CL:AIRE

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CL:AIRE research bulletins describe specific, practical aspects of research which have direct application to the characterisation, monitoring or remediation of contaminated soil or groundwater. This bulletin describes weathered hydrocarbon transformation and its implications for bioremediation, analysis and risk assessment (outputs from LINK Bioremediation Project - BIOREM35).

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Bioremediation of heavy hydrocarbons - reducing uncertainty in meeting risk-based targets: laboratory to field scale (PROMISE Project)

1. INTRODUCTION

Globally, petroleum hydrocarbons are a widely utilised resource, but their use has resulted in contamination through accidental spillage and leakage (Pollard et al., 1994). Certain components of petroleum contamination may pose risks to human health, property, watercourses, ecosystems, and other environmental receptors (Nathanail et al., 2007). There are a plethora of approaches to, and techniques available for, the remediation of contaminated land (Biowise, 2000; Whittaker et al., 1995; Wood, 1997). One's choice of approach depends on a number of environmental, economic and human health considerations among others (Environment Agency, 2005). In the context of this research, biopiling and windrow turning of impacted soil are important engineered systems that allow *ex situ* but often on-site remediation of hydrocarbon impacted soils (Figure 1).

Figure 1: Biopile treatment unit of the field trial carried out during PROMISE project (©Remedios Ltd).

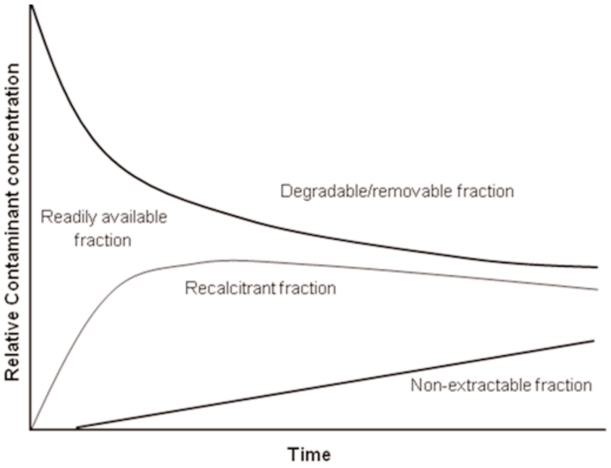

Figure 2: Bioavailability changes of petroleum hydrocarbons over time (extracted from Semple et al., 2003).

While on-site/*ex situ* remediation is recognised as a cost effective technology and its application is increasing, its performance continues to be represented by reference to reductions in the hydrocarbon 'load' in soils being treated, rather than reductions in the risks posed by hydrocarbon contamination. Risk assessment is a well-established and important tool for environmental management decisions, which is commonly used in petroleum hydrocarbon contaminated land management (Brassington et al., 2007). It is employed as a means of assessing and managing potential impacts to human and ecosystem health (Vegter et al., 2002). Several risk-based frameworks for petroleum hydrocarbons in soil have been published under the auspices of the American Society for Testing and Materials (ASTM, 1994), Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG, 1999), the Canadian Council of Ministers of the Environment (CCME, 2000), the American Petroleum Institute (API, 2001), the Massachusetts Department of Environmental Protection (MaDEP, 2002) and the Environment Agency of England and Wales (Environment Agency, 2005), each reflecting national legislation, a range of expert judgements and socioeconomic issues (Wang et al., 1998; Vik et al., 2001). Typically these frameworks adopt a three tiered approach with increasingly sophisticated levels of data collection and analysis, as an assessor moves through the tiers. However, these frameworks are not always supported by suitable and robust analytical protocols, especially in the case of matrices contaminated with weathered hydrocarbons. The composition of petroleum hydrocarbon products can vary substantially depending on the nature, composition, and degree of processing of the source material. Once released to the environment, petroleum products are subject to physical, chemical and biological processes (weathering) that further change their composition, toxicity, availability and distribution (partitioning) within the environment (Figure 2).

The extent of weathering experienced is particularly important when characterising petroleum contamination prior to remediation and land management assessment. Also, a comparison of reference analytical methods used for petroleum risk assessment protocols (Brassington et al., 2007) highlights the need for practical and simple extraction procedures that allow a better characterisation of both aliphatic and aromatic hydrocarbon fractions within oil-contaminated samples, including soil and sediment samples with high moisture levels. In a UK context, the development of novel methods should also allow the identification of risk-indicator compounds within each hydrocarbon fraction, and the monitoring of recalcitrant biomarkers to enable verification of treatment success. Historically, inadequate characterisation and verification has contributed to a lack of investor and, on occasion, regulatory confidence in technology, creating a barrier to securing an established market (CIEF, 2002). Specifically, the Urban Task Force (1999) considered UK brownfield investment to be held back by low confidence in remedial technology, inconsistent technical advice and the absence of publicised successes.

A criticism of risk assessment may be that it makes use of modelled data and as such could be inherently conservative in adoption. To address this, in addition to risk assessment procedures, the team developed and applied a suite of ecotoxicity assays to assess hazard and the changes in hazard associated with treatment. While these may be more reflective of ecological assessment, their sensitivity and rapid response make them suitable for monitoring changes associated with the treatments applied (Figure 3).

This research project, LINK Bioremediation BIOREM35 and CL:AIRE Research Project RP18 offered an opportunity to integrate biological, chemical and ecotoxicological diagnostics, hazard and risk assessment and the transfer of laboratory findings to the field scale (Figure 3). Most importantly the steps enhanced confidence and certainty on the adoption of techniques to meet risk derived criteria.

