On the way forward into bioaccessibility implementation

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The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvellous structure of reality.

Albert Einstein 1879 - 1955

CONTENTS

ABSTRACT

This thesis presents an investigation into the bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) from both laboratory and regulatory perspectives. The concept of bioaccessibility, its applications and incorporation within broad aspects of risk-based contaminated land management is reviewed (Chapter 1). A novel approach (subcritical water extraction) developed to reflect the PAH-bioaccessible fraction is presented (Chapter 2 and 3). Subcritical water extraction at the temperature of 160 ºC predicted bioaccessibility to microorganisms of ^{14}C-phenanthrene-associated activity for majority of determinations in dissimilar spiked soils (Chapter 2). Subcritical water extractions reflected PAH desorption processes (Chapter 3) and bioaccessibility to microorganisms of PAHs in multi-contaminant matrices, both spiked and genuinely contaminated, was reflected by subcritical water extraction yet not predicted directly (Chapter 4). A comparison of suitability for bioaccessibility prediction of four different non-exhaustive techniques is also presented along with a cost-benefit analysis for each method (Chapter 4). A range of hydrocarbon impacted soils and sediments, both spiked and genuinely contaminated, were used within these investigations. Results indicated that a non-exhaustive aqueous hydroxypropyl-β-cyclodextrin extraction provided the best prediction of PAH bioaccessibility to microorganisms but at the greatest cost.

A questionnaire- and interview-based survey was undertaken to better understand real-world implementation of bioaccessibility by decision-makers (Chapter 5). Limitations hampering the use of bioaccessibility as a decision-support tool within contaminated land decision-making were identified. The guidelines used for evaluation of PAH-contaminated land and the extent of bioremediation application in

order to regenerate PAH-contaminated land were also investigated. Findings of the questionnaire indicated that bioaccessibility has been perceived as a useful tool that facilitates contaminated land management by 70.2% of English and Welsh Contaminated Land Officers participating in the survey. Lack of statutory guidance was indicated by 78.2% of the survey participants as the factor hampering implementation of bioaccessibility. ‘Never’ was the most common (44.4%) answer to the question regarding the frequency of using bioremediation in order to clean-up PAH-contaminated land.

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I would never be where I am without them.

INTRODUCTION

CONTAMINATED LAND: Scale of the problem and legislation

Industrialization that has occurred since the mid-nineteenth century has left a legacy of land contamination. For example, in England and Wales contaminated land has resulted from a multitude of large-scale industrial uses, such as gasworks, mining, foundries, chemical production and shipbuilding facilities, in addition to smaller urban activities, such as petrol stations, dry-cleaning ventures and transport-associated facilities (Wild and Jones, 1995; Rivett et al., 2002). The exact identification and evaluation of contaminated sites can be problematic due, for instance, to a lack of records on the location of sites where wastes were deposited, prior to the introduction of a waste licensing regime in 1976 (Rivett et al., 2002). It has been suggested that more than 100 000 contaminated sites may exist in England and Wales (Rivett et al., 2002). Of these sites, from 5000 to 20 000 sites may pose a 'significant possibility of significant harm' to human health and the environment (Hankard et al., 1999). These sites have been estimated to amount to 300 000 hectares or approximately 2% of the land area of England and Wales (Environment Agency, 2009).

In the 1990s, an increased demand for housing, coupled with the need to protect rural areas, triggered the re-use of 'brownfield' land. Brownfield land is defined as a site that is, or was, occupied by a permanent structure (excluding agricultural or forestry buildings), and has become vacant, under-used or derelict hence may be contaminated (ASC, 2008). Currently, development of brownfield land is the United Kingdom Government's priority (ASC, 2008). Setting the growth targets for new homes to three million by 2020, the Government has stated that brownfield development is expected to provide a significant proportion of the land needed (ASC, 2008). As some of the brownfield sites might be contaminated there is a need for their appropriate assessment and possible remediation.

In the United Kingdom, contaminated land is regulated by several interlocking legislative regimes. These include: Part IIA of the Environment Act 1995 (hereafter 'Part IIA'), the Town and Country Planning Act 1990, and; Integrated Pollution Prevention and Control, and waste management licensing. Part IIA and the planning regime are the two main policy processes constituting the core of the contaminated land regulatory system. Whilst the planning regime relating to the land use change is the principal regime that most of the remediation and development of contaminated sites is considered under (Environment Agency, 2009), Part IIA, addressing the current use of the land, provides a statutory definition of contaminated land. Furthermore, Part IIA embodies a set of formalized concepts and principles, such as 'significant pollutant linkage' (see below), which are also required to be applied by the planning system. Part IIA statutorily defines contaminated land as '…any land which appears to the local authority in whose area it is situated to be in such a condition, by reasons of substances in, on or under the land, that:

a. significant harm is being caused or there is a significant possibility of such harm being caused or
b. pollution of controlled waters is being or likely to be caused' (Defra, 2006).

This definition of contaminated land stipulates that harm to health and the environment arises not from the mere presence of contaminating substances but from a 'significant pollutant linkage' (Catney et al., 2006). Thus, for a site to be determined as contaminated, the source of contamination in, on or under the land must be demonstrated to pose a 'significant possibility of significant harm' to the receptor (humans, ecosystem, buildings or controlled waters) by means of a pathway. This risk-based approach underpins the United Kingdom's contaminated land regulation. Risk-based approaches offering improved regulatory efficiency, through proportionality of regulatory enforcement, target resources toward the greatest threats to the environment (Evans et al., 2006). A risk-based approach to contaminated land prevents disproportionate effects of the land blight and prioritizes remediation needs, thereby avoiding the unsustainable removal of contaminants from soil, irrespective of the risk they pose. Also, risk-based approaches can generate new insights about risk and improve decision-making (Rothstein et al., 2006). From a contaminated land perspective, risk-based regulation has provided incentives for improving knowledge on site-investigations and chemical analyses, contaminants fate, transport and toxicity effects (Rothstein et al., 2006). Furthermore, the contaminated land regime has recently moved into a new era where environmental decisions must be 'socially-robust' (transparent and participative towards society) within a context of sustainable development (Gibbons, 1999; Pollard et al., 2004).

The ongoing evolution of the contaminated land regulation was also evident throughout the course of this research. In 2008, soil guideline values were withdrawn and the new 'Guidance on the legal definition of contaminated land' was published by the Department for Environment Food and Rural Affairs (DEFRA, 2008) along with the new model for calculating soil assessment criteria (Environment Agency, 2009). The literature review on the contaminated land regime in the United Kingdom (Chapter 1) describes contaminated land guidance before the major modifications, whilst Chapter 5 and the Appendix present the most recent situation and the English and Welsh Local Authorities perspectives towards contaminated land regulations.

POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR INTERACTIONS WITH SOIL

This thesis is mainly concerned with a class of organic contaminants known as polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous in the environment and are continuously generated by incomplete combustion of organic materials from both natural sources, such as volcanic eruptions and forest fires, and anthropogenic activities (fossil fuel combustion). In addition, PAHs have been naturally produced from geochemical processes, such as thermal geologic reactions associated with fossil fuel formation (Achten and Hofmann, 2009) and from biogenic production by living organisms, such as plants, termites and/or microorganisms associated with them (Bandowe et al., 2009). It is, however, the anthropogenic source that is the major cause of environmental pollution from PAHs and the focus of remediation activities (Bamforth and Singleton, 2005). PAHs belong to a class of organic compounds that consist of two or more fused benzene rings and/or pentacyclic molecules arranged in a variety of structural configurations (e.g. linear, angular, cluster). In addition to their pervasiveness, PAHs are of concern due to their genotoxic properties. In 1761, physician John Hill first recognized the link between the snuff and nasal cancer (Cerniglia, 1984). Following Hill’s findings, research into PAH toxicity continued, resulting in the identification of their carcinogenic, mutagenic and teratogenic properties (Fisher, 1999). Consequently, 16 PAHs have been classified by the United States Environmental Protection Agency as ‘priority pollutants’, and have become a focus for scientific research.

The fate of PAHs in the environment is primarily governed by their physico-chemical properties. Some of the key physico-chemical properties of PAHs include their octanol-water partition coefficient (Kow) and vapor pressure. The octanol-water partition coefficient (usually expressed logarithmically) is the ratio of a concentration of a compound in octanol and a concentration in water, at a given temperature, and is used as a measure of PAH hydrophobicity. In general, as the molecular weight of PAHs increases together with the log Kow value, the aqueous solubility of PAHs decreases. The vapor pressure (tendency of molecules or atoms to escape from liquid or solid) of PAHs is rather low, except for 2-rings PAHs, therefore, most PAHs do not tend to readily volatilize. On account of their physico-chemical properties, PAHs persist in the environment with soil being their main repository (Semple et al., 2005).

In aquatic environments, PAHs become rapidly associated with sediments where they may become buried, re-suspended or/and bioaccumulated (Cerniglia, 1992; Bamforth and Singleton, 2005). Mechanisms governing the fate of PAHs in the soil environment are also relevant in controlling PAH behavior in sediments yet may differ with respect to the extent of the process (Overcash et al., 2005). For instance, sediments exhibit lower extents of sorption of organic contaminants into the matrix than soils (Overcash et al., 2005).

When PAHs enter soil, they are subjected to a number of processes, for instance volatilization and leaching, in addition to sequestration, diffusion and entrapment, which control the environmental persistence of these compounds (Figure 1).

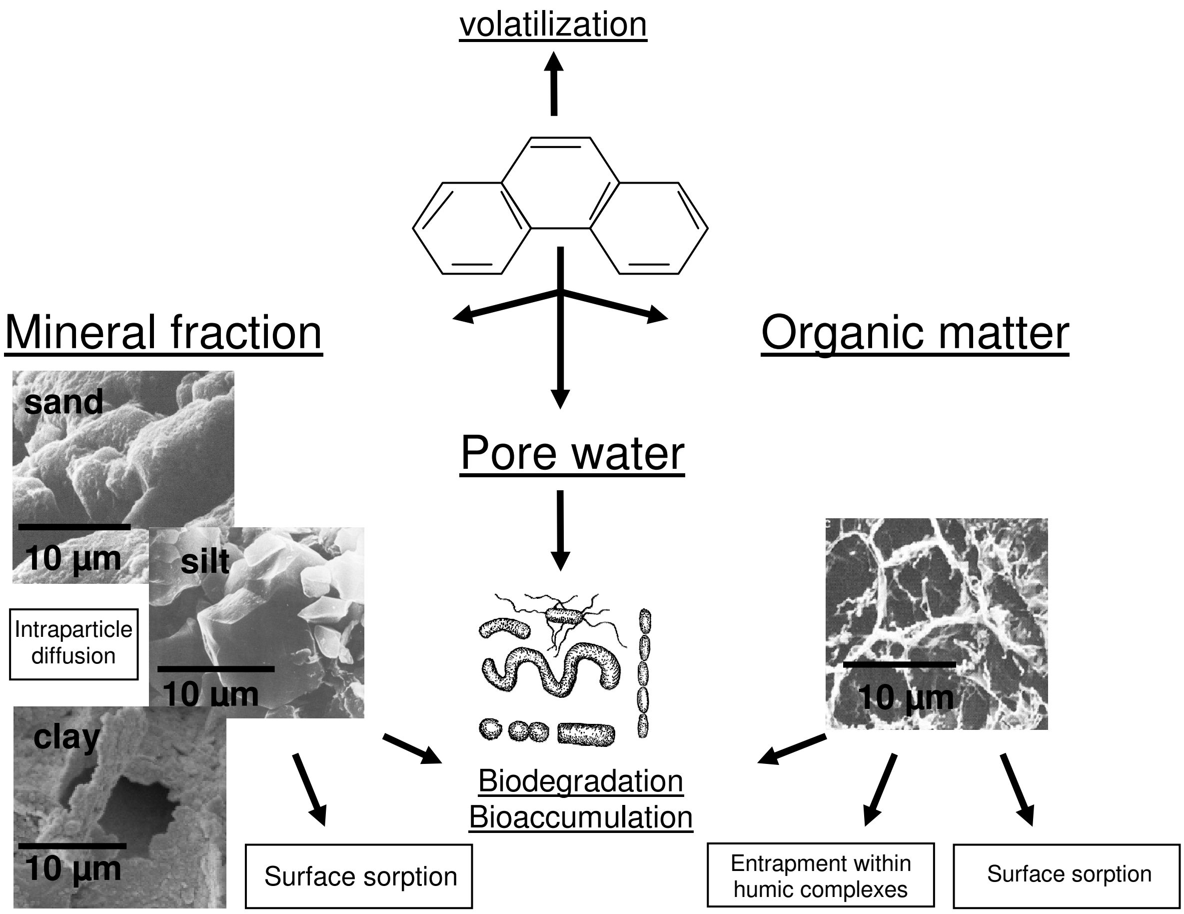

FIGURE 1. Intra-soil processes governing polycyclic aromatic hydrocarbon fate (adapted from Reid et al., 2000). Photos courtesy of Dr. Keith Tovey, University of East Anglia.

The biochemical persistence of PAHs arises from dense clouds of π-electrons on both sides of the ring structure, making them resistant to nucleophilic attack. It has now also been widely accepted that as the contaminant-soil contact time increases, contaminants become progressively sorbed to soil constituents and/or are trapped within the pores, and are being gradually sequestered within the matrix, a process named as ‘ageing’ (Alexander, 2000). Briefly, the ultimate result of contaminants ageing is the movement of compounds from accessible compartments (such as soil solution) into sites within the matrix that are not readily accessed by microorganisms or by higher organisms (Alexander, 2000). These complex contaminant-matrix interactions have been shown to be controlled by a number of factors including, *inter* *alia*, quantity and quality of organic matter (Pignatello and Xing, 1996; Xing and Pignatello, 1997), soil inorganic constituents with particular reference to nanoporosity (Nam and Alexander, 1998), soil processing by microorganisms (Carmichael and Pfaender, 1997), alternate events of wetting and drying (White et al., 1997) and contaminant concentration (Divincenzo and Sparks, 1997). Collectively, ageing is believed to be governed by the physical and chemical properties of PAHs and soil type (Alexander, 2000). Although sorption to mineral components of soil (Bamforth and Singleton, 2005) and mineral intra-particle entrapment (Chung and Alexander, 1999) do occur, it is generally believed that sorption to organic matter is the dominant process governing PAH sequestration (Pignatello, 2000).

Because hydrophobic non-polar PAHs preferentially partition to solid phases (depending on the compounds physico-chemical properties, concentration, time, presence of co-contaminants and soil texture), and, due to PAH loss from the system, only a fraction of total PAH content in soil will be readily available for biological transformation. Nevertheless, biodegradation by catabolically-competent microbial communities has been shown to play a significant role in PAH dissipation from the environment (Cerniglia, 1992). Collectively, the extent of biodegradation depends on the physical availability of the compounds on the one hand and the engagement of catabolically active degraders on the other. Physical availability of PAHs is controlled primarily by their aqueous solubility and the partition co-efficient. Thus, lower molecular weight PAHs are potentially more prone to be microbially transformed than higher molecular weight PAHs. In addition, biodegradation depends on the intrinsic potential of microorganisms to biodegrade a particular compound (Heitkamp and Cerniglia, 1989). The microbial degradation of naphthalene, phenanthrene and anthracene has been well documented contrary to the scarcity of research relating to the microbial degradation of higher molecular weight PAHs. PAHs with more than three benzene rings have, however, been shown to undergo co-metabolic transformations (Heitkamp and Cerniglia, 1989). Factors controlling microbial recalcitrance to biodegrade organic contaminants include: a lack of enzymes necessary to alter chemical structure of the contaminant, limited uptake into microbial cells, which limits intracellular decomposition processes, a lack of regulatory proteins that can recognize the contaminant and induce enzyme synthesis, and/or toxicity of the contaminant affecting microorganisms with catabolic potential towards a particular contaminant (Overcash et al., 2005). In addition to recalcitrance, other factors limiting the microbial degradation of PAHs include: restricted microbial movement, low concentration of contaminants, temperature, pH, nutrient availability and toxicity of end-products and the presence of other co-contaminants, such as cyanide and heavy metals (Amor et al., 2001; Bamforth and Singleton, 2005).

BIOAVAILABILITY AND BIOACCESSIBILITY: Methodology and legislative challenges

Partitioning processes and the principles of PAH dissipation in the environment have been the rationale for the formulation of bioavailability and bioaccessibility concepts. These concepts exist in many different contexts, such as human risk assessment or ecological risk assessment, leading therefore to a multitude of definitions for these terms. For the purpose of this study, a semantic definition derived by Semple et al. (2004) has been employed. The authors presented bioavailability as the fraction of contaminant 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). Bioaccessibility relates to the compound which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical. In other words, the term bioaccessibility embraces both bioavailability and potential availability of the compound over a wider time span (Semple et al., 2004).

Evaluation of microbial bioaccessibility may have direct implications when assessing the potential success of bioremediation to regenerate contaminated land. Moreover, bioaccessibility data may assist in evaluating exposure of ecological receptors to soil contaminants and hence facilitate a more proportionate definition of 'significant possibility of significant harm' within risk assessments.

Traditional exhaustive extraction techniques based on harsh organic solvents (e.g. Soxhlet extraction) have failed to predict the bioaccessibility of PAHs in soils (Reid et al., 2000). Indeed, these vigorous extractions tend to overestimate the biodegradable fraction of contaminants (Alexander, 2000). Although reliable, biological tests to evaluate bioaccessibility are often time-consuming and laborious. As a consequence, alternative chemical, non-exhaustive extraction techniques aiming to determine bioaccessible contaminant fractions have been investigated. Approaches that have been widely researched include: non-exhaustive extractions with organic solvents (Kelsey et al., 1997; Liste and Alexander, 2002; Tang et al., 2002); aqueous based extractions, such as the use of hydroxypropyl-ß-cyclodextrin (Reid et al., 2000; Stokes et al., 2005; Allan et al., 2007); solid phase extraction using sorbents, for instance Tenax (Cornelissen et al., 1997, 1998).

Although there are apparent advantages of using these chemical proxies to evaluate bioaccessibility, no single method stands out as an uncontested best option. Since bioaccessibility is organism- and contaminant-dependent, and can vary in different soils on the account of their different properties, one method to assess bioaccessibility is not, and could not be, all encompassing. Important considerations whilst selecting appropriate methods to assess bioaccessibility include method relevance to target receptor, reproducibility, ease of operation and costs. These aspects are compared and discussed in Chapter 4.

There are a number of challenges in conveying bioavailability/bioaccessibility philosophy into legislative framework. Firstly, in the United Kingdom, there are no officially accepted methods to derive bioavailability/bioaccessibility values in the context of human health risk assessment. The Environment Agency has been widely criticized for ‘sitting on the fence’ approach regardless of substantial research supporting PBET testing as a reflection of the bioavailable fraction (e.g BGS, 2007).

Secondly, there are uncertainties associated with methodologies and a lack of guidance on how to deal with these uncertainties (Saikat et al., 2007). Indeed, similarly to guidance on standard statistical processing of data from contaminated land assessment, a standardized approach to dealing with uncertainties associated with bioaccessibility data is necessary. Some authors have pointed out other factors hampering bioavailability/bioaccessibility implementation, such as scarcity of time- and cost-effective methods (Reichenberg and Mayer, 2006) and a lack of statutory guidance on the use of bioaccessibilty data (Environment Agency, 2006). Contaminated Land Officers, being under auspices of the Environment Agency and being publicly liable for their decisions, need legislative support in decision-making for contaminated land. It can be therefore argued that legislative regulation of bioaccessibility application may play a significant role in hampering the implementation of bioaccessibility data. The discussion on the challenges that regulators, policy-makers and the research community face with respect to bioavailability and bioaccessibility is extended in the literature review (Chapter 1) and in the outcomes of the survey with Contaminated Land Officers from England and Wales (Chapter 5 and the Appendix).

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OBJECTIVES

This study was designed to investigate various aspects behind the broad concept of bioaccessibility. From the perspective of pragmatic evaluation of PAH bioaccessibility to microorganisms, a new methodological approach was investigated. A new method to assess PAH bioaccessibility to microorganisms is desired on account of the need for a rapid, cheap and environmentally-acceptable chemical approach to substitute for more expensive and time-consuming biological tests.

Subcritical water extraction has been previously demonstrated to be a successful technique to selectively and non-exhaustively extract organic contaminants, including PAHs. Selective extractions of PAH with subcritical water were demonstrated to be controlled by PAH aqueous solubility and were dependent on PAH log Kow. On account of this, subcritical water extraction has been shown to reflect PAH desorption processes. It is also well established that microbial biodegradation of PAH is controlled by PAH desorption. It was therefore hypothesised that non-exhaustive subcritical water extraction, by reflecting PAH desorption, would also reflect PAH biodegradation. To date, no such a comparison of PAH-subcritical water extraction extent with PAH bioaccessibility to microorganisms exists. Furthermore, this investigation is significant in that subcritical water extraction has never been performed using commercially available instruments. In this study, an Accelerated Solvent Extractor (Dionex ASE 200), which is widely available for standard pressurised solvent extractions, was employed.

Despite a variety of proposed chemical methods to evaluate microbial bioaccessibility this concept is present more within scientific and technical debates rather than constituting a practical tool to routinely support decision-making on contaminated land. It has been recognized the lack of time- and cost-efficient methods as one of the primary obstacles that hampers bioaccessibility from being operationally applied. It is therefore necessary to delineate an approach that not only precisely evaluates bioaccessibility *per se* via simulating processes that occur in environment but that is also time- and cost-effective. The objective of this study was to compare four different non-exhaustive extraction methodologies (subcritical water extraction, the use of aqueous solution of cyclodextrins, surfactant extraction and butanol extraction) developed to reflect bioaccessibility to microorganisms. An evaluation comprising not only the accuracy of bioaccessibility prediction but also an appraisal of other crucial economical and practical facets behind various extraction techniques is presented. No similar comparison including such a spectrum of methodological approaches along with a broader analysis of the key factors governing a choice and validation of the method has been previously undertaken.

The concept of bioaccessibility is present also within the regulatory perspective, mainly in the context of human health risk assessment. The use of bioaccessibility is, however, not regulated at the Environment Agency or at the level of any other authoritative body. The use of bioaccessibility is ‘allowed’ yet not officially supported by the Environment Agency. It was therefore the objective of this study to investigate the views of Contaminated Land Officers within English and Welsh Local Authorities on the practical implementation of bioaccessibility within their day-to-day decision-making. It was hypothesised that lack of standardized central guidance from an authoritative body hampers the implementation of bioaccessibility as a decision-support tool for contaminated land. It was also the objective of this study to investigate the degree to which the Officers perceive bioaccessibility as a useful tool that facilitates contaminated land management. Familiarity with the concept of bioaccessibility, its acceptance by decision-makers and the need for bioaccessibility data within contaminated land decision-making were evaluated. In addition, both the questionnaire and interviewing, provided contextual aspects regarding social and institutional divers influencing decision-makers' perspective. To date, no such a collation of decision-makers' opinion exists.

On account of decision-makers' leeway in using guideline values to evaluate land contaminated with PAHs, it was the objective of this study to determine the most commonly used models and the reasons for their selection. As bioaccessibility to microorganisms has a direct application in assessing the bioremediation potential, the frequency of using bioremediation as a clean-up technique in Local Authorities' jurisdiction areas to regenerate PAH-contaminated land was also evaluated.

OVERVIEW AND CHAPTER CONCLUSIONS

This thesis is presented as a collation of five manuscripts; either published (Chapter 2), in press (Chapter 1 and 4), under review (Chapter 3) or in draft (Chapter 5). Appended is a report based on the survey on Contaminated Land Officers in England and Wales, submitted to the Environment Agency, Defra and disseminated to Local Authorities in the United Kingdom. The following summaries serve to introduce the scope of each Chapter, the key findings and an indication of their significance.

CHAPTER 1 (literature review): Bringing bioavailability into contaminated land decision-making: The Way Forward?

This review describes a risk-based contaminated land regime using the regulation in the United Kingdom as a case study. Risk-based approaches provide flexible frameworks and intrinsically stimulate scientific advances to improve accuracy of risk-assessments. Bioavailability and bioaccessibility concepts constituting decision-support tools for contaminated land assessment are reviewed. Different contexts of the use of bioavailability and bioaccessibility are presented along with interpretations of the terms and their applications across different disciplines. Critical discussion on advantages and limitations of bioavailability and bioaccessibility is provided and the gaps in the state-of-knowledge are highlighted. It should be noted that contaminated land regulations are continuously evolving. This review was undertaken and submitted before recent changes to the contaminated land regulation. These changes do not however affect the nature of the analysis presented within the review. All terms and official documents that this review refers to were in force whilst this research was undertaken.

CHAPTER 2: Environmentally friendly assessment of organic compound bioaccessibility using subcritical water

Chapter 2 presents an investigation into a novel non-exhaustive extraction technique developed in order to reflect the fraction of PAH bioaccessible to microorganisms. While subcritical water extraction has been successfully applied to predict long-term release rates of hydrophobic organic compounds (HOCs) no comparative studies between subcritical water extractions of HOCs and their bioaccessibility to microorganisms have been undertaken. Subcritical water extractions have been performed using Accelerated Solvent Extractor designed for rapid extractions. Five different temperatures of subcritical water extractions (namely 40, 80, 120, 160, 180 and 200 ºC) were investigated for three different extraction times (5, 10 and 20 minutes) under moderate pressure of 103 bar. Extraction extents of ^{14}C-phenanthrene associated activity were investigated in two dissimilar soils at three different ageing times (14, 28 and 49 days). The results from chemical extractions were correlated with biological estimations of bioaccessibility from respirometry assays. For the majority of determination, no significant ($p > 0.05$) difference between subcritical water-extracted ^{14}C-phenanthrene-associated activity at 160 ºC and the fraction mineralized by catabolically active *Pseudomonas* sp. was observed.

These results are significant in that water used as a solvent for extraction of hydrophobic contaminants represents an environmentally favourable approach over more conventional organic solvent based techniques.

CHAPTER 3: Subcritical water extraction as a potentially ‘greener’ approach to assess organic contaminant partitioning

In this Chapter further investigations towards PAH-subcritical water extractions were undertaken. Four different soils and sediments, both spiked and historically contaminated, were used in addition to multi-contaminant reference material. Matrices were extracted with subcritical water and Accelerated Solvent Extractor at four different temperatures, namely 50, 100, 150 and 200 ºC for 10 min under moderate pressure (103 bar). Concurrent with increasing temperatures to 150 °C there was an increase in PAH extraction efficiencies. For the majority of determinations no significant ($p > 0.05$) differences between extractions at 150 °C and 200 °C were observed. Results showed, regardless of matrix complexity, that as the molecular weight of PAH increased, along with decreasing aqueous solubility, there was a decrease in PAH extractability. Selective subcritical water extraction of PAHs dependant on their octanol-water partition coefficients hence aqueous solubilities was observed. Varied extraction efficiencies of PAHs at the same extraction conditions reflected dissimilarities between environmental matrices used in this experiment.

The results of this work indicated that it was possible to reflect PAH desorption processes in multi-contaminant matrices using subcritical water extraction and potentially reflect bioaccessibility of PAH in mixtures. This method is advantageous in that it is fast (rapid Accelerated Solvent Extraction and shorter sample preparation as the drying step is not required), cheap, water-based hence ‘green’ (on account of lower organic solvent demand) and therefore, arguably, more environmentally acceptable.

CHAPTER 4: Beyond contaminated land assessment: on costs and benefits of bioaccessibility prediction

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This study presents a comprehensive appraisal of four non-exhaustive extraction methods dedicated to evaluate PAH bioaccessibility to microorganisms. Both novel techniques (subcritical water extraction and surfactant Brij 700 extraction) as well as previously demonstrated approaches (hydroxypropyl-β-cyclodextrin and butanol extractions) were compared not only from the perspective of accuracy of bioaccessibility prediction but also incorporating other crucial appraisal criteria (such as cost of the method, extraction time and environmental-friendliness of the method). Whilst the use of cyclodextrins was the best predictor of the bioaccessible fraction to microorganisms for the majority of compounds (in 78% of cases investigated), other methods appeared more cost- and time-effective. The use of B700 and butanol were the cheapest methods with butanol being additionally the most rapid technique. Yet their prediction of bioaccessibility in 56% and 52% of determinations respectively precluded suitability of these methods for bioaccessibility reflection in this study. While subcritical water extraction did not provide a direct (1:1) prediction of PAH bioaccessibility to microorganisms it reflected PAH desorption processes and hence provided a refection of the fraction of PAH bioaccessible to microorganisms.

In the context of contaminated land management these results can assist in establishing cost-benefit trade-offs of different non-exhaustive extraction techniques and contribute to tailoring information on contaminant bioaccessibility to support risk evaluation on contaminated sites.

CHAPTER 5: ‘The Two Cultures’? Towards a better understanding of bioaccessibility implementation

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This Chapter presents real-world circumstances of practical utilization of bioaccessibility information within decision-making for contaminated land. Bioaccessibility has existed as a concept and a decision-support tool for over a decade yet its incorporation into contaminated land decision-making in the United Kingdom is not statutorily defined. This survey-based study presents a collation of views of 151 Local Authorities (40.3%) from England and Wales regarding approaches to the practical application of bioaccessibility and constraints associated with its implementation. The majority of respondents (70.2%) perceived bioaccessibility as a useful tool that facilitates contaminated land management. Whilst necessity to access more information regarding bioaccessibility was indicated by 76.8% of participants, a need for more research for under-investigated contaminants, such as benzo[*a*]pyrene was emphasised. Lack of statutory guidance was indicated by 78.2% of respondents as the factor hampering application of bioaccessibility data. A dearth of central guidance and, as a consequence, a lack of standardized approach to bioaccessibility application throughout England and Wales were reported.

The results of this work assist in bridging the gap between science and policy through recognition of real-world priorities from decision-makers’ perspective. Action priorities for both research community and policy-makers in order to improve the quality of contaminated land management are highlighted. On the basis of this study a report has been prepared (Appendix) to inform the Environment Agency, Defra and other relevant bodies about the findings of this survey.

Authorship statement – Agnieszka Latawiec

On the way forward into bioaccessibility implementation

The table below provides an account of the candidate's involvement in experimentation, data processing and manuscript production with respect to Chapters/Papers that comprise this thesis.

Chapter	Chapter/paper title and status	Contribution to data and processing	Contribution to writing
1	Bringing bioavailability into contaminated land decision-making: The Way Forward? In press in: Critical Reviews in Environmental Science and Technology	Literature review was solely undertaken and written by Agnieszka Latawiec.	First author. Lead the preparation of this manuscript with review being provided by co-authors (Supervisors) Swindell, Simmons, Reid.
2	Environmentally friendly assessment of organic compound bioaccessibility using subcritical water Published in: Environmental Pollution 156 (2008) 467-473	All experimental work and data processing was solely undertaken and written by Agnieszka Latawiec.	First author. Lead the preparation of this manuscript with review being provided by co-authors (Supervisors) Swindell and Reid.
3	Subcritical water extraction as a potentially 'greener' approach to assess organic contaminant partitioning Under review in: Environmental Pollution	All experimental work and data processing was solely undertaken and written by Agnieszka Latawiec.	First author. Lead the preparation of this manuscript with review being provided by Reid (Supervisor).
4	Beyond contaminated land assessment: on costs and benefits of bioaccessibility prediction In press in: Environment International	All experimental work and data processing was solely undertaken and written by Agnieszka Latawiec.	First author. Lead the preparation of this manuscript with review being provided by Reid (Supervisor).
5	'The Two Cultures'? Towards a better understanding of bioaccessibility implementation	The questionnaire and interviews were solely undertaken, analysed and written by Agnieszka Latawiec.	First author. Lead the preparation of this manuscript with review being provided by the Supervisors.

Agnieszka E. Latawiec (23 April 2009)

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The work presented here portrays the concept of bioaccessibility within the broad context of both a laboratory evaluation and a pragmatic implementation within contaminated land decision-making.

To date, non-exhaustive subcritical water extraction of PAHs using an Accelerated Solvent Extractor (ASE 200) in order to reflect the microbial-bioaccessible fraction has not been investigated. The results presented here are the first successful attempts correlating PAH-subcritical water extractability and PAH bioaccessibility to microorganisms. Collectively, subcritical water extraction at 160 ºC directly predicted microbial bioaccessibility of ^{14}C-phenanthrene-associated activity whilst paralleled microbial bioaccessibility of PAHs in mixtures. The results of this work indicated that subcritical water extractions using the ASE 200 reflected PAH desorption processes in dissimilar multi-contaminant genuinely contaminated matrices. The results of this study, developing operationally for the first time subcritical water extraction method with commercially available equipment, could pave the way for further research. Yet the results from this study are only initial attempts to compare PAH-subcritical water extraction using a limited number of matrices. It would therefore be desirable to investigate different matrices where particle size distribution, organic matter content and co-contaminants may influence the extent of extractions. Further work to expand on the results from this study could include:

- Testing subcritical water extraction technique against complex matrices from different contaminated sources, as these are likely to display a range of intra- and inter-class contaminant interactions.

On the basis of desorption based subcritical water extractions of PAHs (revealed in this study) and given previous research demonstrating that changes in the subcritical water flux altered the extraction extents, it would be valuable to investigate dynamic aspects of sub-critical water extraction using the ASE 200 instrument. This could be achieved by increasing the number of 'extraction cycles' within the extraction. Further work could therefore include:

- Investigating the influence of alterations of the ASE 200 parameters on subcritical water extraction efficiencies of PAHs.

