R.S., Titah, H.S., Latif, M.T., Abasa, A.R., Husin, A.K., Hanima, R.F. & Ayub, R. 2016. Screening and identification of plants at a petroleum contaminated site in Malaysia for phytoremediation. Journal of Environmental Science and Management, 19, 27–36.

Igunnugbemi, O.O. 2013. Biodegradation of aliphatic and polycyclic aromatic hydrocarbons in petroleum oil-contaminated soils. Lancaster University. (At: https://onesearch.lancaster-university.uk/primo-explore/fulldisplay?docid=44LAN_ALMA_DS2189824420001221&context=L&vid=LUL_VU1&lang=en_US&search_scope=LSCOP_44LAN_LANUL_LIB&adaptor=Local Search Engine&tab=books&query=any,contains,Biodegradation of . Accessed: 7/2/2018).

Ijah, U.J.J. 1998. Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. Waste Management, 18, 293–299.

Ilori, M.O.N. & Amund, D.I. 2000. Degradation of anthracene by bacteria isolated from oil polluted tropical soils. Zeitschrift fur Naturforschung - Section C Journal of Biosciences, 55, 890–897.

Ite, A.E., Adebisi, O.O., Hanney, N.F. & Semple, K. 2016. The Effect of Rhizosphere Soil and Root Tissues Amendment on Microbial Mineralisation of Target C – Hydrocarbons in Contaminated Soil. International Journal of Environmental Bioremediation & Biodegradation, 4, 21–34.

Janbandhu, A. & Fulekar, M.H. 2011. Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. Journal of Hazardous Materials, 187, 333–340.

Jiao, X.C., Xu, F.L., Dawson, R., Chen, S.H. & Tao, S. 2007. Adsorption and absorption of polycyclic aromatic hydrocarbons to rice roots. Environmental Pollution, 148, 230–235.

Jidere, C.M., Akamigbo, F.O.R. & Ugwuanyi, J.O. 2012. Phytoremediation potentials of cowpea (Vigina unguiculata) and maize (Zea mays) for hydrocarbon degradation in organic

and inorganic Manure-Amended tropical typic paleustults. International Journal of Phytoremediation, 14, 362–373.

Jiradecha, C., Urgun-Demirtas, M. & Pagilla, K. 2006. Enhanced electrokinetic dissolution of naphthalene and 2,4-DNT from contaminated soils. Journal of Hazardous Materials, 136, 61–67.

John, R.C., Essien, J.P., Akpan, S.B. & Okpokwasili, G.C. 2012. Polycyclic aromatic hydrocarbon-degrading bacteria from aviation fuel spill site at Ibeno, Nigeria. Bulletin of Environmental Contamination and Toxicology, 88, 1014–1019.

Johnsen, A.R., De Lipthay, J.R., Reichenberg, F., Sørensen, S.J., Andersen, O., Christensen, P., Binderup, M.L. & Jacobsen, G.S. 2006. Biodegradation, bioaccessibility, and genotoxicity of diffuse polycyclic aromatic hydrocarbon (PAH) pollution at a motorway site. Environmental Science & Technology, 40, 3293–3298.

Johnsen, A.R., Wick, L.Y. & Harms, H. 2005. Principles of microbial PAH-degradation in soil. Environmental Pollution, 133, 71–84.

Joner, E.J., Corgié, S.C. & Leyval, C. 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biology and Biochemistry, 34, 859–864.

Joner, E.J., Johansen, A., Loibner, A.P., Cruz, M.A. Dela, Szolar, O.H.J., Portal, J.M. & Leyval, C. 2001. Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. Environmental Science & Technology, 35, 2773–2777.

Joner, E.J. & Leyval, C. 2003. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environmental Science & Technology, 37, 2371–2375.

Jones, D. 1998. Organic acids in the rhizosphere - a critical review. Plant and Soil, 205, 25–44.

Jones, K.C., Alcock, R.E., Johnson, D.L., Semple, K.T. & Woolgar, P.J. 1996. Organic chemicals in contaminated land : analysis, significance and research priorities. Land Contamination and Reclamation, 4, 189–198.

Jones, D.L. & Brassington, D.S. 1998. Sorption of organic acids in acid soils and its implications in the rhizosphere. European Journal of Soil Science, 49, 447–455.

Jones, D.L. & Darrah, P.R. 1992. Re-sorption of organic components by roots of Zea mays L. and its consequences in the rhizosphere - I. Re-sorption of 14C labelled glucose, mannose and citric acid. Plant and Soil, 143, 259–266.

Jones, D.L. & Darrah, P.R. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil, 166, 247–257.

Jones, D.L.L., Dennis, P.G.G., Owen, a. G.G. & van Hees, P. a. W. a W. 2003. Organic acid behavior in soils - misconceptions and knowledge gaps. Plant and Soil, 248, 31–41.

Jones, R.K., Sun, W.H., Tang, C.-S. & Robert, F.M. 2004. Phytoremediation of petroleum hydrocarbons in tropical coastal soils. II. Microbial response to plant roots and contaminant. Environmental Science and Pollution Research iIternational, 11, 340–346.

Jonker, M.T.O., Van Der Heijden, S.A., Kreitinger, J.P. & Hawthorne, S.B. 2007. Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with solid-phase microextraction. Environmental Science & Technology, 41, 7472–7478.

Jonker, M.T.O., Hoenderboom, A.M. & Koelmans, A.A. 2004. Effects of sedimentary sootlike materials on bioaccumulation and sorption of polychlorinated biphenyls. In: Environmental Toxicology and Chemistry, pp. 2563–2570.

Jonker, M.T.O. & Koelmans, A.A. 2002. Extraction of polycyclic aromatic hydrocarbons from soot and sediment: Solvent evaluation and implications for sorption mechanism. Environmental Science & Technology, 36, 4107–4113.

Jørgensen, K.S., Puustinen, J. & Suortti, A.M. 2000. Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. Environmental Pollution, 107, 245–254.

Juhasz, A.L., Aleer, S. & Adetutu, E.M. 2014. Predicting PAH bioremediation efficacy using bioaccessibility assessment tools: Validation of PAH biodegradation-bioaccessibility correlations. International Biodeterioration and Biodegradation, 95, 320–329.

Juhasz, A.L. & Naidu, R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. International Biodeterioration & Biodegradation, 45, 57–88.

Juo, A.S.R. & Franzluebbers, K. 2003. Tropical soils : properties and management for sustainable agriculture. Oxford University Press.

Kaimi, E., Mukaidani, T., Miyoshi, S. & Tamaki, M. 2006. Ryegrass enhancement of biodegradation in diesel-contaminated soil. Environmental and Experimental Botany, 55, 110–119.

Kanaly, R. a & Harayama, S. 2000. Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria MINIREVIEW Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria. Journal of Bacteriology, 182, 2059–2067.

Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K. & Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change, 5, 588–595.

Kelsey, J.W., Kottler, B.D. & Alexander, M. 1997. Selective chemiscal extractants to predict bioavaiability of soil-aged organic chemicals. Environmental Science & Technology, 31, 214–217.

Khan, S., Afzal, M., Iqbal, S. & Khan, Q.M. 2013. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere, 90, 1317–1332.

Khan, M.I., Cheema, S.A., Shen, C., Zhang, C., Tang, X., Malik, Z., Chen, X. & Chen, Y. 2011. Assessment of pyrene bioavailability in soil by mild hydroxypropyl-β- cyclodextrin extraction. Archives of Environmental Contamination and Toxicology, 60, 107–115.

Khan, M.I., Cheema, S.A., Shen, C., Zhang, C., Tang, X., Shi, J., Chen, X., Park, J. & Chen, Y. 2012a. Assessment of phenanthrene bioavailability in aged and unaged soils by mild extraction. Environmental Monitoring and Assessment, 184, 549–559.

Khan, M.I., Cheema, S.A., Tang, X., Shen, C., Sahi, S.T., Jabbar, A., Park, J. & Chen, Y. 2012b. Biotoxicity assessment of pyrene in soil using a battery of biological assays. Archives of Environmental Contamination and Toxicology, 63, 503–512.

Khodadoust, A.P., Reddy, K.R. & Narla, O. 2006. Cyclodextrin-Enhanced Electrokinetic Remediation of Soils Contaminated with 2,4-Dinitrotoluene. Journal of Environmental Engineering, 132, 1043–1050.

Ko, S.O., Schlautman, M.A. & Carraway, E.R. 1999. Partitioning of hydrophobic organic compounds to sorbed surfactants. 2. Model development/predictions for surfactant-enhanced remediation applications. Environmental Science & Technology, 32, 2776–2781.