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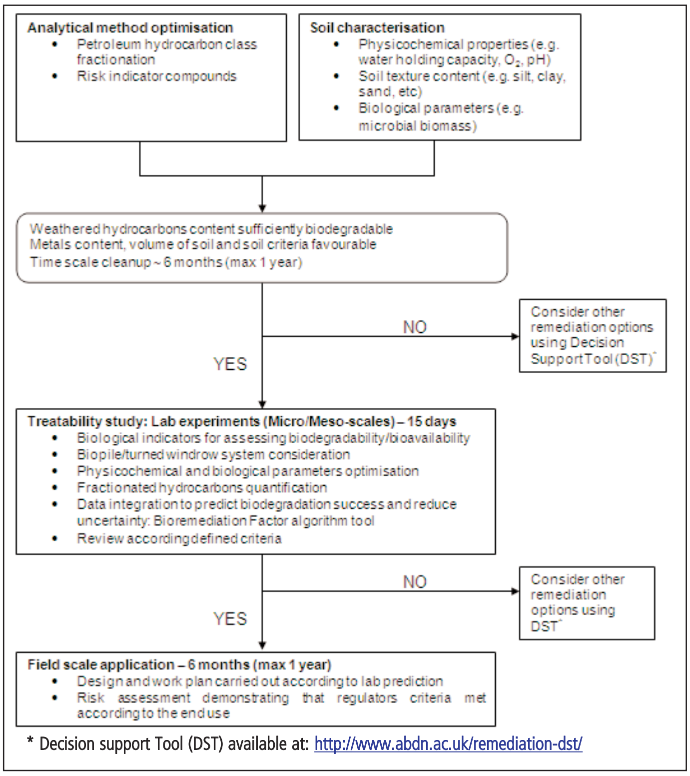


* Decision support Tool (DST) available at: http://www.abdn.ac.uk/remediation-dst/

Figure 3: On-site/*ex situ* remediation criteria decision tree. Note that all parameters in the decision tree boxes should be 'yes', either before or after optimisation. If not, other options should be considered.

2. DEVELOPMENT OF A NOVEL AND BESPOKE TECHNIQUE FOR QUANTIFICATION OF FRACTIONATED HYDROCARBONS AND USE IN A RISK-BASED CONTEXT

There is a need to have a practical, simple and widely adopted procedure to characterise and quantify aliphatic and aromatic hydrocarbon fractions in oil-contaminated samples (Brassington et al., 2007; Environment Agency, 2005). This must be effective across all textures of soil under wide moisture ranges. In a UK context, the development of methods should also allow the identification of risk-indicator compounds within each hydrocarbon fraction, and the monitoring of recalcitrant biomarkers to enable verification of treatment success.

Concerns exist over the performance of the current reference methods used, specifically in terms of poor extraction efficiencies and analytical losses imparted by sample handling. The alteration of chemical composition with time may also affect the accuracy of final measurements and lead to misrepresentations of human health risk. In this respect, the impact of calibration on final measurement needs to be evaluated for a range of weathered products.

There has been no specification or adoption of recommended analytical procedures for petroleum hydrocarbons and individual laboratories often have their own in-house methods. Furthermore, the framework itself notes that currently adopted methods for petroleum hydrocarbon analysis may not be suitable for the heavier compounds or weathered hydrocarbon (Environment Agency, 2005). These observations further highlight the need to develop a suitable and robust analytical procedure to inform risk assessment.

Soxhlet extraction is a widely used, benchmarked, exhaustive and easily standardised technique for the extraction of petroleum hydrocarbon contaminated soils (Shu et al., 2003). Disadvantages, including lengthy extraction times, degradation of thermally labile compounds, use of large volumes of organic solvents and the need to concentrate samples have resulted in the investigation into alternative exhaustive and robust methods (Whittaker et al., 1995; Hawthorne et al., 2000; Hollender et al., 2003). Ultrasonic extraction has been investigated elsewhere and has potential for wider use in this area of analysis (Banjoo et al., 2005). Ultrasonication is a quick, easy and cost-effective method that is now widely used in environmental analysis. However, analytical procedures using ultrasonication vary not only in the method used (e.g. type and volume of solvents, cycle duration etc.), but also in the type of ultrasonic apparatus used (sonic probe or ultrasonic bath). Some of the more detailed investigations have shown that ultrasonic methods have the potential to produce equivalent or higher efficiencies than currently used methods such as Soxhlet (Banjoo et al., 2005). Conversely, other investigations have shown the opposite to this with lower efficiencies compared to alternative methods (Song et al., 2002). It was concluded that if sonication is to be used in place of traditional methods, it needs to be clearly defined and optimised.

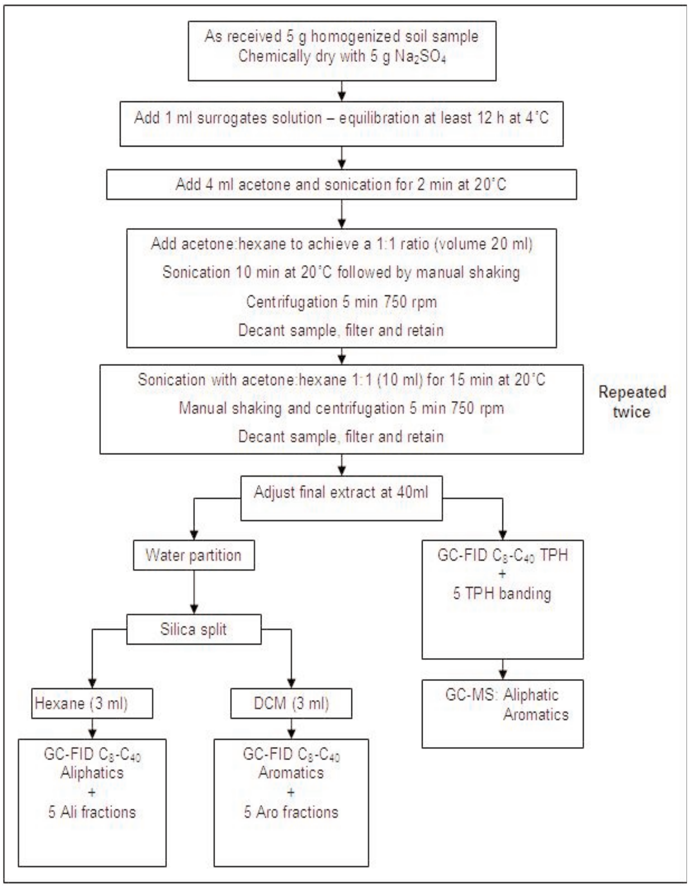


Figure 4: Overview of the analytical process recommended for analysing soil contaminated with weathered hydrocarbons (reproduced from Risdon et al., 2008). GC-FID: gas chromatography coupled to Flame Ionisation Detector; GC-MS: gas chromatography coupled to Mass Spectrometer.

Here a novel solvent ultrasonic extraction procedure for soils contaminated with weathered hydrocarbons is presented, allowing petroleum hydrocarbon class fractionation and identification of risk-indicator compounds (Figure 4). This method has been developed by and through the company, TES Bretby and is a competitive market technique.