Subcritical water extraction extents of PAHs from genuinely contaminated matrices obtained in this study were relatively low and did not directly corresponded to microbial bioaccessibility. It has been also previously demonstrated that extents of biotransformation of PAHs by other environmental receptors (e.g. earthworms) are lower than biodegradation. Future study could therefore:

- Compare static subcritical water extraction extents using the ASE 200 at extraction conditions applied within this study with different environmental receptors, such as earthworms in order to directly predict bioaccessibility.

On the account of practicality, time effectiveness and the evidence that subcritical water extractions using the ASE 200 reflect PAH desorption processes further work could include:

- Broadening investigations on suitability of subcritical water for bioaccessibility prediction to other organic contaminants (e.g. pesticides).

This study was a successful collaboration with decision-makers. It was apparent from the questionnaire and interviews with Contaminated Land Officers that there is a need for more knowledge transfer from academia to regulatory bodies. Equally decision-makers perspective from the real-world circumstances may have a significant impact on the direction of research. This may result in a more appropriate decision-making on the one hand and a more applied science on the other. It is therefore recommended to:

- Provide regulators the access to recent scientific publications and further investigate the priorities and day-to-day circumstances of contaminated land management from regulators' perspective.

CHAPTER 1

Bringing bioavailability into contaminated land decision-making: The Way Forward?

Bringing bioavailability into contaminated land decision-making: The Way Forward?

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ABSTRACT

Beyond the current regulatory regime there is ongoing research into the environmental fate of pollutants that could potentially be integrated into contaminated land decision-making. In an era of great demand for decision-support tools it is increasingly urgent for scientists to develop reliable methodology assisting sustainable land management and for policy makers to adopt these developments. This is true notably for widespread land contamination with polycyclic aromatic hydrocarbons. Recently published research on bioavailability offers complementary approach into contaminated land evaluation as well as the assistance in the development of remediation strategies. Here, these studies are reviewed and a critical discussion on the implementation of bioavailability across different disciplines within contaminated land management is presented. Scientific gaps are identified and transdisciplinary research confronting key normative questions facing regulators is recommended. Discussion is presented with reference to the United Kingdom's contaminated land regime. This regime is risk-based and as a consequence the general principles, concerns and chemistry behind bioavailability processes discussed in this review are applicable to risk-based approaches elsewhere.

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1. INTRODUCTION

Economies worldwide depend directly and indirectly on ecosystem services.[51] Soil is one of the key components of the natural environment and performs a plethora of crucial environmental, economic and social functions.[7] Largely non-renewable, soil provides up to 95 % of all human sustenance.[9] However, due to ubiquitous land contamination and the global population increase, viable land area *per capita* is steadily decreasing. As natural capital and ecosystem services become more strained, their value is expected to increase.[15,51] Thus, maximising soil utility whilst seeking to proportionately manage land contamination poses a complex challenge for integrated land management.

Increasing land scarcity has resulted in re-development of brownfield sites (for instance to meet housing growth targets) and has gradually raised the issue of contaminated land up the political agenda.[10] Soils have been subject to diffuse contaminant input from both natural and anthropogenic sources in addition to localised sources of contamination including, *inter alia*, accidental spillages, deliberate disposal and dumping.[44] Whilst initial research into soil contamination has focused on inorganic pollutants, organic contaminants are now receiving increasing attention.[62]

This review primarily considers the environmental fate and legislative management of polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of organic chemicals composed of fused benzene rings and/or pentacyclic molecules (Figure 1), primarily formed during the incomplete combustion of fossil fuels.[76] The United States Environmental Protection Agency (US EPA) since the late 1970s has regarded 16

PAHs to be of substantial concern to human health.[65] Scientific Committee on Food (SCF), a European Commission body, identified 15 PAHs that possess both genotoxic and carcinogenic properties.[75] In the United Kingdom (UK), PAHs are regarded as potential organic contaminants for the assessment of industrial land[30] and are considered as risk-critical components on many contaminated sites.[8] The evidence of carcinogenicity of some PAHs,[39] their prevalence[76] and existing gaps in our understanding of their environmental fate[27] warrant inclusion of PAHs in risk evaluation for contaminated sites. Not only there is scarcity of information on contemporary levels of PAHs in soils[68] but also there is still relative paucity of information on the toxicity, distribution and availability of organic contaminants in the environment.[8] Moreover, putative interactions within the group of organic contaminants and their synergistic impact on biological endpoints provide an impetus for further research and consequently its legislative implications.[45] PAHs, particularly those of higher molecular weight, are persistent and hence accumulate in the environment, with soil being the main repository.[66] Wild and Jones (1995)[76] estimated that over 90% of the environmental burden of PAHs in the UK resides in soils. As a consequence, soil contamination with organic contaminants is of critical concern, is the key driver for soil remediation activities[58] and a challenge for complex legislative management.

NAPHTHALENE
Molecular weight (g mol $^{-1}$) 128.17
Aqueous solubility at 25 °C (mg L $^{-1}$) 31. 7

ACENAPHTYLENE
Molecular weight (g mol $^{-1}$) 152.2
Aqueous solubility at 25 °C (mg L $^{-1}$) 16.1

PHENANTHRENE
Molecular weight (g mol $^{-1}$) 178.23
Aqueous solubility at 25 °C (mg L $^{-1}$) 1.3

BENZO[a]PYRENE
Molecular weight (g mol $^{-1}$) 252.32
Aqueous solubility at 25 °C (mg L $^{-1}$) 0.003

Figure 1. Chemical structures and physical properties of representative polycyclic aromatic hydrocarbons (PAHs).

As a point of reference this review introduces the UK risk-based contaminated land regime. It should be noted that risk-based approaches to contaminated land assessment form the foundations of contaminated land regulation in other countries throughout Europe and the United States of America and are applicable and transferable to other countries.[53] In this review, firstly, contaminated land assessments that depend upon comparing environmental concentrations with toxicology-based model calculations are presented and, secondly, approaches that relate to direct measurements on-site are discussed. Finally, critical discussion of both means is presented. Although this review focuses on PAHs, other compounds are mentioned, where relevant.

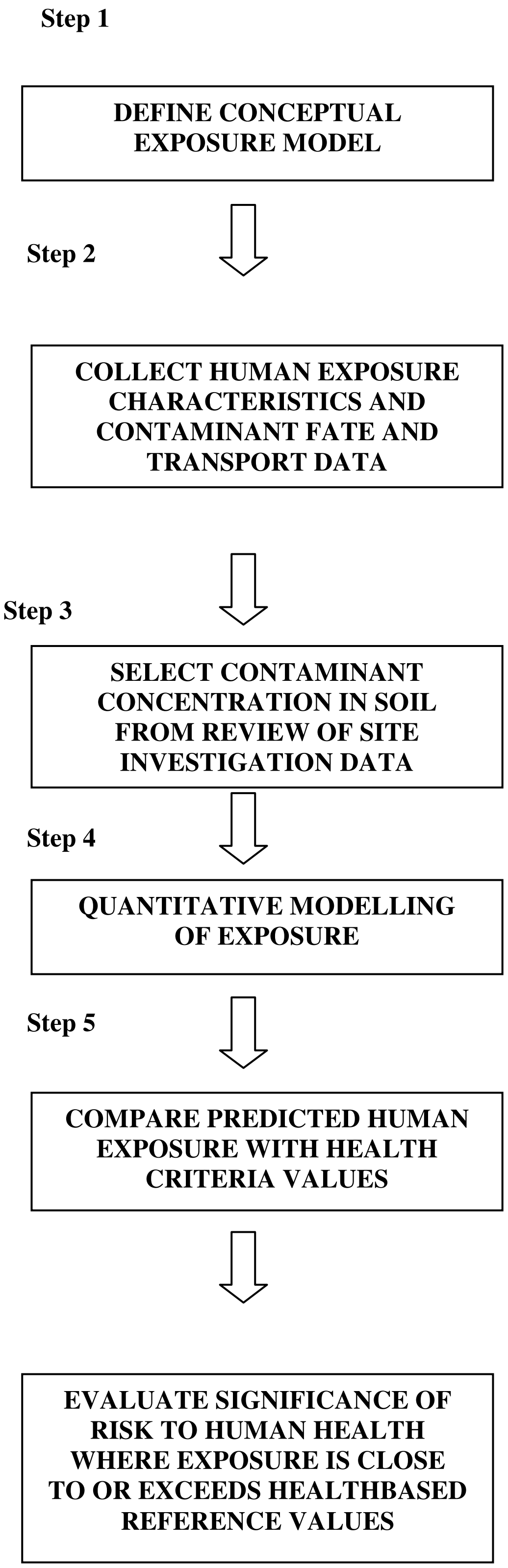
2. CURRENT APPROACH TO THE LAND CONTAMINATION ASSESSMENT

2.1. The United Kingdom contaminated land policy

Contaminated land regime in the UK is derived from Part IIA of the Environmental Protection Act (EPA) 1990. Part IIA was interjected to the Act (section 57) in 1995 and along with Statutory Guidance, was introduced in England, Wales and Scotland. UK contaminated land risk-based regulation is reflected by the classification of a site as contaminated on the basis that the contamination poses an 'unacceptable' risk to the defined receptor (humans, controlled waters, ecosystems, buildings) in the context of the current use of the land. Hence, the regime follows the 'pollutant linkage' paradigm that requires a contaminant source, receptor and a pathway (capable of exposing the receptor) to be identified. Collectively, Part IIA stipulates that harm to the receptor arises not from the mere presence of a contaminant of concern in soil but that a receptor is exposed to a 'significant possibility of significant harm.' It should be emphasized that in the UK there are no statutorily defined standards for contaminant concentrations in soil that compel immediate remediation. As a consequence, risk needs to be assessed on a site-by-site basis in the context of specific land circumstances.[22] These are rationalised within a 'conceptual exposure framework' (Figure 2).

'Suitable for use' doctrine is a fundamental component of UK land regulation that underpins the risk-based approach.[22] The concept of 'suitable for use' entails the identification and removal of unacceptable risks from contaminated land, the reclamation of land into beneficial use, and controlling cost burdens through proportionality and economical sustainability. The one exception to the 'suitable for use' approach to regulatory action applies where contamination has resulted from a specific breach of an environmental licence or permit. In such circumstances it is required, under the relevant regulatory regime, to remove the contamination completely.[22]

Under Part IIA, Local Authorities (LAs) are primary regulators of contaminated sites. LAs identify contaminated land and act as an enforcing authority, with the exception of sites designated as 'Special Sites', which are the responsibility of the Environment Agency (EA). However, responsibility rests with the LAs to determine whether a site meets the Part IIA 'Special Site' definition (e.g. a site where pollution is actively entering controlled waters or a site containing explosives).



Key Differences Between Soil Guideline Values and Site-Specific Assessment Criteria

Soil Guideline Values	Site-Specific Criteria
Conceptual model derived not for a specific site but for a broad range of sites within a land-use category. Precautionary assumptions ensure that the model is protective of most, if not all, sites within the category.	Conceptual model based on a real site, taking account of specific circumstances and more targeted data collection.
Human exposure characteristics taken from generic data sets. Contaminant fate and transport parameters and algorithms are based on generic site conditions and simplifications of real behaviour.	Generic data sets are used in conjunction with site-specific information. For example, rather than modelling plant uptake of contaminants, it is possible to measure its value.
A worst-case situation must be assumed, for example, the contamination is at or near to the surface with a uniform concentration across the site. A seed value for this concentration is used.	Real site data can be reviewed and analysed with exposure models tailored accordingly. A range of contaminant concentrations might be used rather than a single value.
Predicted human exposures to a contaminant from soil are generated.	Predicted human exposures can be modelled, supported by potential measurements of impacts through health monitoring.
Predicted human exposures to soil contaminants from step 4 are compared directly with relevant health criteria values.	Predicted human exposures to soil contaminants from step 4 are compared directly with relevant health criteria values.
If predicted exposure from step 4 exceeds the relevant health criteria value in step 5, then steps 3 to 6 are repeated with a new seed soil concentration of the contaminant. Where the predicted exposure from step 4 equals the health criteria value in step 5, then the soil concentration of the contaminant is used as the basis of the Soil Guideline Value.	If predicted exposure from step 4 exceeds the relevant health criteria value in step 5, then further action is required. This may include remedial treatment or the refinement of the conceptual model through collection of further site information.

Figure 2. Schematic overview of the steps in quantitative site-specific risk assessment, illustrating the differences between Soil Guideline Values and site-specific assessment criteria (adopted from CLR 10).[29]

In the UK, liability for paying the remediation costs is assigned in the first instance (Class A liability group) to the original polluter (person that caused or knowingly permitted the contaminants to be in, on or under the land) following the 'polluter pays principle' (PPP). If the Class A persons cannot be identified, responsibility falls next on the current landowner or the land occupier (Class B). This is derived from the traditional principle of English property law *caveat emptor* ('let the buyer beware') that passes liability with the title of the property to a new owner.[10] Remediation of sites of which ownership cannot be identified (orphan sites) becomes the responsibility of the LA.

2.2. Decision-making within contaminated land assessment

In the UK a scientifically-based framework for the practical assessment of historically contaminated land relies both on Part IIA (relates to the current use of the land) and the Town and Country Planning Acts (planning regime is employed when there is a change in the land-use). This framework is differentiated on the basis of the particular receptor exposure to contaminants. Smith et al. (2005)[69] indicated that in the UK the protection of human health has been the major driver for the identification of contaminated land.

2.2.1. Human health risk assessment and Soil Guideline Values (SGVs)

A series of key reports considering human health risk identification from contaminated sites have been published by both the Department for Environment, Food and Rural Affairs (DEFRA) and the EA.[25,27,29,30] The package consists of four major Contaminated Land Reports (CLR 7-10), supported by collation of toxicological data (TOX reports) for priority contaminants and the Soil Guideline

Values (SGVs) for some of these contaminants (Table 1).[*] As the CLR framework regards chronic exposure other transient risks are regulated by Control of Substances Hazardous to Health (COSHH).[25]

Although, the UK was one of the first countries to propose criteria for concentrations of certain contaminants in soil in the context of land development and the SGVs are advised to be used as a bench mark, the term ‘Soil Guideline Value’ is defined neither in the Environmental Protection Act nor in the Statutory Guidance. Its use is not a legal requirement; however, it is likely to become a standard tool for contaminated land assessments in practice.[52]

SGVs are defined as ‘intervention values’ beyond which risks from exposure to contaminants would be ‘unacceptable’ and, thus could lead to a significant possibility of significant harm.[25] This requires not only scientific (toxicological) information on the health effects, but also judgement on what constitutes an ‘unacceptable’ risk in the context of soil not being a sole exposure source to the contaminant. Furthermore, SGVs are intended to be used to inform the selection of remediation standards or target values for individual sites.

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[*] It should be noted that contaminated land regulations are subject to continuous evolvement. This review was undertaken and submitted before recent changes to the contaminated land regime in England. These changes do not however affect the nature of the analysis presented here. All terms and official documents that this review refers to were in force whilst this research was undertaken.

Table 1. Hierarchy of the key documents regarding human risk assessment from land contamination

CLR 7 Assessment of Risks to Human Health from Land Contamination: An Overview of the Development of Soil Guideline Values and related Research. This serves as an introduction to the other reports in this series. It sets out the legal framework, definition of contaminated land under Part IIA, the development and use of SGVs and references to related research
CLR 8 Priority Contaminants Report. Identifies priority contaminants, on the basis that they are likely to be present on many current or former sites affected by industrial or waste management activity in the UK in sufficient concentrations to cause harm; and that they pose a risk to humans, buildings, water or ecosystems. It also indicates which contaminants are likely to be associated with particular industries.
CLR 9 Contaminants in Soils: Collation of Toxicological Data and Intake Values for Humans. This report sets out the approach to the selection of tolerable daily intakes and Index Doses for contaminants to support the derivation of SGVs
CLR 10 TOX. These reports detail the derivation of Tolerable Daily Intakes or Index Doses for the contaminants for which SGVs have been determined.
CLR 10 Contaminated land Exposure Assessment Model (CLEA): Technical Basis and Algorithms. Describes the conceptual models for each standard land-use that are used to derive the SGVs.
CLR 10 SGV. These reports set out the derivation of Soil Guideline Values

The starting point in the development of human health risk criteria thus SGVs is the identification of ‘intakes’ that do not pose a significant risk of causing significant harm, i.e. a tolerable daily intake (TDI). TDIs are based on toxicological information and are derived as the greatest tolerable value of the compound (No Observed Adverse Effect Level, NOAEL). NOAEL is derived from experimental animal studies and epidemiological studies and subsequently transformed to meet human health criteria by the incorporation of safety factors (usually 100; 10 x 10 for inter and intra species variations, respectively) and, as a consequence, equals TDI.

In derivation of SGVs the tolerable daily soil intake (TDSI) value is taken into consideration and is calculated by incorporating background exposure (mean daily intake; MDI) from non-soil sources. MDI is estimated from published information on ambient air concentrations and average concentrations measured in water and food products.[27]

For some substances, such as genotoxic carcinogens that include some PAHs, no threshold is set below which adverse effects may not occur. Therefore, it is conservatively assumed that they carry a putative risk at any level of exposure and they are characterised by the minimal risk level dose; Index Dose (ID). Consequently, TDSI for carcinogens (such as benzo[*a*]pyrene) equals the ID. As a result, the setting of remediation targets for these compounds would follow the principle of ‘as low as reasonably practicable’ (ALARP).

The Contaminated Land Exposure Assessment Model (hereafter CLEA) provides algorithms and technical basis for the contaminated land assessment and for derivation of SGVs.[29] It produces the conceptual model for a number of generic 'standard' land-uses (residential, allotments and commercial/industrial) expected to be representative of a range of site conditions. CLEA predicts the amount of the contaminant to which humans might be exposed based on a given concentration of the contaminant in the soil. This predicted exposure is compared to health criteria values (HCV). As the conceptual model is often a simple exemplification of the hypothesised relationship within the pollutant linkage paradigm in some circumstances a further, detailed site-specific assessment is required (Figure 2).

In summary, a SGV is the concentration of a contaminant in soil where the estimated average daily exposure to a chemical from soil (ADE) for the critical receptor in each standard land-use equals the relevant HCV (TDSI or ID). ADE incorporates chemical exposure rate, frequency and duration, human body weight and averaging time comprising three exposure pathways, namely: inhalation, oral intake, and dermal route.

2.2.2. Assumptions and limitations of the CLR framework

Assessing human exposure to contaminants in soil is a highly complicated process that involves not only understanding of the fate and transport of chemicals in the environment but also social aspects of human behaviour and variability within human population. The probabilistic CLEA model[29] overcomes these facets of variability and uncertainty by replacing some single-value parameters in the exposure assessment with a family of values selected from a defined probability distribution (e.g. body weight, respiration rate, body surface area). Repeating the simulation, the probabilistic model builds a range of predicted exposures allowing a better understanding of the sensitivity of the assessment. The value to be subsequently chosen as SGV is the 95th percentile of the distribution of average daily exposures (the highest value left when the top 5% of numerically sorted set of collected data is discarded).

For standard commercial and industrial land-use, a working female adult is assumed to be the critical receptor, whereas for the residential and allotment land-uses, a female child from birth to six years (assuming higher chance of soil ingestion and low body weight). However, lead assessment is based on an exposure period from birth to two years, as this is believed to be the critical time when lead most affects intellectual and behavioural development.[29]

When modelling SGV for a specific land-use the contaminant and the site characterisation, total porosity and enrichment factor predicting the vapour transport and plant uptake, are taken into account. For organic compounds, in the absence of Henry's law constant, an aqueous solubility is used in the CLEA model and subsequently incorporated into dermal and vapour exposure assessment. However, in contrast to the well investigated environmental fate of pesticides and growth regulators[29] the behaviour of organic compounds, as those found on former industrial sites (along with PAHs) is more obscure. It is suggested that the contaminants most likely to be absorbed via dermal route are those with a high solubility in both fats and water.[74] However, although hydrophobic organic compounds (HOC) more readily penetrate through the *stratum corneum* (skin's outer layer) they are also more likely to be strongly absorbed within the soil. All these factors contribute to extreme complexity of partitioning modelling of organic compounds.

There is a considerable amount of literature on the epidemiology of workers exposed to complex mixtures of PAHs. Examples for aluminium production[5] and coke production[55] demonstrated numerous incidences of lung and skin tumours. It has not however been possible to assess with confidence the contribution of the individual PAH to the observed cancer burden. Consequently, at a 1990 meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that PAHs should be considered as a separate class. Both the WHO and the US EPA have attempted to estimate the human cancer risks from ingested benzo[*a*]pyrene by the low-dose extrapolation of the dose-response curves of the fore-stomach tumours seen in dietary studies in rodents on the assumption that humans, mice and rats exhibit the same quantitative susceptibility to cancer potency. Exposure to benzo[*a*]pyrene has also been used as an indicator of exposure to PAHs in general.[28,57] In 2002 an International Agency on Research on Cancer (IARC) Working Group classified naphthalene as 'possibly carcinogenic to humans' based on 'inadequate evidence' in humans yet 'sufficient' in laboratory animals. No good-quality oral carcinogenicity data were identified for naphthalene and consequently it was concluded that tumours (following inhalation) did not arise from a direct genotoxic mechanism. Instead, the US EPA applied a 3000 and 300 uncertainty factor to NOAEL and LOAEL (lowest observed adverse effect level) values, respectively in order to derive threshold health criteria. Both approaches resulted in a health criteria value of 20 $\mu g\ kg^{-1}\ day^{-1}$ and are adopted as both oral TDI and TDSI (as MDI equals 0.1 $\mu g\ kg^{-1}\ day^{-1}$).[34]

To date, no SGV exists for any of the PAHs. SGV 2 and SGV 19 for naphthalene and benzo[*a*]pyrene, respectively are currently being prepared[26,28] based on the aforementioned toxicological information.

Quantitative generic guidelines based upon standard assumptions serve as an encouraging, transparent and consistent approach for land contamination evaluation. Guideline values, if appropriately used, may simplify decision making for LAs and reduce costs of risk assessments.[53] Together with a careful assessment of the available scientific information and a general application of precautionary approach they contribute to a proper land contamination assessment. However, the SGV may also be controversial. As Nathanail and Earl (2001)[53] accurately pictured, unlike a fish out of water, a guideline value separated from its explanatory text does not die. Out of context, it exists as unintentional 'magic number' that improperly used might lead to misinterpretation. This is true especially as the current SGVs serve also as remediation target values. Notably, not every exceedance of the SGV has to lead to the decision to remediate the site. This decision should involve detailed site conceptual model in order to identify significant pollutant linkage and cost-benefit analyses in accordance with the ALARP principle. A decision to remediate in order to meet improperly applied guideline values may indeed entail unreasonable cost, use of energy resources or/and landfill space which is at odds with sustainability priorities.

2.2.3. Other models

In the absence of statutory binding generic assessment criteria (as SGV) regulators may search for other models to evaluate land contamination. This is true also for PAHs that are commonly present at contaminated sites in mixtures. US EPA guidelines or RISC-HUMAN based on the National Institute of Public Health and Protection (RIVM) in the Netherlands can also be used to estimate human exposure from contaminants from soil, sediment and groundwater.[34] Among others, ConSim model has been designed specifically to deal with problems associated with assessing the risk to groundwater by leaching contaminants whilst Sniffer offers a method to derive site-specific human health assessment criteria for both metals/metalloids and organics.[47]

2.3. Ecological risk assessment

Screening soil quality under Part IIA can be performed on the basis of the ecological receptor exposure. Terrestrial ecological risk assessment is an advancing discipline, inherently complex due to a necessity for multi-species analyses.[69] There are three different ecological risk assessment endpoints, namely individual, population and entire ecosystem. Although endangered species are being protected as individuals, decisions for most species are based on population level effects (abundance, mortality and reproduction). Within Part IIA protected ecological receptors are designated habitats and species (Site of Special Scientific Interest) that currently encompass more than 7% of the land in England and Wales.[69]

In keeping with human health risk assessment approaches the Environment Agency offers a package of reports regarding ecological risk assessment.[31,32,37] Similarly, these documents are not statutorily binding. The definition of contaminated land under Part IIA may cause problems when attempting to derive quantitative criteria for ecological harm.[37] The identification and assessment of the significant possibility of ecological harm for a particular site is not straightforward and is subject to interpretations. Species, present at a particular site, might have adapted with respect to a particular stressor. Thus, in some circumstances, even when the significant possibility of significant harm is established the remediation activity itself may result in more harm to the ecosystem.

2.4. Soil Framework Directive

The new emerging Soil Framework Directive[12] seeks to establish the legislation management of risks associated with land contamination at a European level.[21] If the proposed directive is adopted, it would be the first soil specific legislation to apply across Europe. The proposal aimed to establish a common strategy for the protection and sustainable use of soil, integration of soil concerns into other policies, preservation of soil functions as well as restoration of degraded soils within a 'suitable for use' (current and approved future use) principle.

A plethora of already existing regulations with respect to land both at European level (for instance, Integrated Pollution Prevention Control Directive, Environmental Liability Directive, Common Agricultural Policy, Strategic Environmental Assessment) and the broad portofolio of UK legislation, at the national level brings into question the necessity of a new Directive.[21,23,24]

As a result of consultation and wider stakeholder discussions, the UK Government concluded that it could not support the proposed Directive, unless significant changes avoiding unnecessary additional administrative burden and disproportionate costs were applied.[24] The European Parliament completed a first reading of the Directive on 13 November 2007 voting it through but with substantial amendments.[24]

3. BIOAVAILABILITY/BIOACCESSIBILITY

Common approach to assessing receptor exposure to soil contaminants, as applied in a regulatory context, assumes that total contaminant concentrations are available for uptake and transformation by living organisms. However, it is widely accepted that as organic compounds persist or 'age' in soils they become progressively less available for uptake and biotransformation and, as a consequence, for exerting toxic effects on living organisms.[1,56,64] It has been shown that commensurate with increasing contaminant-soil contact time, a decrease in residues accessible for degradation was observed.[67] Transformations of both organic and inorganic contaminants in soil may result in their mobility being lower within the environment, which in turn might lead to their reduced availability to humans. Therefore, risk assessments could be optimised by employing both exposure estimation based on the effective concentration (effective dose) of the contaminant and existing intrinsic toxicity data.[43]

Although availability concepts are being increasingly recognised to play a critical role in risk assessment, standards regarding how to translate this information into risk-based decision making are scarce.[50] Also, multiplicity of bioavailability and bioaccessibility definitions[27,43,64] may lead to confusion and hamper their applications across different disciplines.

3.1. Advances in human health risk assessment

With respect to human health risk assessment the bioavailable fraction is defined as the fraction of the chemical that can be absorbed by the body through the gastrointestinal system, the pulmonary system and the skin, into the blood and lymphatic system, and equals 'uptake.'[27] Two operational definitions of bioavailability exist, namely absolute (defined as the fraction or percentage of an external chemical dose that reaches the systemic circulation) and relative bioavailability.[38] The latter compares absolute bioavailabilities of different forms of a contaminant or for the different exposure media containing the contaminant. Relative bioavailability is important for the risk assessment of land contamination, where matrix effects can substantially alter the bioavailability of a soil-associated contaminant.

Determining bioavailability requires *in vivo* testing of either human volunteers or an appropriate surrogate animal model with oral uptake physiology similar to that of humans. Such assessments are costly and are associated with ethical concerns. To our knowledge, only one soil sample, containing elevated lead levels, has been studied in terms of human bioavailability.[49] Simulation of the dissolution of soil contaminants in the human gastrointestinal tract in laboratory tests (*in vitro*) has therefore been suggested to provide an upper limit of human oral bioavailability, namely the bioaccessibility. Thus, the bioaccessible fraction is defined as the fraction, which occurs as a vapour or is released during processes like digestion into solution ('intake').[38]

The ultimate aim of developing *in vitro* bioaccessibility methods is to reduce the need for human and animal testing in routine risk assessments. The principle underlying *in vitro* method development is that bioavailability depends on the rate and extent of release/solubility of a contaminant into an extraction solvent resembling human gut fluid. Most of the UK laboratories use a slightly modified version of a method originally described by Ruby et al. (1996),[59] known as the 'physiologically based extraction test' (PBET). In this test, soils contaminated with metal species are incubated at 37 °C in an acid solution for a specified period, in order to mimic the conditions experienced by food within the stomach. The pH of the solution is then increased to a value near neutral, with incubation continuing for a further period of time in order to mirror residence time in the small intestine. Enzymes and organic acids are also added to simulate gastric and small-intestinal fluids. In addition to the stomach and intestine phases, some *in vitro* methods also include a saliva phase.[35]

Evidence of a strong correlation between *in vitro* bioaccessibility and *in vivo* bioavailability data for different soil types is considered as a key condition for acceptance of *in vitro* bioaccessibility methods in human health risk assessments. Therefore, the absence of reference materials containing a range of *in vivo* data with which to validate bioaccessibility results also reduces the confidence of the results generated with *in vitro* methods.[36] Furthermore, differences in types of *in vitro* methods, operating procedures and reporting of results could contribute to a large variation in bioaccessibility data.[35]

The Environment Agency of England and Wales already incorporates bioavailability and bioaccessibility into human health risk assessments provided the information is based on substantial research. To-date, sufficient data have been presented only for lead and benzene. Consequently, guideline values are based on the contaminant concentration in soil that will result in a given amount of lead in the blood following ingestion or for benzene following inhalation.[27] Contaminant partitioning has also been taken into consideration when estimating plant uptake using generic regression equations[60] that incorporate information on contaminant ageing, soil type and compound hydrophobicity. This is subsequently incorporated into a human exposure model.

One of the important assumptions of bioaccessibility testing with respect to humans is that it represents exposure only through the oral ingestion pathway.[33] However, ingestion may not be the primary pathway for some contaminants, for example volatile organic compounds. It is noteworthy that there is dearth of widely acceptable methods for estimating bioavailability of organic contaminants such as PAHs from soils.[38] This is primarily due to the complex metabolism of most organic compounds.

3.2. Other developments related to bioavailability/bioaccessibility

Semantic definitions of bioavailability and bioaccessibility as given by Semple et al. (2004)[63] may serve as a background to advances in methodology within ecological risk assessments and to remediation strategy improvements. In this context, the bioavailable fraction can be defined as that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time. On the other hand, bioaccessibiliy relates to the compound which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical. In other words, the term bioaccessbility embraces both bioavailability and potential availability of the compound over a wider time span.

A plethora of methods shown to reflect the bioavailable/biaccessible fraction[63] have been reported[14,17,48,56] and differentiated on the basis of whether the method employs biological assays or a chemical approach. Clearly, both approaches have their advantages and constraints (Table 2). Nevertheless, the preference for a method to be rapid, precise, ethical and reasonably cheap resulted in the recent development of chemical-based approaches that could subsequently replace time-consuming biological assays.

Table 2. Comparison of the methods to assess organic contaminant bioavailability

Biological assay	**Chemical method**
Longer	Quicker
Variability	More precise
Require appropriate biota	May require specialist and equipment
Involves living organisms	Environmental burden (use of solvents)
Ethical issues	No ethical issues
Usually more expensive	Usually cheaper

There are a number of organic solvent extractions applied in a non-exhaustive manner in order to reflect contaminant bioavailability.[2,11,46] These studies showed that the extents of PAHs removed by mild extraction decreases with increased ageing time. Good correlations were evident between the amounts of unaged and aged PAHs assimilated by *Eisenia foetida* and the 95% ethanol extraction.[73] Similarly, Liste and Alexander (2002)[48] showed no significant ($p < 0.05$) difference between butanol extraction and worm uptake of chrysene.

A need to develop a method that reflects complexity of interactions between soil, the soil biota in question and the physico-chemical properties of a range of organic contaminants, has led however to investigation of alterative approaches that rely upon desorption of contaminant into aqueous phase. One of the first examples published by Cornelissen et al. (1997, 1998)[13,14] predicted PAH microbial accessibility (degradation) using solid phase extraction (Tenax). Tenax has been shown to extract the rapidly desorbing fraction (via the aqueous phase) and to reflect the microbially available fraction within the initial desorbing phase. More recent studies[18] showed that the residual 3- and 4-ring PAH concentrations after a Tenax extraction were comparable with the residual concentrations after 21 d of biodegradation. It has also been demonstrated that Tenax could be used to predict the extent of microbial degradation of total petroleum hydrocarbons (TPH).[16] Cuypers et al. (2000, 2001)[16,17] have also presented a persulfate oxidation method to assess the microbially available fraction. This method, based on selective chemical oxidation, removes the bioavailable contaminants from the amorphous (also called 'soft' or 'rubbery') soil/sediment organic matter (SOM). Under optimised conditions residual concentrations of PAHs strongly correlated with residual concentrations after biodegradation.[16,17] A novel approach using hydroxypropyl-β-cyclodextrin (HPCD) based on the principle of desorption to the aqueous phase has recently been developed.[54,56,71] The unique property of the hydrophilic HPCD molecule and its hydrophobic organic cavity provided the hypothesis that these compounds would extract labile soil-associated PAHs, whereas sequestered contaminants would not readily transfer to the aqueous phase.[56] The HPCD-based extraction method, relying on contaminant mass transfer, has been successfully applied to predict microbial degradation in single and multiple contaminant systems involving a range of PAHs under both laboratory[3,42,56,71] and real world conditions.[18,41]

Recently, the International Organization for Standardization (ISO) has released a guidance for the selection and application of methods to measure bioavailability in soil and soil materials.[43] It was created as a response to an increasing demand for a validated pool of methods to be used in soil assessments and promotes the development and the introduction of the bioavailability concept for a particular receptor in the context of specific site circumstances.