Ko, S.O., Schlautman, M.A. & Carraway, E.R. 2000. Cyclodextrin-enhanced electrokinetic removal of phenanthrene from a model clay soil. Environmental Science & Technology, 34, 1535–1541.

Kong, H., Sun, R., Gao, Y. & Sun, B. 2013. Elution of polycyclic aromatic hydrocarbons in soil columns using low-molecular-weight organic acids. Soil Science Society of America Journal, 77, 72–82.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, 165, 382–396.

Lara-Severino, R. del C., Camacho-López, M.A., Casanova-González, E., Gómez-Oliván, L.M., Sandoval-Trujillo, Á.H., Isaac-Olivé, K. & Ramírez-Durán, N. 2016. Haloalkalitolerant Actinobacteria with capacity for anthracene degradation isolated from soils close to areas with oil activity in the state of Veracruz, Mexico. International Microbiology, 19, 15–26.

Lateef, B.S. & Oluwafemi, S.O. 2014. Fluorene biodegradation potentials of Bacillus strains isolated from tropical hydrocarbon-contaminated soils. African Journal of Biotechnology, 13, 1554–1559.

Leahy, J.G. & Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews, 54, 305–315.

Lee, P.H., Doick, K.J. & Semple, K.T. 2003. The development of phenanthrene catabolism in soil amended with transformer oil. FEMS Microbiology Letters, 228, 217–223.

Leemhuis, H., Kelly, R.M. & Dijkhuizen, L. 2010. Engineering of cyclodextrin glucanotransferases and the impact for biotechnological applications. Applied Microbiology and Biotechnology, 85, 823–835.

Lei, L., Suidan, M.T., Khodadoust, A.P. & Tabak, H.H. 2004. Assessing the bioavailability of PAHs in field-contaminated sediment using XAD-2 assisted desorption. Environmental Science & Technology, 38, 1786–1793.

Leigh, M.B., Fletcher, J.S., Fu, X. & Schmitz, F.J. 2002. Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environmental Science & Technology, 36, 1579–1583.

Lerch, R.N., Thurman, E.M. & Kruger, E.L. 1997. Mixed-mode sorption of hydroxylated atrazine degradation products to sell: A mechanism for bound residue. Environmental Science & Technology, 31, 1539–1546.

Li, S. & Purdy, W.C. 1992. Cyclodextrins and their applications in analytical chemistry. Chemical Reviews, 92, 1457–1470.

Li, T., Yuan, S., Wan, J., Lin, L., Long, H., Wu, X. & Lu, X. 2009. Pilot-scale electrokinetic movement of HCB and Zn in real contaminated sediments enhanced with hydroxypropyl-β-cyclodextrin. Chemosphere, 76, 1226–1232.

Li, T., Yuan, S., Wan, J. & Lu, X. 2010. Hydroxypropyl-??-cyclodextrin enhanced electrokinetic remediation of sediment contaminated with HCB and heavy metals. Journal of Hazardous Materials, 176, 306–312.

Ling, W., Ren, L., Gao, Y., Zhu, X. & Sun, B. 2009. Impact of low-molecular-weight organic acids on the availability of phenanthrene and pyrene in soil. Soil Biology and Biochemistry, 41, 2187–2195.

Ling, W., Sun, R., Gao, X., Xu, R. & Li, H. 2015. Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil. European Journal of Soil Science, 66, 339–347.

Liste, H.H. & Alexander, M. 1999. Rapid screening of plants promoting phenanthrene degradation. Journal-of-Environmental-Quality, 28, 1376–1377.

Liste, H.H. & Alexander, M. 2002. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere, 46, 1011–1017.

Liu, S.L., Luo, Y.M., Cao, Z.H., Wu, L.H., Ding, K.Q. & Christie, P. 2004. Degradation of benzo[a]pyrene in soil with arbuscular mycorrhizal alfalfa. Environmental Geochemistry and Health, 26, 285–293.

Loethen, S., Kim, J.M. & Thompson, D.H. 2007. Biomedical applications of cyclodextrin based polyrotaxanes.

Loftsson, T. & Brewster, M.E. 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. Journal of Pharmaceutical Sciences, 85, 1017–1025.

Loftsson, T., Leeves, N., Bjornsdottir, B., Duffy, L. & Masson, M. 1999. Effect of cyclodextrins and polymers on triclosan availability and substantivity in toothpastes in vivo. Journal of Pharmaceutical Sciences, 88, 1254–1258.

Lohmann, R., Macfarlane, J.K. & Gschwend, P.M. 2005. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York Harbor sediments. Environmental Science & Technology, 39, 141–148.

Louvel, B., Cébron, A. & Leyval, C. 2011. Root exudates affect phenanthrene biodegradation, bacterial community and functional gene expression in sand microcosms. International Biodeterioration and Biodegradation, 65, 947–953.

Lucas-Abellán, C., Fortea, I., Gabaldón, J.A. & Núñez-Delicado, E. 2008. Encapsulation of quercetin and myricetin in cyclodextrins at acidic pH. Journal of Agricultural and Food Chemistry, 56, 255–259.

Luo, L., Zhang, S., Zhang, L. & Christie, P. 2011. Effect of phosphate on phenanthrene sorption in soils. Journal of Colloid and Interface Science, 353, 275–280.

Luthy, G.R., Ainken, R.G., Brusseau, L.M., Cunningham, D.S., Gschwend, M.P., Pignatello, J.J., Reinhard, M., Traina, J.S., Jr, Weber, J.W. & Westall, C.J. 1997. Critical review sequestration of hydrophobic organic contaminants by geosorbents. American Chemical Society, 31, 3341–3347.

Luthy, R.G., Dzombak, D.A., Peters, C.A., Roy, S.B., Ramaswami, A., Nakles, D. V. & Nott, B.R. 1994. Remediating tar-contaminated soils at manufactured gas plant sites. Environmental Science & Technology, 28.

Ma, L., Zhang, J., Han, L., Li, W., Xu, L., Hu, F. & Li, H. 2012. The effects of aging time on the fraction distribution and bioavailability of PAH. Chemosphere, 86, 1072–1078.

Macleod, C.J.A. & Semple, K.T. 2002. The adaptation of two similar soils to pyrene catabolism. Environmental Pollution, 119, 357–364.

Macleod, C.J.A. & Semple, K.T. 2003. Sequential extraction of low concentrations of pyrene and formation of non-extractable residues in sterile and non-sterile soils. Soil Biology and Biochemistry, 35, 1443–1450.

Maddela, N.R., Masabanda, M. & Leiva-Mora, M. 2015a. Novel diesel-oil-degrading bacteria and fungi from the Ecuadorian Amazon rainforest. Water Science and Technology, 71, 1554–1561.

Maddela, N.R., Scalvenzi, L., Pérez, M., Montero, C. & Gooty, J.M. 2015b. Efficiency of Indigenous Filamentous Fungi for Biodegradation of Petroleum Hydrocarbons in Medium and Soil: Laboratory Study from Ecuador. Bulletin of Environmental Contamination and Toxicology, 95, 385–394.

Madrid, F., Rubio-Bellido, M., Villaverde, J., Tejada, M. & Morillo, E. 2016. Natural attenuation of fluorene and pyrene in contaminated soils and assisted with hydroxypropyl-β-cyclodextrin. Effect of co-contamination. Science of the Total Environment, 571, 42–49.

Magdolna Nagy, Z., Gruiz, K., Molnár, M. & Fenyvesi, É. 2013. Comparative evaluation of microbial and chemical methods for assessing 4-chlorophenol biodegradation in soil. Periodica Polytechnica: Chemical Engineering, 57, 25–35.

Mahanty, B. & Kim, C.G. 2016. Effect of soil acidification on n-hexane extractable PAH fractions. European Journal of Soil Science, 67, 60–69.

Mahmoudi, N., Slater, G.F. & Juhasz, A.L. 2013. Assessing limitations for PAH biodegradation in long-term contaminated soils using bioaccessibility assays. Water, Air, and Soil Pollution, 224.

Maila, M.P. & Cloete, T.E. 2002. Germination of Lepidium sativum as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. International Biodeterioration & Biodegradation, 50, 107–113.

Makhadmeh, I.M. 2001. Ecophysiology of the Plant ± Rhizosphere System. Plant Production, 9, 1–9.

Maliszewska-Kordybach, B. & Smreczak, B. 2000. Ecotoxicological activity of soils polluted with polycyclic aromatic hydrocarbons (pahs) - effect on plants. Environmental Technology (United Kingdom), 21, 1099–1110.