A sequential ultrasonic solvent extraction method has been evaluated using four different soil matrices i) silty soil, ii) clay soil, iii) sandy soil and iv) a granular matrix comprising ash, brick and concrete fragments selected to represent the surface soils found at many industrial sites (made ground). Each soil matrix was spiked with a mixture of diesel and lubricating oil at levels corresponding to 20% (10,000 mg/kg) and 80% (40,000 mg/kg) of the concentration range typical to environmental soil samples (API, 2001; Sarkar et al., 2005). In order to evaluate the recovery from the extraction method, dried samples were spiked with a surrogate solution containing o-terphenyl (oTP), squalane (Sq), heptamethylnonane (HMN) and 2-fluorobiphenyl (2-Fb).

Analyses of kerosene range organic compounds (KRO), diesel range organic compounds (DRO), mineral oil range organic compounds (MRO) and Texas risk carbon banding fractions show relative standard deviation (RSD) values ranging between 2% and 13% with the highest overall degree of variability occurring when analysing the lightest fractions including DRO C_{10}-C_{24} ranges and TEXAS1-TPH C_8-C_{10} ranges (Table 1). With the exception of the made ground soil, a higher degree of precision was typically observed when extracting higher concentrations of hydrocarbons. It is only when analysing the aromatic fraction (Table 2) that RSD values rise above 20%. The greatest degree of variability was observed when extracting low concentrations of the C_8-C_{10} range for all of the soils tested, possibly due to volatile losses or thermal decomposition of compounds.

The method meets Environment Agency mCerts performance targets (30% bias, 15% precision) and is compatible with the existing UK risk framework. The method also provides practical assistance to the contaminated land sector and is currently included in the draft being developed by the Standing Committee of Analysts on the determination of hydrocarbon compounds in soils and associated materials (SCA, 2009).

The method is faster than a typical Soxhlet method, allowing 24 (or more depending upon size of centrifuge and sonic bath) samples to be extracted in ca. 70 minutes. If no clean-up/fractionation step is required, extracts can be directly analysed by gas

Table 1: Texas Risk, DRO, KRO and MRO bandings, mean concentration extracted, precision and bias for soils spiked with 20% (10,000 mg/kg) and 80% (40,000 mg/kg) of typical hydrocarbon concentration found in environmental samples (adapted from Risdon et al., 2008).

		Silty soil		Clay soil		Sandy soil		Made ground	
Carbon Band	Spike Level	Mean mg/kg	Precision mg/kg (%RSD)	Mean mg/kg	Precision mg/kg (%RSD)	Mean mg/kg	Precision mg/kg (%RSD)	Mean mg/kg	Precision mg/kg (%RSD)
Texas TPH C_8-C_{10}	20%	224	12(5)	234	30(13)	231	28(12)	228	23(10)
	80%	669	23(3)	673	50(7)	655	31(5)	642	36(6)
Texas TPH C_{10}-C_{12}	20%	531	23(4)	550	33(6)	536	25(5)	556	21(4)
	80%	1593	51(3)	1697	108(6)	1633	41(2)	1690	109(6)
Texas TPH C_{12}-C_{16}	20%	2317	111(5)	2383	163(7)	2328	100(4)	2502	114(5)
	80%	7173	282(4)	7615	529(7)	7340	161(2)	7832	517(7)
Texas TPH C_{16}-C_{21}	20%	2363	61(2)	2523	178(7)	2445	76(3)	2653	83(3)
	80%	6783	254(4)	7352	534(7)	6919	173(2)	7531	318(4)
Texas TPH C_{21}-C_{35}	20%	4304	204(5)	4173	337(8)	3880	170(4)	4471	126(3)
	80%	12143	354(3)	12100	799(7)	11461	454(4)	12678	412(3)
DRO C_{10}-C_{24}	20%	8401	1095(13)	6574	545(8)	6468	318(5)	7052	228(3)
	80%	18957	642(3)	19801	1528(8)	19135	317(2)	20677	1006(5)
KRO C_8-C_{14}	20%	1663	65(4)	1641	148(9)	1639	75(5)	1724	66(4)
	80%	4963	180(4)	4986	510(10)	5005	171(3)	5201	375(7)
MRO C_{22}-C_{34}	20%	3933	159(4)	3708	328(9)	3527	138(4)	4123	120(3)
	80%	11415	331(3)	10862	776(7)	10451	545(5)	11513	425(4)

RSD: Relative Standard Deviation

Table 2: Aliphatic and aromatic fractions, mean concentration extracted, precision and bias for TPH, DRO, KRO, MRO and Texas risk bandings (adapted from Risdon et al., 2008).

	Aliphatic hydrocarbons		Aromatic hydrocarbons	
Carbon Band	Mean mg/kg	Precision mg/kg (%RSD)	Mean mg/kg	Precision mg/kg (%RSD)
TPH	160	24(18)	166	27(16)
DRO	83	13(15)	84	14(16)
KRO	31	5(15)	12	2(14)
MRO	77	13(17)	89	15(17)
$>C_8$-$>C_{10}$*	10	2(16)	-	-
$>C_{10}$-$>C_{12}$	10	2(15)	8	2(22)
$>C_{12}$-$>C_{16}$	21	3(16)	29	6(20)
$>C_{16}$-$>C_{21}$	22	4(18)	33	5(16)
$>C_{21}$-$>C_{35}$	77	14(18)	94	16(17)

*PAH mix used to spike the soil contained no aromatic PAH $>C_8$-C_{10} fraction

chromatography coupled to mass spectrometry (GC-MS) without further evaporation. Soxhlet methods can take between 2-24 hours (depending on the protocol), often produce relatively dirty samples requiring sample clean up, and often require sample evaporation. Soxhlet also requires more glassware that is fragile and comparatively expensive to that used in sonication. Soxhlet extraction protocols can require up to 250 ml of extracting solvent, whereas the sonication method described here uses 40 ml. Lower solvent consumption per sample not only reduces costs but reduces the environmental impact of their subsequent disposal.

The ultrasonic solvent extraction method described here is an improvement on conventional methods, as it saves time and lowers solvent consumption. Further, there are no evaporation steps preventing the potential loss of front end hydrocarbon bands and the use of water partitioning facilitates an effective solvent exchange prior to fractionation. The method involves fewer handling steps and disposable apparatus eliminates potential cross contamination. Importantly, it is easy and simple to reproduce.