4. DISCUSSION

Information on contaminants dissipation from soils, conceptualised employing ‘ageing’ processes (Figure 3) and contaminant loss (for instance, via volatilization, photolysis, leaching and degradation) may facilitate a more proportionate risk assessment. Models incorporating total contaminant concentrations may overestimate risks and represent a highly conservative approach. On the other hand, endeavours to apply bioavailability/bioaccessibility face difficulties already at the stage of an explicit defining of these terms. A wide range of ways to explain bioavailability/bioaccessibility concepts, even if inherent for an emerging field of research, may lead to confusion and obscurity. As the terms ‘bioavailability’ and ‘bioaccessibility’ are already commonly used in human health risk assessments,[27,35] care should be taken when employing these terms across other fields such as contaminants partitioning and contaminant availability to other biota. At the moment, it therefore might be necessary to specify the receptor and the context in which the terms bioavailability/bioaccessibility are used (‘bioavailable for’).

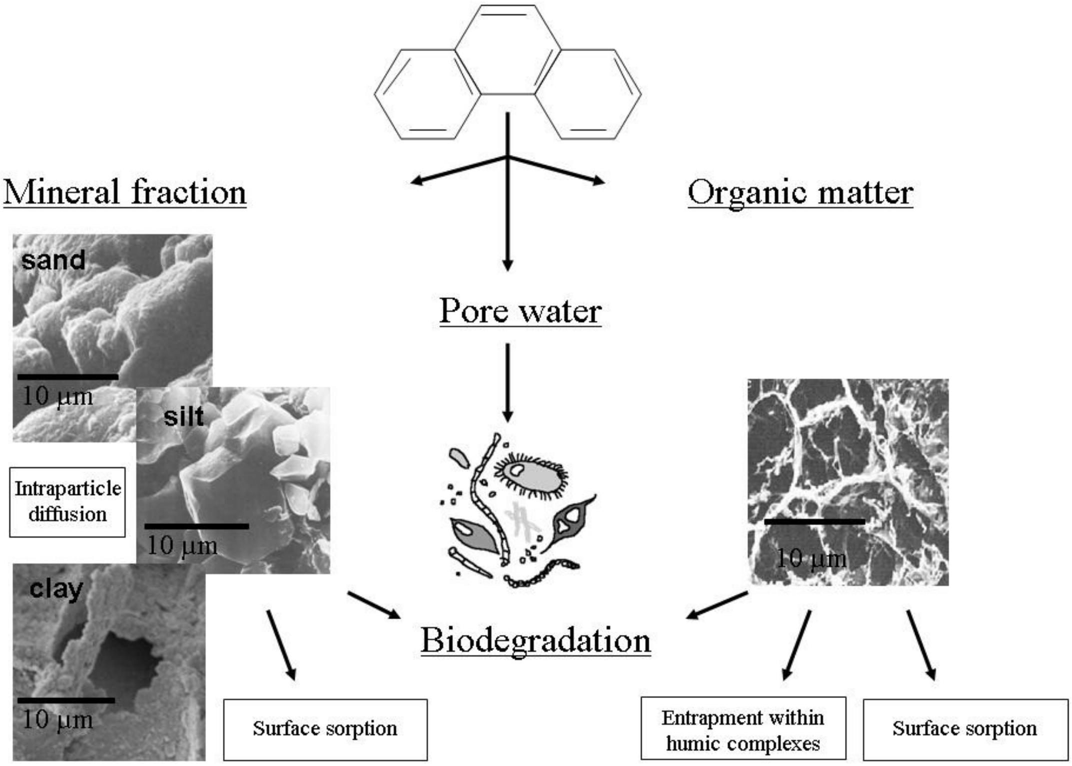

Figure 3. Ageing processes of organic compounds in soils (adapted from Reid et al., 2000).[56] Photos courtesy of Dr. Keith Tovey, University of East Anglia.

4.1. Bioavailability/bioaccessibility vs. Soil Guideline Values and site-specific human health risk assessment

Fundamentally, decisions regarding risk associated with contaminated land can be supported through the application of SGVs or through site-specific assessments that currently might incorporate information on contaminant bioavailability. SGVs-based human health risk assessments ought to encompass variability between individuals and must be applicable across a range of soil types and site conditions. Therefore, regardless of the existing literature supporting natural degradation and dispersion processes that lead to a gradual reduction in contaminant concentrations over time, this generic assessment criteria adopts precautionary, ‘no degradation’ position. In 2006 The Cabinet Office Soil Guideline Value Task Force in response to concerns about the conservative derivation of SGVs released a document ‘Soil Guideline Values: The Way Forward.’[20] It was implied that SGVs are not proportionate or realistic, representing an obsolete and archaic benchmark. So far, there has been no consensus with respect to bioavailability in relation to this document.

At the moment the EA does not support inclusion of bioavailability/bioaccessibility into derivation of SGVs for most compounds due to large uncertainties and gaps in its scientific validity.[20] There is still no convincible method that would enable to reduce the level of conservatism within SGVs derivation without causing a potential risk to human health. Considering PAHs in human health risk assessment appears to be even more complicated. As possible carcinogens, they carry putative risk at even slight levels of exposure[27] and commonly occur in mixtures.

Studies evaluating the geochemical parameters of soil in relation to bioaccessibility and bioavailability in humans are still in their infancy. Animal studies that have been conducted to assess bioavailability and/or to validate *in vitro* methods often limit the number of samples used (because of the costs involved and practicality) and generally do not involve the geochemical characterisation of samples. In addition, many studies have used artificially-contaminated samples (spiked samples) instead of genuinely-contaminated matrixes. That may limit our understanding of real-world circumstances.[35] There are some other knowledge gaps to be filled. Two main problems with validation of bioaccessibility are a limited confidence in the generation of data due to the large variation in results obtained from laboratories on the same soils, and a lack of evidence that methods and data are being correlated with appropriate robust bioavailability (*in vivo*) data to address population variability. To date, only one soil sample, containing elevated lead levels, has been studied in terms of human bioavailability.[49] The results showed that absorption was approximately an order of magnitude different (26.2% vs. 2.5%) between the fasted and fed states, respectively. There might be other co-factors influencing different absorption rates between different individuals. That might result in problems with extrapolation of bioavailability data to a larger, diverse population.

Yet due to widespread presence of many contaminants in the environment at levels exceeding guideline values at many investigated sites, there is a need for decision-support tools in deciding what actually constitutes a 'significant possibility of significant harm' to a specific receptor. This is true especially as some guidelines values are often below ambient contaminant concentrations stemming from natural sources. Remediation of all sites is virtually not possible especially in the sustainability context. Information on contaminant bioavailability may serve this additional tool within site-specific risk assessments facilitating proportionate risk evaluation and cost-effective land management. Whilst there is relatively robust research on bioaccessibility of heavy metals and arsenic the information on bioaccessibility of PAHs is scarce. PAHs are widely distributed in the environment and due to transportation present both at industrial sites and in pristine soils. Benzo[*a*]pyrene is prevalent at elevated concentrations elsewhere. Therefore, there is a need for extensive research on PAH bioavailability/bioaccessibility with an emphasis on the fate of benzo[*a*]pyrene being often a surrogate for PAH to assess human exposure[57] and the contaminant of a critical concern. This information could support regulators' decision-making on sites where PAHs are found.

Regardless of significant number of studies that uncovered the principles of contaminants behaviour in soil systems, detailed predictive models of such soil-contaminant systems are still in their early development.[25,50] Contaminant availability should be therefore considered with caution, on site-to-site basis. On the other hand, uncertainties associated with bioaccessibility should not hamper implementation of scientific developments into decision-making. Focusing on scientifically supported trends and peer-reviewed academic advances can not only stimulate further research on bioavailability and bioaccessibility but also serve alternative pool of available solutions, challenging and improving *status quo*.

4.2. Other applications of bioavailability/bioaccessibility

Assessing availability of contaminants to soil biota may indirectly, or indeed directly, indicate the significant possibility of significant harm to ecological receptors. Critical factors in selecting species as indicators of possible harm within ecological risk assessment are their ecological relevance, importance and sensitivity. In general, for organic contaminants bacteria and earthworms are chosen for bioavailability assessments. Bacterial biodegradation rates are usually higher than earthworms bioaccumulation rates.[71,73] However, Tang et al. (1998)[72] showed that following microbial degradation, earthworms were still capable of assimilating PAHs. The results suggested that extensive biodegradation by microorganisms does not necessarily remove the entire bioavailable fraction of an aged compound. Therefore, toxicological assays should be specific to individual groups of organisms, typically those of highest importance or interest rather than constrained to the species for which contaminant bioavailability is the highest.[73]

Within ecological risk assessment it has been recommended that background levels of contaminants should be considered in the derivation of soil screening values.[37] This is controversial as ambient concentrations of a number of contaminants have increased both from natural and human activities (e.g. lead). Current ecosystems have evolved and adapted to these changes and external remedial intervention could deteriorate rather than improve. In such circumstances bioavailability/bioaccessibility evaluation could assist as a decision-support tool to assess receptors’ exposure and establishing ‘significant possibility of significant harm’.

Information on contaminant bioavailability can be a very powerful tool both within ecological risk assessment and in assisting selection of the remediation approach. Real-world implementation of contaminant bioavailability data (including PAHs) along with chemical measurements of bioavailability (for example using Tenax), toxicological assays (including bioassays with nematodes) and site ecology (field inventory of soil ecology) has already been reported[40] and resulted in sustainable solutions within ecological risk assessments. These comprehensive solutions encompassed not only biochemical processes in contaminated matrixes but also involved wider context of complexity of whole ecosystem protection resulting in a more realistic risk assessment and preventing endangered species impairment. Indeed, contaminated land management often entails complex circumstances (for instance protection of ecologically valuable sites) as well as social aspects (desire for green spaces) and it is important to incorporate these into decision-making.

Bioavailability data may also serve the information about biodegradation endpoint. This is remarkably useful when selecting bioremediation as a remediation technique for sites contaminated with organic contaminants. Most of the studies on bioavailability consider however only individual organic compounds whereas industrially contaminated sites are predominantly contaminated by mixtures of compounds. Co-contaminants such as benzene, toluene, ethylene and xylene (BTEX) and aliphatic hydrocarbons can hinder remediation of PAH.[6] BTEX and aliphatic hydrocarbons are readily biodegradable *in situ* which results in the depletion of available oxygen and the onset of anaerobicity. Details regarding the efficiency and scale of PAH degradation in anaerobic conditions is limited and recent studies of the mechanisms of PAH anaerobic degradation are still tentative.[6] Heavy metals, often present at contaminated sites along with organic contaminants, may inhibit microbial degradation and affect biodegradation rates.[4,61] In addition, the presence of PAHs in multicomponent mixtures causes interactive effects, which can either increase or decrease the rate of degradation of an individual PAH.[19] The effects of metal toxicity on organic pollutant biodegradation as well as interactions within PAHs group have not been adequately defined quantitatively and qualitatively and there is a need for research in that field.

Incorporating the information on contaminant bioavailability/bioaccessibility may facilitate selection of remediation technique, saving time and money. Nevertheless, as biodegradation evaluation entails burden of variability, the soil remediated on the basis of bioavailability should be post-monitored, where possible. This is due to uncertainty regarding the fate of residues left on-site. Indeed, it is possible that non-available compound is released to the soil solution when on-site circumstances change which could pass on a legacy of contamination (assuming that 'significant possibility of significant harm' resulted).

5. CONCLUSIONS – TRANSLATING SCIENCE INTO POLICY

Breakdown of communication between the sciences and humanities as developed by Snow in the concept of 'Two Cultures' was perceived as a main hindrance for solving environmental problems already in 1959.[70] There is now a need for bridging scientific research and social science to avoid dispute: 'Why does the policy-maker not do anything?' against 'Why the researcher can not deliver a science?' This is also true for managing contaminated land where solving environmental problems entails often conflict of interests.

When making environmental decisions it is necessary to achieve a balance between over- and under-protection. Under-protection causes potential risk, whereas over-protection results in extensive costs not only in financial terms but also in the context of what the society has to give up in return (opportunity costs). Advances in scientific research on contaminants behaviour in soils contribute to a continuous increase in tools available to support risk-based approach within sustainable contaminated land decision-making. Risk-based regulatory regimes undoubtedly facilitate the incorporation of emerging scientific advances into environmental decision-making. Yet including information on bioavailability/bioaccessibility into risk assessments is not intended to replace other approaches. Bioavailability/bioaccessibility data increases the set of tools available to support evaluation of contaminated land and may provide a way forward towards the cost-benefit trade-off. Collectively, information on contaminants bioavailability/bioaccessibility offers potential benefits for decision-making and its incorporation into risk assessments may contribute to a more transparent and realistic contaminated-site assessment, facilitating effective land management and promoting sustainable land regeneration.

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CHAPTER 2

Environmentally friendly assessment of organic compound bioaccessibility using subcritical water

Environmentally friendly assessment of organic compound bioaccessibility using subcritical water

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ABSTRACT

The evaluation of microbial availability of contaminants is of a high importance for better reflecting the processes governing contaminant fate in the soil and for establishing the risk associated with contaminated sites. A subcritical water extraction technique was assessed for its potential to determine the microbially-degradable fraction of ^{14}C-phenanthrene-associated activity in two dissimilar soils at three different ageing times (14, 28 and 49 days). For the majority of determination, no significant ($p > 0.05$) difference between subcritical water-extracted ^{14}C-activity at 160 ºC and the fraction mineralized by catabolically active *Pseudomonas* sp. was observed. Collectively, results suggested that the subcritical water extraction technique was an appropriate technique for predicting the biodegradable fraction of phenanthrene-associated ^{14}C-activity in dissimilar soils following increasing soil-contaminant contact time.

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1. INTRODUCTION

Soils represent a major global reservoir for organic contaminants (Semple et al., 2005) that enter the soil as a consequence of incomplete combustion of organic materials, from both anthropogenic activities and natural processes (Wild and Jones, 1995). Among scientists and regulators there is concern regarding the potential of organic chemicals to accumulate in the environment, to a level that results in negative impacts on soil biological functions, its fertility and productivity (Fisher, 1999). Many organic compounds are of interest due to their persistence and putative carcinogenic properties (Wild and Jones, 1995). However, as organic compounds persist in soil they become progressively less available for uptake and biotransformation (Alexander, 2000). These processes, collectively termed 'ageing' are primarily governed by sequestration mechanisms that involve diffusion into soil pores, partitioning into soil organic matter and/or strong surface sorption (Hatzinger and Alexander, 1995). Moreover, the environmental fate of contaminants is determined by the physico-chemical characteristics of a compound in addition to its volatilization and leaching from the soil (Xing and Pignatello, 1997; Semple et al., 2003).

The United Kingdom's definition of contaminated land (Part IIA of the Environmental Protection Act 1990) advocates a risked-based approach to contaminated land assessment. Implicit to the need to establish a 'significant pollutant linkage', there is a necessity to consider the available fraction of a contaminant as this is the fraction that represents a risk to living organisms (Alexander and Alexander, 2000). A greater understanding of partitioning processes may also contribute to accurate evaluation of potential contaminant pathways affecting other receptors, such as controlled waters (Environment Agency, 2006).

Biological assays are frequently used to assess the available fraction of contaminants in soil (Menzie et al., 2000) yet they are often time-consuming and expensive (Alexander, 2000). Non-exhaustive (mild) extraction techniques offer an alternative approach. A number of mild-extraction techniques shown to reflect the bioavailable/bioaccessible (as defined by Semple et al., 2004) fraction of organic contaminants have recently been developed (Cuypers et al., 2002; Liste and Alexander, 2002; Allan et al., 2006). Nevertheless, further work is required to correlate chemical extraction extents with microbial degradation in order to resolve the applicability of techniques with respect to compound availability assessment (Swindell and Reid, 2006). A further motivation to the adoption of non-exhaustive methods is the desire to reduce the use of hazardous organic solvents. This has led to the recent development of a variety of new extraction approaches and to the investigation of alternative extraction fluids (Yang et al., 1997).

Water is a natural solvent, widely available in a high state of purity (Deng et al., 2004). It is non-toxic and hence its disposal results in little burden to the environment (Smith, 2006). Yet at ambient temperature the dielectric constant of water is approximately 80 (Uematsu and Franck, 1980), precluding efficient solvation of low-polarity organic compounds (Hawthorne et al., 1994). The high polarity of water can be significantly lowered by increasing its temperature (subcritical water when $T < 374$ ºC) under moderate pressures to maintain its liquid state (Miller et al., 1998). At a temperature of around 200 ºC, water under pressure has similar properties to an organic solvent, such as methanol (Miller and Hawthorne, 1998) hence it has the ability to extract non-polar organic compounds. By altering extraction conditions, namely by selecting an appropriate extraction temperature, it is therefore possible to generate an aqueous solvent with a range of properties that correspond to different proportions of methanol and water. Furthermore, there is significant attention in the literature on the effect of moisture in the extracted sample on the extraction yields when using organic solvents (Handley, 1999). Research has indicated substantial differences in extraction efficiency between samples containing different amounts of water (Heemken et al., 1997). Sample moisture content is not a cause for concern when using water as a solvent and, as a consequence, a drying step is not required. In addition, in natural systems PAH desorption occurs principally in aqueous phases (Johnson and Weber, 2001). Thus, it may be preferable to adopt an approach that employs solutions likely to occur naturally in the soil when incorporating chemical extraction technique within contaminant availability assessment. The use of water as an extraction solvent has also received recent attention due to an increasing demand for versatile analytical methodologies and costs reduction (Smith, 2006).

While the use of superheated water as an extraction method has been successfully applied to predict rates of long-term release of hydrophobic organic compounds (Miller et al., 1998; Hawthorne et al., 2000; Johnson and Weber, 2001), to our knowledge, no comparative studies between HOC extractability using superheated water and HOC accessibility to microorganisms for biodegradation have been undertaken.

The aim of the present study was to correlate organic contaminant (phenanthrene) extraction using superheated water with the extent of microbial biodegradation in the soil. To this end this study sought to: (i) determine extraction efficiencies under different extraction conditions for two dissimilar soils; (ii) establish trends in compound partitioning with ageing; (iii) establish if a consistent relationship between extractability and microbial bioaccessibility (biodegradation) existed.

2. MATERIALS AND METHODS

2.1. Chemicals

Phenanthrene (purity > 98%) and its radiolabelled analogue [^{14}C-9]-phenanthrene (radio-chemical purity > 98%, 55.7 mCi mmol^{-1}) were purchased from Sigma Aldrich Ltd., UK. Methanol used to prepare spiking solutions was obtained from Fisher Scientific UK. Sample oxidizer scintillation cocktails (Carbosorb-E$^{\mathrm{TM}}$, Permafluor-E$^{\mathrm{TM}}$), Combustaid$^{\mathrm{TM}}$ and the liquid scintillation cocktails (Ultima Gold and Ultima Gold XR) were purchased from Perkin Elmer Life Sciences, UK. 'Pico' glass scintillation vials (7 ml) were supplied by Cranberra Packard, UK. Sodium hydroxide and GF/A filter paper were obtained Merck, UK and Whatman, UK, respectively. All salts used to prepare the inorganic minimal basal salts solution (MBS) were provided by BDH chemicals, UK.

2.2. Soil collection, spiking and storage

Two dissimilar soils (A horizon), Moulton sandy loam and Sheringham loam were collected from sites in Norfolk (UK) at NGR grid references TG 199 073 and TG 113 355, respectively. Prior to spiking, soils were air-dried for 10 d and subsequently homogenised by sieving (2 mm). The soils texture (based on Eldridge, 1980; Hoge et al., 1984) along with other soils properties are listed in Table 1. Organic matter content was determined by mass loss on ignition at 450 °C. Water holding capacity (WHC) and moisture content were determined by oven drying at 70 ºC, to constant mass.

Table 1. Soil properties

Soil series	Grid reference	% Sand[a]	% Silt[a]	% Clay[a]	% LOI[b]	pH
Moulton	TG199073	63[c]	25[c]	12[c]	3.3	7.7
Sheringham	TG113355	47[d]	44[d]	9[d]	3.4	6.6

[a] Values for residue after ashing
[b] LOI: loss on ignition (dry weight basis)
[c] Data from Hodge et al. (1984)
[d] Data from Eldridge (1980)

Spiking solutions containing a mixture of $^{12}C/^{14}C$-phenanthrene were added to the soils following a single-step spiking/re-hydrating (60% WHC) procedure described elsewhere (Reid et al., 1998). Spiking standards delivered a phenanthrene concentration of 100 mg kg $^{-1}$ and a ^{14}C-activity of 64 and 58 Bq g $^{-1}$ to Moulton and Sheringham soil, respectively, relative to soil dry weight. Control treatments with re-hydrated soils containing only ^{12}C-phenanthrene (100 mg kg $^{-1}$) were also prepared. Blending was carried out using a stainless steel spatula in glass beakers while distilled water was used in the re-hydration stage. After thorough mixing of the soils (357 g d.w.), the treatments were incubated within sealed amber glass jars (125 ml, Fisher Scientific UK), in the dark at 15 ºC for 14, 28 and 49 days. The soils were not sterilised in order to reflect processes occurring in the natural environment. Phenanthrene availability was therefore not altered by possible changes in the soil structure and contaminant loss from biodegradation by indigenous microflora was not prevented.

2.3. Determination of total ^{14}C-phenanthrene-associated activity in soils

At each sampling time, total ^{14}C-activity remaining in the soil was determined by sample oxidation. Soil samples (2.0 g; n=10) were packed into cellulose combustion cones and combusted using a Packard 307 sample oxidiser (3 min combustion time).

Combustaid (100 µl) was added to each combustion cone. $^{14}CO_2$ released during combustion was trapped in 10 ml of Carbosorb-E (> 95% trapping efficiency) and eluted using Permafluor-E scintillation cocktail (10 ml). The obtained solutions were counted on a Canberra Packard Tri-Carb 2900TR liquid scintillation counter (LSC), using standard calibration and quench-correction techniques.

2.4. Superheated Water Extraction Procedure

An operationally defined extraction scheme was designed. Extractions were performed on Accelerated Solvent Extractor (ASE 200, Dionex (UK) Ltd.) on 5 g samples (Dionex Application Note 313, 316) in 33 ml stainless steel cells. Cellulose disks were placed at the outlet end of each extraction cell. To prevent a blockage of the extraction system the soil samples were mixed with Ottawa quartz sand (Fisher Scientific UK). Extractions were conducted using miliQ water as a solvent at six different temperatures; 40, 80, 120, 160, 180 and 200 ºC under the pressure of 1500 psi. For every temperature three static times were applied (5, 10 and 20 min). Static time (or extraction time) was defined as the contact time of the soil sample with the solvent (water) within the extraction cell. Each extraction condition was replicated four times. The extracted analytes were purged from the sample cell using pressurized nitrogen at 150 psi for 120 s to ensure complete transfer from the cells to the collection vials. After cooling, Ultima Gold XR (15 ml) was added to 5 ml of each extract. Specific quench curves that corrected background interferences were applied for both soils for each extraction condition and ^{14}C-radioactivity of extracts was determined by LSC counting. Data relating to ASE-extractability have been presented relative to total ^{14}C-activity obtained by sample oxidation at a given sampling time.

Following the extraction, soil sub-samples were collected from the cell and combusted to verify ^{14}C-activity mass balances.

2.5. Determination of microbial degradability of ^{14}C-residues in soil

Determination of the mineralization of the ^{14}C-contaminant was used as a measure of the microbial bioaccessible fraction of phenanthrene in the soils (Reid et al., 2001). Respirometry assays that provided optimal biodegradation conditions were performed using modified Schott bottles (250 ml) sealed with a Teflon-lined screw cap. A $^{14}CO_2$ trap that was fixed to the cap consisted of a 7 ml 'Pico' glass scintillation vial containing 1 M sodium hydroxide (1 ml) loaded onto a GF/A filter paper. The respirometers containing mineral basalt salts (MBS, 30 ml) were autoclaved prior to adding the soil (10 g, 4 replicates). The MBS medium consisted of (g l^{-1}): 0.6 KNO_3, 0.3 NaCl, 0.15 $MgSO_4.7H_2O$, 0.25 KH_2PO_4, 0.6 $(NH_4)_2SO_4$, 0.75 K_2HPO_4 and 1 ml of trace element solution (g l^{-1}; 0.002 LiCl, 0.003 KBr, 0.003 KI, 0.004 $SnCl_2$, 0.008 $CaSO_4$, 0.01 $ZnSO_4$, 0.01 $Al_2(SO_4)_3$, 0.01 NiCl, 0.01 $CoSO_4$, 0.03 $FeSO_4$, 0.06 $MnCl_2$ (Skerman, 1967). An inoculum (2 ml) of catabolically active *Pseudomonas* sp. (13 x 10^6 – 17 x 10^6 cells per g of the soil, d.w.) was added to the slurries at the start of the assay (see below). The respirometers were agitated on a flat bed rotary shaker (Denley) at 100 rpm. $^{14}CO_2$ release due to catabolism of microbially accessible ^{14}C-phenanthrene was trapped upon reaction with the NaOH within the 7 ml vial. The traps were changed periodically and the $^{14}CO_2$ evolution was monitored until mineralization plateaued (~ 21 d). Ultima Gold scintillation cocktail (6 ml) was added to the removed traps and their ^{14}C-radioactivity assessed by LSC. Mineralization of phenanthrene was performed at each ageing time in the soil slurries with and without the addition of *Pseudomonas* sp.

2.6. Inoculum preparation

Phenanthrene degrading bacterial inoculum was isolated on selective agar from a genuinely polluted site. Agar (2%) plates with the colonies were routinely stored in the incubator at 20 ºC. Prior to the analyses, a catabolically active (with respect to phenanthrene) bacterial inoculum (identified by sequence of the 16S rDNA as *Pseudomonas putida*, 100% sequences BLAST), was cultured on phenanthrene (200 mg l^{-1}) in MBS solution at 20 ± 2 ºC (Reid et al., 2000). After four days of incubation (exponential growth phase) on an orbital shaker at 100 rpm, the culture was centrifuged at 5000 *g* for 20 min. The supernatant was discarded and cells re-suspended in MBS. This procedure was repeated to ensure thorough washing of the cells. Harvested cells were collected into a single stock solution and 2 ml was inoculated into the respirometers. Plate counts (Claus, 1989) performed on 2% agar in MBS solution with phenanthrene added as the sole carbon source ensured adequate cell density in inocula added to the respirometers.

2.7. Statistical analysis

Following blank-correction, statistical analysis of the results was performed using SPSS 12.0.1 for Windows. The statistical significance of the superheated water extraction parameters influence on extraction efficiencies was determined using a General Linear Model (GLM; post hoc Tukey test, type III or IV Sum of Squares). If normality assumptions, based on residuals analysis, were violated square roots of the dependent variables were used. To assess ageing and partitioning significance, t-test and Mann-Whitney Test, for parametric and non-parametric data respectively, were applied. Each statistical test was performed at the 95% confidence interval with the significance level at 0.05 unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1. Temporal ^{14}C- activity loss following ageing

The total amount of ^{14}C-activity remaining in the two soils was determined at each sampling time. Significant ($p < 0.001$) decreases in ^{14}C-phenanthrene-associated residual activity were observed between 14 and 28 d of ageing time for both Moulton and Sheringham soil (Figure 1). Similarly, after 49 d, in both soils there was a significant ($p < 0.001$) decline in ^{14}C-activity as compared to 14 d. In addition, there was a more pronounced decrease (29%) in ^{14}C-activity between 28 d and 49 d in the Moulton soil as compared to the Sheringham soil (3%). Collectively, within the 49 d of ageing time during this experiment a greater loss in ^{14}C-activity was observed in the Moulton soil (41%) than in the Sheringham soil (20%).

These results support other studies that show decline in the contaminant concentration as the contact time between the organic compound and the soil increased (Jones et al., 1996; Liste and Alexander, 2002; Swindell and Reid, 2006). Based on the finer texture of the Sheringham soil (Table 1), it was inferred that phenanthrene partitioning into the Sheringham soil would be greater than in Moulton soil and, as a consequence, phenanthrene loss would be hampered. Indeed, after 49 d there was a greater decline in the total ^{14}C-phenanthrene associated activity in the Moulton soil samples (41%) as compared to the Sheringham soil (20%). These losses might be attributed to higher volatilization from Moulton soil and/or microbial activity (Reid et al., 2000; Semple et al., 2003).

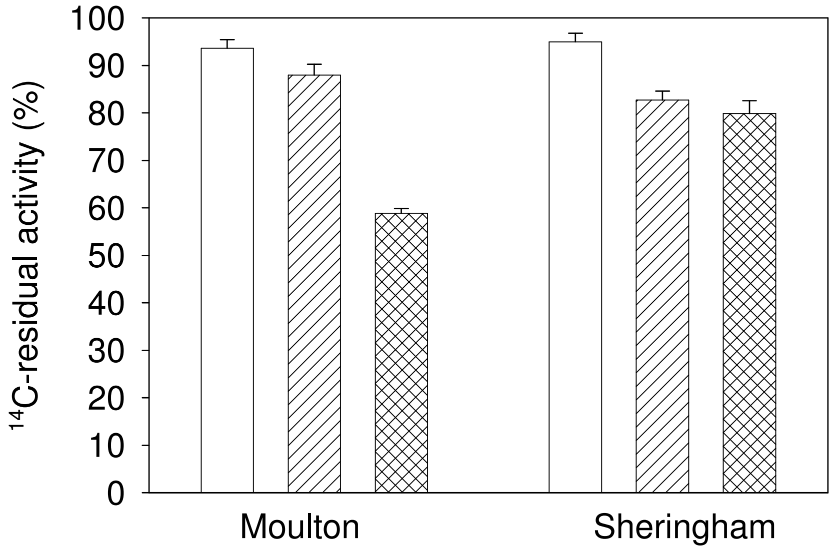

Figure 1. Residual ^{14}C-activity remaining (% relative to initial activity) after 14 (white), 28 (hatched) and 49 (cross hatched) days of ageing time for two dissimilar soils. Error bars represent standard errors (n = 60 for Moulton soil and n = 20 or n = 30 for Sheringham soil).

3.2. Effect of superheated water extraction parameters on extractable ^{14}C-phenanthrene associated activity

3.2.1. Temperature

There was a positive relationship between temperature and ^{14}C-phenanthrene extractability at each ageing time for both soil types (GLM, $r^2 > 0.890$ and $r^2 > 0.883$ for Moulton and Sheringham, respectively; Figure 2). These results are in agreement with trends described elsewhere (Hawthorne et al., 1994; Yang et al., 1997; Miller et al., 1998; Crescenzi et al., 2000) indicating an increase in the extractable PAH fraction as water temperature increased. It may be conjectured that by increasing the extraction temperature while maintaining the solvent in a liquid state (Richter et al., 1996) it was possible to decrease the magnitude of hydrogen bonding and dipolar interactions occurring on the high energy surfaces of the soil sample, thus enhance diffusion. At the lowest extraction temperature of 40 °C the amount of extracted ^{14}C-associated activity was less than 10%. This corroborated previous findings that the high dielectric constant of water at lower temperatures precluded efficient dissolving of hydrophobic organic compound (Hawthorne et al., 1994)

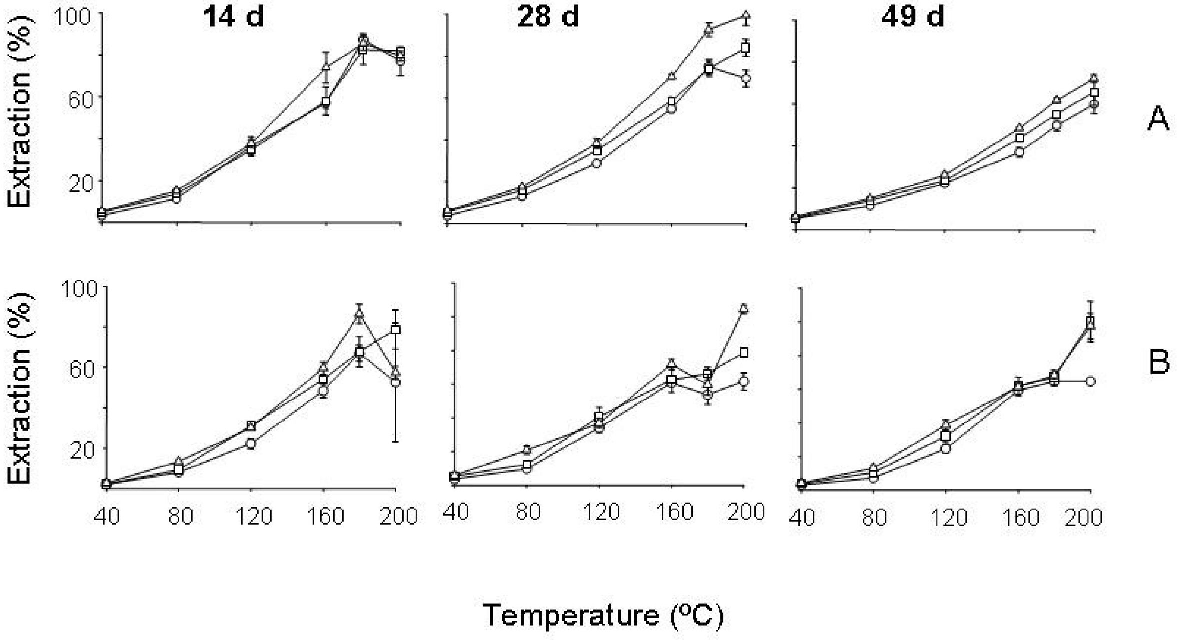

Figure 2. Superheated water-extractable ^{14}C-activity (%) at the temperature of 40, 80, 120, 160, 180 and 200 °C, relative to the total ^{14}C-activity from sample oxidisation in Moulton (A) and in Sheringham (B) soil at the three ageing times (14 d, 28 d and 49 d) with static times (min) of 5 (circle), 10 (square) and 20 (triangle). Error bars, where evident, represent standard error (n = 4).