Mar Sojo, M., Nuñez-Delicado, E., García-Carmona, F. & Sánchez-Ferrer, A. 1999. Cyclodextrins as activator and inhibitor of latent banana pulp polyphenol oxidase. Journal of Agricultural and Food Chemistry, 47, 518–523.

Marchand, L., Sabaris, C.Q., Desjardins, D., Oustrière, N., Pesme, E., Butin, D., Wicart, G. & Mench, M. 2016. Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons. Environmental Science and Pollution Research, 23, 3120–3135.

Margesin, R., Hämmerle, M. & Tscherko, D. 2007. Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: Effects of hydrocarbon concentration, fertilizers, and incubation time. Microbial Ecology, 53, 259–269.

Margesin, R. & Schinner, F. 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56, 650–663.

Martin, B.C., George, S.J., Price, C.A., Ryan, M.H. & Tibbett, M. 2014. The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: Current knowledge and future directions. Science of the Total Environment, 472, 642–653.

Martínez, V.E. & López, F. 2001. Efecto de hidrocarburos en las propiedades físicas y químicas de suelo arcilloso. Terra Latinoamericana.

Matamala, R. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science, 302, 1385–1387.

Maturi, K. & Reddy, K.R. 2006. Simultaneous removal of organic compounds and heavy metals from soils by electrokinetic remediation with a modified cyclodextrin. Chemosphere, 63, 1022–1031.

Mccray, J.E. & Brusseau, M.L. 1999. Cyclodextrin-enhanced in situ flushing of multiple-component immiscible organic liquid contamination at the field scale: Analysis of dissolution behavior. Environmental Science & Technology, 33, 89–95.

McKelvie, J.R., Wolfe, D.M., Celejewski, M., Simpson, A.J. & Simpson, M.J. 2010. Correlations of Eisenia fetida metabolic responses to extractable phenanthrene concentrations through time. Environmental Pollution, 158, 2150–2157.

Means, J.C., Wood, S.G., Hassett, J.J. & Banwart, W.L. 1980. Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environmental Science & Technology, 14, 1524–1528.

Meng, L., Qiao, M. & Arp, H.P.H. 2011. Phytoremediation efficiency of a PAH-contaminated industrial soil using ryegrass, white clover, and celery as mono- and mixed cultures. Journal of Soils and Sediments, 11, 482–490.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2004a. Phytoremediation in the tropics-the effect of crude oil on the growth of tropical plants. Bioremediation Journal, 8, 177–184.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2004b. Phytoremediation of petroleum-contaminated soils in the tropics - Pre-selectlon of plant species from eastern Venezuela. Journal of Applied Botany and Food Quality, 78, 185–192.

Merkl, N., Schultze-Kraft, R. & Infante, C. 2005. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water, Air, and Soil Pollution, 165, 195–209.

Miranda, R.D.C., De Souza, C.S., Gomes, E.D.B., Lovaglio, R.B., Lopes, C.E. & Sousa, M.D.F.V.D.Q. 2007. Biodegradation of diesel oil by yeasts isolated from the vicinity of Suape Port in the State of Pernambuco -Brazil. Brazilian Archives of Biology and Technology, 50, 147–152.

Miya, R.K. & Firestone, M.K. 2000. Phenanthrene-degrader community dynamics in rhizosphere soil from a common annual grass. Journal of Environment Quality, 29, 584.

Miya, R.K. & Firestone, M.K. 2001. Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. Journal of Environment Quality, 30, 1911–1918.

Mokany, K., Raison, R.J. & Prokushkin, A.S. 2006. Critical analysis of root: Shoot ratios in terrestrial biomes. Global Change Biology, 12, 84–96.

Molina-Barahona, L., Rodríguez-Vázquez, R., Hernández-Velasco, M., Vega-Jarquín, C., Zapata-Pérez, O., Mendoza-Cantú, A. & Albores, A. 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Applied Soil Ecology, 27, 165–175.

Molnár, M., Leitgib, L., Gruiz, K., Fenyvesi, E., Szaniszló, N., Szejtli, J. & Fava, F. 2005. Enhanced biodegradation of transformer oil in soils with cyclodextrin--from the laboratory to the field. Biodegradation, 16, 159–168.

Morais, D., Pylro, V., Clark, I.M., Hirsch, P.R. & Tótola, M.R. 2016. Responses of microbial community from tropical pristine coastal soil to crude oil contamination. PeerJ, 4, e1733.

Morillo, E., Pérez-Martínez, J.I. & Ginés, J.M. 2001. Leaching of 2,4-D from a soil in the presence of β-cyclodextrin: Laboratory columns experiments. Chemosphere, 44, 1065–1069.

Morillo, E., Sánchez-Trujillo, M.A., Villaverde, J., Madrid, F. & Undabeytia, T. 2014. Effect of contact time and the use of hydroxypropyl-β-cyclodextrin in the removal of fluorene and fluoranthene from contaminated soils. Science of the Total Environment, 496, 144–154.

Mosinger, J., Tománková, V., Němcová, I. & Zýka, J. 2001. Cyclodextrins in analytical chemistry. Analytical Letters, 34, 1979–2004.

Moubasher, H.A., Hegazy, A.K., Mohamed, N.H., Moustafa, Y.M., Kabiel, H.F. & Hamad, A.A. 2015. Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. International Biodeterioration & Biodegradation, 98, 113–120.

Mueller, K.E. & Shann, J.R. 2006. PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees. Chemosphere, 64, 1006–1014.

Mueller, K.E. & Shann, J.R. 2007. Effects of tree root-derived substrates and inorganic nutrients on pyrene mineralization in rhizosphere and bulk soil. Journal of Environment Quality, 36, 120.

Muijs, B. & Jonker, M.T.O. 2011. Assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments. Environmental Science & Technology, 45, 3554–3561.

Mulligan, C.N., Yong, R.N. & Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. Engineering Geology, 60, 193–207.

Muratova, A.Y., Dmitrieva, T. V., Panchenko, L. V. & Turkovskaya, O. V. 2008. Phytoremediation of oil-sludge-contaminated soil. International Journal of Phytoremediation, 10, 486–502.

Muratova, A.Y., Golubev, S.N., Merbach, W. & Turkovskaya, O. V. 2009a. Biochemical and physiological peculiarities of the interactions between Sinorhizobium meliloti and Sorghum bicolor in the presence of phenanthrene. Microbiology, 78, 308–314.

Muratova, A., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A., Hirche, F., Merbach, W. & Turkovskaya, O. 2009b. Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of Sorghum bicolor (L.) Moench. Environmental and Experimental Botany, 66, 514–521.

Muratova, A., Hübner, T., Tischer, S., Turkovskaya, O., Möder, M. & Kuschk, P. 2003. Plant–rhizosphere-microflora association during phytoremediation of PAH-ontaminated soil. International Journal of Phytoremediation, 5, 137–151.

Muratova, A., Pozdnyakova, N., Golubev, S., Wittenmayer, L., Makarov, O., Merbach, W. & Turkovskaya, O. 2009c. Oxidoreductase activity of sorghum root exudates in a phenanthrene-contaminated environment. Chemosphere, 74, 1031–1036.

National Academies. 2003. Bioavailability of contaminants in soils and sediments: Processes, tools, and applications.

Nélieu, S., Delarue, G., Ollivier, E., Awad, P., Fraillon, F. & Pelosi, C. 2016. Evaluation of epoxiconazole bioavailability in soil to the earthworm Aporrectodea icterica. Environmental Science and Pollution Research, 23, 2977–2986.

Nichols, T.D., Wolf, D.C., Rogers, H.B., Beyrouty, C. a. & Reynolds, C.M. 1997. Rhizosphere microbial populations in contaminated soils. Water, Air, & Soil Pollution, 95, 165–178.

Nortcliff, S. 2010. Soils of the Tropics. pp. 1–15. Springer, Berlin, Heidelberg.

Northcott, G.L. & Jones, K.C. 2001. Partitioning, extractability, and formation of non extractable PAH residues in soil.2. Effects on compund dissolution bahaviour. Environmental Science & Technology, 35, 1111–1117.

Nwanna, I.E.M., George, G.O. & Olusoji, I.M. 2006. Growth study on chrysene degraders isolated from polycyclic aromatic hydrocarbon polluted soils in Nigeria. African Journal of Biotechnology, 5, 823–828.

Nwinyi, O.C., Kanu, I.A., Tunde, A. & Ajanaku, K.O. 2014. Characterization of diesel degrading bacterial species from contaminated tropical ecosystem. Brazilian Archives of Biology and Technology, 57, 789–796.