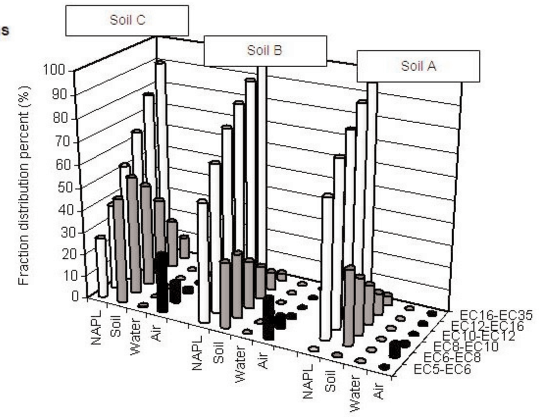
3. RELATING EMPIRICAL HYDROCARBON ANALYSIS TO MULTIMEDIA FATE MODELS

The dominance of the non aqueous phase liquid (NAPL) and its association with hydrophobic contaminants is theoretically established but rarely incorporated into the exposure assessment tools used to derive soil screening levels and guideline values. This is an oversight that has an impact on the certainty associated with site assessment and remedial end-point predictions. Its significance comes into play when one considers the residual risk posed by post-treatment residues.

Fugacity models (Mackay, 2001) and their application to characterising the fate relationships between environmental media have received comprehensive coverage (Gobas and Muir, 2004). Level I fugacity calculations illustrate basic equilibrium partitioning behaviour. In contrast, Level II calculations account for advection and reactions in the form of residence times and half-lives respectively within environmental media. The calculations assume an environment in equilibrium, but under conditions of steady-state flow; i.e. the amount entering the environment is mass-balanced by the amount lost to the combined mechanisms of flow, reaction or degradation. Whilst progress has been made in modelling the distribution of potentially toxic organic chemicals between solid, liquid (water) and gas phases within the soil or sediment (and hence provide an approximation of potential exposure), there has been less attempt to include the source term (oil) in fugacity models (Walter et al., 2000; Nieman, 2003). Thus, the relationships between chemical presence, toxic response, bioavailability and risk for weathered hydrocarbons have yet to be fully elucidated and coupled into a meaningful risk management framework.

In this research, a Level II fugacity model was developed to include four phases in the soil matrix, namely: air, water, mineral soil and NAPL (Pollard et al., 2008). The fugacity modelling confirmed the propensity for petroleum hydrocarbon fractions and risk critical compounds to be preferentially partitioned to the NAPL and soil phases (Figure 5). When organic carbon content in soil was higher than 7% w/w (biopile soil C), the chemical mobility of the aliphatic (up to EC8) and aromatic (up to EC21) hydrocarbon fractions was further reduced by 50% (Figure 5). Biodegradation was

Aliphatic fractions



Aromatic fractions

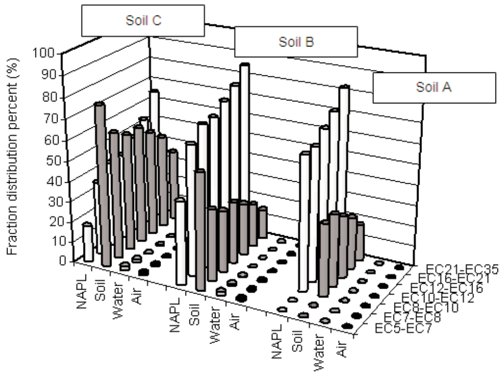


Figure 5: Estimated mass distribution of the petroleum hydrocarbon fractions among biopile compartments (% organic carbon in soil A, B and C: 4.9, 6.0 and 13.2%, respectively).

the dominant removal process, except for the volatile aromatic fractions. The rates of degradation for the aliphatic fractions were up to three times faster than the aromatic ones, especially for the heavier hydrocarbon fractions. Dominance and persistence of the heavier aromatic fractions (> EC12-16) with soil concentration of ca. 10,000 mg/kg required > 31 months to be fully eliminated from the biopile systems. However, modelled depletion times for contaminants in the context of authentic biopiling are immaterial and thus research efforts should be focused on the likely exposures of humans and other receptors to residual saturation at hydrocarbon-contaminated sites (Table 3).

Table 3: Representative estimated rates and persistence of hydrocarbon fraction and indicator transformation in biopile soil B.

Hydrocarbon fractions and indicators	% loss by degradation	% loss by volatilisation	Persistence in each environmental compartment (h)				Overall residence time (days)
			Air	Water	Soil	NAPL	
Aliphatic							
EC 5-6	79	21	59	6	783	3522	182
EC>6-8	88	12	44	6	864	3890	200
EC>8-10	97	3	34	6	633	2849	147
EC>10-12	99	1	25	6	600	2698	139
EC>12-16	100	0	18	6	600	2698	138
EC>16-35	100	0	12	6	1123	5053	258
Aromatic							
EC 5-7	9	91	2	0	31	140	7
EC>7-8	29	71	52	6	1039	4674	240
EC>8-10	73	27	31	6	1382	6217	318
EC>10-12	74	26	19	6	1942	8741	446
EC>12-16	89	11	12	6	1491	6708	342
EC>12-16	94	6	18	6	2755	12424	634
EC>21-35	99	1	21	6	4155	18755	956
Indicator compounds							
Naphthalene	61	39	16	6	2597	11686	596
Acenaphthene	86	14	1	6	1039	4674	238
1-Methylnaphthalene	84	16	7	6	1039	4674	239
Phenanthrene	92	8	26	6	4155	18755	956
Pyrene	98	2	7	6	4155	18755	955
Fluoranthene	98	2	31	6	4155	18755	956
Benzo(a)pyrene	100	0	7	6	4155	18755	955
Benzo(b)fluoranthene	100	0	19	6	4155	18755	956
Benzo(k)fluoranthene	100	0	7	6	4155	18755	955
Chrysene	99	1	7	6	4155	18755	955
Dibenzo(a,h)anthracene	100	0	7	6	4155	18755	955

Table 4: GC-FID quantification of aliphatic and aromatic fractions of 11 soils contaminated with weathered hydrocarbons.