In five out of six comparisons (Figure 2), there were no significant differences in the amounts of ^{14}C-activity extracted employing higher temperatures (160, 180 and 200 °C) for a specific static time (5 min). Furthermore, extractions extents were inconsistent at higher temperatures (160, 180 and 200 °C), with the exception of Moulton soil after 49 d of ageing time. Two possibilities might account for this: 1) thermal degradation of phenanthrene at 180 °C and 200 °C, as suggested for particular contaminant concentrations by Yang and Hildebrand (2006), or 2) slow sequestration of the compound within the soil pores. The former explanation was rejected on account of mass balances performed at the harshest extracted conditions; these resulted in > 90 and > 99% recovery for Moulton and Sheringham soil, respectively. In support of the latter hypothesis, it is suggested that relatively unaged compound (14 d) would have been largely available for extraction at 160 ºC. As a consequence, the higher extraction temperatures of 180 ºC and 200 ºC resulted in only minimal additional extractability. It was therefore anticipated that as the compound became sequestered within the soil matrix (Hatzinger and Alexander, 1995) with increased ageing (49 d) an opportunity could have existed for incrementally greater extraction efficiency within increasing temperatures from 160 to 200 ºC. Indeed, extraction efficiency was observed to increase significantly ($p < 0.001$) within the highest temperatures in the Moulton soil after 49 d of ageing time. It is possible that 49 d of the phenanthrene-soil contact time was enough for the compound to be diffused into soil pores, adsorbed across a range of sorption sites and/or partitioned into soil matrix in Moulton samples but not long enough for the Sheringham soil (see also section 3.3.).

3.2.2. Static time

Operating at the particular temperature, three static times were applied; 5, 10 and 20 min. Regression equations (1) – (6) illustrate interaction between the temperature (T), static time (S) and the extractability.

(1) $\% \text{ ext} = -27.640 + 0.549T + 0.304S, r^2 = 0.913$

(2) $\%\text{ext} = -33.155 + 0.536T + 0.881S, r^2 = 0.915$

(3) $\% \text{ ext} = -20.515 + 0.381T + 0.477S, r^2 = 0.931$

(4) $\% \text{ ext} = -27.360 + 0.471T + 0.425S, r^2 = 0.821$

(5) $\% \text{ ext} = -28.448 + 0.478T + 0.842S, r^2 = 0.869$

(6) $\% \text{ ext} = -36.558 + 0.547T + 0.651S, r^2 = 0.866$

Equations (1), (2) and (3) reflect processes observed in the Moulton soil samples after 14 d, 28 d and 49 d of ageing, respectively. Similarly, equations (4), (5) and (6) represent Sheringham soil at the same three ageing times. Slopes in the regression equations indicate that the influence of static time is less significant than the effect of temperature on the extraction at 14 d of ageing time for both Moulton (T = 0.549; S = 0.304) and Sheringham (T = 0.471; S = 0.425); Equations (1) and (4). As the ageing time increased there was an increase in the effect of the static time on extraction efficiencies; Equations (2), (3), (5) and (6). These observations cannot be complemented by any extensive previous research as, to the authors' knowledge, static time coupled with rising temperatures of superheated water has not been investigated. However, increasing the static time at elevated temperatures can allow analyte, entrapped within soil sample matrix, to diffuse into the extraction solvent (Dionex Technical Note 208). Indeed, most post hoc analyses of Moulton data (Figure 2) revealed that as the extraction conditions became harsher (with an increasing static time up to 20 min) there was an increase in the amount of the compound extracted. Such a relationship consistent over a range of extraction temperatures was not observed in the Sheringham soil samples (Figure 2, post hoc analyses).

In summary, superheated water liberated a span of ^{14}C-phenanthrene-associated activity (extraction values in the elutant ranged from 5% to 100% depending on extraction conditions; Figure 2). These values represent extraction efficiencies obtained when applying both mild and rigorous extraction techniques. Moreover, higher temperatures result in a decrease of the surface tension of the liquid solvent and its cohesive energy density thereby reducing its viscosity (Edge et al., 2006). In this study, this was reflected by an increase in the amount of water eluted from the cell as the temperature increased, from 10 ml to 40 ml in each collection vial.

3.3. Biodegradation assays and the relationship between ^{14}C-residues bioaccessibility and superheated water extractability

Commensurate with increasing ageing, a decrease in residues accessible for microbial degradation was observed in both soils (Table 2). This is in agreement with other studies (Singleton, 1994; Smith et al., 1999; Dictor et al., 2003) that report decrease in biodegradation with increasing ageing time. Significant decreases ($p < 0.001$) in extent of mineralization in Moulton soil samples between 14 d and 49 d, and 28 d and 49 d of ageing time were observed for inoculated samples. In Sheringham assays, even after 49 d of ageing time, the mineralization values remained high (~ 68%), concordant with the previous observations regarding extractability. Differences in the extents of mineralization between Moulton and Sheringham support previous hypothesis regarding sequestration and are in accordance with findings of others that bioavailability/bioaccessibility is soil-dependent (Hatzinger and Alexander, 1995; Chung and Alexander, 1998; Smith et al., 1999). Although observations on total contaminant loss (Figure 1) may suggest higher sequestration in Sheringham than in Moulton soil, this is not supported by both extractability and mineralization studies.

Higher biodegradation values as observed for the Sheringham soil would suggest higher amounts of phenanthrene to be available. Similar findings that showed higher mineralization extents in loam than in sandy soil following similar ageing intervals have previously been reported (Chung and Alexander, 1998).

Table 2. Mineralization (%) of ^{14}C-phenanthrene associated activity in two dissimilar soils with and without the addition of catabolically active bacterial inoculum. Values are means (n = 4 and n = 3 for inoculated and non-inoculated assays, respectively) ± standard error

Ageing time (days)	Moulton		Sheringham	
	bacteria	no bacteria	bacteria	no bacteria
14	64.97 ± 5.3a	23.14 ± 3.9a	79.84 ± 1.2a	21.34 ± 5.1a
28	53.62 ± 3.5a	55.45 ± 2.2b	67.55 ± 3.3a	71.13 ± 6.5b
49	23.19 ± 0.1b	24.00 ± 0.9a	67.89 ± 4.7a	64.14 ± 4.6b

a/b comparison between values within assay over ageing time; the same letter within a column represents a no significant difference.

Biodegradation tests verifying the catabolic potential of intrinsic fauna revealed the presence of microbes able to mineralize phenanthrene in the both soils (Table 2, 'no bacteria'). There were significant increases in their catabolic competence between 14 d and 28 d of ageing in both Moulton and Sheringham soils. This may be attributed to induction of the specific enzymes enabling incorporation of the organic compound into oxidation processes (Reid et al., 2001) and optimisation of catabolic activity of indigenous microorganisms to degrade PAHs. Indeed, it has been shown that it may take up to several weeks or months to establish potent phenanthrene degrading microbial consortium in the soil (Macleod and Semple, 2006). In support of this, relatively low losses of phenanthrene within the first two weeks after spiking (6.8 and 5.3% for Moulton and Sheringham soil, respectively; Figure 1) suggest that no extensive mineralization has taken place during the initial ageing time.

Relating the superheated water extractability of ^{14}C-phenanthrene-associated activity with the mineralizable fraction allowed the assessment of the ability of water-based extraction to predict microbial degradation (Figure 3). Following ageing, in four out of six comparisons, there were no significant differences between the extraction at 160 ºC and the biodegradation values. Extractions at 40 and 80 ºC underestimated mineralization endpoints in both soils at each ageing time, whereas extraction performed at 180 and 200 ºC overestimated bioaccessibility (Moulton soil) and represented harsher extraction conditions. Among static times, 10 min showed the closest relationship with the mineralized fraction. However, the consistency of the superheated water extraction (160 ºC, 10 min static time) and its high correlation to biodegradable fraction (Pearson or Spearman coefficients for parametric and non-parametric data, respectively) was no longer evident in the Moulton soil after 49 d. The extraction overestimated the amount of mineralized fraction by about 20%. Discrepancy between the extraction value at 160 ºC and the mineralized fraction was also observed in the Sheringham samples after 14 d. The extraction significantly underestimated the mineralization value by about 25%.

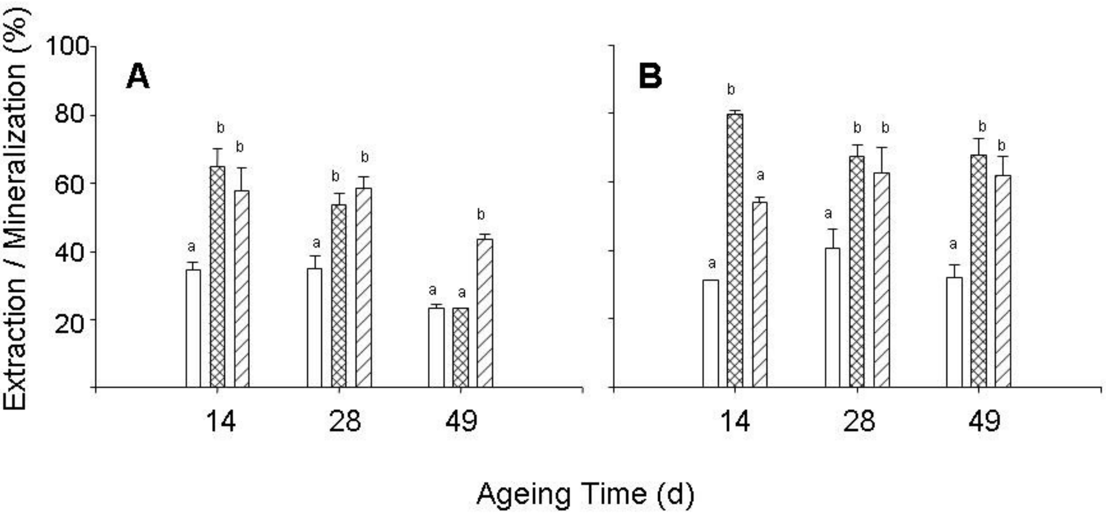

Figure 3. Comparison of the superheated water-extractable ^{14}C-activity (10 min static time) and mineralization values in Moulton (A) and Sheringham (B) soil after 14, 28 and 49 days of ageing time. Bars represent extraction at 120 ºC (white), mineralization extents (cross hatched) and extraction at 160 ºC (hatched); n = 4. Error bars represent standard errors. The same letter within ageing period indicates no significant difference.

Collectively, our results support superheated water extraction as a means of predicting phenanthrene bioaccessibility. This research however represents a first step in developing a new technique for the assessment of HOC bioaccessibility. There is a need to extend the investigation to a mixture of compounds from genuinely polluted soils in order to reflect real world conditions. It is possible that the temperature of subcritical water extraction that reflects bioavailable/bioaccessible fraction may differ depending on analyte physico-chemical properties, ageing time, presence of co-contaminants and soil types. Nevertheless, consistent observation (with respect to ^{14}C-phenanthrene-associated activity) of no significant differences between the superheated water extraction and the biodegradable fraction at different ageing times in dissimilar soils warrants further research towards finding an appropriate and environmentally-friendly procedure.

4. CONCLUSIONS

In an era of environmental pollution and increasing public awareness, soil quality is emerging as an issue of vital importance to regulators, scientists and developers for effective land management. Consequently, there is a need for tools to predict the fraction of contaminant available to biota hence to measure the risk posed by contaminants in soils. To-date, inadequate scientific understanding of soil contamination-related processes has hampered the widespread consideration of bioavailability/bioaccessibility processes in remedial decision making (Kreitinger et al., 2007). Also, despite a common use of bioremediation, it has been observed that some fraction of PAH remains undegraded on account of their low bioavailability/bioaccessibility (Cuypers et al., 2000). Thus, applying bioavailability/bioaccessibility when considering bioremediation as a cleaning-up technique may assist in the land management and reduce costs of remediation.

Within the context of overall site management, chemical measurements of contaminant availability are not intended to replace other site-management approaches, rather they are a means to broaden the range of available options. This investigation is a step forward in finding an appropriate rapid tool for the assessment of the bioaccessible fraction of soil associated contaminant.

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CHAPTER 3

Subcritical water extraction as a potentially 'greener' approach to assess organic contaminant partitioning

Subcritical water extraction as a potentially ‘greener’ approach to assess organic contaminant partitioning

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ABSTRACT

A rapid sequential subcritical (superheated) water extraction method for polycyclic aromatic hydrocarbons contaminated soil and sediment is presented. Decreasing the polarity of water by successive increase of the extraction temperature from 50 °C to 200 °C at moderate pressure (103 bar) enabled selective, non-exhaustive extractions to be performed. Concurrent with increasing temperatures to 150 °C there was an increase in PAH extraction efficiencies. For the majority of determinations no significant differences between extractions at 150 °C and 200 °C were observed. Varied extraction efficiencies of PAHs at the same extraction conditions reflected dissimilarities between environmental matrices used in this experiment. Selective subcritical water extraction of PAHs dependant on their octanol-water partition coefficients was observed. The water-based approach to organic contaminant partitioning assessment presented here arguably evokes less burden to the environment. This technique may be applicable in evaluation of risks associated with contaminated sites and in assessments of their bioremediation potential.

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1. INTRODUCTION

Contaminated sites worldwide contain an array of hydrophobic organic contaminants (Jones, 1996) that represent a potential risk to living organisms. It has been widely accepted, that the traditional exhaustive techniques quantitatively extracting the 'total' amount of the target compound from contaminated samples overestimate the magnitude of the environmental and societal problems associated with the residues of organic pollutants (Alexander, 2000). Intra-matrix processes, collectively named 'ageing', promote contaminant sequestration within soil and sediment particles primarily via diffusion into pores, partitioning into organic matter and/or surface sorption (Hatzinger and Alexander, 1995). Less exhaustive techniques have therefore been recently investigated aiming to extract the bioavailable fraction of contaminants (Cuypers et al., 2000; Reid et al., 2000a; Allan et al., 2006). The desire to minimize the use of hazardous organic solvents (Yang et al., 1997), a need for costs reduction and increasing demand for versatile extraction techniques (Smith, 2006) have been the primary rationales behind development of alternative non-exhaustive extraction procedures.

Water has been perceived as 'the ultimate green solvent' being cheap, non-toxic and non-flammable (Smith, 2006). Water is recyclable and its disposal has been regarded as benign with little effect on the environment. Yet at ambient temperatures water is too polar to efficiently extract most of the non-ionic organic compounds associated with contaminated solids (Hawthorne et al., 1994). By increasing the temperature of water under moderate pressures (50-200 bar), to maintain its liquid state, water hydrogen bonding weakens, and the polarity decreases drastically (Smith, 2006). At the temperature of 250°C and a pressure of 50 bar the polarity of water, described by its dielectric constant (ε), equals 27, which falls between those of ethanol (ε = 24) and methanol (ε = 33) (Yang et al., 1997; Miller et al., 1998).

Polycyclic aromatic hydrocarbons (PAHs) are of significance at many contaminated sites (such as gas works, foundries) on account of carcinogenicity of some PAHs (Fisher, 1999). Aqueous solubilities of PAHs at 25 ºC varies from milligrams per litre to less than nanograms per litre and decrease rapidly with PAH molecular weight (MW). For instance, the solubility of naphthalene (MW = 128 g mol^{-1}, log Kow = 3.37) is 31 mg L^{-1} whereas benzo[*ghi*]perylene (MW = 276 g mol^{-1}, log Kow = 6.50) is 0.0003 mg L^{-1} (Environment Agency, 2003).

While subcritical water (T < 374 °C) has previously been reported to efficiently extract PAHs from different environmental matrices (Hawthorne et al., 1994; Yang et al., 1997; Miller and Hawthorne, 1998), feasibility of using Accelerated Solvent Extractor (ASE) 200 and subcritical water to extract PAHs from dissimilar matrices under different extraction conditions has not been determined. To these ends this study sought to investigate the effect of four different ASE extraction temperatures on the extraction extents of different molecular weight PAHs from contaminated soil and sediment (both spiked and genuinely contaminated materials were used) using subcritical water. ASE 200 is a commercially available instrument that allows pressurised liquid extraction (PLE), usually with conventional organic solvents. It was hypothesised that by sequentially raising the subcritical water extraction temperature within ASE extractions it would be possible to generate a spectrum of non-exhaustive extraction conditions and, as a consequence, reflect desorption processes of various MW PAHs. A novel, quick, simple and potentially environmentally acceptable extraction technique to assess labile fractions of hydrophobic organic contaminants is presented.

2. MATERIALS AND METHODS

2.1. Chemicals

All solvents were HPLC grade. Acetone used to prepare spiking solutions, dichloromethane (DCM) and hydrochloric acid (~ 36% analytical grade reagent) were purchased from Fisher Scientific UK, whilst isopropyl alcohol was provided by Sigma Aldrich Ltd UK. Fluorene, phenanthrene and pyrene (purity > 98%) were purchased from Sigma Aldrich Ltd UK. Hydromatrix was provided by Varian (Surrey, UK). Florisil (60-100 mesh) used for in-cell clean up (Hubert et al., 2000; Hildebrandt et al., 2007) within DCM extractions was provided by Promochem, Germany. Copper (general purpose grade) was purchased from Fisher Scientific. TCL Polynuclear Aromatic Hydrocarbon Mix used to prepare standards for GC-MS analyses was supplied by Supelco, USA.

2.2. Samples

For the purpose of this study five different matrices were used: spiked soil and sediment, reference material and genuinely contaminated soil and sediment. Pristine soil (A horizon) and sediment from the River Yare were collected at the University of East Anglia (UEA) at NGR grid references TG 199 073 and TG 191 070, respectively. Prior to spiking, both materials were dried for 14 days and homogenised by sieving (2 mm). Spiking solutions containing fluorene, phenanthrene and pyrene were added into the soil and sediment following single-step spiking/re-hydrating (60% WHC) procedure described elsewhere (Allan et al., 2007) and delivered concentrations of

100 mg kg^{-1}, 500 mg kg^{-1} and 250 mg kg^{-1}, respectively, relative to dry weight. After thorough mixing the treatments (250 g) were tumbled in amber jars (500 mL) in the dark at 15 °C on the end-over-end rotor for 21 days. Matrices were not sterilized thus contaminants degradation by indigenous flora was not precluded. Reference material (RM) was a multi-contaminant matrix made by homogenising (ball-milling) soils containing different concentrations of diesel, lubricating oil and PAH (Hickman et al., 2008). This homogenised material (500 g) was then tumbled in the amber glass at 15 °C for 14 days on an end-over-end mechanical rotor and stored in the dark until used. Genuinely contaminated soil and sediment were collected at the grid references NZ 290 630 and NZ 292 63, respectively, from the former tar works in Newcastle (hereafter TW). Samples were air-dried for 14 days in the fume cupboard under light vacuum and subsequently homogenised by sieving (2 mm). PAH concentrations in the spiked and genuinely contaminated and in the reference material matrices are presented in Table 1, whilst properties of soils and sediments (particle size distribution, organic matter (loss on ignition), % C) are presented in Table 2.

2.3. Determination of total concentrations

Total residual concentrations were evaluated by PLE at each sampling time with ASE 200 (Dionex Corp.). On the bottom and on the top of each extraction cell, 2 and 1 GF/B filters (Dionex) were placed, respectively. Due to the presence of sulphur, which can cause damages within the ASE extraction system, activated copper was placed on the bottom of the extraction cells containing genuinely contaminated samples. The activation procedure of copper is described elsewhere. Prior to sample addition, extraction cells were also loaded with ~ 2 g of cleaning agent (Florisil). Each sample (~ 5 g) was additionally mixed with Florisil (5 g and 10 g for UEA and TW samples, respectively) and the drying agent (Hydromatrix). Remaining head space of the cell was filled with Hydromatrix. Different proportions of cleaning and drying agents in the extractions cells were dictated by differences in samples moisture. TW samples were virtually dry, thus Hydromatix was primarily used in order to fill the dead space of the extraction cell. UEA samples carried greater moisture as a result of the spiking/re-hydrating procedure and therefore more Hydromatrix (~ 3 g) was mixed with the sample. Samples were extracted using DCM (n = 3). Extraction conditions were adopted from EPA Method 3545 and are similar to those employed by others (Schantz et al., 1997; Mielke et al., 2001); 100 °C, 5 min equilibration (heat time), 5 min static (extraction) time, 10.3 MPa (103 bar), 60% flush volume, 1 static cycle. The extracted analytes were purged from the sample cell using pressurized nitrogen at 10 bar for 120 s to ensure complete transfer from the cells to the collection vials.

Table 1. PAH: Total residues extracted with DCM. Retention Time (RT) and Mass-Charge Ratio (*m/z*) applied during GC-MS analyses

PAH (log Kow)	UEA		RM	TW		RT (min)	*m/z*
	soil	sediment		soil	sediment		
	mean, mg kg^{-1} (SD)		mean, mg kg^{-1} (SD)	mean, mg kg^{-1} (SD)			
naphthalene (3.37)	nd	nd	3.72(0.04)	1.48(0.17)	3.74(0.06)	7.313	128
acenaphthene (3.92)	nd	nd	0.18(0.01)	1.45(0.42)	10.8(2.51)	9.925	153
acenaphthylene (4.00)	nd	nd	0.12(0.002)	0.11(0.04)	0.33(0.08)	9.654	152
fluorene (4.18)	60(8.7)	83(28)	0.25(0.04)	1.38(0.33)	14.47(2.56)	10.791	166
anthracene (4.54)	nd	nd	0.70(0.05)	2.84(0.94)	5.69(1.49)	13.240	178
phenanthrene (4.57)	324(49)	400(102)	4.67(1.02)	9.7(3.11)	81.29(16.4)	13.105	178
pyrene (5.18)	146(25)	205(75)	2.33(0.21)	10.10(3.47)	40.36(6.57)	16.839	202
fluoranthene (5.22)	nd	nd	3.85(0.19)	13.13(4.38)	62.88(11.62)	16.269	202
chrysene (5.70)	nd	nd	1.49(0.09)	6.53(2.2)	10.20(1.63)	19.998	228
benzo[*b*]fluoranthene (5.80)	nd	nd	1.64(0.11)	7.13(1.18)	5.67(0.24)	23.103	252
benzo[*a*]anthracene (5.91)	nd	nd	1.50(0.14)	8.21(2.35)	12.77(2.62)	19.910	228
benzo[*k*]fluoranthene (6.00)	nd	nd	0.62(0.05)	3.37(0.83)	2.38(0.37)	23.215	252
benzo[*a*]pyrene (6.04)	nd	nd	0.27(0.01)	9.40(1.33)	4.46(0.03)	24.340	252
benzo[*ghi*]perylene (6.50)	nd	nd	0.75(0.06)	5.34(0.81)	2.00(0.5)	32.360	276
indeno[*123cd*]pyrene (6.65)	nd	nd	0.88(0.13)	5.70(0.96)	1.89(0.24)	30.513	276
dibenzo[*ah*]anthracene (6.75)	nd	nd	0.49(0.08)	2.52(0.94)	1.08(0.10)	30.864	278
∑ PAH	530	688	23	88	260		

nd – not determined

Table 2. Samples properties

Series	% sand	% silt	% clay	% LOI	% C
UEA soil	88	12	0	3.3	2.12
UEA sediment	100	0	0	1.65	1.15
TW soil	83	17	0	7.47	3.35
TW sediment	66	33.5	0.5	3.89	2.33

LOI – loss on ignition at 450 °C (dry weight basis)

2.4. Subcritical water extraction

Water extractions were performed in a similar manner to conventional PLE using ASE 200 (described above) at four different extraction temperatures; 50, 100, 150 and 200 °C. Based on the authors' previous work (Latawiec et al., 2008) a 10 min static time was selected for all water extractions in this study. A flush volume of 20% was used to prevent possible errors in the system occurring due to high water viscosity at lower temperatures. For dispersion of matrices and to prevent blockages in the extraction system samples were mixed with Ottawa quartz sand (20-30 mesh, Fisher Scientific UK). Extractions were conducted using Milli-Q water obtained from Milli-Q systems manufactured by Millipore, USA. All matrices were extracted in triplicate.

2.5. Liquid-liquid extraction

After cooling in the collection vial, each extract (20-40 mL depending on the temperature of extraction) was transferred into a pear shaped separating funnel (100 mL, Scientific Laboratory Supplies UK). The empty collection vials were then rinsed with 2 x 2 mL of DCM and these washes collected in the separating funnel. An additional 5 mL of DCM was added into the funnel that was subsequently gently agitated in order to facilitate the transfer of extracted PAHs from water into the organic phase. After ~ 5 min, DCM deposited as the lower phase in the funnel, was flushed through DCM pre-wetted GF/A filter paper (Whatman UK) into the amber volumetric flask (25 mL). This liquid-liquid extraction procedure was subsequently repeated twice with 8 mL of DCM to ensure complete transfer of PAHs from water to DCM. Prior to method development the efficiency of PAHs recoveries within triplicated liquid-liquid exchange from water into DCM was determined using cumulative curves (data not presented). Volumetric flasks were made up to 25 mL with DCM. During each separation 3 drops of isopropyl alcohol were added to promote definitive boundary between water and DCM.

2.6. Analytical Procedure

Quantification of target PAHs in all extracts was performed using GC-MS fitted with a mass selective detector (Perkin Elmer, Clarus 500). Compound separation was carried out using a fused silica capillary column (Perkin Elmer Elite 5MS, 30 m) coated with 5% diphenyl and 95% Dimethyl Polysiloxane stationary phase (0.25 mm i.d. x 0.25 mm film thickness). Mass spectrometer operated at 70 eV in positive ion mode using selective ion response (SIR). The carrier gas was helium (CP grade, BOC UK) at a constant flow of 1 mL min^{-1}. Autosampler injections (1 µL) were performed in the 1:10 split ratio. The oven temperature was programmed as follows: 35 °C (holding time 1.5 min) raised to 100 °C at gradient of 25 °C min^{-1}, then at 15 °C min^{-1} to 190 °C (2 min hold) and finally ramped at 10 °C min^{-1} to 270 °C and held for 15 min. Total run time was 35 min. The injector, transfer and ion source temperatures were set at 180, 280 and 180 °C, respectively with the detector voltage at 450 V. Identification of PAHs was made by integrating peak areas at specific *m/z* (Table 1) using Turbomass Software provided with the instrument and by comparison of these peaks with the response of a known concentration of PAHs. Analytical parameters such as detection limit and quantification of PAHs were determined using standard solutions and appropriate standard calibration curves. Calibration standard with known concentration was inserted every 6 samples to control any possible machine drift within a run. Consistency of GC-MS responses has been also cross-experiments confirmed to provide comparability of the results.

2.7. Statistical analysis

All statistical analyses were performed using SPSS 16.0 for Windows. Statistical significance of the influence of extraction temperature on extraction efficiencies of different compounds was determined at 95% confidence interval with the significance level at 0.05 unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1. Effect of subcritical water temperature on extractions from spiked soil and sediment

There was a positive linear relationship between the temperature of extraction and the extraction efficiencies for fluorene ($r^2 = 0.75$, $p < 0.001$) and phenanthrene ($r^2 = 0.85$, $p < 0.001$) from the spiked soil (Figure 1). Pyrene (of greater Kow, Table 1) was extracted above the detection limit from the UEA soil only at 200 °C.

In the spiked sediment it was observed that relationships between extraction efficiency and temperature for fluorene and phenanthrene followed quadratic regression (with r^2 values of 0.916 and 0.997, respectively; $p < 0.001$). From the quadratic regression line it was predicted that 160 °C (fluorene) and 170 °C (phenanthrene) were maximum extraction temperatures after which no further increases in extractions would be observed. In addition, no significant increase in the extractions of pyrene from the spiked sediment between 150 °C and 200 °C was observed. Mann-Whitney tests also showed no significant differences between extractions of fluorene, phenanthrene at 150 °C and 200 °C from both UEA soil and UEA sediment. Similar no significant differences between extractions with ASE subcritical water at 160, 180 and 200 °C for ^{14}C-phenanthrene associated activity have previously been described (Latawiec et al., 2008). It is suggested that contaminants become successively sequestered within dissimilar soils pores, hence the opportunity could exist for incrementally greater extractions efficiencies as extraction conditions become harsher at higher temperatures. This could explain a more gradual release of contaminants from the soil (loamy sand) than from the sediment (sand) on account of different proportions of sand and silt in these matrices (Chung and Alexander, 1998; Chung, 1999). Furthermore, on the account of high total loading of PAHs in spiked matrices it was possible that at higher temperatures PAHs reached the maximum water solubility within the extraction cell and additional increase of the extraction temperature brought only little or indeed no increase in the extraction efficiencies. There is however paucity of research on PAHs solubility in subcritical water and on various factors controlling these solubilities, such as ratio of sample, dispersing agent and water volume within the ASE extraction cell (Andersson et al., 2005) to warrant unequivocal discussion.

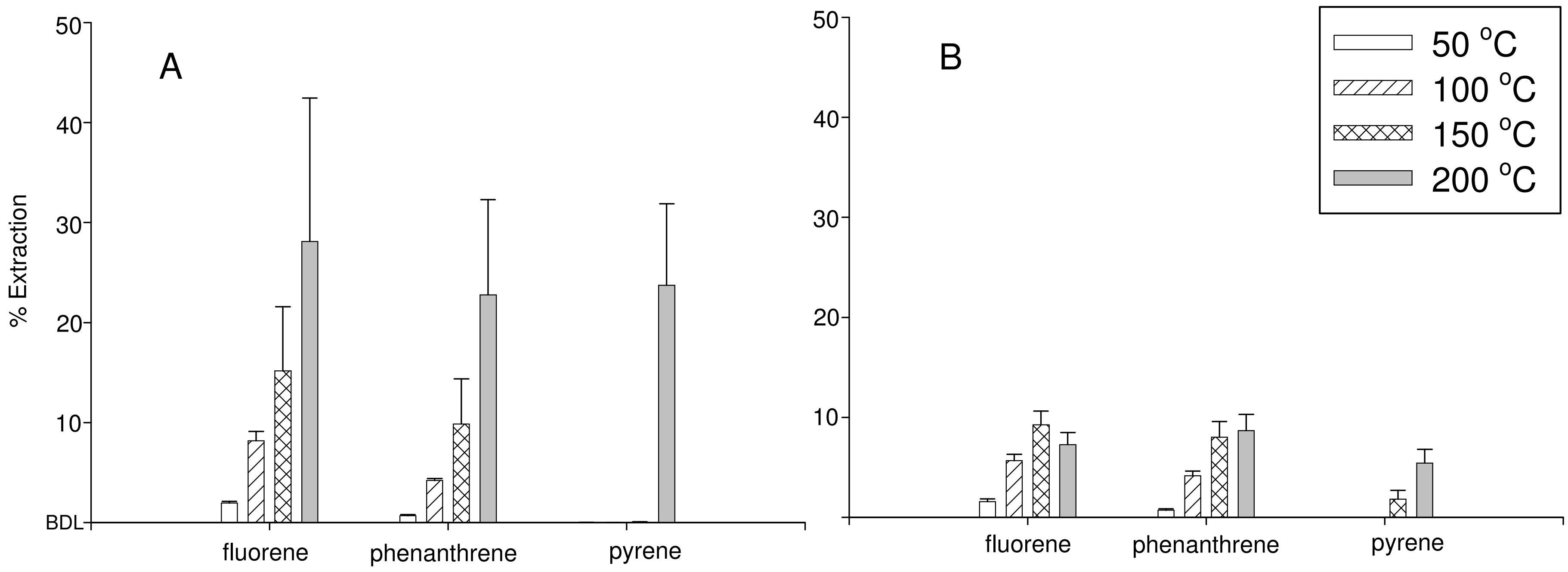

Figure 1. Effect of extraction temperature on the extraction efficiencies of fluorene, phenanthrene and pyrene from spiked soil (A) and spiked sediment (B) at 50 ºC (white bars), 100 ºC (hatched bars), 150 ºC (cross bars) and 200 ºC (grey bars) using subcritical water at 103 bar. Recoveries (%) are relative to values from DCM extraction. Error bars represent standard errors (n = 3). BDL – below detection limit.

3.2. Effect of subcritical water temperature on the extraction efficiencies from the reference material and genuinely contaminated matrices. Selectivity of subcritical water

The effect of four different extraction temperatures on the efficiency of extraction of 16 PAHs from the reference material is presented in Figure 2, whilst from genuinely contaminated soil and sediment in Figures 3 and 4, respectively. With the exception of naphthalene, as the temperature of extraction increased from 50 °C to 200 °C there was an increase in the extent of extraction of PAHs from the reference material. Considering naphthalene it is possible that volatilization may have resulted in losses of this the most volatile of the compounds tested and, as a consequence, lower extraction efficiency at 200 ºC being observed (Burkhardt et al., 2005).