Obayori, O.S., Ilori, M.O., Adebusoye, S.A., Oyetibo, G.O. & Amund, O.O. 2008. Pyrene-degradation potentials of Pseudomonas species isolated from polluted tropical soils. World Journal of Microbiology and Biotechnology, 24, 2639–2646.

Obire, O. & Nwaubeta, O. 2002. Effects of refined petroleum hydrocarbon on soil physicochemical and bacteriological characteristics. Journal of Applied Sciences and Environmental Management, 17, 47–67.

Obuekwe, I.S. & Semple, K.T. 2013a. Impact of Al and Fe on the development of phenanthrene catabolism in soil. Journal of Soils and Sediments, 13, 1589–1599.

Obuekwe, I.S. & Semple, K.T. 2013b. Impact of Zn and Cu on the development of phenanthrene catabolism in soil. Environmental Monitoring and Assessment, 185, 10039–10047.

Oburger, E., Kirk, G.J.D., Wenzel, W.W., Puschenreiter, M. & Jones, D.L. 2009. Interactive effects of organic acids in the rhizosphere. Soil Biology and Biochemistry, 41, 449–457.

OECD. 2006. OECD Test Guideline 208: Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test. Guidelines for the Testing of Chemicals, Terrestrial Plant Test Seedling Emergence and Seedling Growth Test, 227, 1–21, (At: http://www.oecd-ilibrary.org/environment/test-no-208-terrestrial-plant-test-seedling-emergence-and-seedling-growth-test_9789264070066-en.).

Ogbonnaya, O.U., Adebisi, O.O. & Semple, K.T. 2014. The impact of biochar on the bioaccessibility of 14 C-phenanthrene in aged soil. Environ. Sci.: Processes Impacts, 16, 2635–2643, (At: http://xlink.rsc.org/?DOI=C4EM00396A.).

Ojeda-Morales, M.E., Domínguez-Domínguez, M., Hernández-Rivera, M.A. & Zavala-Cruz, J. 2015. Biosurfactant production by strains of azospirillum isolated from petroleum-contaminated sites. Water, Air, and Soil Pollution, 226.

Okere, U. V. & Semple, K.T. 2012. Biodegradation of PAHs in "Pristine" soils from different climatic regions. Journal of Bioremediation & Biodegradation, s1, 1–11.

Okolo, J.C. 2005. Enhancing crude oil degradation in a sandy soil: Effects of addition of poultry manure. Global Journal of Environmental Sciences, 4, 47–53.

Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede, T.O. 2007. The Effects of Spent Engine Oil on Soil Properties and Growth of Maize (Zea mays L .) No of seedlings that survived x 100 No of seedlings contaminat ed. Journal of Applied Sciences and Environmental Management, 11, 147–152.

Olson, P.E., Castro, A., Joern, M., Duteau, N.M., Pilon-Smits, E.A.H. & Reardon, K.F. 2007. Comparison of plant families in a greenhouse phytoremediation study on an aged polycyclic aromatic hydrocarbon-contaminated soil. International Journal of Environmental Quality, 36, 1461–1469.

Onifade, A.K. & Abubakar, F.A. 2007. Characterization of hydrocarbon-hegrading microorganisms isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. Research Journal of Microbiology, 2, 149–155, (At: http://www.scialert.net/abstract/?doi=jm.2007.149.155. Accessed: 25/1/2018).

Oonnittan, A., Shrestha, R.A. & Sillanpää, M. 2008. Effect of cyclodextrin on the remediation of hexachlorobenzene in soil by electrokinetic Fenton process. Separation and Purification Technology, 64, 314–320.

Ortega-Calvo, J.J., Harmsen, J., Parsons, J.R., Semple, K.T., Aitken, M.D., Ajao, C., Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R., Peijnenburg, W.J.G.M., Römbke, J., Streck, G. & Versonnen, B. 2015. From bioavailability science to regulation of organic chemicals. Environmental Science & Technology, 49, 10255–10264.

Ortega-González, D.K., Zaragoza, D., Aguirre-Garrido, J., Ram??rez-Saad, H., Hern??ndez-Rodr??guez, C. & Jan-Roblero, J. 2013. Degradation of benzene, toluene, and xylene isomers by a bacterial consortium obtained from rhizosphere soil of Cyperus sp. grown in a petroleum-contaminated area. Folia Microbiologica, 58, 569–577.

Osuji, L.C. & Nwoye, I. 2007. An appraisal of the impact of petroleum hydrocarbons on soil fertility : the Owaza experience. African Journal of Agricultural Research, 2, 318–324.

Osuji, L.C., Udoetok, I.A. & Ogali, R.E. 2006. Attenuation of petroleum hydrocarbons by weathering: A case study. Chemistry and Biodiversity, 3, 422–433.

Oudot, J., Dupont, J., Haloui, S. & Roquebert, M.F. 1993. Biodegradation potential of hydrocarbon-assimilating tropical fungi. Soil Biology and Biochemistry, 25, 1167–1173.

Ouvrard, S., Lapole, D. & Morel, J.L. 2006. Root exudates impact on phenanthrene availability. Water, Air, and Soil Pollution: Focus, 6, 343–352.

Ouvrard, S., Leglize, P. & Morel, J.L. 2014. PAH Phytoremediation: Rhizodegradation or Rhizoattenuation? International Journal of Phytoremediation, 16, 46–61.

Oyelami, A.O., Okere, U. V., Orwin, K.H., De Deyn, G.B., Jones, K.C. & Semple, K.T. 2013. Effects of plant species identity, diversity and soil fertility on biodegradation of phenanthrene in soil. Environmental Pollution, 173, 231–237.

Palittapongarnpim, M., Pokethitiyook, P., Upatham, E.S. & Tangbanluekal, L. 1998. Biodegradation of crude oil by soil microorganisms in the tropic. Biodegradation, 9, 83–90.

Panchenko, L., Muratova, A. & Turkovskaya, O. 2017. Comparison of the phytoremediation potentials of Medicago falcata L. And Medicago sativa L. in aged oil-sludge-contaminated soil. Environmental Science and Pollution Research, 24, 3117–3130.

Papadopoulos, A., Paton, G.I., Reid, B.J. & Semple, K.T. 2007. Prediction of PAH biodegradation in field contaminated soils using a cyclodextrin extraction technique. Journal of Environmental Monitoring, 9, 516.

Parrish, Z.D., Banks, M.K. & Schwab, A.P. 2005. Effect of root death and decay on dissipation of polycyclic aromatic hydrocarbons in the rhizosphere of yellow sweet clover and tall fescue. Journal of Environment Quality, 34, 207.

Pepper, I.L., Gerba, C.P. & Brusseau, M.L. 2006. Environmental and Pollution Sience. Elsevier/Academic Press.

Pérez-Armendáriz, B., Martínez-Carrera, D., Calixto-Mosqueda, M., Joel, A. & Rodríguez-Vázquez, R. 2010. Filamentous fungi remove weathered hydrocarbons from polluted soil of tropical México. Revista Internacional de Contaminacion Ambiental, 26, 193–199.

Pérez-Hernández, I., Ochoa-Gaona, S., Adams Schroeder, R.H., Rivera-Cruz, M.C. & Geissen, V. 2013. Tolerance of four tropical tree species to heavy petroleum contamination. Water, Air, and Soil Pollution, 224.

Pham, T.D., Shrestha, R.A. & Sillanpaa, M. 2010. Removal of hexachlorobenzene and phenanthrene from clayey soil by surfactant- and ultrasound-assisted electrokinetics. Journal of Environmental Engineering-Asce, 136, 739–742.

Phillips, L.A., Greer, C.W., Farrell, R.E. & Germida, J.J. 2009. Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments. Applied Soil Ecology, 42, 9–17.

Phillips, L.A., Greer, C.W. & Germida, J.J. 2006. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biology and Biochemistry, 38, 2822–2833.

Pignatello, J.J. 1998. Soil organic matter as a nanoporous sorbent of organic pollutants. Advances in Colloid and Interface Science, 76–77, 445–467.

Pignatello, J.J. & Xing, B. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. Environmental Science & Technology, 30, 1–11.

Pilon-Smits, E. 2005. Phytoremediation. Annual Review of Plant Biology, 56, 15–39.

Prokop, Z., Nečasová, A., Klánová, J. & Čupr, P. 2016. Bioavailability and mobility of organic contaminants in soil: new three-step ecotoxicological evaluation. Environmental Science and Pollution Research, 23, 4312–4319.

Qi, Q. & Zimmermann, W. 2005. Cyclodextrin glucanotransferase: From gene to applications. Applied Microbiology and Biotechnology, 66, 475–485.