Hydrocarbon fractions		Concentration (mg/kg dry weight soil)										
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11
Aliphatic	C5-C6	-	-	-	-	-	-	-	-	-	-	-
	C6-C8	-	-	-	-	-	-	-	-	-	-	-
	C8-C10	<4	<4	<4	<4	<4	<13.8	<13.8	<13.8	<11.2	<4.5	<13.1
	C10-C12	19	6.82	64.6	41.1	63.2	107	32.5	67.3	30.3	25.4	59.6
	C12-C16	752	308	1370	777	1280	1130	1560	715	714	629	1258
	C16-C21	1810	730	2980	1500	2690	1840	3980	1706	3454	2893	3379
	C21-C35	1530	608	1800	1090	1800	2710	7480	3990	2718	6051	8987
	C35-C44	nd	nd	nd	nd	nd	nd	nd	nd	1595	1006	5339
Aromatic	C5-C7	-	-	-	-	-	-	-	-	-	-	-
	C7-C8	-	-	-	-	-	-	-	-	-	-	-
	C8-C10	<4	<4	<4	<4	<4	<13.8	<13.8	<13.8	<4	<4	<4
	C10-C12	<4	<4	15.1	5.62	12.1	156	<13.8	<13.8	7.4	<4	5.76
	C12-C16	68.3	133	618	228	449	2580	399	216	2880	139	173
	C16-C21	624	714	2250	855	2010	5990	1730	982	3797	753	716
	C21-C35	1080	894	2110	1070	2280	5570	3450	2690	5333	888	867
	C35-C44	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not determined; "-" not detected

Table 5: List of polycyclic compounds detected in soils contaminated with weathered hydrocarbons using GC-MS. A total ion profile between a mass range of 45-450 has been reconstructed and target PAH have been identified accordingly to their quantitative ion (*m/z*).

Compounds	Abb[1]	Carbon number	Q_{ion}[2] (*m/z*)	CAS[3]	Non-cancer target organs/systems or effects[4]
Polycyclic aromatic hydrocarbons					
Naphthalene	N	10	128	91-20-3	Body weight
C1-Naphthalenes	N1	11	142		
C2-Naphthalenes	N2	12	156		
C3-naphthalenes	N3	13	170		
C4-naphthalenes	N4	14	184		
Acenaphthylene	ANY	12	152	208-96-8	Liver
Acenaphthene	ANA	12	154	83-32-9	Liver
Fluorene	F	13	166	86-73-7	Blood
C1-Fluorenes	F1	14	180		
C2-Fluorenes	F2	15	194		
C3-Fluorenes	F3	16	208		
Phenanthrene	P	14	178	85-01-8	Kidney
Anthracene	A	14	178	120-12-7	None specified
C1-Phenanthrenes/anthracenes	P1	15	192		
C2-Phenanthrenes/anthracenes	P2	16	206		
C3-Phenanthrenes/anthracenes	P3	17	220		
Fluoranthene	FL	16	202	206-44-0	Blood-kidney-liver
Pyrene	PY	16	202	129-00-0	Kidney
C1-Fluoranthenes/pyrenes	FL1	17	216		
C2-Fluoranthenes/pyrenes	FL2	18	230		
C3-Fluoranthenes/pyrenes	FL3	19	244		
Benz(a)anthracene*	BA	18	228	56-55-3	
Chrysene*	C	18	228	218-01-9	
C1-Chrysenes/benz(a)anthracenes	C1	19	242		
C2-Chrysenes/benz(a)anthracenes	C2	20	256		
C3-Chrysenes/benz(a)anthracenes	C3	21	270		
C4-Chrysenes/benz(a)anthracenes	C4	22	284		
benzo(b)fluoranthene*	BBF	20	252	205-99-2	
benzo(k)fluoranthene*	BKF	20	252	207-08-9	
benzo(e)pyrene	BEP	20	252	192-97-2	Nasal
Perylene*	PER	20	252	198-55-0	
benzo(a)pyrene*	BAP	20	252	50-32-8	
indeno(1,2,3-c,d)pyrene*	IN	22	276	193-39-5	
Dibenz(a,h)anthracene*	DBA	20	278	53-70-3	
benzo(g,h,i)perylene	BPE	22	276	191-24-2	Neurological
Dibenzo(a,l)pyrene*	DBP	24	302	191-30-0	
Single PAHs					
2-Methylnaphthalene	2-MN	11	142	91-57-6	Nasal
1-Methylnaphthalene	1-MN	11	142	90-12-0	Nasal
Oxygen heterocycles					
Dibenzofuran	DBF		168	132-64-9	None specified
Sulfur heterocycles					
Dibenzothiophene	D	14	184	132-65-0	
C1-Dibenzothiophenes	D1	13	198		
C2-Dibenzothiophenes	D2	14	212		
C3-Dibenzothiophenes	D3	15	226		
C4-Dibenzothiophenes	D4	16	240		

[1] Abb: Abbreviation

[2] Q_{ion}: Quantitative ion

[3] CAS: Chemical Abstracts Service

[4] the default target organ(s)/systems are those reported to occur at the doses used to derive the reference dose (US) or tolerable daily intake (UK). Non-default target organ(s)/systems or effects may be justified through a detailed toxicological analysis of the chemicals at a specific site.

* denotes a carcinogen

The implications of the fugacity modelling developed here are important for risk analysts and remediation engineers because the fate of the target compounds can be predicted and explained. The overriding dominance of the NAPL phase for hydrophobic contaminants is well established, but is rarely incorporated into exposure tools. Hence, the tools used to derive soil guideline values do not account for the strength of this association and likely over-estimate the soil bound fraction.

Most importantly, if used correctly such an approach could enhance confidence and reduce uncertainty in the development of remedial end-points for remediation of heavy and weathered hydrocarbons.

4. RISK CRITICAL "INDICATOR COMPOUNDS" IN WEATHERED HYDROCARBONS AND PROPOSITION OF A RISK CRITICAL FRACTIONS SET

The UK approach to human health risk assessment of petroleum hydrocarbons encourages the adoption of a combined indicator and fraction approach in a tiered risk-based framework (Environment Agency, 2005). Specific indicator compounds should be assessed because these are often the key risk drivers at petroleum contaminated sites. The assessment of fractions should facilitate a more representative picture of risk at sites where the origin of the petroleum hydrocarbon contamination may be unclear (Environment Agency, 2005).

Table 6: List of aliphatic hydrocarbons identified including branched alkanes (*).