As the subcritical water temperature increased there was an increase in the number of PAHs extracted above detection limits from: 5 compounds at 50 °C to 8 compounds at 100 °C to 11 compounds at 150 °C and finally to 14 compounds at 200 °C (Figure 2). It was anticipated that due to selectivity of subcritical water that higher MW PAHs, such as 5 rings PAH (dibenzo[*ah*]anthracene) and 6 rings PAHs (benzo[*ghi*]perylene, indeno[*123cd*]pyrene) were not quantitatively extracted at lower temperatures and were detectable only at 200 °C. Lower MW PAHs, for instance 3 rings acenaphthylene and anthracene were however also not extracted to concentrations above detection limits at 50 and 100 °C, whereas 3 rings acenaphthene was detected only at 200 °C. This may be explained by low concentrations of these lower MW PAHs in the reference material (Table 1). Indeed, fluorene and benzo[*a*]pyrene (concentrations of 0.25 and 0.27 mg kg^{-1}, respectively) were also not detected at any extraction temperature. It has been previously reported (Langenfeld et al., 1993;

Hawthorne et al., 1994; Barthe and Pelletier, 2007) that lower concentrations of compounds result in a more pronounced sorption, thus ‘tight’ sequestration within matrices and consequently in low extraction efficiencies of mild extractions. In addition, PAHs release from the reference material during extractions may also have been retarded due to sorption into black carbon present in this matrix (Koelmans et al., 2006; Hickman et al., 2008).

To further assess the effect of subcritical water temperature on the extraction of PAHs two historically contaminated matrices were investigated. As the temperature of extraction increased from 50 °C to 100 °C and subsequently to 150 °C there was an increase in the PAHs extraction extents from contaminated soil (intercepts of regression lines were: 14.9, 17.6, 35 for 50 ºC, 100 ºC and 150 ºC, respectively). Further increase of the extraction temperature to 200 °C did not result in significant increase in the extraction efficiencies of PAHs (with the exception of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene; $p = 0.05$).

It has been previously demonstrated that PAHs can be subject to thermal degradation at higher subcritical water temperatures (Andersson et al., 2003; Yang and Hildebrand, 2006). Andersson *et al.* (2003) demonstrated that at the temperatures above 200 °C (240 min extraction time) thermal degradation is likely to govern decrease of PAHs recoveries from subcritical water extraction. It was also suggested by the authors that the longer static extraction time the higher possibility of thermal degradation. Nevertheless, in the experiments presented in this manuscript 10 min static extraction time was employed and, as a consequence, likelihood of thermal degradation would have been limited (Hawthorne et al., 1994).

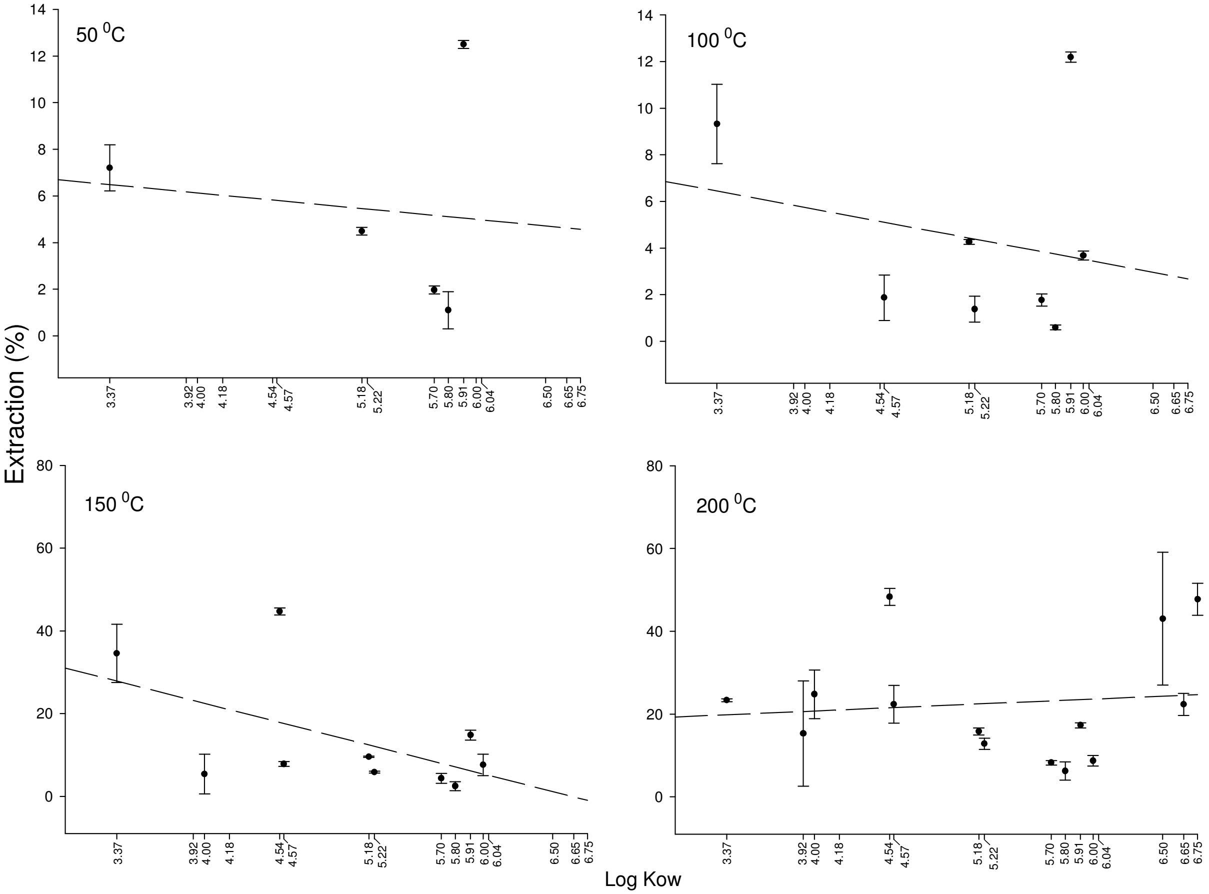

Figure 2. Effect of extraction temperature on the extraction efficiency of PAHs from the reference material using subcritical water at 103 bar. 16 PAHs are arranged according to increasing log Kow values (see table Table 1). Percent recoveries are relative to values from DCM extraction. Error bars represent standard errors (n = 3). Missing point indicates values below detection limit. Regression equations are: 50 °C: y = 8.4 - 0.57x ($r^2 = 0.02$); 100 °C: y = 10 -1.11x ($r^2 = 0.56$); 150 °C: y = 56.6 - 8.54x ($r^2 = 0.28$); 200 °C: y = 14.99 + 1.44x ($r^2 = 0.01$).

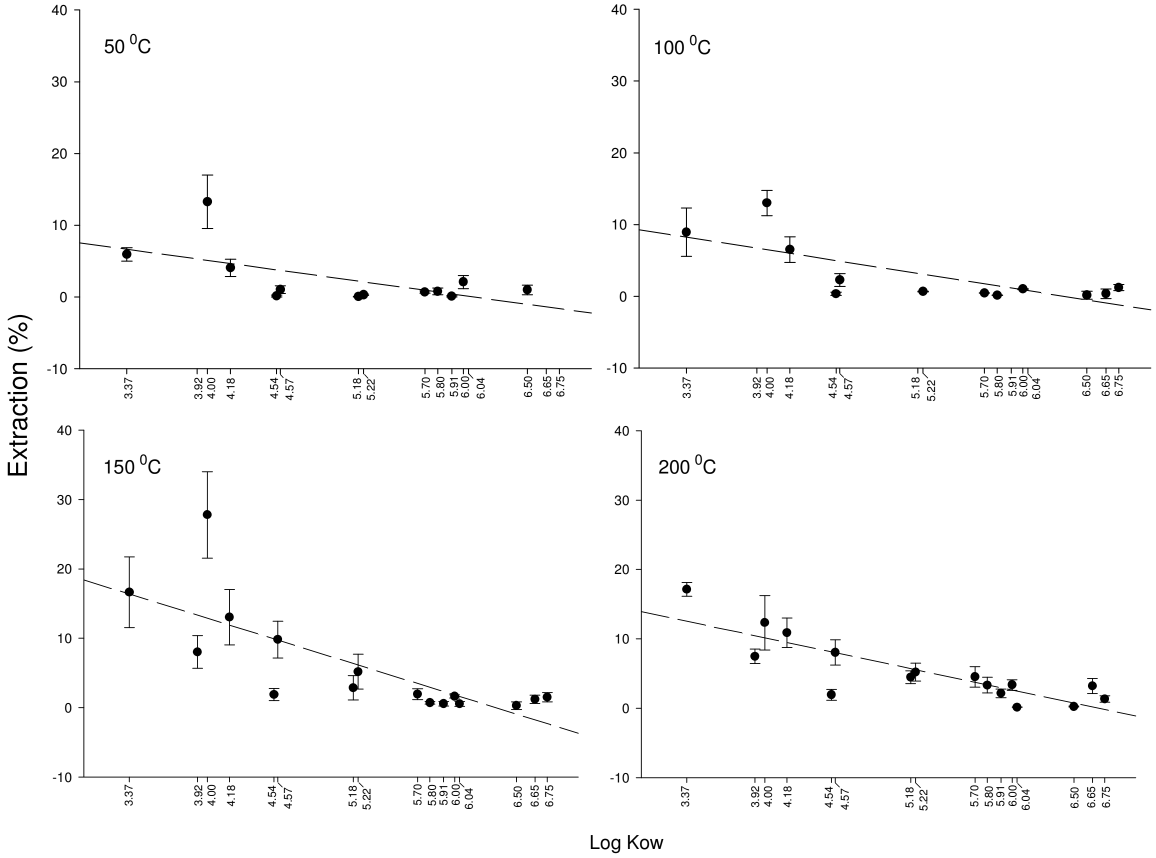

Figure 3. Effect of extraction temperature on the extraction efficiency of PAHs from genuinely contaminated soil using subcritical water at 103 bar. 16 PAHs are arranged according to increasing log Kow values (see Table 1). Percent recoveries are relative to values from DCM extraction. Error bars represent standard errors (n = 3). Missing point indicates values below detection limit. Regression equations are: 50 °C: $y = 14.9 - 2.45x$ ($r^2 = 0.36$); 100 °C: $y = 17.6 - 2.78x$ ($r^2 = 0.55$); 150 °C: $y = 35 - 5.53x$ ($r^2 = 0.58$); 200 °C: $y = 25.2 - 3.77x$ ($r^2 = 0.71$).

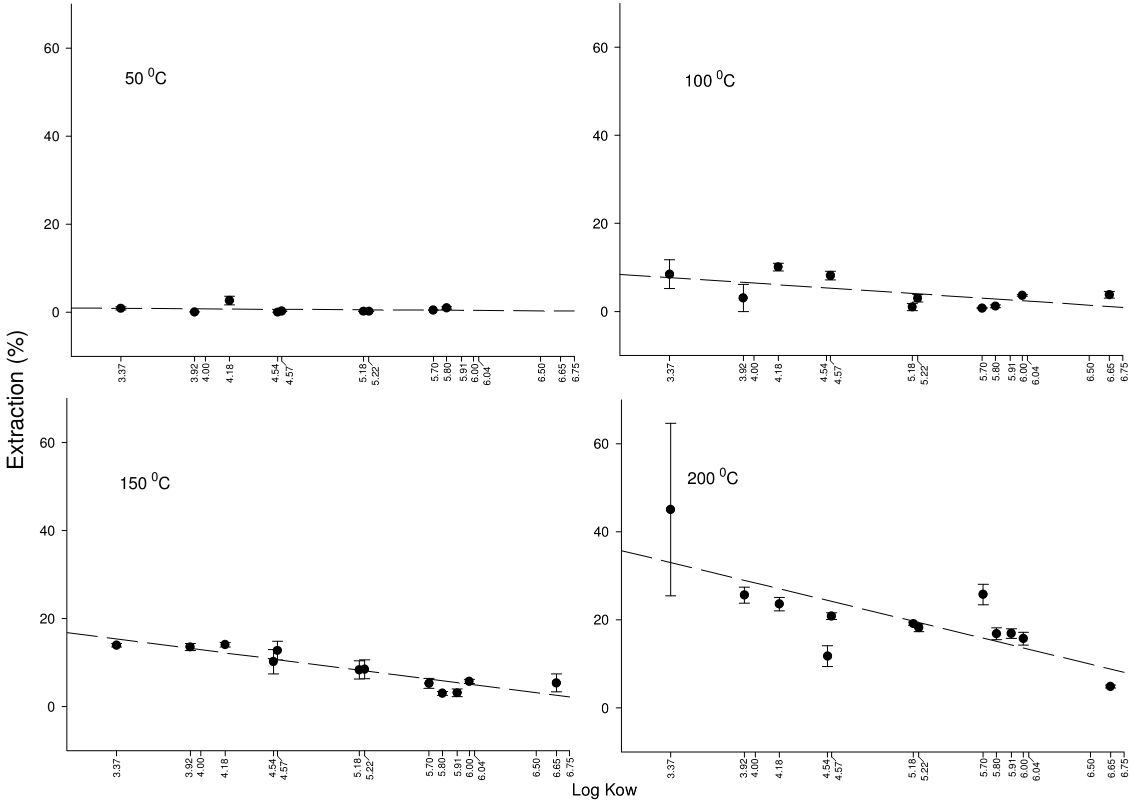

Figure 4. Effect of extraction temperature on the extraction efficiency of PAHs from genuinely contaminated sediment using subcritical water at 103 bar. 16 PAHs are arranged according to increasing log Kow values (see Table 1). Percent recoveries are relative to values from DCM extraction. Error bars represent standard errors (n = 3). Missing point indicates values below detection limit. Regression equations are: 50 °C: $y = 1.5 - 0.18x$ ($r^2 = 0.03$); 100 °C: $y = 14.4 - 2.01x$ ($r^2 = 0.37$); 150 °C: $y = 28.4 - 3.89x$ ($r^2 = 0.83$); 200 °C: $y = 57.9 - 7.37x$ ($r^2 = 0.55$).

Considering extractions from TW sediment there was a gradual increase in the extraction efficiencies of the order of 3% at 50 °C, to ~ 10% at 100 °C, ~ 20% at 150 °C and finally to a range between 4 and 45% at 200 °C. Also, in 7 out of 8 extraction temperatures (regression equations; Figure 3 and Figure 4) there was a decrease in the extraction efficiencies as the log Kow of PAH increased. This is concordant with the research of others (Hawthorne et al., 1994; Yang et al., 1997; Hartonen, 2000) and corroborates selectivity of subcritical water as an extraction solvent. As extraction temperature increased the selectivity of subcritical water to resolve partitioning of dissimilar PAHs increased. Selectivity was apparent from the gradient of regression lines relating to the relationship between compound properties (log Kow) and extraction efficiencies. These regression gradients were observed to increase with increasing extraction temperature thus supporting greater differentiation between compounds extraction efficiencies at higher temperatures. In the case of TW soil regression line slopes were -2.45 (at 50 ºC), -2.78 (at 100 ºC), -5.53 (at 150 ºC), -3.77 (at 200 ºC), whereas -0.18 (at 50 ºC), -2.01 (at 100 ºC), -3.89 (at 150 ºC), -7.37 (at 200 ºC) for TW sediment. The results from simple spiked matrices (Figure 1) additionally corroborated selectivity of subcritical water extractions wherein pyrene was detected only at higher temperatures.

In addition, whilst ranges of PAHs extractions were similar at 50 °C, 100 °C and 150 °C for soil and sediment, at the highest temperature of 200 °C higher extractions of PAHs from the TW sediment than from TW soil were observed. These differences may reflect different degrees of sequestration within organic matter and/or different total concentrations of PAHs in TW soil and sediment. It is well established that organic matter fraction is a main sorbent for hydrophobic molecules as PAHs, unless this fraction is limited (Pignatello and Xing, 1996; Alexander, 2000; Pignatello, 2000; Reid et al., 2000b). It was noted that TW soil samples were richer in organic matter than TW sediment (Table 1), which may have led to a more extensive entrapment of PAHs within soil organic matter and, as a result, lower extraction efficiencies (Hatzinger and Alexander, 1995; Alexander, 2000). Furthermore, lower concentrations of PAHs in soil compared with sediment may additionally account for their stronger 'binding' and lower extraction efficiencies at 200 °C. Lack of detection of acenaphtyhlene, benzo[*ghi*]perylene, dibenzo[*ah*]anthracene (present at low concentrations) after subcritical water extractions from the TW sediment further supports this interpretation.

To summarise differences between extractions from dissimilar samples used in this study K-means cluster analysis was performed (Table 3). Cluster analysis (segmentation or taxonomy analysis) enabled identification of homogenous subgroups of cases (where a case equals each PAH extraction at particular temperature) within population of all PAHs extractions. Final cluster centres representing average extraction efficiency on cluster members indicated significant differences within all PAHs extractions for particular matrix. These statistical outputs complement aforementioned selective extraction of PAHs. It can be also observed that as the extraction efficiency increased there was a decrease in total PAHs extracted.

Differences in extractions for different matrices used in this experiment reflected different concentrations of contaminants, dissimilar textures hence different extents of pores penetration by subcritical water, various organic matter contents and, possibly, presence of co-contaminants (in the case of genuinely contaminated samples). Changes in the slopes of regression equations (Figure 2, 3 and 4) dependant on the extraction temperature additionally illustrate selectivity of subcritical water as the extraction solvent for PAHs. Collectively, across matrices at a particular temperature there was a decrease in PAH extraction as their log Kow increased (along with increasing MW and decreasing aqueous solubility of PAH).

Table 3. Final cluster centres and number of cases* associated with each cluster

	Final cluster centres (number of cases)				Total cases
RM	3.83 (16)	12.42 (12)	28.17 (5)	45.95 (4)	37
TW soil	1.15 (36)	6.51 (12)	13.76 (7)	27.79 (1)	56
TW sediment	2.25 (22)	12.09 (14)	22.19 (6)	45.04 (1)	43

* Each case represents extraction of particular PAH at each temperature

4. CONCLUSIONS

Raising the temperature of subcritical water with ASE increased the extraction efficiencies of PAHs and allowed their selective extraction dependant on PAH log Kow. The strength of the inverse relationship between extraction extent and the log Kow value was evident for the real-world matrices on account of sorption-desorption processes controlling contaminants release hence extractions. It has been shown previously that the upper operating temperature limit (200 °C) precluded effective extraction of non-polar high molecular weight PAHs using subcritical water without the addition of co-solvent (Ramos et al., 2002; Burkhardt et al., 2005). Conversely, it has been shown that extraction only with water at ambient conditions underestimates bioavailability to microorganisms (Allan, et al., 2006). Non-exhaustive extraction technique presented here could therefore potentially mimic PAH desorption in aqueous media that has been shown to govern contaminant bioavailability (Reid et al., 2000b). Moreover, by altering extraction conditions with ASE 200 it was possible to generate a range of conditions of subcritical water extraction and control the degree of extraction exhaustiveness. While in this study ASE 200 performed extractions in a 'static' mode it can also be converted into a 'dynamic' system, wherein the number of extraction cycles is increased. This could overcome putative solubility issues and/or desorption limitations and increase extraction extents.

Because most existing environmental methods generally use labour intensive, exhaustive Soxhlet extraction it has become imperative to implement more efficient environmentally-friendly and environmentally-relevant methods. Despite uncontested desorption-based rationale behind the use of subcritical water to determine contaminant partitioning, subcritical water relevance for bioavailability reflection has not been operationally defined. It has been suggested that the lack of time- and cost-efficient method may hamper the application of partitioning data into contaminated land decision-making (Reichenberg and Mayer, 2006). The extraction method presented here is quick (rapid ASE extraction and shorter sample preparation as the drying step is not required), cost-effective (data available from the authors), water-based thus ‘green’ (on account of lower organic solvent demand) and therefore, arguably, more environmentally acceptable. Notably, given various alternatives for subsequent quantification of the subcritical water-extracted analytes this method could be not only ‘green’ from the extraction step perspective but also potentially environmentally benign at the subsequent analysing stage (Smith, 2008). This technique may increase the set of tools available to assess contaminant partitioning and assist in evaluation of risks associated with contaminated sites.

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CHAPTER 4

Beyond contaminated land assessment: on costs and benefits of bioaccessibility prediction

Beyond contaminated land assessment: on costs and benefits of bioaccessibility prediction

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ABSTRACT

Advances towards sustainable land management necessitate application of a broader portofolio of decision-support tools that improve evaluation of contaminated land. Over the last decade regulators have directed concerted effort towards rationalization of risk-based contaminated land policies recognizing bioavailability and bioaccessibility as concepts to be incorporated into risk assessments. The desire for a precise and rapid method to inform consideration of bioavailability and bioaccessibility to support risk assessment of contaminated land has never been greater. This study presents a comprehensive appraisal of both emerging non-exhaustive extraction techniques (subcritical water extraction and Brij 700 extraction) developed to reflect polycyclic aromatic hydrocarbon (PAH) bioaccessibility to microorganisms as well as formerly demonstrated methodologies (the use of cyclodextrins and butanol extraction). Application of unified evaluation criteria across different techniques enabled comparison not only from the bioaccessibility prediction perspective but also analysis of economical (cost of extraction) and practical (such as extraction time) measures. Whilst the use of cyclodextrins was the best predictor of the bioaccessible fraction for the majority of compounds, other methods appeared more cost- and time-effective. Juxtaposition of the techniques presented in this study assists establishing cost-benefit trade-offs of different non-exhaustive extraction techniques and contributes to tailoring information on contaminant bioaccessibility to support risk evaluation on contaminated sites.

1. INTRODUCTION

Bioavailability and bioaccessibility concepts have been perceived as a pivotal issue within considerations of dissipation and fate of organic contaminants in the environment on account of evidence of contaminant sequestration and 'ageing' processes (Stucki and Alexander, 1987; Alexander, 2000; Reid et al., 2000a; Allan et al., 2007). Mechanisms governing contaminant availability and the consequences of ageing for distribution of organic contaminants within environmental matrices have been broadly described elsewhere (White et al., 1997; Alexnader, 2000; Pignatello, 2000; Reid et al., 2000a). There is an extensive amount of research relating both to pragmatic evaluations of contaminant availability across a range of receptors (Carmichael et al., 1997; Cuypers et al., 2001; Johnson et al., 2002; Allan et al., 2007; Kreitinger et al., 2007) and to theoretical deliberations on bioavailability/bioaccessibility terminology (Semple et al., 2004; ISO, 2006). In this manuscript the syntactical term 'bioaccessibility' akin to the concept ratiocinated by Semple et al. (2004) was adopted. The bioaccessible fraction provides a reference not only to the amount of a substance readily available to an organism at a given instant (bioavailability) but also to the fraction potentially available over time (Semple et al., 2004). Indeed, on account of risk-based approaches that pervade contaminated land regulatory regimes elsewhere (Nathanail and Earl, 2001) an approach that comprises evaluation of the fraction transformed by microorganisms at the particular time and set environmental conditions may be a priori incorrect. It has been also previously demonstrated (Huesemann et al., 2004) that abiotic desorption of PAHs can be significantly higher than the fraction biodegraded by microorganisms (due to recalcitrance of PAHs). This freely soluble and/or putatively available fraction may present a potential risk to living organisms over time and/or under altered environmental conditions. Thus, from the risk assessment perspective and for the purpose of this research it appears more relevant to adopt the approach that comprises not only the instantaneous bioavailable quantity but also the fraction released from the matrix via abiotic mechanisms (e.g. abiotic desorption, volatilization), collectively named here 'bioaccessible' (see also section 4.1.).

It is widely accepted that 'total' contaminant concentrations measured by exhaustive means bear little relevance to actual risks that contaminants possess towards living organisms (Kelsey et al, 1997; Allan et al., 2007). Alternative non-exhaustive approaches have therefore been explored in order to evaluate the bioavailable/bioaccessible contaminant fraction by means of both bioassays and chemical proxies (biomimetics). The desire for a method to be rapid, precise, cheap, ethical, user-friendly and environmentally benign has resulted in recent interest and development of alternative, non-biological approaches. Both non-exhaustive solvent extractions (Kelsey et al., 1997; Tang and Alexander, 1999; Liste and Alexander, 2002) as well as desorption based techniques (Cornelissen et al., 1998; Cuypers et al., 2000; Reid et al., 2000b; Allan et al., 2007) aiming to assess bioavailable/bioaccessible contaminant fraction have previously been presented.

Despite a plethora of research, a multitude of discussions and a variety of proposed methodologies, bioavailability and bioaccessibility concepts are present more within scientific and technical debates rather than constituting a practical tool to routinely support decision-making on contaminated land. Indeed, Reichenberg and Mayer (2006) stated the lack of time- and cost-efficient methods as one of the primary obstacles that hampers bioavailability [and by extension bioaccessibility] from being operationally applied. It is therefore necessary to delineate an approach that not only precisely evaluates bioavailability/bioaccessibility *per se* via simulating processes that occur in environment but that is also time- and cost-effective.

A wide range of properties of different PAHs (Bamforth and Singleton, 2005), their environmental significance (Wenzl et al., 2006), complexity of intra- and inter- class interactions (Jones and de Voogt, 1999; Dean-Ross et al., 2002), prevalence and persistence (Wild and Jones, 1995) and, as a consequence, overall environmental risk were key factors in determining the selection of PAHs for this research. Furthermore, ubiquity of PAHs in the environment drives an increasing demand for a more proportionate risk assessment within decision-making on land contaminated with these compounds (Brassington et al., 2007).

This study was designed to compare four different non-exhaustive extraction methodologies, namely subcritical water extraction (Latawiec et al., 2008), the use of aqueous solution of cyclodextrins (Reid et al., 2000b; Allan et al., 2007), surfactant extraction (Brij 700) (Barthe and Pelletier, 2007) and organic solvent (butanol) extraction (Liste and Alexander, 2002) from both spiked and genuinely contaminated soil and sediments. Extraction extents were subsequently compared with values obtained from slurry biodegradation assays. An evaluation comprising not only the accuracy of bioaccessibility prediction but also an appraisal of other crucial economical and practical facets behind various extraction techniques is presented. To the authors' knowledge no similar comparison including such a spectrum of methodological approaches along with a broader analysis of the key factors governing a choice and validation of the method has been undertaken.

2. BACKGROUND

The use of subcritical water extraction as a means of assessing microbial bioaccessibility of PAHs has not been extensively studied. While promising applications of subcritical water to predict long-term release rates of hydrophobic organic compounds have been reported previously (Johnson and Weber, 2001;

Hawthorne et al., 2002), to the authors' knowledge only one successful attempt to correlate subcritical water and mineralization extents in spiked soils exists (Latawiec et al., 2008). It was therefore the intention to further investigate subcritical water extractions and its correlation to microbial degradation in historically contaminated samples.

An aqueous-based cyclodextrin (HPCD) extraction technique was proposed by Reid et al. (2000b) and subsequently refined by Stokes et al. (2005) and Allan et al. (2006) as a mimic of the mass transfer processes that govern contaminant availability to microorganisms. Hydroxypropyl-β-cyclodextrin is a cyclic oligosaccharide comprising of seven α-1,4-linked glucose units presenting a hydrophilic exterior (rendering it water soluble) and a toroidal-shape apolar cavity (enabling the formation of 1:1 inclusion complexes with hydrophobic organic moiety), thereby increasing organic compound aqueous solubility. In accordance with the above, HPCD has been successfully applied to predict microbial degradation in single and multiple contaminant systems involving a range of PAHs under both laboratory (Stokes et al., 2005; Allan et al., 2006) and real world conditions (Cuypers et al., 2002; Hickman et al., 2008).

An alternative to subcritical water extraction and HPCD water-based method is the use of aqueous solutions of surfactants. Barthe and Pelletier (2007) presented the first attempt to assess PAH bioavailability with surfactant Brij 700 (B700). B700 (poly(oxyethylene)(100)stearyl ether) is a water soluble non-ionic high molecular weight surfactant. Hydrophobic organic compounds preferentially partition and are incorporated into the hydrophobic core of the micelle (aggregation of surfactant molecules), which may result in the increase in organic compound aqueous solubility. In addition, poly(oxyethylene) chain was believed to show similarities with natural biosurfactants produced by bacteria found in genuinely contaminated soils (Barthe and Pelletier, 2007).

Extractions with butanol (BuOH) developed to non-exhaustively extract contaminants from soils have shown inconsistent results. While some authors have successfully correlated phenanthrene recoveries from butanol extractions with biodegradation (Kelsey et al., 1997; Liste and Alexander, 2002) others have shown that butanol acted as an exhaustive extractant and overestimated microbial degradation (Macleod and Semple, 2003; Juhasz et al., 2005).

In order to establish ‘total’ contaminant concentrations exhaustive extraction techniques have been traditionally used. These harsh techniques, such as Soxhlet extraction, shake extraction or pressurised liquid extraction (PLE) are based on the release of compounds following interactions with selected, usually organic, solvents (Swindell and Reid, 2007a). For the purpose of this study PLE was adopted with dichloromethane (DCM) as a solvent for exhaustive PAH extractions (Schantz et al., 1997).

3. MATERIALS AND METHODS

3.1. Samples

Pristine soil (A horizon) and sediment from the River Yare were collected at the University of East Anglia (UEA) at National Grid References TG 199 073 and TG 191 070, respectively. Prior to spiking, both materials were dried for 14 days and homogenised by sieving (2 mm). Spiking solutions containing fluorene, phenanthrene, pyrene and benzo[*a*]pyrene were added to the soil and sediment following single-step spiking/re-hydrating (60% WHC) procedure (Allan et al., 2007) and delivered concentrations of 100 mg kg^{-1}, 500 mg kg^{-1}, 250 mg kg^{-1} and 50 mg kg^{-1}, respectively, relative to dry weight. After thorough mixing the treatments (250 g) were tumbled in amber jars (500 ml) in the dark at 15 °C on the end-over-end rotor for 21 days. Matrices were not sterilized thus degradation of contaminants by indigenous flora was not prevented. Genuinely contaminated soil (NGR NZ 290 630) and sediment (NGR NZ 292 631) used in this study were collected from the former tar works in Newcastle (hereafter TW). TW soil samples were collected from a site, densely vegetated with semi-mature and mature trees, adjacent to the River Tyne, whereas TW sediment was sampled from the River Tyne foreshore. The foreshore was visibly contaminated with non-aqueous phase liquid (NAPL) seepage. Samples were air-dried for 14 days in the fume cupboard under light vacuum and subsequently homogenised by sieving (2 mm). Soil and sediment properties (particle size distribution, organic matter measured as loss on ignition at 450 ºC and % C, % H, %N determined using a Carlo Erba EA1108 Elemental Analyzer) are presented in Table 1.

Table 1. Properties of the soil and sediment matrices.

	% sand	% silt	% clay	%LOI[a]	% C	% H	% N
UEA soil	88	12	0	3.30	2.12	0.3	0.20
UEA sediment	100	0	0	1.65	1.15	0.07	0.04
TW soil	83	17	0	7.47	3.35	0.5	0.20
TW sediment	66	33.5	0.5	3.89	2.33	0.2	0.06

[a] – Loss on ignition

3.2. Subcritical water extraction

Water extractions were performed similarly to conventional pressurised liquid extraction (PLE; section 3.6.) using Accelerated Solvent Extractor 200 (ASE 200; Dionex corp.) under the extraction temperature of 200 °C. Based on the authors previous work (Latawiec et al., 2008) 10 min static (or extraction) time was selected for all water extractions in this study. For dispersion of matrices and to prevent blockages in the extraction system samples were mixed with Ottawa quartz sand (20-30 mesh, Fisher Scientific UK). Extractions were conducted using Milli-Q water obtained from

Milli-Q systems manufactured by Millipore, USA. After cooling in the ASE collection vial, each extract (n = 3) was transferred into a pear shaped separating funnel (100 ml, Scientific Laboratory Supplies UK). The empty collection vials were then rinsed with 2 x 2 ml of DCM and these washes collected in the separating funnel. An additional 5 ml of DCM was added into the funnel that was subsequently gently agitated in order to facilitate the transfer of extracted PAHs from water into the organic phase. After ~ 5 min, DCM deposited as the lower phase in the funnel, was flushed through DCM pre-wetted GF/A filter paper (Whatman UK) into the amber volumetric flask (25 ml, Fisherbrand UK). This liquid-liquid extraction procedure was subsequently repeated twice with 8 ml of DCM to ensure complete transfer of PAHs from water to DCM. Prior to the method development, the efficiency of PAHs recoveries within triplicated liquid-liquid exchange from water into DCM was determined using cumulative curves (data not presented). Volumetric flasks were made up to 25 ml with DCM and 1 ml of the extract was carried forward to quantification. During each separation 3 drops of isopropyl alcohol were added to promote definitive boundary between water and DCM.