Radwan, S.S., Al-Awadhi, H., Sorkhoh, N. a. & El-Nemr, I.M. 1998. Rhizospheric hydrocarbon-utilizing microorganisms as potential contributors to phytoremediation for the oil Kuwaiti desert. Microbiological Research, 153, 247–251.

Ramirez, N. & Cutright, T.J. 2001. Sorption-desorption of pyrene for Colombia and New Mexico soils. Polycyclic Aromatic Compounds, 18, 273–292.

Ramsay, J.A., Robertson, K., VanLoon, G., Acay, N. & Ramsay, B.A. 2005. Enhancement of PAH biomineralization rates by cyclodextrins under Fe(III)-reducing conditions. Chemosphere, 61, 733–740.

Rasse, D.P., Rumpel, C. & Dignac, M.F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil, 269, 341–356.

Reddy, K.R. 2013. Electrokinetic remediation of soils at complex contaminated sites: Technology status, challenges, and opportunities. Coupled Phenomena in Environmental Geotechnics, 131–147.

Reddy, K.R., Ala, P.R., Sharma, S. & Kumar, S.N. 2006. Enhanced electrokinetic remediation of contaminated manufactured gas plant soil. Engineering Geology, 85, 132–146.

Reid, B.J., Jones, K.C. & Semple, K.T. 2000a. Bioavailability of persistent organic pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environmental Pollution, 108, 103–112.

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D. & Semple, K.T. 2001. A simple 14C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. FEMS Microbiology Letters, 196, 141–146.

Reid, B.J., Semple, K.T. & Jones, K.C. 1998. Prediction of bioavailability of persistent organic pollutants by a novel extraction technique. Contaminated Soil ’98, Proceedings of the International FZK/TNO Conference on Contaminated Soil, 6th, Edinburgh, May 17-21, 1998, 2, 889–890.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2000b. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental Science & Technology, 34, 3174–3179.

Reid, B.J., Stokes, J.D., Jones, K.C. & Semple, K.T. 2004. Influence of hydroxypropyl-β-cyclodextrin on the extraction and biodegradation of phenanthrene in soil. Environmental Toxicology and Chemistry, 23, 550–556.

Reilley, K.A., Banks, M.K. & Schwab, A.P. 1996. Organic chemicals in the environment: Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality, 25, 212–219.

Rentz, J.A., Alvarez, P.J.J. & Schnoor, J.L. 2004. Repression of Pseudomonas putida phenanthrene-degrading activity by plant root extracts and exudates. Environmental Microbiology, 6, 574–583.

Rentz, J.A., Alvarez, P.J.J. & Schnoor, J.L. 2005. Benzo[a]pyrene co-metabolism in the presence of plant root extracts and exudates: Implications for phytoremediation. Environmental Pollution, 136, 477–484.

Rezek, J., in der Wiesche, C., Mackova, M., Zadrazil, F. & Macek, T. 2008. The effect of ryegrass (Lolium perenne) on decrease of PAH content in long term contaminated soil. Chemosphere, 70, 1603–1608.

Rhodes, A.H., Carlin, A. & Semple, K.T. 2008a. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science & Technology, 42, 740–745.

Rhodes, A.H., Dew, N.M. & Semple, K.T. 2008b. Relationship between cyclodextrin extraction and biodegradation of phenanthrene in soil. Environmental toxicology and chemistry / SETAC, 27, 1488–95.

Rhodes, A.H., Hofman, J. & Semple, K.T. 2008c. Development of phenanthrene catabolism in natural and artificial soils. Environmental Pollution, 152, 424–430.

Rhodes, A.H., McAllister, L.E., Chen, R. & Semple, K.T. 2010a. Impact of activated charcoal on the mineralisation of 14C-phenanthrene in soils. Chemosphere, 79, 463–469.

Rhodes, A.H., McAllister, L.E. & Semple, K.T. 2010b. Linking desorption kinetics to phenanthrene biodegradation in soil. Environmental Pollution, 158, 1348–1353.

Rhodes, A.H., Riding, M.J., McAllister, L.E., Lee, K. & Semple, K.T. 2012. Influence of activated charcoal on desorption kinetics and biodegradation of phenanthrene in soil. Environmental Science & Technology, 46, 12445–12451.

Richnow, H.H., Eschenbach, A., Mahro, B., Kästner, M., Annweiler, E., Seifert, R. & Michaelis, W. 1999. Formation of nonextractable soil residues: A stable isotope approach. Environmental Science & Technology, 33, 3761–3767.

Riding, M.J., Doick, K.J., Martin, F.L., Jones, K.C. & Semple, K.T. 2013. Chemical measures of bioavailability/bioaccessibility of PAHs in soil: Fundamentals to application. Journal of Hazardous Materials, 261, 687–700.

Rivera-Cruz, M.D.C., Ferrera-Cerrato, R., Sánchez-García, P., Volke-Haller, V., Fernández-Linares, L. & Rodríguez-Vázquez, R. 2004. Descontaminación de suelos con petróleo crudo

mediante microorganismos autóctonos y pasto alemán [echinochloa polystachya (H.B.K.) hitchc.]. Agrociencia, 38, 1–12.

Riveroll-Larios, J., Escalante-Espinosa, E., Fócil-Monterrubio, R.L. & Díaz-Ramírez, I.J. 2015. Biological Activity Assessment in Mexican Tropical Soils with Different Hydrocarbon Contamination Histories. Water, Air, & Soil Pollution, 226, 353.

Rizzo, A.C.L., Da Cunha, C.D., Santos, R.L.C., Santos, R.M., Magalhães, H.M., Leite, S.G.F. & Soriano, A.U. 2008a. Preliminary identification of the bioremediation limiting factors of a clay bearing soil contaminated with crude oil. Journal of the Brazilian Chemical Society, 19, 169–174.

Rizzo, A.C.L., Santos, R.M., Soriano, A.U., Landa, F. & Keite, S.G.F. 2008b. Monitored natural attenuation of tropical petroleum-bearing soils. In: International UFZ-Deltares/TNO Conference on Soil-Water Systems.

Rohrbacher, F. & St-Arnaud, M. 2016. Root exudation: The ecological driver of hydrocarbon rhizoremediation. Agronomy, 6, 19.

Rojas-Avelizapa, N., Olvera-Barrera, E. & Fernández-Linares, L. 2005. Feasibility study of bioremediation of a drilling-waste-polluted soil: Stimulation of microbial activities and hydrocarbon removal. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 40, 2189–2201.

Roldan, F., Maldonado, C., Guevara, C. & Cubillos, A.M. 2010. Natural attenuation of oily sludge used for the maintenance of an unpaved road in Arauca (Colombia). Bioremediation Journal, 14, 81–91.

Rorison, H. & Rorbinson, D. 1986. Mineral nutrition. In: Methods in Plant Ecology (eds. Moore, P.D. & Chapman, S.D.). Blackwell Scientific Publications, London, United Kingdom.

Rostami, I. & Juhasz, A.L. 2013. Bioaccessibility-based predictions for estimating PAH biodegradation efficacy - Comparison of model predictions and measured endpoints. International Biodeterioration and Biodegradation, 85, 323–330.

Ruiz-Marín, A., Zavala-Loria, J.C., Canedo-López, Y. & Cordova-Quiroz, A. V. 2013. Tropical bacteria isolated from oil-contaminated mangrove soil: bioremediation by natural attenuation and bioaugmentation. Revista Mexicana de Ingeniería Química, 12, 553–560.

Rutherford, P.M., Gray, M.R. & Dudas, M.J. 1997. Desorption of [14C]naphthalene from bioremediated and nonbioremediated soils contaminated with creosote compounds. Environmental Science and Technology, 31, 2515–2519.

Sabaté, J., Viñas, M. & Solanas, A.M. 2006. Bioavailability assessment and environmental fate of polycyclic aromatic hydrocarbons in biostimulated creosote-contaminated soil. Chemosphere, 63, 1648–1659.

Saenger, B.W. 1980. Cyclodextrin Inclusion Compounds in Research and Industry. 8, 344–362.

Salam, L.B., Obayori, O.S. & Olatoye, N.O. 2014. Biodegradation of anthracene by a novel actinomycete, Microbacterium sp. isolated from tropical hydrocarbon-contaminated soil. World Journal of Microbiology and Biotechnology, 30, 335–341.

Saleha, H. 2008. Literature overview: Microbial metabolism of high molecular weight polycyclic aromatic hydrocarbons. Remediation Journal, 18, 131–161.

Salt, D.E., Smith, R.D. & Raskin, I. 1998. Phytoremediation. Annual Review of Plant Biology, 49, 643–668.