Compounds	Carbon number	Mass	Q_{ion}(1) (m/z)	CAS(2)	Non-cancer target organs/systems or effects(3)
Decane	10	142	57	124-18-5	Small neuro behavioural effects
Undecane	11	156	57	1120-21-4	
Dodecane	12	170	57	112-40-3	
Tridecane	13	184	57	629-50-5	
Tetradecane	14	198	57	629-59-4	
Pentadecane	15	212	57	629-62-9	
Hexadecane	16	226	57	544-76-3	Effect of liver function
Heptadecane	17	240	57	629-78-7	
Pristane*	19	268	57	1921-70-6	Plasmacytomas
Octadecane	18	254	57	593-45-3	
Phytane*	20	282	57	638-36-8	
Nonadecane	19	268	57	629-92-5	
Eicosane	20	282	57	112-95-8	
Heneicosane	21	296	57	629-94-7	
Docosane	22	310	57	629-97-0	
Tricosane	23	324	57	638-67-5	
Tetracosane	24	228	57	646-31-1	
Pentacosane	25	352	57	629-99-2	
Hexacosane	26	366	57	630-01-3	
Heptacosane	27	380	57	593-49-7	
Octacosane	28	394	57	630-02-4	
Nonacosane	29	408	57	630-03-5	
Triacontane	30	422	57	638-68-6	
Hentriacontane	31	436	57	630-04-6	
Dotriacontane	32	450	57	544--85-4	
Tritriacontane	33	464	57	630-05-7	
Tetratriacontane	34	478	57	14167-59-0	
Pentatriacontane	35	492	57	630-07-9	
Hexatriacontane	36	506	57	630-06-8	
Heptatriacontane	37	520	57	7194-84-5	
Octatriacontane	38	534	57	7194-85-6	
Nonatriacontane	39	548	57	7194-86-7	
Tetracontane	40	562	57	4181-95-7	

[1] Q_{ion}: Quantitative ion

[2] CAS: Chemical Abstracts Service, a division of the American Chemical Society, assigns these identifiers to every chemical that has been described in the literature to make database searches more convenient, as chemicals often have many names.

[3] the default target organ(s)/systems are those reported to occur at the doses used to derive the reference dose (US) or tolerable daily intake (UK). Non-default target organ(s)/systems or effects may be justified through a detailed toxicological analysis of the chemicals at a specific site.

Table 7: Petroleum hydrocarbon fractions proposed as candidate indicator fractions for weathered hydrocarbons (threshold indicator fractions are in bold).

Aliphatic Fractions	Aromatic Fractions
EC >12-16	EC >12-16
EC >16-35	EC >16-21
EC >35-44	EC >21-35
	EC >35-44
EC >44-70	

Table 8: List of potential indicator compounds for weathered hydrocarbons.

Potential indicator compounds for weathered hydrocarbons contaminated soils	
Naphthalene	Aromatic > EC10-EC12
Acenaphthene 1-methylnaphthalene	Aromatic > EC12-EC16
Pyrene Phenanthrene	Aromatic > EC16-EC21
Fluoranthene Benz[a]anthracene* Benzo[b]fluoranthene* Benzo[k]fluoranthene* Benzo[a]pyrene* Benzo[ghi]perylene Chrysene* Dibenz[a,h]anthracene* Indeno[1,2,3-c,d]pyrene 5-methylchrysene	Aromatic > EC21-EC35

*Non-threshold indicator compound, also known to possess some genotoxic carcinogenic potential

Based on analyses of contaminated soils by weathered hydrocarbons carried out during the PROMISE project (Tables 4, 5 and 6), a set of hydrocarbon fractions (Table 7) and indicator compounds (Table 8) for weathered hydrocarbons has been identified by reference to Environment Agency recommendations.

As already demonstrated by the petroleum hydrocarbons fractions analysis in Table 4, the distribution of aliphatic compounds (or alkanes) for weathered hydrocarbons ranges between C10 and C40 including pristane and phytane the two branched alkanes currently found in petroleum hydrocarbons (Table 6).

5. APPLICATION OF DIAGNOSTIC STRATEGY FOR HAZARD ASSESSMENT AND REMEDIATION MONITORING USING ECOLOGICAL INDICATORS

Widely used soil ecotoxicity assays were selected at this stage (Table 9) based on their ease of execution and representation of different ecological soil organisms. The assays serve two primary purposes: (i) assessing changes in hazard as a function of treatment and time and (ii) determining the significance of biological processing in remediation progress.

Table 9: Ecotoxicological Tests – selected species are in bold.

Test	Species considered
Earthworm survival	*Eisenia fetida*, ***Lumbricus terrestris, Lumbricus rubellus***
Seed germination	***Brassica alba*** **mustard white**, *Triticum aestivum* (Consort) wheat, *Pisum sativum*, pea
Luminescence-based bacterial biosensors	Metabolic: *Vibrio fischeri Escherichia coli* HB101, ***Pseudomonas putida*** **F1 Tn5** Catabolic: ***Escherichia coli*** **HMS174,** *Escherichia coli* DH5α, *Pseudomonas putida* TVA8

Earthworm survival and seed germination should only be used at the start and end of the remediation process due to the greater volumes of soil required. For all of the assays performed, the non-hydrocarbon control soil used was a freely draining, agricultural loamy sand from NE Scotland (Boyndie Association/Corby Series, Scotland) with a sand:silt:clay ratio of approximately 80:15:5.

Earthworm survival

Earthworms were selected because they are probably the most important invertebrate soil toxicity test. There is a significant amount of data about their response to hydrocarbons and the steps required to optimise the assay. While a range of species was tested, *Lumbricus* were the preferred receptor for a 14 days assay (Langdon et al., 1999; Shakir et al., 2002). For each replicate microcosm, 5 earthworms were used, which were incubated individually for 2 weeks at 15°C, 80% humidity and no light. Lids were fitted loosely to minimise evaporation.

Seed germination

Seed germination is simple to perform and relatively sensitive to hydrocarbons. Of the seeds selected, mustard seeds were most sensitive to hydrocarbon contamination (Saterbak et al., 2000), which was confirmed in initial trials. The seeds were germinated at 25°C, 80% humidity and a light:darkness cycle of 16:8 hours for 4 days.

Luminescence-based bacterial biosensors

Remedios and the University of Aberdeen have pioneered the application of microbial-based luminescence sensors in hydrocarbon impacted soils. These sensors are able to assess hazard and may also be used to monitor remediation progress.

Two different types of bacterial biosensors were used: metabolic (for the assessment of microbial toxicity) and catabolic (for the assessment of bioavailable hydrocarbons), which were *Pseudomonas putida* F1 Tn5 and *Escherichia coli* HMS174, respectively (Selifonova and Eaton, 1996; Weitz et al., 2001). Cells for the assays were freeze-dried using standard procedures (Bundy et al., 2001) and stored at -20°C to ensure the reproducibility of the assay. Standard procedures for the assay are well established and have been widely reviewed (Bundy et al., 2001). Controls for the metabolic sensor were non-contaminated soils while for the catabolic sensor, these were the compounds capable of maximum induction of the sensor.