3.3. HPCD extraction

Samples were extracted using an aqueous solution of hydroxypropyl-β-cyclodextrin (HPCD) by shake extraction (Reid et al., 2000b; Allan et al., 2006). Samples (3 g; n = 3) were weighted into Teflon centrifuge tubes and 60 mM of HPCD solution added (30 ml). The tubes were sealed and placed horizontally on a flat bed rotary shaker. The tubes were shaken at 100 rpm for 20 h at ambient temperature before centrifugation at 5000 x *g* (Sigma Laboratory centrifuge, 4K15) for 20 min. The supernatant was discarded and the resulting pellet was shaken with 30 ml of distilled water for 10 s and centrifuged again. This procedure was repeated to ensure complete removal of remaining HPCD solution. The pellets were then transferred with the drying agent

(Hydromatrix, Varian UK) into the ASE cells and extracted with DCM as described below (section 3.6.).

3.4. Brij 700 extraction

Surfactant solutions were prepared using distilled water to reach a concentration of 5.25 mM (Barthe and Pelletier, 2007). Samples (3 g; n = 3) were transferred into Teflon tubes and 30 ml of surfactant solution added to each tube. The tubes were shaken on the rotary shaker at 100 rpm for 16 h at ambient temperature before centrifugation at 5000 x *g* for 20 min. The supernatant was discarded and the resulting pellet rinsed twice with distilled water (30 ml) for 10 s and centrifuged again. As aforementioned, this ensured removal of possible remnants of the extracting solution from the sample. The sample was subsequently processed as described above for the HPCD extraction pellet and as given in the section 3.6.

3.5. Butanol extraction

The butanol (butan-1-ol or BuOH) extraction was adapted from the method described by Liste and Alexander (2002). Samples (3 g; n = 3) were weighted into polypropylene copolymer (PPCO) centrifuge tubes and butanol (4.5 ml) was then added. Mass and volume were kept in agreement with 10:15 sample to solvent ratio used by Liste and Alexander (2002). The mixture was suspended using a vortex mixer for 120 s. The tubes were centrifuged at 5000 x *g* to separate samples and the butanol. It has been previously shown (Northcott and Jones, 2003) that centrifugation achieved better recoveries of PAH than a filtration procedure, as used by Liste and Alexander (2002) and was therefore the preferred separation technique. The supernatants were discarded and the centrifuge pellets were transferred into the ASE cells to extract the residual concentrations (section 3.6.).

3.6. Exhaustive extraction - PLE

Residual concentrations of PAHs in post-extraction pellets and 'total' contaminants concentrations in all matrices were evaluated using standard US EPA 3545 method (US EPA, 1995). Extractions were performed with ASE 200 under standard conditions (100 ºC, 103 bar, 5 min equilibration time, 5 min static time, 60% flush, 1 static cycle) using dichloromethane (DCM) as the extraction solvent. An in-cell clean-up technique using Florisil, described elsewhere (Hubert et al., 2000; Hildebrandt et al., 2007), was employed within all DCM extractions. Throughout the experiment, all samples were extracted in triplicate.

3.7. Slurry assays

Slurry assays designed to evaluate both biotic (microbial degradation) and abiotic (e.g. volatilization) losses of contaminants were carried out in 250 ml Duran glass bottles containing 10 g of contaminated material (n = 3) and 30 ml of sterile mineral basal salts (MBS). MBS medium was produced by dissolving the following in 1 l of deionised water: 0.6 g KNO_3, 0.3 g NaCl, 0.15 g $MgSO_4.7H_2O$, 0.25 g KH_2PO_4, 0.6 g $(NH_4)_2SO_4$, 0.75 g K_2HPO_4; and 1 ml of trace element solution obtained by dissolving 0.002 g LiCl, 0.003 g KBr, 0.003 g KI, 0.004 g $SnCl_2$, 0.008 g $CaSO_4$, 0.01 g $ZnSO_4$, 0.01 g $Al_2(SO_4)_3$, 0.01 g NiCl, 0.01 g $CoSO_4$, 0.03 g $FeSO_4$, 0.06 g $MnCl_2$ in 1 l of deionised water (Skerman, 1967). Treatments containing spiked matrices were then inoculated with 2 ml of catabolically competent (towards phenanthrene) *Pseudomonas sp.* (13 x 10^6 – 17 x 10^6 cells per g of the soil, d.w.) at the start of the assay. Bacterium inocula were produced from enrichment culturing from PAH-contaminated soil described elsewhere (Reid et al., 2000b; Allan et al.; 2007; Hickman et al., 2008). Simultaneously, non-inoculated spiked matrices were tested for biodegradation extents to verify hypothesised no significant difference between treatments with and without additional bacteria added (after Allan et al., 2007). All slurries were agitated at 100 rpm on an orbital shaker for 28 d. It has been previously shown (Saponaro et al., 2002) that extending slurry tests time over 25 d does not bring significant additional abatement of PAHs. Bottles were loosely fitted with Teflon-lined screw caps to allow oxygen exchange. Treatments were run in a fume cupboard under light vacuum away from sunlight at ambient temperature (20 ± 2 ºC). Following 28 d incubation period slurries were filtrated under light vacuum and the filter cake retained for subsequent PAHs quantification. To this end the filtration cakes were dried with Hydromatrix and transferred into the ASE extraction cells, and extracted with DCM as described above (section 3.6.).

3.8. PAHs analysis

Quantification of target PAH in all extracts was performed using GC-MS fitted with a mass selective detector (Perkin Elmer, Clarus 500). Compound separation was carried out using a fused silica capillary column (Perkin Elmer Elite 5MS, 30 m) coated with 5% diphenyl and 95% Dimethyl Polysiloxane stationary phase (0.25 mm i.d. x 0.25 mm film thickness). Mass spectrometer operated at 70 eV in positive ion mode using selective ion response (SIR). The carrier gas was helium (CP grade, BOC UK) at a constant flow of 1 ml min^{-1}. Autosampler injections (1 µl) were performed in the 1:10 split ratio. The oven temperature was programmed as follows: 35 °C (holding time 1.5 min) raised to 100 °C at gradient of 25 °C min^{-1}, then at 15 °C min^{-1} to 190 °C (2 min hold) and finally ramped at 10 °C min^{-1} to 270 °C and held for 15 min. Total run time was 35 min. The injector, transfer and ion source temperatures were set at 180, 280 and 180 °C, respectively with the detector voltage at 450 V. Identification of PAHs was made by integrating peak areas at specific *m/z* using Turbomass Software provided with the instrument and by comparison of these peaks with the response of a known concentration of PAHs. Analytical parameters such as detection limit and quantification of PAHs were determined using standard solutions (Supelco, USA) and appropriate standard calibration curves. Calibration standard with known concentration was inserted every 6 samples to control any possible machine drift within the run. Consistency of GC-MS responses has been also cross-experiments confirmed to provide comparability of the results.

3.9. Data processing and statistical analysis

Extraction extents from non-exhaustive techniques and the results from the slurry test presented throughout this manuscript are relative to the 'total' DCM-extractable PAH concentrations at each sampling point (section 3.6.). Statistical analysis of the results was performed using SPSS 16.0 for Windows. Statistical significance was determined at 95% confidence interval with the significance level at 0.05.

4. RESULTS AND DISCUSSION

4.1. Slurry assays

Concordat with other researchers (Cerniglia, 1992; Hickman et al., 2008) and in accordance with the section 1 of this manuscript, the term bioaccessibility will be used hereafter interchangeably with contaminant losses evaluated by slurry assays. Notably, biodegradation of PAHs, especially those of low molecular weight (MW), is believed to account for a majority of these losses (Cerniglia, 1992).

The results of slurry tests are presented in Table 2 and 3, for UEA and TW samples, respectively. As the octanol-water partition coefficient and the MW of PAHs increased (along with the increase in their hydrophobicity) there was a decrease in PAH bioaccessible fraction in both UEA soil and UEA sediment (Table 2). This is congruent with the research of others (Bamforth and Singleton, 2005; Johnsen et al., 2005; Semple et al., 2007; Hickman and Reid, 2008) and corroborates mechanisms behind PAH ageing and fate comprehensively discussed elsewhere (*sensu lato* Providenti et al., 1993). Environmental persistence of PAH increases commensurately with their MW due to increase in lipophicility and as a resultant of the resonance energies of fused benzene rings of higher MW PAHs, and high activation energies that, on the other hand, retard PAH biological transformations and control PAH recalcitrance (Cerniglia, 1992). It is also well established that sorption of hydrophobic compounds from aqueous solution or at high relative humidity is dominated by organic matter (OM), unless that fraction is very small (Pignatello, 2000). Manilal and Alexander (1991) showed that the sorption of phenanthrene and its retention within organic fraction were the major factors influencing the rate of phenanthrene transformation by microorganisms. In addition, Chung and Alexander (1998) highlighted the importance of nanopores associated with silt and their large surface area delivering potential sorption sites. UEA sediment constitutes exclusively of sand and contains relatively little OM, which likely curbed PAH entrapment (Carmichael and Pfaender, 1997). In accordance with the above, significant differences between bioaccessibility of pyrene and benzo[*a*]pyrene for UEA soil and UEA sediment were observed. It can be conjectured that these hydrophobic compounds were subject to ageing processes and sequestration to a greater extent within UEA soil than within UEA sediment.

It was observed that the bioaccessibility values for all PAHs in both UEA soil and UEA sediment were generally high; it should, however, be noted that these matrices were spiked. Thus, it appears that 21 d of contaminant-matrix contact time before commencement of the slurry assays might have not been enough for the contaminant to be extensively aged. As a consequence, substantial amounts of freely soluble contaminants remained in the solution and/or have been transformed by biota and/or desorbed from the matrices via other abiotic mechanisms (Huesemann et al., 2004). It was also possible that lower MW PAH, such as fluorene and phenanthrene, were extensively biodegraded by adapted catabolically active or/and added microbial consortia (no significant differences between test slurries with and without bacteria added; data not included). Furthermore, although UEA soil contains more (3.30%) OM than UEA sediment (1.65%) the most abundant fraction in these matrices is sand established to weakly retain organic contaminants. In addition, it has been previously reported (Hawthorne et al., 1994) that high total PAH concentrations (as applied for spiked matrices in this study) render their weaker 'binding' within the sorption sites that, as a consequence, might have led to elevated contaminants losses observed in our experiments.

Table 2. Biaccessibility (loss) and PAH extraction extents attributed to subcritical water extraction at 200 ºC, HPCD extraction, B700 and BuOH extraction in spiked matrices. All values relative to initial DCM-extractable residues

	UEA soil (% ± SE)					UEA sediment (% ± SE)				
	Loss	H_2O	HPCD	B700	BuOH	Loss	H_2O	HPCD	B700	BuOH
Fluorene	95±1	28±4	88±2	60±7	63±15	98±1	7±1	93±2	94±1	84±1
Phenanthrene	94±2	23±9	85±4	38±13	62±15	97±1	9±2	93±3	93±1	84±1
Pyrene	70±3	24±8	71±5	51±8	64±14	89±2	5±1	78±1	93±1	80±1
Benzo[*a*]pyrene	69±2	BDL	73±5	73±3	58±8	86±1	BDL	71±1	95±0.5	75±3

BDL – below detection limit

It was observed that genuinely contaminated samples exhibited diverse extents of PAH loss after 28 d of slurry test (Table 3). Collectively, as the PAH MW increased there was a decrease in PAH bioaccessible fraction. Inconsistency noted for acenaphthylene is attributed to low total concentrations of this compound at the beginning of the assay (0.11 mg kg $^{-1}$ and 0.33 mg kg $^{-1}$ for TW soil and TW sediment, respectively; Table 3). In addition to strong sorption of contaminants at low environmental concentrations, biodegradation is also contaminant- and degrader-dependent (Boethling and Alexander, 1979). Contrary to other well investigated lower MW PAHs little is known about acenaphthylene biodegradation (Pinyakong et al., 2004) both in the terms of catabolic genes responsible of acenaphthylene biotransformation and multi-contaminant interactions.

It was noted that in 8 out of 10 comparisons (Table 3), losses of PAHs observed for TW soil were lower than PAH losses from the TW sediment. TW soils samples origin from woodland and, as a consequence, were richer in soil OM (7.47 %). It was therefore suggested that a more extensive sequestration within TW soil occurred as compared to TW sediment, which may have led to relatively lower contaminants losses from TW soil (Hatzinger and Alexander, 1995; Pignatello, 2000). Additionally, the presence of NAPL, observed at time of sample collection, could have affected PAH fate in the TW sediment (Pollard et al., 2008). It is proposed that the enhanced loss of PAHs from TW sediment may be also due to competitive sorption for a limited number of sorption sites between PAH residues and NAPL and, to a lesser extent, due to displacement of these residues from sorption sites by the NAPL (Swindell and Reid, 2007b).

Table 3. Bioaccessibility (loss) and PAH extraction extents attributed to subcritical water extraction at 200 ºC, HPCD extraction, BuOH and B700 extraction in genuinely contaminated matrices. All values relative to initial DCM-extractable residues

	TW soil (% ± SE)						TW sediment (% ± SE)					
	initial concentration (mg kg^{-1} ± SE)	Loss	H_2O	HPCD	B700	BuOH	initial concentration (mg kg^{-1} ± SE)	Loss	H_2O	HPCD	B700	BuOH
Naphthalene	1.48±0.17	64±5	17±1	32±12	20±5	41±5	3.74±0.06	44±1	45±20	ND	ND	ND
Acenaphthene	1.45±0.42	61±5	8±1	50±9	32±5	64±5	10.8±2.51	81±1	26±2	61±2	38±17	47±5
Acenaphthylene	0.11±0.04	24±4	12±4	43±5	39±10	52±5	0.33±0.08	41±2	26±4	33±3	ND	30±6
Fluorene	1.38±0.33	64±5	11±2	49±9	34±6	65±3	14.47±2.56	89±1	24±1	80±1	64±9	58±4
Anthracene	2.84±0.94	60±5	2±1	55±10	21±3	73±3	5.69±1.49	80±1	12±2	79±2	67±10	65±3
Phenanthrene	9.7±3.11	46±7	8±2	42±11	26±3	64±6	81.29±19.4	86±2	21±1	81±1	71±8	58±4
Pyrene	10.10±3.47	43±5	5±1	41±8	ND	65±5	40.36±6.57	42±1	19±0.5	66±1	70±8	66±3
Fluoranthene	13.13±4.38	49±4	5±1	47±9	4±4	68±5	62.88±11.62	72±1	18±1	74±1	70±8	65±4
Chrysene	6.53±2.2	13±8	4±1	41±10	ND	64±3	10.20±1.63	33±10	26±2	71±4	ND	64±6
Benzo[*a*]anthracene	8.21±2.35	35±7	2±1	53±7	15±4	72±3	12.77±2.62	55±12	17±1	75±3	70±7	72±3

ND – not determined

4.2. Bioaccessibility prediction of fluorene, phenanthrene, pyrene and bezno(a)pyrene in spiked soil and sediment

Extraction extents from spiked matrices interpreted as deviations from the values obtained from slurry tests (difference of the means) are presented in the Figure 1. It was observed that subcritical water extraction at the conditions applied within this study significantly underestimated contaminant loss and was too mild to successfully predict (by means of no significant difference) the bioaccessible fraction for each compound in both UEA soil and UEA sediment. There are different factors that potentially might have contributed to low subcritical water extraction efficiencies. For instance, high loading of PAH in spiked matrices might have rendered maximum water solubility within the extraction cell and, as a consequence, further contaminant desorption may have been limited. Given the scarcity of research on PAH solubility in subcritical water and on various factors controlling this solubility (Andersson et al., 2005), such as ratio of sample, dispersing agent and water volume within the ASE extraction cell, further discussion would be clearly speculative. Degradation of thermally stable PAHs was unlikely to occur within subcritical water experiments presented in this manuscript primarily due to short (10 min) extraction time applied within the experiments (Hawthorne et al., 1994).

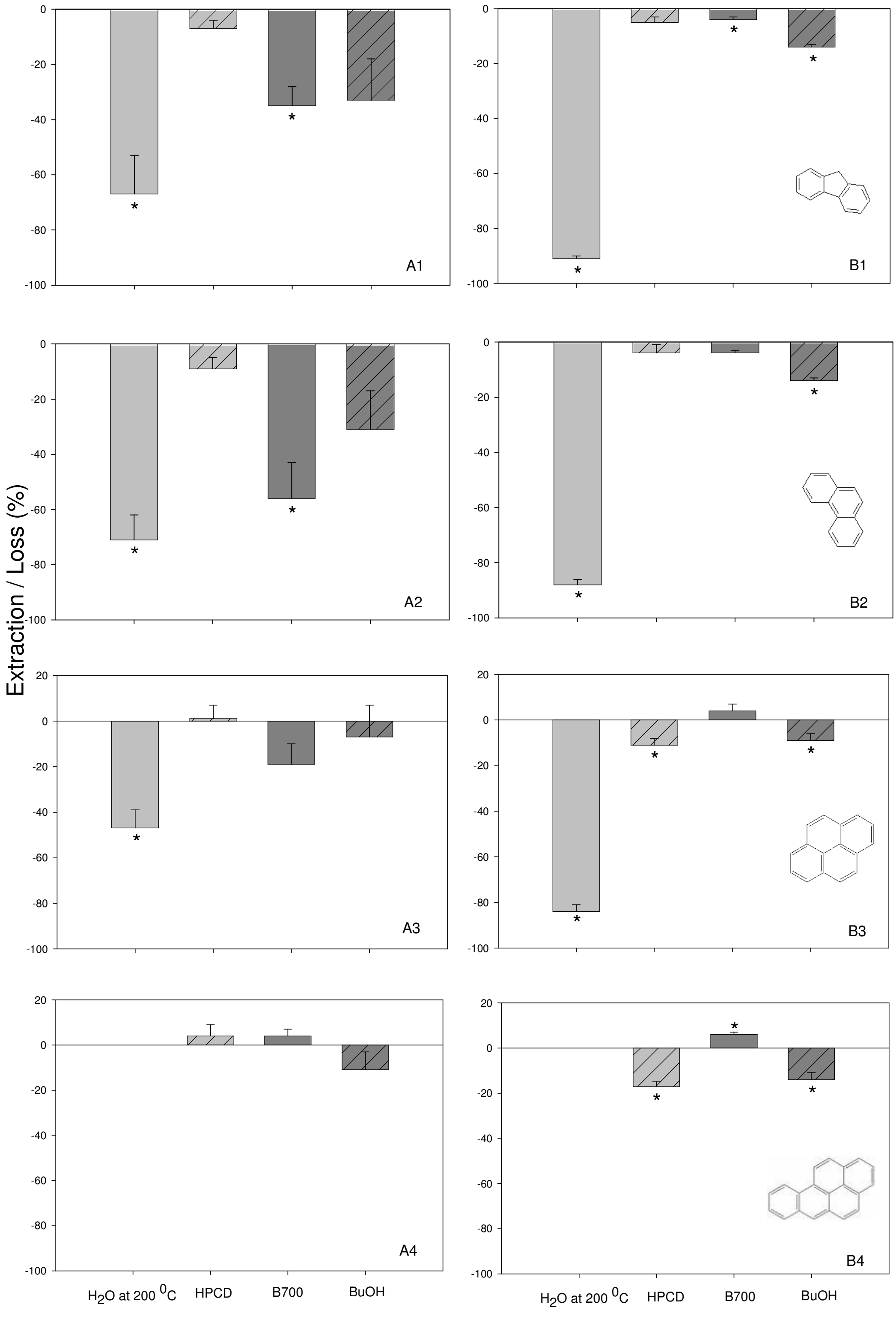

Figure 1. Difference between extraction extent and the bioaccessibility value (loss) obtained from 28 d slurry test for subcritical water extraction at 200 ºC (grey bars), cyclodextrin extraction (hatched grey bars), Brij 700 extraction (dark grey bars) and butanol (dark grey hatched bars) for UEA soil (A) and UEA sediment (B). Numbers 1-4 associated with sample identification (A and B) correspond to fluorene, phenanthrene, pyrene and benzo[*a*]pyrene, respectively. Missing bars for benzo[*a*]pyrene indicate values below detection limit after subcritical water extraction. * indicates significant difference between loss and extraction means.

Considering PAH in both tested matrices it was noted that performance of other extraction methods did not consistently over- or underestimated bioaccessibility in UEA soil and UEA sediment. For example, no consistency across different PAH and dissimilar matrices with respect to B700 extractions was observed (Figure 1).

Results presented in Figure 1 corroborate findings of others (e.g. Allan et al., 2007) that supported the use of HPCD as means to reflect bioaccessible fraction of PAHs. The discrepancy observed for pyrene and benzo[*a*]pyrene in the UEA sediment may be explained by previously reported steric constraints associated with the cyclodextrin molecule (Cuypers et al., 2002; Stokes et al., 2005). It is possible that higher MW PAHs may be physically too large to fully fit the HPCD cavity and form 1:1 inclusion complex, which can result in poorer extraction efficiencies although the formation of 2:1 complexes have been established for compounds of larger molecular volume (Wang and Brusseau, 1995). The differences in HPCD extractions observed for UEA soil and UEA sediment (Table 2) and consequently differences in deviations from the bioaccessibility value can be explained by different textures of these matrices and different OM contents. It can be anticipated that ageing processes in UEA soil were more advanced due to finer texture and higher amount of soil OM than in UEA sediment. As a result, higher HPCD extractions from UEA sediment than from UEA soil were observed for each PAH with an exception of benzo[*a*]pyrene (Table 2). This also corroborates differences in bioaccessibility for UEA soil and UEA sediment (section 4.1.).

It has been previously suggested that PAH bioavailability to both earthworms and bacteria could be determined, under different extraction conditions, using BuOH (Kelsey et al., 1997; Liste and Alexander, 2002). In our experiments with spiked samples butanol underestimated (significantly in UEA sediment) bioaccessiblity of these compounds (Figure 1). It can be also noticed that although relative extents from butanol extractions are lower than HPCD extraction extents (in 6 out of 8 comparisons; Table 2) the relative deviations (underestimations) from bioaccessibility are more pronounced for butanol than for HPCD (in 6 out of 8 comparisons; Figure 1). Similar observations of a more rigorous extractions performed by HPCD solution as compared to butanol extraction for a similar soil type has previously been reported (Swindell and Reid, 2006). Conversely, it is possible that butanol enabled a more rigorous extractions of higher MW PAHs (such as pyrene and benzo[*a*]pyrene) as compared with HPCD and, as a consequence, better approximated bioaccessibility of these PAHs in the UEA sediment.

4.3. Bioaccessibility prediction in genuinely contaminated samples

Comparison of the extraction extents using different non-exhaustive extraction techniques presented as deviations from bioaccessible contaminant fraction (difference of the means) for genuinely contaminated samples are shown in the Figure 2. Concordant with the findings from spiked matrices, subcritical water extraction at 200 ºC underestimated (significantly for 14 out of 20 PAHs) bioaccessible fraction for both TW soil and TW sediment. Moreover, as PAH log Kow increased there was a general decrease in the subcritical water-extractable fraction (Table 3). Similar findings have previously been reported (Hawthorne et al., 1994). It should be noted that based on the analyses of the regression line slopes of subcritical water extractions and bioaccessibility plotted against PAH log Kow, the extractions were analogous to the bioaccessible fraction (Table 3). It was inferred that subcritical water extraction enabled selective PAH extraction dependant of PAH log Kow and, as a consequence, paralleled the bioaccessible fraction. It is also noteworthy that extraction conditions of ASE 200 can be modified (for instance by changing static extraction mode into dynamic mode), which may potentially overcome putative solubility/desorption constraints within the ASE cell and lead to higher extraction extents.

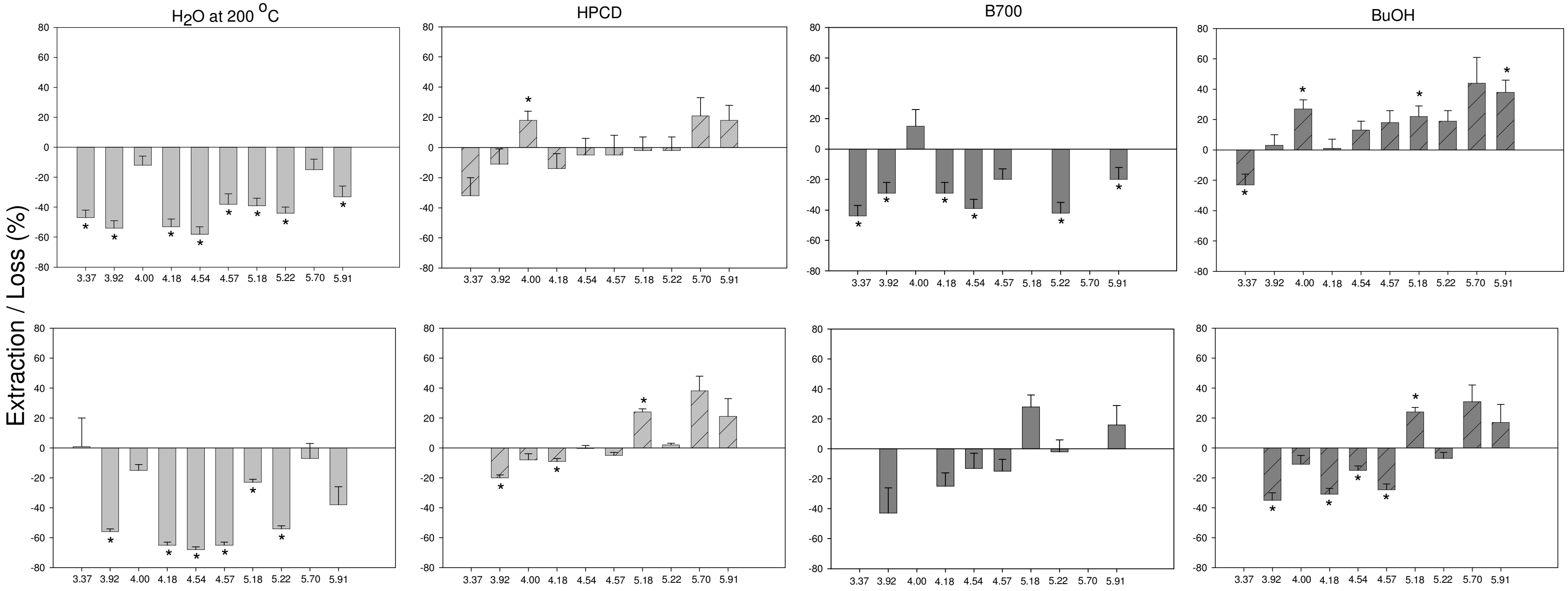

Figure 2. Difference between extraction extent and the bioaccessibility value (loss) obtained from 28 d slurry test for subcritical water extraction at 200 ºC (grey bars), cyclodextrin extraction (hatched grey bars), Brij 700 extraction (dark grey bars) and butanol (dark grey hatched bars) for TW soil (upper frames) and TW sediment (lower frames) for 10 different PAHs. PAHs are arranged according to increasing Log Kow as follows: naphthalene 3.37; acenaphthene 3.92; acenaphthylene 4.0; fluorene 4.18; anthracene 4.54; phenanthrene 4.57; pyrene 5.18; fluoranthene 5.22; chrysene 5.70; benzo[*a*]anthracene 5.91. Missing bars indicate values below detection limit. * indicates significant difference between loss and extraction means.

It was observed that HPCD successfully predicted bioaccessible fraction for 9 out of 10 PAHs in the TW soil. Inconsistency observed for the acenaphthalene can be explained by low total concentration of this contaminant (discussed above), which may have resulted in its limited desorption. HPCD can potentially overcome this strong 'binding' and, as a consequence, extracted more contaminant fraction than the fraction obtained from the slurry test. It was also noted, that deviations from the bioaccessible fraction predicted by HPCD for TW sediment were relatively low despite indications of significant difference, influenced by standard errors, for three compounds (namely acenaphthene, fluorene and pyrene). In addition, HPCD overestimated the bioaccessible fraction of PAH characterised by higher log Kow in both TW soil and TW sediment. It is possible that higher MW PAH (higher log Kow) due to recalcitrance and ageing were not degraded and/or abiotically removed from these long-aged matrices yet extracted by HPCD solution.

In general, the extents of extractions with the surfactant Brij 700 underestimated the bioaccessible fraction in both TW soil and TW sediment. These results were not surprising as B700 has been previously shown to reflect the extents of PAH bioaccumulation in worms (Barthe and Pelletier, 2007), which are usually lower than biodegradation values. Regardless of chemical rationale underpinning endeavours to correlate surfactants extractions with bioavailable/bioaccessible fraction there is paucity of successful application of surfactant as a proxy of PAH bioavailability/bioaccessibility (Cuypers et al., 2002). B700 extraction extents were inconsistent across different matrices used in our study and irrespective of PAH MW (Table 2 and 3) corroborating findings of others (e.g. Cuypers et al., 2002).

For the majority of determinations for TW soil butanol extractions overestimated PAHs bioaccesisbility whilst both significantly under- and overestimated PAHs bioaccessibility in TW sediment. Interestingly, bioaccessibility of most of the lower log Kow PAHs were underestimated by both HPCD and butanol extractions yet the extents of under- and overestimation by butanol were generally higher when compared to HPCD extractions. Similar findings were reported by Juhasz et al. (2005) where, first, butanol extraction underestimated biodegradation of 3-ring PAH, whereas overestimated of 4,5,6 rings PAH, second, HPCD overestimated bioaccessibility of higher MW PAH. It has been proposed (Barthe and Pelletier, 2007) that large HPCD molecules may not reach nanopores in which PAH are entrapped which may reduce HPCD efficiency to capture and extract these compounds. Other authors (Swindell and Reid, 2006) have reported that butanol exhibited relatively higher extractions than HPCD in aged samples with the discrepancies between extraction extents from butanol and HPCD increasing with the increase in PAH hydrophobicity; an observation relevant to the TW soil samples in our study (Table 3).

4.4. Method appraisal

Comparison of the extractions techniques employed in this study is presented in Table 4. Based on the indicators of method appropriateness for bioavailability assessment (after Reichenberg and Mayer, 2006; and Swindell and Reid, 2007a) four primary criteria for the appraisal of the techniques have been selected. These were: cost of the method per sample, extraction time, requirement of organic solvent and the accuracy of bioaccessibility reflection (indicated by no significant difference between extraction extent and bioaccessibility value).

Total cost comprised of operating costs (extracting and dispersing agents, electricity and gas consumption) and equipment costs evaluated per sample. In these calculations the following operating costs were used: subcritical water extraction: Milli-Q water processing (£0.003), Ottawa sand (£0.07), gas consumption (£0.2), electricity consumption (£0.033); HPCD extraction: HPCD powder (£2.92), water distillation (£0.0015), centrifuge energy cost (£0.012), shaker energy cost (£0.008); B700 extraction: B700 powder (£0.04), water distillation (£0.0015), centrifuge energy cost (£ 0.012), shaker energy cost (£0.007); BuOH extraction: BuOH (£0.25), centrifuge energy cost (£0.003). Equipment costs per sample associated with the capital costs of laboratory apparatus and consumables indispensable to perform extractions were equal for HPCD and B700 (£0.63) and included: shaker (£0.105), centrifuge (£0.34), Teflon tubes (£0.185), whereas for BuOH included: vortex mixer (£0.0074), centrifuge (£0.113), polypropylene copolymer tubes (£0.002) and for subcritical water extraction included: capital equipment cost of ASE 200 (£1.3), extraction cell- and collection assembly-associated costs (£0.90). It can be noticed that for both ASE and B700 equipment costs account for the majority of the total costs, whereas operating costs constitute most of the total costs of HPCD and BuOH extractions.

Table 4. Comparison of the non-exhaustive extraction techniques in terms of cost, time, use of organic solvent and reflection of bioaccessibility.

	Subcritical water	HPCD	B700	BuOH
Total cost per sample (£)	2.51	3.57	0.69	0.38
Operating cost per sample (£)	0.31	2.94	0.06	0.25
Equipment cost per sample (£)	2.20	0.63	0.63	0.12
Time (h)	0.55	21	17	0.33
Organic solvent required	No	No	No	Yes
Bioaccessibility reflection (%)*	23	78	56	52

* incidences of no significant difference between loss from the slurry test and extraction extent

Calculations were made on the basis of experiments presented in this study for an intra-comparative purpose only and may not represent the market price. For instance, costs of extracting agents can vary depending on the supplier. Similarly, majority of extractions presented above were water-based and the costs of water were limited to its distillation or processing in the Mili-Q system. The cost of water per litre in the United Kingdom can be complicated to establish and it is often (as in this study) assumed as zero, although the true economic value of this natural resource is higher. Notwithstanding that the actual costs *per se* may diverge from the values calculated here due to different prices of commodities throughout the world, applying uniform criteria across different techniques performed within this study enabled comparative assessment of various non-exhaustive approaches.

The time needed for each extraction (Table 4) does not include samples preparation as well as post-extraction and analytical procedures (with the exemption of separation assumed as intrinsic step following the extractions). It should be also noted that these times provide a point of reference as to how long a method takes to perform. This time does not reflect time staff committed to the extraction; in the case of HPCD and B700 extractions once samples are prepared they can be left unattended for shaking.

It can be concluded that for the purpose of chemical reflection of bioaccessible fraction to microorganisms the use of cyclodextrin was the most successful of all techniques (78%; Table 4). Moreover, water-based techniques can be perceived as more environmentally acceptable over organic solvent-based methods for which environmental burden and costs associated with post-extraction disposal can be significant. In this study disposal costs being largely dependent on the post-extraction procedures dictated by a plethora of possible analytical methods for compound quantification, were not considered.