Samanta, S.K., Singh, O. V & Jain, R.K. 2002. Polycyclic aromatic hydrocarbons : environmental pollution and bioremediation. Trends in Biotechnology, 20, 243–248.

Sander, M. & Pignatello, J.J. 2007. On the reversibility of sorption to black carbon: Distinguishing true hysteresis from artificial hysteresis caused by dilution of a competing adsorbate. Environmental Science & Technology, 41, 843–849.

Sangabriel, W., Ferrera-Cerrato, R., Trejo-Aguilar, D., Mendoza-López, M.R., Cruz-Sánchez, J.S., López-Ortiz, C., Delgadillo-Martínez, J. & Alarcón, A. 2006. Tolerancia y capacidad de fitorremediación de combustóleo en el suelo por seis especies vegetales. Revista Internacional de Contaminacion Ambiental, 22, 63–73.

Schmidt, M.W.I. & Noack, A.G. 2000. Black carbon in soils and sediments' Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles, 14, 777–793.

Schneider, R.P., Morano, S.C., Gigena, M.A.C., Missawa, S.K., Rocha, R.C.S., Da Silva, L.R., Ellert, N., Kataoka, S., Katsuragi, C., Da Silva Rosa, C. & De Oliveira Filho, L.C. 2006. Contamination levels and preliminary assessment of the technical feasibility of employing natural attenuation in 5 priority areas of Presidente Bernardes Refinery in Cubatão, São Paulo, Brazil. Environmental Monitoring and Assessment, 116, 21–52.

Schneiderman, E. & Stalcup, A.M. 2000. Cyclodextrins: A versatile tool in separation science. In: Journal of Chromatography B: Biomedical Sciences and Applications, pp. 83–102.

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L. & Carreira, L.H. 1995. Phytoremediation of organic and nutrient contaminants. Environmental Science & Technology, 29, 318–323.

Schwab, a. P., Al-Assi, a. a. & Banks, M.K. 1998. Adsorption of Naphthalene onto Plant Roots. Journal of Environment Quality, 27, 220.

Semple, K.T., Dew, N.M., Doick, K.J. & Rhodes, A.H. 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environmental Pollution, 140, 164–172.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. & Harms, H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science & Technology, 38, 228A–231A.

Semple, K.T., Doick, K.J., Wick, L.Y. & Harms, H. 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. Environmental Pollution, 150, 166–176.

Semple, K.T., Morriss, a. W.J. & Paton, G.I. 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54, 809–818.

Semple, K.T., Reid, B.J. & Fermor, T.R. 2001. Impact of composting strategies on the treatment of soil contaminated with diesel fuel.pdf. Environmental Pollution, 112, 269–283.

Semple, K.T., Riding, M.J., McAllister, L.E., Sopena-Vazquez, F. & Bending, G.D. 2013. Impact of black carbon on the bioaccessibility of organic contaminants in soil. Journal of Hazardous Materials, 261, 808–816.

Shieh, W.J. & Hedges, A.R. 1996. Properties and applications of cyclodextrins. Journal of Macromolecular Science - Pure and Applied Chemistry, 33, 673–683.

Shin, D. & Nam, K. 2014. Potential use of a self-dying reporter bacterium to determine the bioavailability of aged phenanthrene in soil: Comparison with physicochemical measures. Journal of Hazardous Materials, 265, 1–7.

Shukla, K.P., Sharma, S., Singh, N.K., Singh, V. & Tiwari, K. 2011. Nature and role of root exudates : Efficacy in bioremediation. African Journal of Biotechnology, 10, 9717–9724.

Siciliano, S.D. & Germida, J.J. 1998. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environmental Reviews, 6, 65–79.

Siddiqui, S. & Adams, W.A. 2002. The fate of diesel hydrocarbons in soils and their effect on the germination of perennial ryegrass. Environmental Toxicology, 17, 49–62.

Singh, A., Kuhad, R.C. & Ward, O.P. 2009. Biological Remediation of Soil: An Overview of Global Market and Available Technologies. pp. 1–19. Springer, Berlin, Heidelberg.

Singh, M., Sharma, R. & Banerjee, U.C. 2002. Biotechnological applications of cyclodextrins. Biotechnology Advances, 20, 341–359.

Sivaraman, C., Ganguly, A. & Mutnuri, S. 2010. Biodegradation of hydrocarbons in the presence of cyclodextrins. World Journal of Microbiology and Biotechnology, 26, 227–232.

Šmídová, K., Hofman, J., Ite, A.E. & Semple, K.T. 2012. Fate and bioavailability of 14C-pyrene and 14C- lindane in sterile natural and artificial soils and the influence of aging. Environmental Pollution, 171, 93–98.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Alder, J. 2006. Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues. Environmental Pollution, 141, 519–525.

Smith, M.J., Flowers, T.H., Duncan, H.J. & Saito, H. 2011. Study of PAH dissipation and phytoremediation in soils: Comparing freshly spiked with weathered soil from a former coking works. Journal of Hazardous Materials, 192, 1219–1225.

Somtrakoon, K., Suanjit, S., Pokethitiyook, P., Kruatrachue, M., Lee, H. & Upatham, S. 2008. Enhanced biodegradation of anthracene in acidic soil by inoculated Burkholderia sp. VUN10013. Current Microbiology, 57, 102–106.

Song, Y., Wang, F., Bian, Y., Kengara, F.O., Jia, M., Xie, Z. & Jiang, X. 2012. Bioavailability assessment of hexachlorobenzene in soil as affected by wheat straw biochar. Journal of Hazardous Materials, 217–218, 391–397.

Song, Y., Wang, F., Yang, X., Liu, C., Kengara, F.O., Jin, X. & Jiang, X. 2011. Chemical extraction to assess the bioavailability of chlorobenzenes in soil with different aging periods. Journal of Soils and Sediments, 11, 1345–1354.

Sopeña, F., Semple, K., Sohi, S. & Bending, G. 2012. Assessing the chemical and biological accessibility of the herbicide isoproturon in soil amended with biochar. Chemosphere, 88, 77–83.

de Souza Pohren, R., de Oliveira Leite, D.A.N., de Franceschi de Angelis, D. & Vargas, V.M.F. 2016. Performance of simulated bioremediation in real samples of soils contaminated with PAHs. Water, Air, and Soil Pollution, 227, 1–14.

Stokes, J.D., Wilkinson, A., Reid, B.J., Jones, K.C. & Semple, K.T. 2005. Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl-β-cyclodextrin extraction technique. Environmental Toxicology and Chemistry, 24, 1325.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2007a. Importance of chemical structure on the development of hydrocarbon catabolism in soil. FEMS Microbiology Letters, 272, 120–126.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2007b. Microbe-aliphatic hydrocarbon interactions in soil: Implications for biodegradation and bioremediation. Journal of Applied Microbiology, 102, 1239–1253.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2008. Linking chemical extraction to microbial degradation of 14C-hexadecane in soil. Environmental Pollution, 156, 474–481.

Stroud, J.L., Paton, G.I. & Semple, K.T. 2009. Predicting the biodegradation of target hydrocarbons in the presence of mixed contaminants in soil. Chemosphere, 74, 563–567.

Sun, S. & Boyd, S.A. 1991. Sorption of polychlorobiphenyl (PCB) congeners by residual PCB-Oil phases in soils. Journal of Environment Quality, 20, 557.

Sun, T.R., Cang, L., Wang, Q.Y., Zhou, D.M., Cheng, J.M. & Xu, H. 2010. Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil. Journal of Hazardous Materials, 176, 919–925.

Sun, M., Fu, D., Teng, Y., Shen, Y., Luo, Y., Li, Z. & Christie, P. 2011. In situ phytoremediation of PAH-contaminated soil by intercropping alfalfa (Medicago sativa L.) with tall fescue (Festuca arundinacea Schreb.) and associated soil microbial activity. Journal of Soils and Sediments, 11, 980–989.

Sun, B., Gao, Y., Liu, J. & Sun, Y. 2012a. The impact of different root exudate components on phenanthrene availability in soil. Soil Science Society of America Journal Abstract - Soil Chemistry, 76, 2041 2050.

Sun, B., Ling, W. & Wang, Y. 2013a. Can root exudate components influence the availability of pyrene in soil? Journal of Soils and Sediments, 13, 1161–1169.

Sun, W.H., Lo, J.B., Robert, F.M., Ray, C. & Tang, C.-S. 2004. Phytoremediation of petroleum hydrocarbons in tropical coastal soils. I. Selection of promising woody plants. Environmental Science and Pollution Research International, 11, 260–266.