Microbial respiration

Microbial respiration is one of the most commonly executed soil assay methods. The CO_2 is a measure of the microbial activity and by relating activity to biomass an estimate of the metabolic quotient can be made. Soils may respire more because of the presence of a substrate, an increasing biomass or indeed a stressed ecosystem so it is important to combine relevant controls with any selected treatment. Measurements were made using a GC-FID with a methaniser from samples that were contained in airtight containers. Appropriate controls and calibrations were also adopted.

Most probable number (MPN counts)

A measure of the change in the total microbial population as a consequence of the presence of hydrocarbons and the performance of degraders are essential. There are various method options to assess this including biochemical, molecular or culturable methods. All have their strengths and limitations. For PROMISE the emphasis was on culture-based approaches. Microbial numbers for both heterotrophic microorganisms and hydrocarbon degraders were estimated using the "most probable number" (MPN) technique (Kirk et al., 2005) using tetrazolium-based enzymatic dyes.

The progress of bioremediation cannot be followed by the measurement of a single parameter. Indeed the success or failure of the process is defined by the bioavailability of the initial material (and metabolites) and the ability for the microbial population to actively degrade these materials. Hence there is a great emphasis on the use of a battery of assays as their mean is likely to be more valuable than that from a single assay. The problem with this approach is that all the data need to be compiled and compared and this involves integrating assessments of different parts of the soil community and processes with each other and chemical characterisation.

Table 10: Soil quality index determined for different biological indicators (modified from Dawson et al., 2008 in light of the findings associated with PROMISE project)

Biological Indicator	Sensitivity	Robustness	Use in Soil Quality Index
Basal Respiration	5	4.5	✓
Dehydrogenase activity	1.5	23.4	✓
P. fluorescens biosensor	0	25.1	✗
E. coli biosensor	0	38.2	✗
L. terrestris CI - Day 14	5	14.4	✓
L. terrestris weight change	4	3.5	✓
Mustard seed germination	2.5	24.8	✓

The research findings demonstrated that a gross reduction of hydrocarbons does not represent environmental improvement, as reported by the biological response. To ease the interpretation, the biological indicator data were transformed into a Soil Quality Index (SQI) which ranked the assays in terms of performance and responsiveness (Table 10). The suitability of individual assays was based on a sensitivity >1 or robustness >20. Sensitivity was the ability of an indicator to determine significant adverse end-points between control and contaminated soils. Robustness reflected consistency of an end-point in the control soil replicates.

One of the key benefits of this hazard-based approach is that it diagnoses in real time the occurrence of metabolites that could pose greater toxicity than the parent molecules. Furthermore, the changes in toxicity and to some extent the bioavailability of fractions within the matrix reflect that some pollutants may be mobilised during the remedial activity.

6. DATA INTEGRATION TO SUPPORT DECISIONS IN REMEDIAL STRATEGIES FOR HEAVY HYDROCARBONS - TESTING THE PERFORMANCE AT FIELD SCALE

Previous studies have often operated in isolation and have failed to consider the reason that remediation is effective or how best to monitor these changes. Based on the knowledge and information gained during the PROMISE project, a diagnostic and risk management protocol for weathered hydrocarbons has been developed (Figure 6).

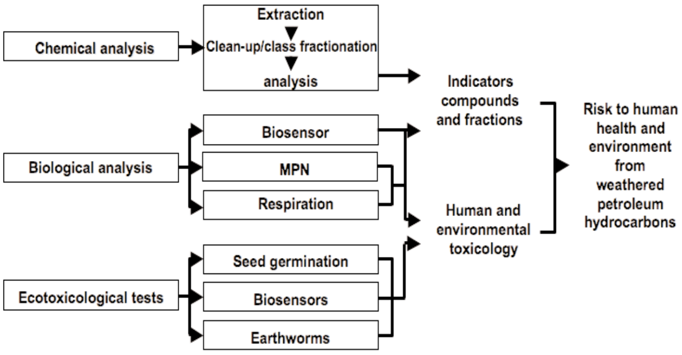


Figure 6: Risk-based approach for sites contaminated with weathered hydrocarbons

To validate the use of this integrated diagnostic approach, a six month field-scale trial was conducted comparing windrow turning and biopiling. The soil (which was of clay loam texture) was contaminated with bunker fuel. This is the first report of a replicated large scale comparison of these two techniques. Three tonne replicates of each soil treatment were set up. The treatments (no amendment, N and P addition, N and P and inoculum addition) were applied both to windrows and biopiles and a fallow non-treatment was also maintained. Each of these treatments was done in replicates. Moisture levels were maintained between 55 and 75% of the maximum water holding capacity by irrigation up to twice weekly. The windrows were turned twice weekly. Air was constantly pumped into the biopiles to ensure that oxygen was not limiting. Nitrogen was added in the form of ammonium nitrate while potassium orthophosphate was added to obtain a C: N: P ratio of 100: 4: 0.33. Further nutrients were added via irrigation as they became limited.

Soil samples for chemical and ecotoxicological assays were collected from each windrow treatment every two weeks for six months including enumeration of total hydrocarbon degraders and total heterotrophs and respiration. Soil samples for chemical analysis were collected at 5 depths, bulked and then homogenised by passing through a 3.25 mm sieve. Samples were then stored in sample bags at 4°C until chemical analysis. The analysis was always carried out within ten days of sampling. Biopile treatments were collected monthly to prevent significant disturbance to the treatment.

Results of this field scale trial showed that biopiling even when optimised was vastly inferior to windrows for these particular soils under the conditions adopted. While nutrient amendments increased the rate of degradation there was no significant advantage in adding inoculum. Most importantly, regular turning was associated with soil structural improvement. This observation has not been reported before and further validates the benefit of windrow turning even for soils with particularly difficult-to-work-with textures.

End-points for human ingestion/dermal contact, water quality leaching and ecological habitat were all found to vary significantly. The nature of the hydrocarbons in the field trial meant that as they were heavy, their toxicity increased as bioremediation progressed. This meant that ecological targets were the most difficult to meet. Water quality and human exposure criteria were more easily met, in part because these failed to account for the significance of metabolites in the measured responses (Table 11).

Table 11: Number of days required for the soil to pass $\text{Human}_{\text{Residential}}$ risk assessment, ecologically defined hazard assessment and water defined criteria (related to environmental quality standards).