The use of B700 and BuOH were undoubtedly cheapest methods with BuOH being additionally the most rapid technique. Yet their prediction of bioaccessibility in 56% and 52% of determinations respectively precluded suitability of these methods for bioaccessibility reflection in this study.

5. CONCLUSIONS

Different non-exhaustive approaches investigated in this study resulted in various feasibilities to predict bioaccessibility of different MW PAHs and in different economical and practical attributes. These results suggest that bioaccessibility assessment falls beyond the realm of a single uncontested approach. Whilst cyclodextrins appeared to be the most accurate predictor of bioaccessible fraction for the majority of PAHs further research towards other techniques can be legitimised on account of their lower cost (e.g. B700) or time-efficiency (e.g. subcritical water). It is also noteworthy, that subcritical water extraction, echoing PAH desorption processes, paralleled bioaccessible contaminant fraction. Overall, for bioaccessibility reflection, an extraction technique that relies on desorption mechanisms would be the most accurate and, perhaps more importantly, consistent over dissimilar environmental matrices.

Requirements of urban development associated with regeneration of brownfield sites entrenched within recent advances towards sustainable land management drives a great demand for improved characterization of contaminated land. Information on contaminant bioaccessibility has been recognized by regulators and decision-makers as an indicator of risks associated with contaminated land. As a consequence, robust methods to assess bioaccessibility are of importance to decision-support. Furthermore, data on bioaccessibility to microorganisms provides a powerful argument when evaluating appropriateness of bioremediation to regenerate contaminated land. Incorporation of bioaccessibility data into decision-support methodologies for contaminated land assessment represents a step towards developing interfaces that will allow more proportionate evaluation of contaminated sites.

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CHAPTER 5

‘The Three Cultures’? Towards a better understanding of bioaccessibility implementation

'The Three Cultures'?
Towards a better understanding of bioaccessibility implementation

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ABSTRACT

Sustainable, proportionate and risk based-approach to contaminated land management pervades contaminated land regimes in many countries. While this approach stipulate national and international priorities, its practical implementation in the United Kingdom (UK) is reliant upon Local Authorities. In the UK bioaccessibility has been recognized as a decision-support tool yet its incorporation into contaminated land decision-making is not statutorily defined. This paper presents the investigation into the views of Local Authorities in England and Wales regarding the practical application of bioaccessibility and constraints associated with its implementation. The research involved an online survey (n = 151, accounting for 40.3% of Authorities) followed by semi-structured interviews (n = 17) with selected respondents. A majority of survey respondents (70%) perceived bioaccessibility to be a useful tool that facilitates contaminated land management. Whilst necessity to access more information regarding bioaccessibility was indicated by 76% of participants, a need for more research into under-investigated contaminants, such as polycyclic aromatic hydrocarbons, was emphasised. Lack of statutory guidance was indicated by 78% of respondents as the main factor hampering the use of bioaccessibility data in regulatory decision-making. Although the research identifies other contributory factors, the lack of central guidance appears to be the main reason for the uneven uptake of bioaccessibility analysis throughout England and Wales. Divergence of policy-maker and decision-maker perceptions of bioaccessibility was indicated by the respondents.

The research brings the voice of front-line regulators into the on-going discussion between policy-makers and scientists on the uses of bioaccessibility. Recognition of the real-world priorities that inform the local decision-makers' perspective may help to bridge the gap between science and policy. This paper concludes by proposing action priorities both for the research community and for policy-makers.

1. INTRODUCTION

Risk-based regulation underpins many contaminated land regimes throughout the world (Nathanail and Earl, 2001; Rothstein et al., 2006; Luo et al., 2008; Naidu et al., 2008a). This approach identifies land as contaminated on the basis of the risk that the contaminants pose to environmental receptors, and not merely on the presence of contaminants of concern (Oberg and Bergback, 2005). Thus, for land to be designated as contaminated a 'significant pollutant linkage' must exist by the presence of a receptor exposed to a contaminant source by means of a pathway (Catney et al., 2006). Principles of contaminant sequestration within a heterogeneous soil matrix, whereby a proportion of the total contaminant present is not readily accessible for transformations (Alexander, 2000), have led to formulations of bioaccessibility concepts. On the account of risk-based contaminated land assessment, these concepts have been perceived as a pragmatic decision-support tool that facilitates a more accurate land evaluation by recognizing that only the 'accessible' fraction may present a risk.

Research on bioaccessibility relating to contaminated land management has attracted both substantial academic (Ruby et al., 1996; Ehlers and Luthy, 2003; Pu et al, 2004; van de Wiele et al., 2004; Palumbo-Roe et al., 2005; Juhasz et al., 2007; Laird et al., 2007; Intawongse and Dean, 2008; Ollson et al., 2009) and regulatory attention (RIVM, 2006; EA, 2007; Saikat et al., 2007; US EPA, 2007). With respect to human health risk assessment, the 'bioaccessible fraction' is defined as the fraction of a substance that is released from the soil, during such processes as digestion into solution making it available for absorption (measured *in vitro*), whilst 'bioavailability' relates to the fraction that reaches the blood system via the gastrointestinal tract (EA, 2002). Bioavailability testing involves *in vivo* models, which raises ethical issues and is too time-consuming and costly to be routinely incorporated into site-specific risk assessments. *In vitro* approaches to estimating the bioaccessible fraction, as a surrogate for bioavailability, have therefore been investigated for more than 10 years (Ruby et al., 1996; Saikat et al., 2007).

Risk-based approaches advocate that only the fraction of contaminant that reaches the central blood circulation may exert adverse effects on human health (Nathanail and Smith, 2007; RIVM, 2006). Therefore, information on contaminant bioavailability and bioaccessibility, contrary to traditional and arguably more conservative 'total' contaminant concentrations, can promote a more proportionate and cost-effective assessment of contaminated land. The primary benefit of measuring bioaccessibility is improving the accuracy of the risk assessment process and prioritizing remedial actions. Indeed, it has been demonstrated that the use of bioaccessibility data prevented unnecessary (and unsustainable) remediation, avoided public anxiety and land blight (Nathanail and Smith, 2007; Naidu et al., 2008a).

Along with the acceptance of the rationale behind the concept of bioaccessibility and its favourable reception by land owners and developers, ambiguity associated with the practical application of bioaccessibility data remains. Issues that are the subject of on-going discussions in relation to the implementation of bioaccessibility include: variability in results obtained from different laboratories on the same sample, lack of a standard method and scarcity of evidence that methods are being correlated with appropriate and robust bioavailability data to address population variability (EA, 2007), and the legal embedding of bioaccessibility within regulatory frameworks (EA, 2006; Latawiec et al., 2009).

Local Authorities are the primary regulators for contaminated land (Defra, 2006). Their experience and expertise is vital to a sustainable management of contaminated land, especially in the context of integrative approach to contaminated land assessment (Pollard et al., 2004). Yet, to our knowledge, no recent research exploring the opinion of decision-makers on the use of bioaccessibility exists. In this study (i) the extent to which scientific research on bioaccessibility has been accepted by Local Authority decision-makers and incorporated into contaminated land assessment practice is investigated, (ii) current limitations in implementing bioaccessibility are identified, (iii) current and potential role of bioaccessibility in contaminated land management (‘do we need bioaccessibility?’) is explored (iv) areas in which academic research could contribute to a better understanding and further utilization of bioaccessibility within contaminated land decision-support are identified.

Current views of Local Authorities in England and Wales on the use of bioaccessibility within contaminated land decision-making are presented. The understanding regulator practices can help to identify significant differences in the perspectives of practitioners, policy-makers and academic researchers. This work offers an account of opinions that can assist in bridging these gaps. Thus, this research has relevance for other countries in the context of integrating of emerging scientific research into risk-based decision-making. Finally, drawing on the insights offered by the study, recommendations in setting action priorities both for the research community and for policy-makers are proposed.

2. METHODS

2.1. Sample

In the United Kingdom, Local Authorities are the primary regulators for contaminated land assessment (Defra, 2006). England and Wales are governed by congruent legislation, in contrary to more autonomous regulations in Northern Ireland and Scotland. This study targeted therefore Local Authority Contaminated Land Officers or their equivalents (the officers who have responsibility for contaminated land in those Authorities where a dedicated post or group does not exist), such as Environmental Health Officers, Planning Officers or Environmental Protection Officers in England and Wales. The invitation to participate in the survey along with a link to an online questionnaire (see below) was sent twice, in August and September 2008, via the Chartered Institute of Environmental Health to its members in 300 Local Authorities in England and Wales. A list of Local Authority contacts with responsibility for contaminated land in all 375 district and unitary Local Authorities in England and Wales (EA, 2006) was subsequently obtained from the Environment Agency. In order to increase the survey coverage and maximize the number of responses, the invitation was sent again in November to all of the contacts on this list. In each mailing, Officers were invited to respond either online, by e-mail, fax, post or over the telephone. It should be noted that a small number of these Local Authorities was not successfully reached (approximately 20). Responses were received from 143 Local Authorities in addition to the 8 Local Authorities that participated in the pilot study conducted in May and June 2008. 143 responses were received from English Local Authorities and 8 from Wales. The proportion of returns corresponded to 40.3 % of all Local Authorities in England and Wales. Although it is possible that there was a self-selection bias towards individuals with greater interest in bioaccessibility, the response rate makes the findings applicable to, at least, 40.3 % of regulators. In addition, responses were received from both rural and urban regions of England (Defra, 2008). Of 117 respondents from England that provided the name of their LA, 44% of the responses were received from urban Local Authorities (major urban, large urban and other urban), whereas 56% of Local Authorities from rural regions responded.

2.2. Survey

The questionnaire (appended at the end of this thesis) consisted of 14 questions and covered topics relating to contaminated land management in the Local Authority's jurisdiction area. The questionnaire was developed on the basis of issues identified from the literature review (e.g. EA, 2002; Ehlers and Luthy, 2003; EA, 2006; BGS, 2007), preliminary interviews with the Officers and discussions with other bodies involved in contaminated land decision-making (such as contaminated land consultancies).

Questions focused more specifically on land contaminated with polycyclic aromatic hydrocarbons (PAHs), including guidelines used for PAH-contaminated land assessment (Question 3) and bioremediation of PAH-contaminated land (Question 4). Three questions (Question 5, 6 and 7) related to the use of bioaccessibility for contaminated land decision-support. Although the questionnaire was anonymous, the respondents were asked to provide background information including the name of their Local Authority, the number of persons in their contaminated land team, the length of time they have worked with contaminated land and the range of tasks they are involved in on a day-to-day basis. These background questions were asked to provide information about the sample and to identify possible variables that might have influenced the experience and views of the respondents, and were used to support the analyses.

Here, the findings of a subset of results relating specifically to the use of bioaccessibility and constraints associated with the implementation of bioaccessibility are reported. The Officers' perspective on the use of bioaccessibility (Question 5) was rated on a 5-point Likert scale (strongly agree, agree, neither agree nor disagree, disagree, strongly disagree) with an additional 'don't know' option. Question relating to constraints upon barriers to the implementation of bioaccessibility and a question relating to bioavailability and bioaccessibility definitions allowed respondents to choose more than one answer: the sum of the results for these questions therefore exceeds 100%. Each question was accompanied by a free text space for respondents to make additional comments.

In order to complement the data generated by the questionnaire-based survey and aid the interpretation of the results, the questionnaire was triangulated with semi-structured personal and telephone interviews (after Arksey and Knight, 2007). 17 interviews were conducted in February and March 2009 with survey respondents who had indicated that they were willing to be approached for more information. The interviews lasted from 25 to 45 minutes of conversation. Each interview focused on 3 key questions relating to 1) the use of bioaccessibility in the Local Authority area, 2) guidance on bioaccessibility and uncertainties associated with bioaccessibility, and 3) need for bioaccessibility data. These questions were further explored by using additional probe questions to achieve greater elaboration and clarifications of the answers given.

2.3. Analysis

SPSS 16.0 for Windows was used for statistical processing of the questionnaire data. Descriptive results are presented as percentage of responses to each question (not necessarily of total number of participants). Results were considered significant at the 95% confidence level ($p < 0.05$). ArcGIS was used to analyze the spatial distribution of the questionnaire responses and to classify the English Local Authorities according to the Department for Environment, Food and Rural Affairs' rural/urban classification scheme (Defra, 2008). To honour assurances of confidentiality given to respondents the spatial analysis is not presented here but the generic results have been used to support the interpretation of the survey findings.

3. RESULTS AND DISCUSSION

3.1. The use of bioaccessibility

The majority of respondents (70.2%) either strongly agreed or agreed that 'bioavailability/bioaccessibility testing is a useful tool that facilitates contaminated land management'. Only 2.4% (corresponding to 3 respondents) either disagreed or strongly disagreed with the statement. The rest of the respondents neither agreed nor disagreed or indicated 'don't know' option (corresponding to 21.8% and 5.6%, respectively). It is noteworthy that all respondents that selected the latter options also neither agreed nor disagreed or selected the ‘don’t know’ option with respect to other questions within the questionnaire. Two reasons might account for this: 1) less familiarity with the concept of bioaccessibility and/or 2) lack of problematic sites within the jurisdiction area. The first explanation is supported by the finding that the majority (79.4%) of respondents who selected ‘neither agree nor disagree’ or ‘don’t know’ in answer to the question about the usefulness of bioaccessibility also strongly agreed or agreed that more information on bioavailability and bioaccessibility was needed. Regarding the second explanation, there are, in many areas throughout England and Wales, as elsewhere, naturally occurring contaminants at levels exceeding guideline values (Juhasz et al., 2007; Nathanail and Smith, 2007; Saikat et al., 2007; Thums et al., 2008). It is virtually impossible to remediate an entire Local Authority’s jurisdiction area and/or to preclude development on the basis of natural abundance of contaminants. The interviews found that bioaccessibility testing has therefore been perceived as a pragmatic, *ad hoc* decision-support tool when dealing, in particular, with naturally occurring contaminants. It was also indicated by 8 out of the 17 interviewees that bioaccessibility data was being applied to so-called ‘grey-zone’ concentrations where the exceedance of the guideline value is not substantial. It has been recognized that measuring bioaccessibility is impractical where the exceedance of the guidelines value is significant, whereby the use of bioaccessibility data would not affect the final designation of the land as contaminated (Naidu et al., 2008b). It has been also previously demonstrated that bioaccessibility data can lead to more cost-effective land management (Nathanail and Smith, 2007). Respondents who considered bioaccessibility to be a useful tool were also more likely to view it as leading to more cost-effective management of contaminated land (Figure 1).

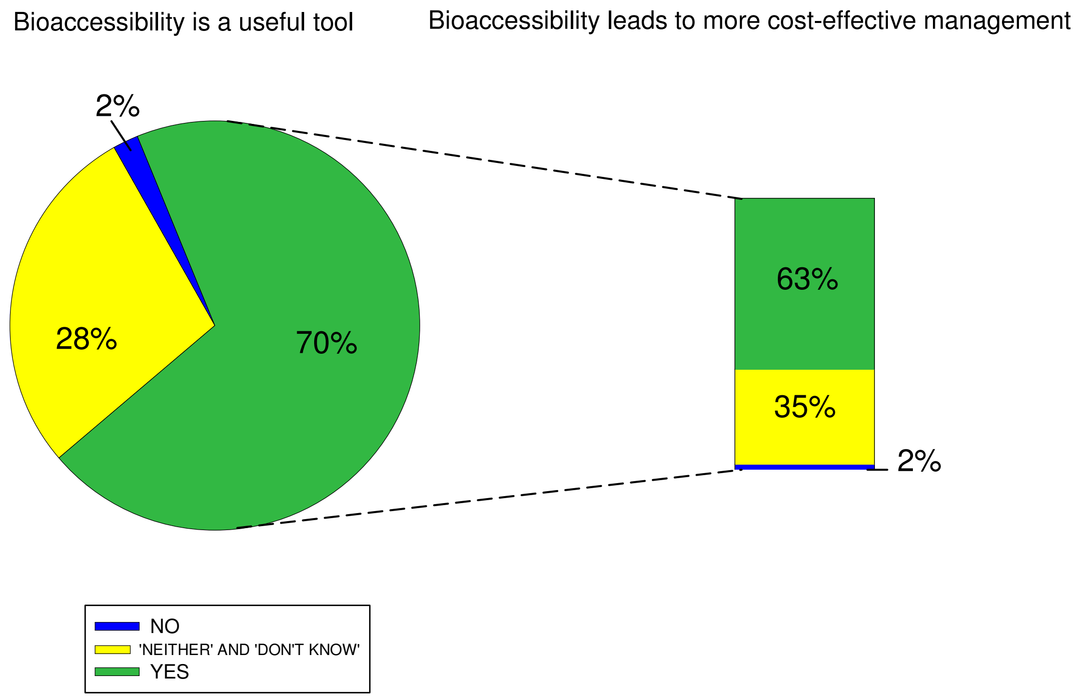

Figure 1. Percent of respondents indicating usefulness of bioaccessibility vs. cost-effectiveness of bioaccessibility.

Information on contaminant bioaccessibility brings additional advantages when dealing with large, open-space areas where a significant amount of soil would have to be removed and/or remediated. Indeed, it was confirmed during the interviews with Officers from urban areas that bioaccessibility data might not be beneficial to smaller contaminated sites (such as gardens attached to houses), where the cost of bioaccessibility testing might surpass the cost of breaking the significant pollutant linkage (e.g. by capping). Seven interviewees pointed to a prevailing concern among some Officers about overestimation of risks when applying current guideline values. The following comment, taken from one of the questionnaires, illustrates a view that was recurrently expressed:

> 'I am firmly of the opinion that our perception of risk is far greater than the actual or true risk posed in many contamination assessments. It is recognized that non consideration of what is and what is not both bioavailable and accessible can lead to gross overestimation. This can result in a number of negative impacts both financially and in terms of human health risk assessment. PAH's B(a)P and Arsenic are the immediate areas most often quoted and referenced in the debate' (Questionnaire, 2008)

From the interviews it also appeared that bioaccessibility could be used not only with respect to (mostly naturally occurring) arsenic and heavy metals but also to other contaminants, both from natural and anthropogenic sources. For example, 65.3% of respondents either strongly agreed or agreed that information on bioaccessibility of benzo[*a*]pyrene, an organic contaminant that belongs to PAHs, is needed. It was elucidated during the interviews that organic contaminants have been generally perceived as under-researched (see also Rivett et al., 2002) and there was a held view among respondents that it is impractical to remediate the land where contamination would inevitably re-appear on account of elevated ambient concentrations from anthropogenic sources. Indeed, ubiquity of organic contaminants, such as PAHs, as a consequence of pervasive and trans-boundary contamination from combustion of organic materials from both anthropogenic sources (fossil fuels) and natural processes (e.g. forest fires), has been previously demonstrated (Jones et al., 1996; Cousins et al., 1997; Jones and Voogt, 1999; Bamforth and Singleton, 2005).

Views on bioaccessibility did not appear to be influenced by the type of area covered by the Authority: statistical analysis of responses to the survey questions found no significant difference between Officers in rural and in urban Authorities in England (Table 1). Similarly, cross-tabulation against other variables such as number of persons in contaminated land team, years of experience with contaminated land or the tasks Officers were involved in revealed no statistically significant differences ($p > 0.05$). It was therefore inferred that complex site-by-site circumstances, due, for instance, to naturally occurring elevated levels of contaminants or increased ambient anthropogenic concentrations and hence the necessity of a subsidiary decision-making tool, are the most likely factors driving the extent of familiarity with and acceptance of bioaccessibility.

Table 1. Number of respondents from rural and urban areas cross-tabulated with the statement relating to the usefulness of bioaccessibility and the information on benzo[*a*]pyrene. P value established on the basis of Pearson Chi-Square test

Bioaccessibility is a useful tool	Rural	Urban	p-value
Strongly agree	14	13	
Agree	27	25	
Neither agree nor disagree	14	6	0.82
Disagree	1	1	
Strongly disagree	0	1	
Don’t know	4	2	
Information on benzo[*a*]pyrene needed			
Strongly agree	15	12	
Agree	27	19	
Neither agree nor disagree	9	11	0.14
Disagree	5	2	
Strongly disagree	0	1	
Don’t know	4	3	

3.2. Bioaccessibility and bioremediation

The bioaccessibility concept has been recognized not only from a human health perspective but also in terms of ecological risk assessment (Ollson et al., 2009) and in the context of applicability of bioremediation techniques (Diplock et al., 2009). Bioremediation is believed to be the most sustainable of all remediation approaches (Zechendorf, 1999; van Dillewijn et al, 2009), whereas the ability of microorganisms to successfully biodegrade PAHs (especially low molecular weight PAHs) is well documented (Cerniglia, 1992; Sepic et al., 1997; Bamforth and Singleton, 2005; Stokes et al., 2005). It is noteworthy that Local Authorities are primarily involved in human health risk assessments. 53.6% of the survey respondents strongly agreed or agreed that 'Information on PAHs bioavailability/bioaccessibility to microorganisms can determine suitability of remediation'. Also, 50.7% of the respondents indicated that bioremediation has been used in their area while 44.4% indicated that it has 'never' been used. Of the respondents reporting the use of bioremediation in their areas, 40.8% reported it has been 'rarely' used (< 10% of sites under remediation), 8.5% answered it has been used 'sometimes' (10-30% of remediated sites), whilst only 1.4% has applied it 'often' (i.e. to more than 30% of sites under remediation). Common opinion among participants was that bioremediation can lead to a more cost-effective and more sustainable remediation (avoiding the 'dig and dump' approach). Yet, time and space constraints, uncertainty associated with residual levels and/or an inability to reduce contamination to the guideline value levels were the main factors perceived as limitations to the adoption of bioremediation. It was also suggested that the current financial crisis may slow down development and encourage developers to undertake more cost-effective albeit less time-efficient bioremediation.

3.3. What are the limitations to the implementation of bioaccessibility data?

In a survey of English and Welsh Local Authorities carried out in early 2005 by the Environment Agency the principal reason given by survey participants for not accepting bioaccessibility assessments was that there was no guidance published by Environment Agency on the use of bioaccessibility (EA, 2006). In our survey, lack of statutory guidance was also indicated as the main reason hampering the use of bioaccessibility (78% of participants; Figure 2). Officers who were interviewed emphasized that not necessarily statutory but that guidance from an authoritative body, such as the Environment Agency, is needed. Recurring opinion of the Officers within the survey was ‘conservatism of contaminated land assessment criteria’ regardless of substantial research in the field of bioaccessibility, both on national and international level; an observation congruent with some authors (BGS, 2007).

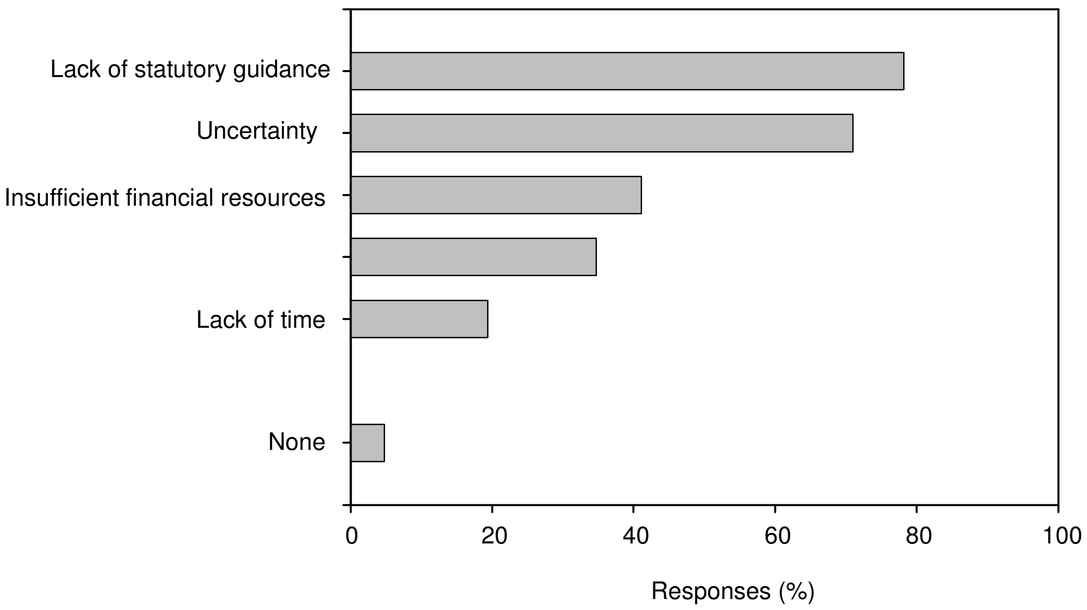

Figure 2. Factors hampering the application of bioaccessibility in Local Authority jurisdiction areas. Bars represent percent of the respondents.

Other reasons indicated as limitations to bioaccessibility application that emerged from the questionnaire included: uncertainty associated with bioavailability/bioaccessibility data (71%), insufficient financial resources available to carry out testing (41.1%), failings in risk assessment reports (34.7%) and a lack of time to analyse the data (19.4%). It was also clear from the interviews that there was an implicit expectation that the guidance would dispel the uncertainties by establishing a standard methodology.

In addition, those respondents who were more confused by the terminology of bioavailability and bioaccessibility were as concerned with the lack of guidance as with uncertainties. Indeed, respondents who indicated confusion with terminology indicated the lack of statutory guidance (71.7%) to be as important a limitation as uncertainty associated with bioaccessibility data (69.9%). Respondents who were familiar with terminological differences indicated the lack of statutory guidance (81.1%) to be the principal limitation, although not necessarily prevention, to the use of bioaccessibility. It was further apparent from the interviews that respondents who were more comfortable with using bioaccessibility data adopted strategies for dealing with the uncertainties. These included: incorporating a ‘worst-case’ scenario (the largest bioaccessibility value); carrying out extra sampling to verify whether data variability was due to heterogeneity of the site or the performance of the method; incorporating information about method robustness from an authoritative source such as the British Geological Survey (BGS); recommendation of a single test throughout all sites (e.g. Physiologically Based Extraction Test for arsenic) to assure consistency across the jurisdiction area; considering trends in data; and looking at vegetable uptake and; considering the use of the site.

In summary, lack of guidance on the use of bioaccessibility was indicated as the main factor hampering the use of bioaccessibility. Local Authorities being under auspices of the Environment Agency and as a liable public body can be legally challenged. Therefore, the guidance would bring more confidence to Officers who were ‘ready to accept bioaccessibility’ (questionnaire results corroborated by 15 out of 17 interviewees) and would standardise the approach to bioaccessibility throughout the country. Some Officers in areas where naturally elevated contaminants have been identified acknowledged their regular use of bioaccessibility data. They perceived the use of bioaccessibility criteria as the only option to prevent remediation of entire Local Authority areas and to allow development.

Uncertainties associated with bioaccessibility along with a lack of robust *in vivo* bioavailability data have been reported as the main limitations to bioaccessibility acceptance by policy-makers (EA, 2002; Interviews, 2009). As uncertainties suggest the need for more research on the one hand and incorporation of already published research into policy-consideration on the other, the latter limitation could appear to be a 'dead-end' statement given its immanent contradiction with respect to animal testing policy (BGS, 2007).

3.4. Recommendations

> 'I think bioaccessibility is useful but there is no certainty of its use in this field [contaminated land] and lack of guidance and information to either support or disagree with using bioaccessibility. If we use it there is no government body to back us up and support us and if we don't use it, what else do we use?' (Questionnaire, 2008)

As corroborated by the interviews, the above statement epitomises the opinion of a number of decision-makers throughout England and Wales. Indeed, only 4.8% of the respondents indicated that they use bioaccessibility data confidently (Figure 2). Given the ubiquity of natural contamination and prevalent elevated ambient concentrations of anthropogenic contaminants there is an uncontested need to equip decision-makers with appropriate management tools. At the moment, for problematic site-specific risk assessments, bioaccessibility data is perceived as the way forward. As bioaccessibility is already in use there is, firstly, an urgent need for straightforward, void of scientific jargon guidance to standardise the application of bioaccessibility data throughout the country and to uniform criteria for development. Secondly, more information, training and availability of successful case studies, together with greater access to research articles, are needed to instil confidence in the use of bioaccessibility data. Indeed, 76.8% of the respondents either strongly agreed or agreed that more information was needed. Participants more familiar with bioaccessibility commented that whilst greater access to information is needed generally, from their perspective as Local Authority regulators new research is critical only for some contaminants, such as PAHs (preferably in mixtures). Whilst there is a need for a standard test for arsenic and metals quoted as extensively investigated, there is also a need for a framework for PAH risk assessment and more research on PAH bioaccessibility. Finally, bringing the knowledge and opinion of Local Authorities into policy-making and into academia, whilst making academic research more available to decision- and policy-makers, can trigger more relevant academic research on the one side, and facilitate better decision-making on the other.

4. CONCLUSIONS

Statutory requirements for the management of contaminated land incorporate qualitative caveats regarding the stringency with which authorities should apply them, stipulating that their application should not entail excessive cost or conflict with ‘overriding public interests’ (Pollard et al., 2004). In this context bioaccessibility offers a decision-support tool. Evaluation of bioaccessibility is however not meant to replace other approaches but to assist decision-makers in situations where alternative solutions are limited. Furthermore, the concepts underpinning the use of bioaccessibility constitute a contaminant behaviour paradigm and it is now widely recognized that the risk from contaminants in the soil arises not from the mere presence of the contaminant of concern but from the ‘significant possibility of significant harm’ that the contaminant poses to environmental receptors. Bioaccessibility embedded within a risk-based approach that provides flexible frameworks and intrinsically stimulates scientific advances to improve accuracy of risk assessments can create strong epistemic and pragmatic circumstances for driving progress in contaminated land risk assessment.

Policy development involving the legal formulation of management objectives via normative concepts such as sustainability, adversity and tolerability, tents to avoid tentative scientific debate and accepts uncertainty with reluctance (Evans et al., 2006). However, for a risk-based system to operate, policy-maker, risk assessor and researcher must effectively communicate. Decision-makers managing land contamination face an array of complex issues and pressures associated with public and financial liability, with the management of different regulatory interfaces, with interpretation of sophisticated analytical data and risk assessment reports, with evaluation of the relative capabilities of remediation technologies and with the maintenance of public confidence in remediation projects. In the face of these challenges and of economic and environmental pressures, it has been indicated by survey participants that consideration of bioaccessibility can aid decision-making, refine risk assessments and facilitate sustainable land management. However, this study indicates that unless a greater commitment is made with respect to developing a standardised perspective on bioaccessibility and securing it within a framework from an authoritative source, the confidence of local regulators in the use of this tool will be undermined and progress in integrating it into contaminated land decision-making will continue to be hampered.

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APPENDIX

Survey on bioaccessibility implementation

Survey on bioaccessibility implementation

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Newcastle City Council and all the Local Authorities that kindly participated in the survey

Keywords:
Bioavailability, Bioaccessibility, Contaminated Land, Local Authorities

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Executive summary

This report details the results of a questionnaire- and interview-based survey of Local Authority Officers in England and Wales. This survey was designed to:

- Explore views on the use of bioaccessibility within contaminated land management.
- Identify current constraints in bioaccessibility implementation.
- Identify areas in which academic research could contribute towards a better understanding and further utilization of bioaccessibility.
- Investigate the extent of use of bioremediation to regenerate PAH-contaminated land.
- Identify which guidelines are commonly used to assess land contaminated with polycyclic aromatic hydrocarbons (PAHs).

Responses were received from 151 Local Authorities, which correspond to 40.3% of all English and Welsh Local Authorities. The results from the questionnaire were complemented by 17 personal or telephone interviews. The most significant findings include:

- The majority of participants (70.2%) perceived bioaccessibility as a useful tool that facilitates contaminated land management.
- Whilst necessity to access more information regarding bioaccessibility was indicated by 76.8% of participants, a need for more research for under-investigated contaminants, such as benzo(a)pyrene was emphasised.
- Lack of statutory guidance was indicated as the main factor hampering application of bioaccessibility data (78.2%).
- CLEA has been indicated by 93.6% of respondents as the most commonly used model for PAH-contaminated land assessment.
- ‘Never’ was the most common (44.4%) answer to the question regarding the frequency of bioremediation use in order to clean-up PAH-contaminated land.

The results of this study have been used to inform Local Authorities, Environment Agency, Department for Environment, Food and Rural Affairs and the research community (further journal articles are in preparation) about the Officers’ views on bioaccessibility application and the real-world circumstances regarding the use of bioaccessibility.

Contents

1. Introduction

Sustainable, proportionate and risk based-approach to contaminated land management pervades contaminated land regimes throughout the world (Rothstein et al., 2006). While this approach stipulates national and international priorities, its practical implementation in the United Kingdom (UK) is reliant upon Local Authorities.

Principles of contaminant sequestration within a heterogeneous soil matrix, whereby a proportion of the total contaminant present is not readily accessible for transformations (Alexander, 2000), have led to formulations of bioaccessibility concepts. On the account of risk-based contaminated land assessment, these concepts have been perceived as a pragmatic decision-support tool that facilitates a more accurate land evaluation, whereby only the 'accessible' fraction may present a risk. In the UK, bioaccessibility has been recognized as a decision-support tool yet its incorporation into contaminated land decision-making is not statutorily defined.