Sun, M., Luo, Y., Christie, P., Jia, Z., Li, Z. & Teng, Y. 2012b. Methyl-β-cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and associated microbial activity in contaminated soil. Journal of Environmental Sciences, 24, 926–933.

Sun, M., Luo, Y., Teng, Y., Jia, Z., Li, Z. & Deng, S. 2013b. Remediation of polycyclic aromatic hydrocarbon and metal-contaminated soil by successive methyl-β-cyclodextrin-enhanced soil washing-microbial augmentation: A laboratory evaluation. Environmental Science and Pollution Research, 20, 976–986.

Supaphol, S., Panichsakpatana, S., Trakulnaleamsai, S., Tungkananuruk, N., Roughjanajirapa, P. & O'Donnell, A.G. 2006. The selection of mixed microbial inocula in environmental biotechnology: Example using petroleum contaminated tropical soils. Journal of Microbiological Methods, 65, 432–441.

Sutton, N.B., Maphosa, F., Morillo, J.A., Al-Soud, W.A., Langenhoff, A.A.M., Grotenhuis, T., Rijnaarts, H.H.M. & Smidt, H. 2013. Impact of long-term diesel contamination on soil microbial community structure. Applied and Environmental Microbiology, 79, 619–630.

Sverdrup, L.E., Hagen, S.B., Krogh, P.H. & van Gestel, C.A.M. 2007. Benzo(a)pyrene shows low toxicity to three species of terrestrial plants, two soil invertebrates, and soil-nitrifying bacteria. Ecotoxicology and Environmental Safety, 66, 362–368.

Swindell, A.L. & Reid, B.J. 2006a. Influence of diesel concentration on the fate of phenanthrene in soil. Environmental Pollution, 140, 79–86.

Swindell, A.L. & Reid, B.J. 2006b. Comparison of selected non-exhaustive extraction techniques to assess PAH availability in dissimilar soils. Chemosphere, 62, 1126–1134.

Swindell, A.L. & Reid, B.J. 2007. The influence of a NAPL on the loss and biodegradation of 14C-phenanthrene residues in two dissimilar soils. Chemosphere, 66, 332–339.

Szejtli, J. 1998. Introduction and general overview of cyclodextrin chemistry. Chemical Reviews, 98, 1743–1754.

Szente, L. & Szejtli, J. 2004. Cyclodextrins as food ingredients. Trends in Food Science and Technology, 15, 137–142.

Tan, K.H. 2009. Environmental Soil Science, Third Edition. CRC Press.

Tang, J., Liste, H.H. & Alexander, M. 2002. Chemical assays of availability to earthworms of polycyclic aromatic hydrocarbons in soil. Chemosphere, 48, 35–42.

Tellechea, F.R.F., Martins, M.A., da Silva, A.A., da Gama-Rodrigues, E.F. & Martins, M.L.L. 2016. Use of sugarcane filter cake and nitrogen, phosphorus and potassium fertilization in the process of bioremediation of soil contaminated with diesel. Environmental Science and Pollution Research, 23, 18027–18033.

Thavamani, P., Megharaj, M. & Naidu, R. 2012. Multivariate analysis of mixed contaminants (PAHs and heavy metals) at manufactured gas plant site soils. Environmental Monitoring and Assessment, 184, 3875–3885.

Thijs, S., Sillen, W., Weyens, N. & Vangronsveld, J. 2017. Phytoremediation: State-of-the-art and a key role for the plant microbiome in future trends and research prospects. International Journal of Phytoremediation, 19, 23–38.

Thompson, O.A., Wolf, D.C., Mattice, J.D. & Thoma, G.J. 2008. Influence of nitrogen addition and plant root parameters on phytoremediation of pyrene-contaminated soil. Water, Air, and Soil Pollution, 189, 37–47.

Tiessen, H., Cuevas, E. & Chacon, P. 1994. The role of soil organic matter in sustaining soil fertility. Nature, 371, 783–785.

Torres, L.G., Climent, M., Saquelares, J., Bandala, E.R., Urquiza, G. & Iturbe, R. 2007. Characterization and treatability of a contaminated soil from an oil exploration zone. International Journal of Environmental Science and Technology, 4, 311–322.

Towell, M.G., Browne, L.A., Paton, G.I. & Semple, K.T. 2011a. Impact of carbon nanomaterials on the behaviour of 14C-phenanthrene and 14C-benzo-[a] pyrene in soil. Environmental Pollution, 159, 706–715.

Towell, M.G., Paton, G.I. & Semple, K.T. 2011b. The biodegradation of cable oil components: Impact of oil concentration, nutrient addition and bioaugmentation. Environmental Pollution, 159, 3777–3783.

Trapp, S. & Karlson, U. 2001. Aspects of phytoremediation of organic pollutants. Journal of Soils and Sediments, 1, 37–43.

Trindade, P.V.O., Sobral, L.G., Rizzo, A.C.L., Leite, S.G.F. & Soriano, A.U. 2005. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: A comparison study. Chemosphere, 58, 515–522.

U. S. Environmental Protection Agency. 2012. OCSPP 850.4100: Seedling Emergence and Seedling Growth. EPA Ecological Effects Test Guidelines.

Uekama, K., Hirayama, F. & Irie, T. 1998. Cyclodextrin drug carrier systems. Chemical Reviews, 98, 2045–2076 ST–Cyclodextrin Drug Carrier Systems.

Uren, N.C. 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: The Rhizosphere: Biochemistry and Organic Substances

at the Soil-Plant Interface (eds. Pinton, R., Varanini, Z. & Nannipieri, P.), pp. 1–21. Marcel Dekker, Inc.

US Environmental Protection Agency. 2013. Priority Pollutant List. (At: https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf. Accessed: 6/2/2018).

Del Valle, E.M.M. 2004. Cyclodextrins and their uses: A review. Process Biochemistry, 39, 1033–1046.

Vasudevan, N. & Rajaram, P. 2001. Bioremediation of oil sludge-contaminated soil. Environment International, 26, 409–411.

Vázquez-Cuevas, G.M. & Semple, K.T. 2016. Measurement of Hydrocarbon Bioavailability in Soil. In: Hydrocarbon and Lipid Microbiology Protocols - Springer Protocols Handbooks (eds. McGenity, T., Timmis, K. & Nogales, B.), pp. 231–246. Springer, Berlin, Heidelberg.

Vázquez-Cuevas, G.M., Stevens, C.J. & Semple, K.T. 2018. Enhancement of14C-phenanthrene mineralisation in the presence of plant-root biomass in PAH-NAPL amended soil. International Biodeterioration and Biodegradation, 126, 78–85, (At: http://dx.doi.org/10.1016/j.ibiod.2017.09.021.).

van Veen, J.A., van Overbeek, L.S. & van Elsas, J.D. 1997. Fate and activity of microorganisms introduced into soil. Microbiology and molecular biology reviews : MMBR, 61, 121–35.

Viglianti, C., Hanna, K., De Brauer, C. & Germain, P. 2006. Removal of polycyclic aromatic hydrocarbons from aged-contaminated soil using cyclodextrins: Experimental study. Environmental Pollution, 140, 427–435.

Villaverde, J. 2007. Time-dependent sorption of norflurazon in four different soils: Use of β-cyclodextrin solutions for remediation of pesticide-contaminated soils. Journal of Hazardous Materials, 142, 184–190.

Villaverde, J., Pérez-Martínez, J.I., Maqueda, C., Ginés, J.M. & Morillo, E. 2005. Inclusion complexes of α- and γ-cyclodextrins and the herbicide norflurazon: I. Preparation and characterisation. II. Enhanced solubilisation and removal from soils. Chemosphere, 60, 656–664.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M., Laiz, L., Saiz-Jimenez, C., Sanchez-Trujillo, M.A. & Morillo, E. 2012. Enhanced mineralization of diuron using a cyclodextrin-based bioremediation technology. Journal of Agricultural and Food Chemistry, 60, 9941–9947.

Villaverde, J., Posada-Baquero, R., Rubio-Bellido, M. & Morillo, E. 2013a. Effect of hydroxypropyl-β-cyclodextrin on diuron desorption and mineralisation in soils. Journal of Soils and Sediments, 13, 1075–1083.

Villaverde, J., Rubio-Bellido, M., Posada-Baquero, R., Madrid, F. & Morillo, E. 2013b. Hydroxypropyl-β-cyclodextrin-based extraction for diuron bioaccessibility in an artificially

contaminated soil. International Journal of Environmental Analytical Chemistry, 93, 1620–1627.

Vogt, K. a, Vogt, D.J. & Bloomfield, J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant and Soil, 200, 71–89.

