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Application of a luminescence-based biosensor for assessing naphthalene biodegradation in soils from a manufactured gas plant

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Exposure of microbial biosensors to cyclodextrin solutions allows the assessment of the degradable fraction of contaminants in soil.

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ABSTRACT

Despite numerous reviews suggesting that microbial biosensors could be used in many environmental applications, in reality they have failed to be used for which they were designed. In part this is because most of these sensors perform in an aqueous phase and a buffered medium, which is in contrast to the nature of genuine environmental systems. In this study, a range of non-exhaustive extraction techniques (NEETs) were assessed for (i) compatibility with a naphthalene responsive biosensor and (ii) correlation with naphthalene biodegradation. The NEETs removed a portion of the total soil naphthalene in the order of methanol > HPCD > βCD > water. To place the biosensor performance to NEETs in context, a biodegradation experiment was carried out using historically contaminated soils. By coupling the HPCD extraction with the biosensor, it was possible to assess the fraction of the naphthalene capable of undergoing microbial degradation in soil.

1. Introduction

It has been suggested that marker-based bacterial biosensors offer new and exciting opportunities for the monitoring of contaminants in the environment because they have been acknowledged to be sensitive, easy to use and reproducible (Huang et al., 2005; Tecon et al., 2006). However, the reality is that although there have been many publications about the construction of such biosensors; there are few environmental applications. The strongest genuine field applied research has been performed by Trang et al. (2005) by making use of microbial biosensors for a comprehensive study of As in Bangladesh drinking waters and Dardenne et al. (2007) for ecological assessment of waters in Belgium. Both manuscripts relate to water samples and not soils and there is a focus on inorganic analytes and not hydrophobic compounds.

Biosensors have been used to either quantify target chemicals in water, sediment and soil samples or to evaluate the relative ecotoxicological impact of such chemicals in these matrices. Microbial, marker-based biosensors have evolved in two ways: (i) measurement of the decrease in a metabolic response (measured by a parameter, such as bioluminescence) to increasing contaminant concentrations (Shaw et al., 2000) and (ii) measurement of the induction of resistance or catabolic activity, contaminant resistance or cellular repair where gene expression increases with rising analyte concentration (Huang et al., 2005; Tecon et al., 2006). Some authors have proposed that a combined approach offers the benefit of generic environmental quality assessment with an estimation of pollutant dose (Paton et al., 2005).

The *lux*-marked bacterial biosensor *Pseudomonas fluorescens* HK44 pUTK21 has been shown to respond sensitively and quantitatively to naphthalene (Heitzer et al., 1992) and a range of other mono and diaromatic compounds (Bundy et al., 2001a,b). Although this biosensor is the most widely cited catabolic-based luminescent biosensor, most studies have focussed on aqueous samples. Indeed, while the value of biosensors in the study of polar organic contaminants in soil has been reported (Shaw et al., 2000), their application to hydrophobic organic contaminants (HOCs) in soil is less common (Semple et al., 2003).

Currently, biosensors need to interface with such target molecules either following a suitable organic solvent extraction step or else directly via the gas phase. Werlen et al. (2004) pioneered a technique that was refined and adopted by various authors including Tecon et al. (2006) and Kohlmeier et al. (2007) which

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shows great promise and sensitivity but has not yet been applied to soil samples.

When making use of organic solvents, biosensors must be exposed to them after appropriate dilution. This approach is problematic for two reasons: (i) the use of organic solvents often has negative adverse effects on biosensors and (ii) the portion of the HOC that partitions into the organic solvent extractions does not reflect HOC bioavailability (Kelsey and Alexander, 1997; Reid et al., 2000), thus undermining the very principle of using biosensors.

In order for an extract to be compatible with a biosensor, it must be predominantly aqueous; thus, organic solvent extracts (e.g. methanol or dimethylsulphoxide) require dilution (typically to $\leq$10%) or the extraction needs to be performed using an aqueous solution with a proportion of solvent present (Bundy et al., 2001a,b). The descriptive term for such solvents is non-exhaustive extraction techniques (NEETs) (Patterson et al., 2004). Aqueous cyclodextrin solutions offer an alternative to the use of organic solvents. Cyclodextrins have a high aqueous solubility, but contain a hydrophobic cavity, allowing the contaminants to be separated from the soil solid phase (Brusseau et al., 1997; Stokes et al., 2005). Recent studies have shown direct relationships between HPCD extraction and microbial degradation of PAHs in soil (Reid et al., 2000; Cuypers et al., 2001; Doick et al., 2005; Allan et al., 2007).

With respect to naphthalene (a representative HOC) the specific aims of this study were (i) to compare the performance of a range of NEETs in their extraction efficiency (ii) to assess the performance of a biosensor when exposed to these NEETs, (iii) to relate naphthalene extractability to its availability for microbial biodegradation and (iv) to validate the coupling of NEETs with bioluminescence-based biosensors in historically contaminated soils.