Research on bioaccessibility in the context of contaminated land management has attracted both substantial academic (Ruby et al., 1996; Ehlers and Luthy, 2003; Pu et al, 2004; van de Wiele et al., 2004; Palumbo-Roe et al., 2005; Juhasz et al., 2007; Laird et al., 2007; Intawongse and Dean, 2008; Ollson et al., 2009) and regulatory attention (RIVM, 2006; EA, 2007; Saikat et al., 2007; US EPA, 2007). With respect to human health risk assessment, the 'bioaccessible fraction' is defined as the fraction of a substance that is released from the soil, during such processes as digestion into solution making it available for absorption (measured *in vitro*), whilst 'bioavailability' relates to the fraction that reaches the blood system via the gastrointestinal tract (EA, 2002). Bioavailability testing involves *in vivo* models, which raises ethical issues and is too time-consuming and costly to be routinely incorporated into site-specific risk assessments. *In vitro* approaches to estimating the bioaccessible fraction, as a surrogate for bioavailability, have therefore been investigated for more than 10 years (Ruby et al., 1996; Saikat et al., 2007).

Risk-based approaches advocate that only the fraction of contaminant that reaches the central blood circulation may exert adverse effects on human health (RIVM, 2006). Therefore, information on contaminant bioavailability and bioaccessibility can promote a more proportionate and cost-effective assessment of contaminated land. Indeed, it has been demonstrated that the use of bioaccessibility data prevented unnecessary (and unsustainable) remediation, avoided public anxiety and land blight (Nathanail and Smith, 2007; Naidu et al., 2008).

Along with the acceptance of the rationale behind the concept of bioaccessibility and its favourable reception by land owners and developers, ambiguity associated with the practical application of bioaccessibility data remains. Issues that are the subject of on-going discussions in relation to the implementation of bioaccessibility include: variability in results obtained from different laboratories on the same sample, lack of a standard method and scarcity of evidence that methods are being correlated with appropriate and robust bioavailability data to address population variability (EA, 2007), and the embedding of bioaccessibility within regulatory frameworks (EA, 2006; Latawiec et al., 2009).

In this study we (i) investigate the extent to which scientific research on bioaccessibility has been accepted by Local Authority decision-makers and incorporated into contaminated land assessment practice, (ii) identify current limitations in implementing bioaccessibility, (iii) explore trade-offs to bioaccessibility adoption along with the current and potential role of bioaccessibility in contaminated land management ('do we need bioaccessibility?') and (iv) identify areas in which academic research could contribute to a better understanding and further utilization of bioaccessibility.

Commonly used guidelines to evaluate PAH-contaminated land are also presented and the frequency of bioremediation used to clean-up PAH-contaminated land is discussed.

2. Research design

In England and Wales Local Authorities are the primary regulators for contaminated land assessment (Defra, 2006). This study targeted therefore Local Authority Contaminated Land Officers or their equivalents, such as Environmental Health Officers, Planning Officers or Environmental Protection Officer. The invitation to participate in the survey along with a link to an online questionnaire (Appendix A) was sent twice, in August and September 2008, via the Chartered Institute of Environmental Health to its members in 300 Local Authorities in England and Wales. A list of Local Authority contacts with responsibility for contaminated land in all 375 district and unitary Local Authorities in England and Wales (EA, 2006) was subsequently obtained from the Environment Agency. In order to increase the survey coverage and maximize the number of responses, the invitation was sent again in November to all of the contacts on this list. In each mailing, Officers were invited to respond either online, by e-mail, fax, post or over the telephone. It should be noted that a small number of these Local Authorities was not successfully reached (approximately 20). Responses were received from 143 Local Authorities in addition to the 8 Local Authorities that participated in the pilot study conducted in May and June 2008. 143 responses were received from English Local Authorities and 8 from Wales. The proportion of returns corresponded to 40.3 % of all Local Authorities in England and Wales. Although it is possible that there was a self-selection bias towards individuals with greater interest in bioaccessibility, the response rate makes the findings applicable to, at least, 40.3 % of regulators. In addition, responses were received from both rural and urban regions of England (Defra, 2008). Of 117 respondents from England that provided the name of their Local Authority, 44% of the responses were received from urban Local Authorities (major urban, large urban and other urban), whereas 56% of Local Authorities from rural regions responded.

The questionnaire consisted of 14 questions and covered topics relating to contaminated land management in the Local Authority's jurisdiction area. The questionnaire was developed on the basis of issues identified from the literature review (e.g. EA, 2002; Ehlers and Luthy, 2003; EA, 2006; BGS, 2007) and preliminary interviews with the Officers. Questions focused more specifically on land contaminated with PAHs, including guidelines used for PAH-contaminated land assessment (Question 3) and bioremediation of PAH-contaminated land (Question 4). Three questions (Question 5, 6 and 7) related to the use of bioaccessibility for contaminated land decision-support. Although the questionnaire was anonymous, the respondents were asked to provide background information including the name of their Local Authority, the number of persons in their contaminated land team, the length of time they have worked with contaminated land and the range of tasks they are involved in on a day-to-day basis. Here, all results are reported and the findings of a subset of results relating specifically to the use of bioaccessibilty and constraints associated with the implementation of bioaccessibility are discussed. The Officers' perspective on the use of bioaccessibility (Question 5) was rated on a 5-point Likert scale (strongly agree, agree, neither agree nor disagree, disagree, strongly disagree) with an additional 'don't know' option. Question relating to constraints upon barriers to the implementation of bioaccessibility and a question relating to bioavailability and bioaccessibility definitions allowed respondents to choose more than one answer: the sum of the results for these questions therefore exceeds 100%. Each question was accompanied by a free text space for respondents to make additional comments. All comments are attached in the Appendix B. The names of the

Local Authorities or any facts that might have led to their identification were removed from the text.

In order to complement the data generated by the questionnaire-based survey and aid the interpretation of the results, the questionnaire was triangulated with semi-structured personal and telephone interviews (after Arksey and Knight, 2007). 17 interviews were conducted in February and March 2009 with survey respondents who had indicated that they were willing to be approached for more information. The interviews lasted from 25 to 45 minutes of conversation. Each interview focused on 3 key questions regarding 1) the use of bioaccessibility in the area of the Local Authority, 2) guidance on bioaccessibility and uncertainties associated with bioaccessibility, and 3) need for bioaccessibility data. These questions were further explored by using additional probe questions to achieve greater elaboration and clarifications of the answers given.

SPSS 16.0 for Windows was used for statistical processing of the questionnaire data. Descriptive results are presented as percentage of responses to each question (not necessarily of total number of participants). Results were considered significant at the 95% confidence level ($p < 0.05$). ArcGIS was used to analyze the spatial distribution of the questionnaire responses and to classify the English Local Authorities according to the Department for Environment, Food and Rural Affairs' rural/urban classification scheme (Defra, 2008). To honour assurances of confidentiality given to respondents the spatial analysis is not presented here and the generic results have been used only to support the interpretation of the survey findings.

3. Results and discussion

The use of bioaccessibility

Question - Please indicate to what extent you agree with each of the following statements relating to contaminated land. Rate on a scale of 1-5 (1 strongly agree, 2 agree, 3 neither agree nor disagree, 4 disagree, 5 strongly disagree)

Table 1. Collation of Officers' views on bioaccessibility.

	1 – strongly agree	**2**	**3**	**4**	**5 – strongly disagree**	**Don't know**	**Response count**
Bioavailability/bioaccessibility testing is a useful tool that facilitates contaminated land management	24.2% (30)	**46.0% (57)**	21.8% (27)	1.6% (2)	0.8% (1)	5.6% (7)	124
Total contaminant concentration is a better guide for decision-making than bioavailability/bioaccessibility data	4.0% (5)	15.3% (19)	**43.5% (54)**	21.8% (27)	7.3% (9)	8.1% (10)	124
Information on benzo(a)pyrene bioavailability/bioaccessibility is needed to support our decision-making	23.4% (29)	**41.9% (52)**	19.4% (24)	7.3% (9)	0.8% (1)	7.3% (9)	124
Information on PAHs bioavailability/bioaccessibility to microorganisms can determine suitability of bioremediation	15.4% (19)	**38.2% (47)**	22.0% (27)	0.8% (1)	0.8% (1)	22.8% (28)	123
Use of contaminant bioavailability/bioaccessibility data leads to more cost-effective site management	15.4% (19)	**38.2% (47)**	30.9% (38)	1.6% (2)	0.8% (1)	13.0% (16)	123
We would need more information on bioavailability/bioaccessibility before deciding if it could help us within risk assessments	26.4% (33)	**50.4% (63)**	13.6% (17)	8.8% (11)	0.0% (0)	0.8% (1)	125

The majority of respondents (70.2%) either strongly agreed or agreed that 'bioavailability/bioaccessibility testing is a useful tool that facilitates contaminated land management' (Table 1). Only 2.4% (corresponding to 3 respondents) either disagreed or strongly disagreed with the statement. The rest of the respondents neither agreed nor disagreed or indicated 'don't know' option (corresponding to 21.8% and 5.6%, respectively). It is noteworthy that all respondents that selected the latter options also neither agreed nor disagreed or selected the 'don't know' option with respect to other questions within the questionnaire.

In many areas throughout England and Wales there are naturally occurring contaminants at levels exceeding guideline values. It is virtually impossible to remediate an entire Local Authority's jurisdiction area and/or preclude development on the basis of natural abundance of contaminants. The interviews found that bioaccessibility testing has therefore been perceived as a pragmatic, *ad hoc* decision-support tool when dealing, in particular, with naturally occurring contaminants. It was also indicated by 8 out of the 17 interviewees that bioaccessibility data was being applied to so-called 'grey-zone' concentrations where the exceedance of the guideline value is not substantial. It has been recognized that measuring bioaccessibility is impractical where the exceedance of the guidelines value is significant, whereby the use of bioaccessibility data would not affect the final designation of the land as contaminated.

The majority (79.4%) of respondents that selected 'neither agree nor disagree' and 'don't know' option in answer to the question about the usefulness of bioaccessibility also strongly agreed or agreed that more information on bioavailability and bioaccessibility was needed. Participants more familiar with bioaccessibility commented that whilst greater access to information is needed generally, from their perspective as Local Authority regulators new research is critical only for some contaminants, such as PAHs (preferably in mixtures).

It has been also previously demonstrated that bioaccessibility data can lead to more cost-effective land management (Nathanail and Smith, 2007). Respondents who considered bioaccessibility to be a useful tool were also more likely to view it as leading to more cost-effective management of contaminated land (Figure 1).

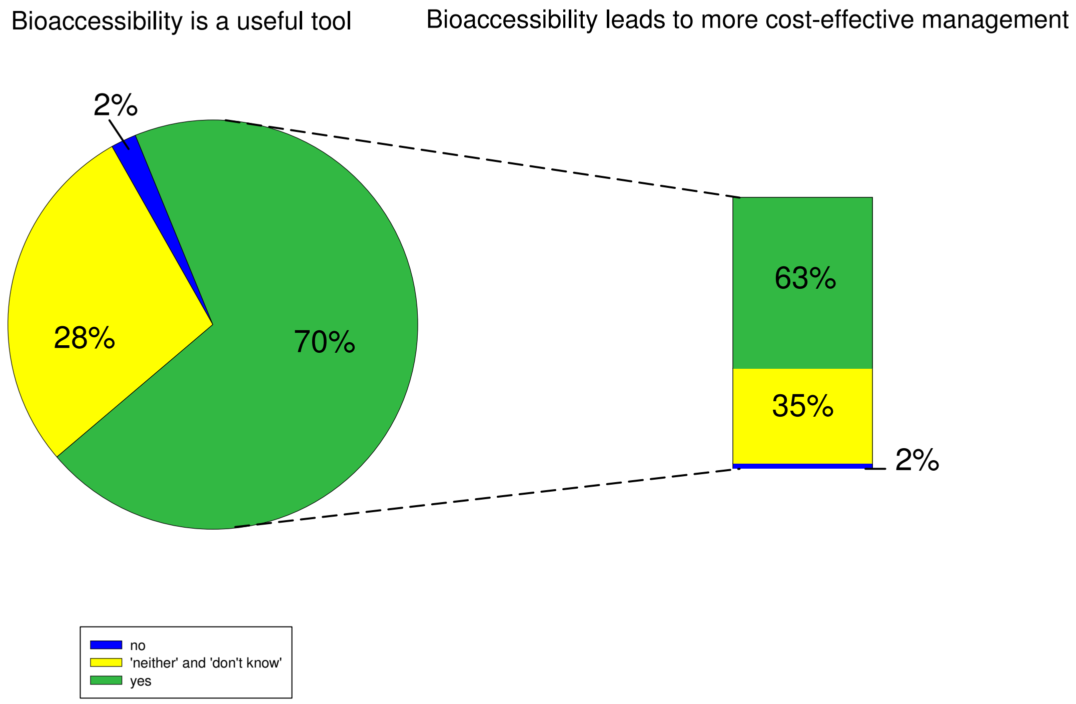

Figure 1. Percent of respondents indicating usefulness of bioaccessibility vs. cost-effectiveness of bioaccessibility.

Information on contaminant bioaccessibility brings additional advantages when considering large, open-space areas where a significant amount of soil would have to be removed and/or remediated. Indeed, it was confirmed during the interviews with Officers from urban areas that bioaccessibility data might not be beneficial to smaller contaminated sites (such as gardens attached to houses), where the cost of bioaccessibility testing might surpass the cost of breaking the significant pollutant linkage (e.g. by capping). Some Officers in areas where naturally elevated contaminants have been identified acknowledged their regular use of bioaccessibility data. They perceived the use of bioaccessibility criteria as the only option to prevent remediation of entire Local Authority areas and to allow development.

Seven interviewees pointed to a prevailing concern among some Officers about overestimation of risks when applying current guideline values. The following comment, taken from one of the questionnaire, illustrates a view that was recurrently expressed:

> 'I am firmly of the opinion that our perception of risk is far greater than the actual or true risk posed in many contamination assessments. It is recognized that non consideration of what is and what is not both bioavailable and accessible can lead to gross overestimation. This can result in a number of negative impacts both financially and in terms of human health risk assessment. PAH's B(a)P and Arsenic are the immediate areas most often quoted and referenced in the debate' (Questionnaire, 2008)

From the interviews it also appeared that bioaccessibility could be used not only with respect to (mostly naturally occurring) arsenic and heavy metals but also to other contaminants, both from natural and anthropogenic sources. For example, 65.3% of respondents either strongly agreed or agreed that information on bioaccessibility of benzo(a)yrene (B(a)P), an organic contaminant that belongs to PAHs, is needed. It was elucidated during the interviews that organic contaminants have been perceived as under-researched (see also Rivett et al., 2002) and there was a held view among respondents that it is impractical to remediate the land where contamination would inevitably re-appear on account of elevated ambient concentrations from anthropogenic sources. Indeed, ubiquity of organic contaminants, as a consequence of pervasive and trans-boundary contamination from combustion of organic materials from both anthropogenic sources (fossil fuels) and natural processes (e.g. forest fires), has been previously demonstrated (Bamforth and Singleton, 2005).

> 'There is no soil guideline value for PAHs/BaP in the UK. We have a TOX report from which the derived SGV is is around 1mg/kg for BaP, this is pretty much the same as the CIEH/LQM GAC. These values are usually well below the background concentrations in urban areas (...) The GAC is a considered to be the highest 'safe' value, what we do not have is knowledge of what value might cause 'harm' of 'significant possibility of harm'. Also lacking is data on land uses other than residential gardens and allotments e.g public open space.' (Questionnaire, 2008)

Views on bioaccessibility did not appear to be influenced by the type of area covered by the Authority: statistical analysis of responses to the survey questions found no significant difference between Officers in rural and in urban Authorities in England (Table 2). Similarly, cross-tabulation against other variables such as number of persons in contaminated land team, years of experience with contaminated land or the tasks Officers were involved in revealed no statistically significant differences ($p > 0.05$). It was therefore inferred that complex site-by-site circumstances, due, for instance, to naturally occurring elevated levels of contaminants or increased ambient anthropogenic concentrations and hence the necessity of a subsidiary decision-making tool, are the most likely factors driving the extent of familiarity with and acceptance of bioaccessibility.

Table 2. Percent of responses from rural and urban areas cross-tabulated with the statement relating to the usefulness of bioaccessibility and the information on benzo(a)pyrene. P-value established on the basis of Pearson Chi-Square test.

Bioaccessibility is a useful tool	Rural	Urban	p-value
Strongly agree	23.7	27.1	0.82
Agree	44.1	52.1	
Neither agree nor disagree	23.7	12.5	
Disagree	1.7	2.1	
Strongly disagree	0	2.1	
Don’t know	6.8	4.2	
Information on benzo(a)pyrene needed			0.14
Strongly agree	25.4	25	
Agree	45.8	39.6	
Neither agree nor disagree	13.5	22.9	
Disagree	8.5	4.2	
Strongly disagree	0	2.1	
Don’t know	6.7	6.2	

Bioaccessibility and bioremediation

The bioaccessibility concept has been recognized not only from a human health perspective but also in terms of ecological risk assessment (Ollson et al., 2009) and in the context of applicability of bioremediation techniques (Diplock et al., 2009). Bioremediation is believed to be the most sustainable of all remediation approaches (van Dillewijn et al, 2009), whereas the ability of microorganisms to successfully biodegrade PAHs (especially low molecular weight PAHs) is well documented (Cerniglia, 1992; Bamforth and Singleton, 2005). It is noteworthy that Local Authorities are primarily involved in human health risk assessments. 53.6% of the respondents strongly agreed or agreed that 'Information on PAHs bioavailability/bioaccessibility to microorganisms can determine suitability of remediation' (Table 1). Also, 50.7% of the respondents indicated that bioremediation has been used in their area while 44.4% indicated that it has 'never' been used (Table 3). Of the respondents reporting the use of bioremediation in their areas, 40.8% reported it has been 'rarely' used (< 10% of sites under remediation), 8.5% answered it has been used 'sometimes' (10-30% of remediated sites), whilst only 1.4% has applied it 'often' (i.e. to more than 30% of sites under remediation).

Table 3. How often has bioremediation been used to clean-up PAH-contaminated land in your area?

	never	rarely (<10% of sites under remediation)	sometimes (10-30% of sites under remediation)	often (>30% of sites under remediation)	don't know	Response Count
frequency	**44.4% (63)**	40.8% (58)	8.5% (12)	1.4% (2)	4.9% (7)	142

Common opinion among participants was that bioremediation can lead to a more cost-effective and more sustainable remediation (avoiding the 'dig and dump' approach). Yet, time and space constraints, uncertainty associated with residual levels and/or an inability to reduce contamination to the guideline value levels were the main factors perceived as limitations to the adoption of bioremediation. It was also suggested that the current financial crisis may slow down development and encourage developers to undertake more cost-effective albeit less time-efficient bioremediation.

What are the limitations to the implementation of bioaccessibility data?

Question - Which, if any, of the following factors hamper the application of bioavailability/bioaccessibility data in your area? (Please choose as many as relevant)

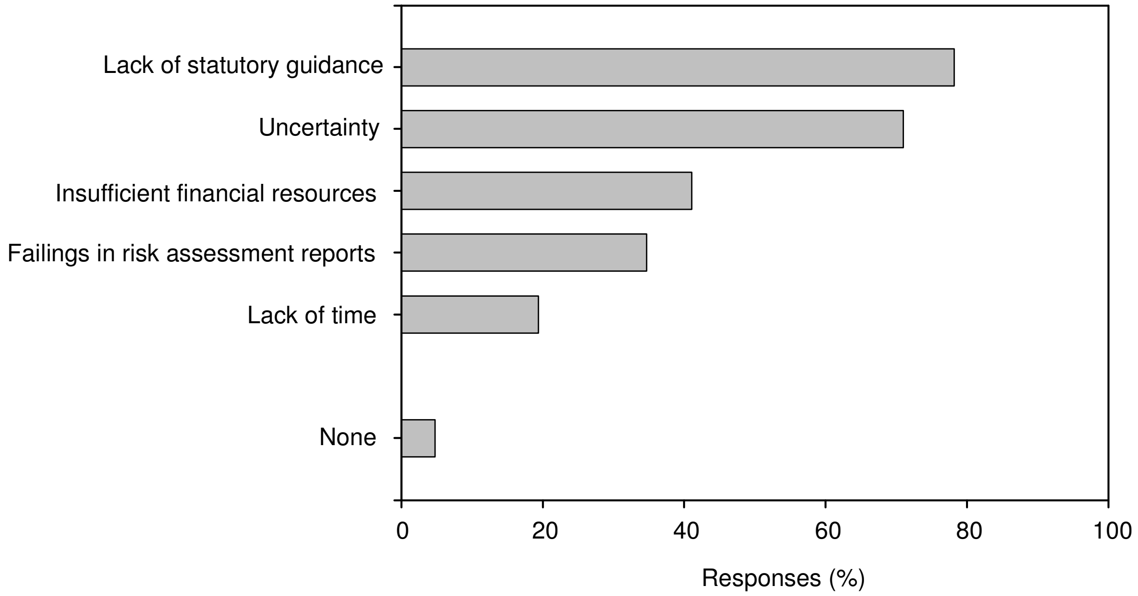

Figure 2. Factors hampering the use of bioaccessibility data. Bars represent percent of responses to each option.

In a survey of English and Welsh Local Authorities carried out in early 2005 by the Environment Agency the principal reason given by survey participants for not accepting bioaccessibility assessments was that there was no guidance published by the Environment Agency on the use of bioaccessibility (EA, 2006). In our survey, lack of statutory guidance was indicated as the main reason hampering the use of bioaccessibility (78% of participants; Figure 2). Officers who were interviewed emphasized that not necessarily statutory but that guidance from an authoritative body, such as the Environment Agency, is needed. Recurring opinion of the Officers within the survey was 'conservatism of contaminated land assessment criteria' regardless of substantial research in the field of bioaccessibility, both on national and international level; an observation congruent with some authors (BGS, 2007).

Other reasons indicated as limitations to bioaccessibility application that emerged from the questionnaire included: uncertainty associated with bioavailability/bioaccessibility data (71%), insufficient financial resources available to carry out testing (41.1%), failings in risk assessment reports (34.7%) and a lack of time to analyse the data (19.4%). It was also clear from the interviews that there was an implicit expectation that the guidance would dispel the uncertainties by establishing a standard methodology.

It was further apparent from the interviews that respondents who were more comfortable with using bioaccessibility data adopted strategies for dealing with the uncertainties. These included: incorporating a ‘worst-case’ scenario (the largest bioaccessibility value); carrying out extra sampling to verify whether data variability was due to heterogeneity of the site or the performance of the method; incorporating information about method robustness from an authoritative source such as the British Geological Survey (BGS); recommendation of a single test throughout all sites (e.g. Physiologically Based Extraction Test for arsenic) to assure consistency across the jurisdiction area; considering trends in data; and looking at vegetable uptake and; considering the use of the site.

Bioavailability and bioaccessibility definitions

Question - Do you make any distinction between the terms bioavailability and bioaccessibility? (Please choose as many as relevant)

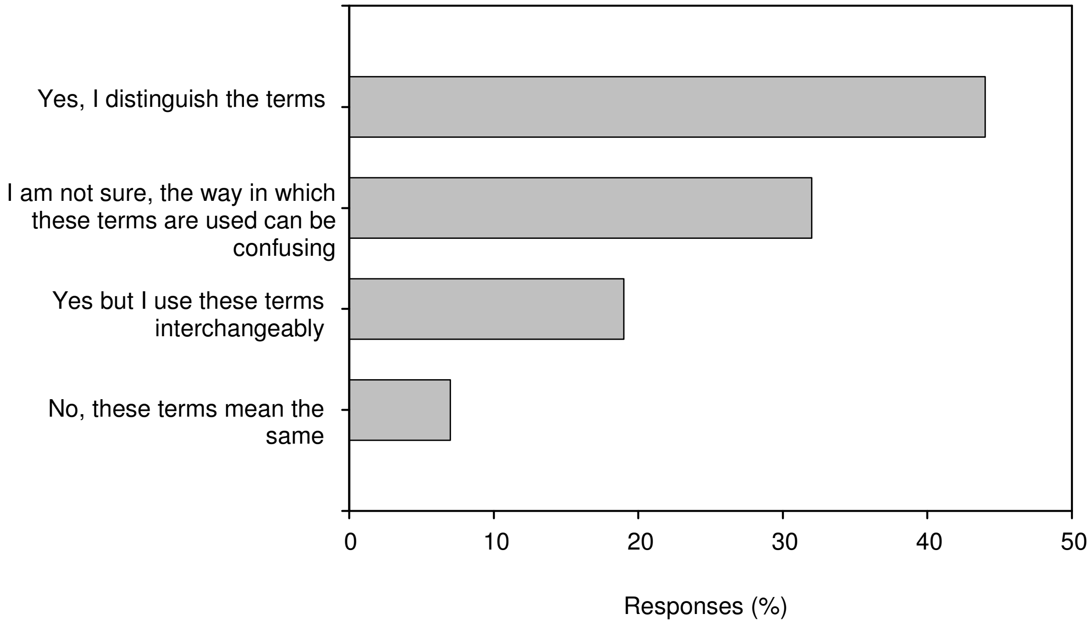

Figure 3. Percent of respondents relating to bioavailability and bioaccessibility terminology.

It is noteworthy, that respondents who were more confused by the terminology of bioavailability and bioaccessibility were as concerned with the lack of guidance as with uncertainties. Respondents who indicated confusion with terminology indicated the lack of statutory guidance (71.7%) to be as important a limitation as uncertainty associated with bioaccessibility data (69.6%). Respondents who were familiar with terminological differences indicated the lack of statutory guidance (81.1%) to be the principal limitation, although not necessarily prevention, to the use of bioaccessibility.

PAH-contaminated land

Question - Please estimate how many sites are officially designated as contaminated in your area.

Question - Of these sites, what proportion is contaminated with polycyclic aromatic hydrocarbons (PAHs; e.g. benzo(a)pyrene)?

These questions were designed to quantify sites contaminated with PAH within England and Wales. Due to inconsistencies within data these questions are not interpreted within this report.

Question - Which guidelines would you accept within risk assessments of sites contaminated with PAHs? (Please choose as many as relevant)

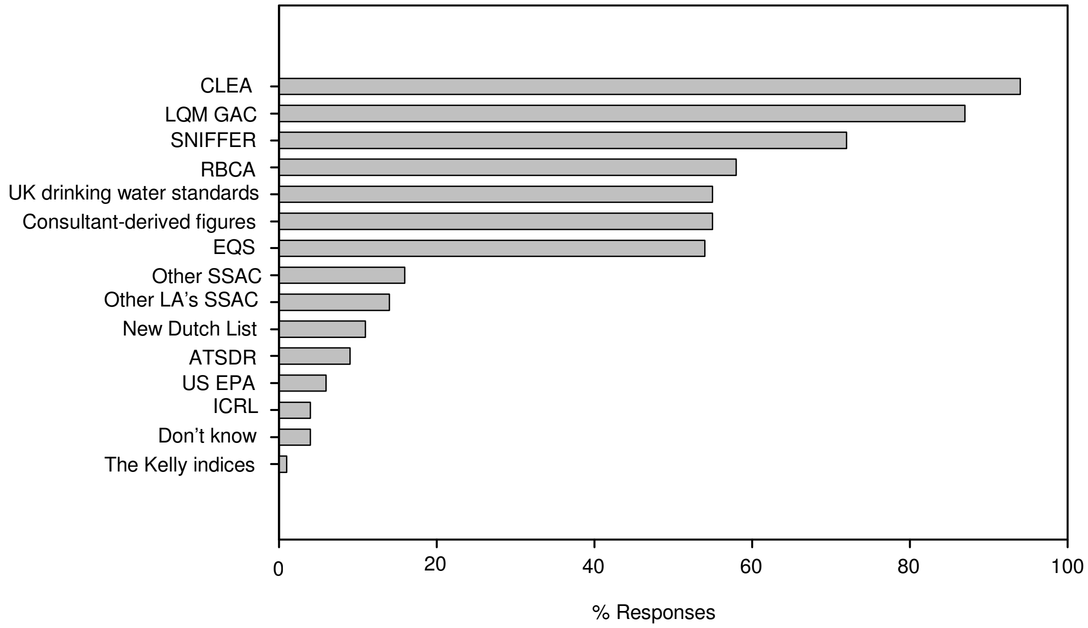

Figure 4. Guidelines used for PAH-contaminated land decision-making.

Most common comments on the use of the guidelines within risk assessments of sites contaminated with PAHs included:

‘Site specific criteria will be accepted where derived from robust, justifiable and relevant risk assessment models which have been adapted to be in line with UK policy.’

‘There is no clear guidance at present on what values to use. LAs are at present basically in a position where we been asked to calculate values for each site with little guidance from DEFRA. This lack of guidance also has implications for our PPS23 work.’

‘I have accepted Site Specific Assessment Criteria for PAH based on Toxicity Equivalent factors as this seems a logical approach. I would also accept generic values derived by large consultancies such as Hydrock, Hyder, Atkins, WSP etc so long as the figures were derived using accepted models (e.g. CLEA, SNIFFER), and do not differ too greatly from those derived by LQM GAC or other transparent approaches. Consultancies seem to favour SNIFFER, though RBCA is increasingly popular for commercial sites (…)’

4. Conclusions and recommendations

> ‘I think bioaccessibility is useful but there is no certainty of its use in this field [contaminated land] and lack of guidance and information to either support or disagree with using bioaccessibility. If we use it there is no government body to back us up and support us and if we don’t use it, what else do we use?’ (Questionnaire, 2008)

As corroborated by the interviews, the above statement epitomises the opinion of a number of decision-makers throughout England and Wales. Indeed, only 4.8% of the respondents indicated that they use bioaccessibility data confidently (Figure 2). Given the ubiquity of natural contamination and prevalent elevated ambient concentrations of anthropogenic contaminants there is an uncontested need to equip decision-makers with appropriate management tools. At the moment, for problematic site-specific risk assessments, bioaccessibility data is perceived as the way forward.

As bioaccessibility is already in use there is, firstly, an urgent need for straightforward, void of scientific jargon guidance to standardise the application of bioaccessibility data throughout the country and to uniform criteria for development. Secondly, more information, training and availability of successful case studies, together with greater access to research articles are needed to instil confidence in the use of bioaccessibility data. Indeed, 76.8% of the respondents either strongly agreed or agreed that more information was needed. Whilst there is a need for a standard test for arsenic and metals quoted as extensively investigated, there is also a need for a framework for PAH risk assessment and more research on PAH bioaccessibility. Finally, bringing the knowledge and opinion of Local Authorities into policy-making and into academia, whilst making academic research more available to decision- and policy-makers, can trigger more relevant academic research on the one side, and facilitate more appropriate decision-making on the other.

The concepts underpinning the use of bioaccessibility constitute a contaminant behaviour paradigm and it is now widely recognized that the risk from contaminants in the soil arises not from the mere presence of the contaminant of concern but from the ‘significant possibility of significant harm’ that the contaminant poses to environmental receptors. Bioaccessibility embedded within a risk-based approach that provides flexible frameworks and intrinsically stimulates scientific advances to improve accuracy of risk assessments can create strong epistemic and pragmatic circumstances for driving progress in contaminated land risk assessment. For a risk-based system to operate, policy-maker, risk assessor and researcher must however effectively communicate.

Statutory requirements for the management of contaminated land incorporate qualitative caveats regarding the stringency with which authorities should apply them, stipulating that their application should not entail excessive cost or conflict with ‘overriding public interests’ (Pollard et al., 2004). In this context bioaccessibility offers a decision-support tool. Evaluation of bioaccessibility is however not meant to replace other approaches but to assist decision-makers in situations where alternative solutions are limited.

Uncertainties associated with bioaccessibility along with a lack of robust *in vivo* bioavailability data have been reported as the main limitations to bioaccessibility acceptance by policy-makers (EA, 2002; Interviews, 2009). As uncertainties suggest the need for more research on the one hand and incorporation of already published research into policy-consideration on the other, the latter limitation could appear to be a 'dead-end' statement given its immanent contradiction with respect to animal testing policy (BGS, 2007).

Decision-makers managing land contamination face an array of complex issues and pressures associated with public and financial liability, with the management of different regulatory interfaces, with interpretation of sophisticated analytical data and risk assessment reports, with evaluation of the relative capabilities of remediation technologies and with the maintenance of public confidence in remediation projects. In the face of these challenges and of economic and environmental pressures, it has been indicated by survey participants that consideration of bioaccessibility can aid decision-making, refine risk assessments and may facilitate sustainable land management.

However, this study indicates that unless a greater commitment is made with respect to developing a standardised perspective on bioaccessibility and securing it within a framework from an authoritative source, the confidence of local regulators in the use of this tool will be undermined and progress in integrating it into contaminated land decision-making will continue to be hampered.

Summary

Most significant findings of this survey include:

- Bioaccessibility is a useful tool that facilitates contaminated land management. 70.2% of the questionnaire participants either strongly agreed or agreed with this statement.
- 65.3% of respondents expressed the need for information on benzo(a)pyrene bioavailability/bioaccessibility to support decision-making on contaminated land.
- Lack of statutory guidance on the use of bioaccessibility was indicated as the main factor hampering the use of bioaccessibility. This was indicated by 78.2% of participants.
- 15 out of 17 interviewees were ‘ready to accept bioaccessibility’, which corroborated findings of the questionnaire.
- There is an outspoken need to equip regulators with decision-support tools especially in the context of elevated natural and ambient contaminants concentrations.

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