	Biopile			Windrow		
	H_R	Eco	Water	H_R	Eco	Water
Control	37	91	91	10	10	12
Nutrient	37	37	37	10	10	10
Nutrient + inoculum	37	37	37	8	8	10
	Control fallow soil					
	H_R	Eco	Water			
	37	91	24			

H_R: Human exposure criteria where residential use was considered as defined in Contaminated Land Exposure Assessment (CLEA) software; Eco: Ecological risk based on bioassay results for hazard assessment; Water: water quality obtained from P20 Environment Agency Excel programme.

7. END-USER AND STAKEHOLDER CONFIDENCE

A decision support tool (DST) was initially designed for sites contaminated with weathered hydrocarbons focusing on site characterisation and a risk-based approach where *ex situ*/on-site remediation is proposed as a treatment option. It introduces contaminated land stakeholders to the range of diagnostic methods developed during the research and the resources available to them. Consistent with the practical aims of this project, the DST has then been broadened to remediation technologies for contaminated land and waters with the perspectives of facilitating and assisting in a transparent decision making process. This tool is based on the current framework used for remediating contaminated land in the UK (Defra and Environment Agency, 2004). It provides a road map of the logical steps involved in the demonstration of risk reduction within process-optimised technology. Inevitably, the DST is an evolving process and as information becomes available the DST will be refined. New technologies and reported successes and failures enable the process to be moderated and evaluated accordingly. The tool is available to the full remediation community and is especially intended to improve regulatory and investor confidence on the capacity of *ex situ*/on-site bioremediation technology to successfully treat contaminated sites with hydrocarbon wastes and to deliver a scientific basis for the UK adoption of a risk-based approach. The tool is available at: http://www.abdn.ac.uk/remediation-dst/

The PROMISE project demonstrated a great number of technical objectives and these have been integrated into a framework to aid stakeholders and the user community. The data which underpin the DST for heavy hydrocarbons reveal that confidence in the process is enhanced when the procedures are adopted (Table 12). Most importantly was the ability to consider the scientific and technical drivers alongside the economic and "wider environmental parameters" (including carbon footprint and life cycle assessment).

Table 12: Output information associated with the field scale experiment and extrapolated from the decision support approach.

Process	Biopiling	Windrow
Preparation	Requirement for effective mixing and screening of the soil in anticipation of an effective remedial management set-up. The clean materials and grossly impacted soils need to be separated and a decision as to their treatments made.	
Construction	A suitable platform that prevents migration of pollution into substrata and is able to cope with traffic.	
Placement	Access to power and pipes for the blowers to be put in place	Accessible units capable of easy turning; hence space is a requirement
SIte Activity	Blowing, suction and capture of volatiles. Covering and water management.	Covering, uncovering and turning. Water management.
Monitoring	Vapour, soil, water for chemical analysis; complimentary microbial assessments.	
Duration	As plant has been removed from site this may be determined by other development issues.	Usually within three months as turning has a significant demand for plant.
End-point	Agreed sample strategy needs to meet risk-defined end-point prior to decommissioning and re-use of soil	
Re-use of materials	Fit for purpose	
Environmental Impact	Use of fuel for compressors and pumps.	Use of fuel for motors used in turning and preparation.
Cost from trial (per tonne*)	£92	£78
Positive aspects from this trial	Easy to set up, minimal maintenance, costing estimated clearly at outset.	Rapidly reached end-point, excellent structural change to soil, error bars of uncertainty in samples reduced with time, cost effective.
Negative aspects from this trial	Poorer end product and took longer to get there, higher cost, more variable samples, difficult to manage physical aspects.	Cessation of turning could have been done much earlier; more space required so does not suit all sites.

* The costs have been extrapolated to the cost per tonne on a 1000 tonnes project and include the rental of equipment and the set-up and decommissioning costs. Licence and uncertainty costs are not included.

The benefits of one approach over another are often difficult to estimate. For example windrow turning would appear to offer a more cost effective approach but there are some weaknesses not least that there would be continual high capital expenditure for the plant associated with the turning process. This would be more cost effective if the plant was actively engaged in other duties on the site and the absolute cost could be shared. In reality the site characteristics as well as the matrix of the soil and the economic status of the operator and the market will define the choice selected.

SUMMARY

- **A novel and robust ultrasonic extraction method** for contaminated soils with weathered hydrocarbons has been developed and optimised during the project. The method covers the determination of total petroleum hydrocarbons (TPH) between nC8 and nC40 and sub-ranges of hydrocarbons in support of the UK regulatory framework. Further modifications to the carbon banding may be made as requested for risk-assessment including ranges known as Texas banding as well as separation of the aliphatic and aromatic fractions. The method can be routinely used for measuring hydrocarbons down to 10 mg/kg in soil. The method has been accredited ISO17025 for TPH analysis, banding and class separation.

- **A diagnostic strategy tool box for ecological hazard assessment** of weathered hydrocarbons has been developed during this research. The tool box highlighted the role of taking a multiple-trophic view in considering both the hazard and remediation of weathered hydrocarbons. The selected bioassay techniques should be used in combination with the chemical analysis to allow ecological relevance and a more focussed understanding of hydrocarbon transformations. The bioassays were selected on their ease of execution and representation of different ecological soil organisms.

- This research has developed **a Level I and II Fugacity model comprising four phases** within the soil matrix, namely: air, water, mineral soil and NAPL and has demonstrated successfully the propensity for risk critical compounds in hydrocarbon-contaminated soils to be preferentially partitioned to the NAPL and soil phases. The fugacity approach demonstrated its suitability for applications to contaminated environments and with further refining could provide a useful tool for the exposure assessment models to generate soil guideline values and to better represent contaminant fate in multimedia systems.

- **A diagnostic and risk strategy protocol for weathered hydrocarbons** has been developed combining chemical, biological and ecotoxicological analysis. The protocol demonstrated that information obtained might be further exploited to assess constraints of bioremediation and most significantly help to identify optimal remediation strategies.

- **A Decision Support Tool** has been developed introducing contaminated land stakeholders to the range of diagnostic methods developed during the research and the resources available to them. The tool kit is an evolving process and as information becomes available it will be refined accordingly. New technologies and reported successes and failures enable the process to be moderated and evaluated accordingly. The tool is available at: www.abdn.ac.uk/remediation-dst/.

- **A website** dedicated to the project has been designed and used to understand stakeholders' requirements, views and aspirations. The website publicises the research programme, the research outcomes and will also advertise new events (e.g. workshops, short course development) on contaminated land related issues. The website is accessible at www.cranfield.ac.uk/sas/resource/research/promise/index.jsp.

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