Volkering, F. & Breure, A.M. 2003. Biodegradation and general aspects of bioavailability. In: PAHs: An ecotoxicological perspective (ed. Douben, P.), p. 391. Wiley.

Van Der Wal, L., Jager, T., Fleuren, R.H.L.J., Barendregt, A., Sinnige, T.L., Van Gestel, C.A.M. & Hermens, J.L.M. 2004. Solid-phase microextraction to predict bioavailability and accumulation of organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil. Environmental Science & Technology, 38, 4842–4848.

Wan, C., Du, M., Lee, D.J., Yang, X., Ma, W. & Zheng, L. 2011. Electrokinetic remediation and microbial community shift of β-cyclodextrin-dissolved petroleum hydrocarbon-contaminated soil. Applied Microbiology and Biotechnology, 89, 2019–2025.

Wan, J., Yuan, S., Chen, J., Li, T., Lin, L. & Lu, X. 2009. Solubility-enhanced electrokinetic movement of hexachlorobenzene in sediments: A comparison of cosolvent and cyclodextrin. Journal of Hazardous Materials, 166, 221–226.

Wang, X. & Brusseau, M.L. 1993. Solubilization of some low-polarity organic compounds by hydroxypropyl- β-cyclodextrin. Environmental Science & Technology, 27, 2821–2825.

Wang, X. & Brusseau, M.L. 1995. Simultaneous complexation of organic compounds and heavy metals by a modified cyclodextrin. Environmental Science & Technology, 29, 2632–2635.

Wang, J.M., Maier, R.M. & Brusseau, M.L. 2005. Influence of hydroxypropyl-β-cyclodextrin (HPCD) on the bioavailability and biodegradation of pyrene. Chemosphere, 60, 725–728.

Wang, J.M., Marlowe, E.M., Miller-Maier, R.M. & Brusseau, M.L. 1998. Cyclodextrin-enhanced biodegradation of phenanthrene. Environmental Science & Technology, 32, 1907–1912.

Wang, C. hui, Wu, S. hua, Zhou, S. lu, Wang, H., Li, B. jie, Chen, H., Yu, Y. na & Shi, Y. xing. 2015. Polycyclic aromatic hydrocarbons in soils from urban to rural areas in Nanjing: Concentration, source, spatial distribution, and potential human health risk. Science of the Total Environment, 527–528, 375–383.

Wang, G., Xu, W., Wang, X. & Huang, L. 2012. Glycine-β-cyclodextrin–enhanced electrokinetic removal of atrazine from contaminated soils. Environmental Engineering Science, 29, 406–411.

Wang, X., Yu, X. & Bartha, R. 1990. Effect of bioremediation on Polycyclic Aromatic Hydrocarbon residues in soil. Environmental Science & Technology, 24, 1086–1089.

Wei, R., Ni, J., Li, X., Chen, W. & Yang, Y. 2017. Dissipation and phytoremediation of polycyclic aromatic hydrocarbons in freshly spiked and long-term field-contaminated soils. Environmental Science and Pollution Research, 24, 7994–8003.

von der Weid, I., Marques, J.M., Cunha, C.D., Lippi, R.K., dos Santos, S.C.C., Rosado, A.S., Lins, U. & Seldin, L. 2007. Identification and biodegradation potential of a novel strain of Dietzia cinnamea isolated from a petroleum-contaminated tropical soil. Systematic and Applied Microbiology, 30, 331–339.

Wenzel, W.W. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant and Soil, 321, 385–408.

White, J.C., Kelsey, J.W., Hatzinger, P.B. & Alexander, M. 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. Environmental Toxicology and Chemistry, 16, 2040–2045.

White, J.C., Mattina, M.I., Lee, W.Y., Eitzer, B.D. & Iannucci-Berger, W. 2003. Role of organic acids in enhancing the desorption and uptake of weathered p ,p -DDE by Cucurbita pepo. Environmental Pollution, 124, 71–80.

White, P.M., Wolf, D.C., Thoma, G.J. & Reynolds, C.M. 2006. Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. Water, Air, and Soil Pollution, 169, 207–220.

Widada, J., Nojiri, H., Kasuga, K., Yoshida, T., Habe, H. & Omori, T. 2002. Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. Applied Microbiology and Biotechnology, 58, 202–209.

Wilcke, W., Amelung, W., Krauss, M., Martius, C., Bandeira, A. & Garcia, M. 2003. Polycyclic aromatic hydrocarbon (PAH) patterns in climatically different ecological zones of Brazil. Organic Geochemistry, 34, 1405–1417.

Wild, S.R. & Jones, K.C. 1995. Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventtory and budget. Environmental Pollution, 88, 91–108.

Wilson, S.C. & Jones, K.C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environmental pollution (Barking, Essex : 1987), 81, 229–249.

Wong, K.W., Toh, B.A., Ting, Y.P. & Obbard, J.P. 2005. Biodegradation of phenanthrene by the indigenous microbial biomass in a zinc amended soil. Letters in Applied Microbiology, 40, 50–55.

Wu, G., Kechavarzi, C., Li, X., Sui, H., Pollard, S.J.T. & Coulon, F. 2013. Influence of mature compost amendment on total and bioavailable polycyclic aromatic hydrocarbons in contaminated soils. Chemosphere, 90, 2240–2246.

Xia, X., Li, Y., Zhou, Z. & Feng, C. 2010. Bioavailability of adsorbed phenanthrene by black carbon and multi-walled carbon nanotubes to Agrobacterium. Chemosphere, 78, 1329–1336.

Xu, S.Y., Chen, Y.X., Wu, W.X., Wang, K.X., Lin, Q. & Liang, X.Q. 2006. Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. Science of the Total Environment, 363, 206–215.

Yan, J., Wang, L., Fu, P.P. & Yu, H. 2004. Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 557, 99–108.

Yang, Y., Chun, Y., Shang, G. & Huang, M. 2004. pH-dependence of pesticide adsorption by wheat-residue-derived black carbon. Langmuir, 20, 6736–6741.

Yang, Y. & Sheng, G. 2003. Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. Environmental Science & Technology, 37, 3635–3639.

Yeung, A.T. & Gu, Y.-Y. 2011. A review on techniques to enhance electrochemical remediation of contaminated soils. Journal of Hazardous Materials, 195, 11–29.

Yeung, P.Y., Johnson, R.L. & Xu, J.G. 1997. Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. Journal of Environment Quality, 26, 1511.

Yoshitomi, K.. & Shann, J.. 2001. Corn (Zea mays L.) root exudates and their impact on 14C-pyrene mineralization. Soil Biology and Biochemistry, 33, 1769–1776.

Yuan, S., Tian, M. & Lu, X. 2006. Electrokinetic movement of hexachlorobenzene in clayed soils enhanced by Tween 80 and β-cyclodextrin. Journal of Hazardous Materials, 137, 1218–1225.

Zavala-Cruz, J., Gavi-Reyes, F., Adams-Schroeder Randy Howard, Ferrera-Cerrato, R., Palma-López, D.J. & Vaquera-Huerta, Humberto Domínguez-Ezquivel, J.M. 2005. Derrames de petróleo en suelos y adaptación de pastos tropicales en El Activo Cinco Presidentes, Tabasco, México. Terra Latinoamericana, 23, 293–302.

Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, T.M., Miltner, A. & Schroth, G. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma, 79, 117–161.

Zhang, Y., Maier, W.J. & Miller, R.M. 1997. Effect of rhamnolipids on the dissolution, bioavailability, and biodegradation of phenanthrene. Environmental Science & Technology, 31, 2211–2217.

Zhang, Z.X., Zhu, Y.X., Li, C.M. & Zhang, Y. 2012. Investigation into the causes for the changed biodegradation process of dissolved pyrene after addition of hydroxypropyl-β-cyclodextrin (HPCD). Journal of Hazardous Materials, 243, 139–145.

Zhu, X., Jin, L., Sun, K., Li, S., Li, X. & Ling, W. 2016. Phenanthrene and pyrene modify the composition and structure of the cultivable endophytic bacterial community in ryegrass (Lolium multiflorum lam). International Journal of Environmental Research and Public Health, 13.

Zhu, Y., Zhang, S., Huang, H. & Wen, B. 2009. Effects of maize root exudates and organic acids on the desorption of phenanthrene from soils. Journal of Environmental Sciences, 21, 920–926.

Zwiernik, M.J., Quensen, J.F. & Boyd, S.A. 1999. Residual petroleum in sediments reduces the bioavailability and rate of reductive dechlorination of aroclor 1242. Environmental Science & Technology, 33, 3574–3578.