2. Materials and methods

2.1. Chemicals

Naphthalene, βCD and HPCD were purchased from Sigma Chemical Company. All solvents were obtained from Rathburn, UK and were of the highest possible grade available (>99%). Teflon centrifuge tubes used in the sequential extraction were obtained from Merck, UK. All growth media were supplied by Oxoid. Reverse osmosis water was used throughout.

2.2. Assay for P. fluorescens HK44 pUTK21

P. fluorescens HK44 pUTK21 was used in a batch-culture assay which was slightly modified from Burlage et al. (1993). Triplicate single colonies were taken from a YEPG plate (0.2 g l^{-1} yeast extract, 2 g l^{-1} peptone, 1 g l^{-1} glucose, 0.2 g l^{-1} NH_4NO_3, 100 ml of pH buffer (added after autoclaving)) and grown overnight (25 °C, 200 rpm) in 10 ml of YEPG medium. An inoculum (1%) was then added to 10 ml of minimal medium (0.2 g l^{-1} $MgSO_4{\cdot}7H_2O$, 0.2 g l^{-1} NH_4NO_3, 100 g l^{-1} buffer (added after autoclaving), 0.1 ml trace element solution) and grown overnight (25 °C, 200 rpm). The overnight culture was then added to 100 ml of M medium, giving a 10% inoculum. This was grown (25 °C, 200 rpm) for 3–4 h, until A_{546} was between 0.35 and 0.40. The cells were then harvested by centrifugation (25 °C, 4000 × *g*, 5 min), resuspended in 10 ml of 0.1 M KCl solution, and used immediately. A 25 μL aliquot of cell suspension was added to each microtitre well using a multichannel pipette, mixed by pipetting for 5 s, sealed with a cover and the plate incubated at 25 °C. The luminescence was read after 60 min exposure.

2.3. Soil sampling, preparation and amendment

Surface horizons (5–15 cm) from Boyndie and Insch soils were sieved field moist through a 10 mm and then through a 2 mm sieve to remove roots and other vascular material. Soil was amended with naphthalene using a single step spiking/rehydration procedure (Reid et al., 1998). Naphthalene was added to soils at concentrations of 100, 200, 400, 600 and 800 mg kg^{-1} of dry weight soil. Soil samples were mixed thoroughly and left for solvent evaporation for 2 days. Control samples were used throughout to assess the effect of the solvent used and soil naphthalene concentration was confirmed analytically.

2.4. Historically contaminated soils

Ten naphthalene-contaminated soils were sampled from a former manufactured gas plant (MGP), located on the coast in northeast Scotland. The soils were part of an ongoing bioremediation strategy and as such the site was well characterised. A detailed chemical and toxicity appraisal was carried out during the remediation and this confirmed naphthalene as the only pollutant to which the biosensor *P. fluorescens* HK44 pUTK21 would respond. Indeed the presence of other PAHs greater than two rings did not induce *P. fluorescens* HK44 pUTK21 and has been shown not to cause microbial toxicity (Bundy et al., 2001a,b, 2003).

2.5. Biodegradation of naphthalene from historically and amended contaminated soils

Ten-gram aliquots of the soils from the former MGP were inoculated with *P. fluorescens* TTC1, a known naphthalene degrader, and left in sealed 250 ml Duran bottles for one month. Triplicate samples of Insch soil were amended with 50, 100, 250 and 500 mg kg^{-1} of naphthalene in a parallel experiment. Every four days the sample was agitated and the jar was opened to replenish the air. The control soils (a clean sand sample from the same MGP site) were amended with 100 mg kg^{-1} of naphthalene, split into two 30 g aliquots, and amended with and without *P. fluorescens* TTC1 to enable an assessment of how long degradation would take to occur. Biodegradation end-point was achieved within the month of the experimental work.

2.6. Extraction of naphthalene from soil using dichloromethane

To quantify the total extractable naphthalene concentration from the PAH-contaminated soils triplicate samples of soil (1.25 g each) were weighed into Teflon centrifuge tubes (35 ml capacity) and dichloromethane (25 ml) added to each. The tubes were sealed and placed on their sides on an orbital shaker (Janke and Kunkel, IKA®-Labortechnik KS 250) and shaken at 150 rpm for 20 h. The tubes were centrifuged at 6500 × *g*.

2.7. Extraction of naphthalene from soil using water, cyclodextrin and methanol

To quantify the extractable naphthalene concentration from the PAH-contaminated soils, triplicate samples of each of the 5 soils (1.25 g each) were weighed into Teflon centrifuge tubes (35 ml capacity) and HPCD or H_2O solutions (25 ml) added to each. In the case of methanol 2.5 ml was added to the tube. The tubes were sealed and placed on their sides on an orbital shaker (Janke and Kunkel, IKA-Labortechnik KS 250) and shaken at 150 rpm for 20 h. The tubes were centrifuged at 6500 × *g*. For the methanol, the extract was diluted to 10% in RO water.

2.8. Analysis of naphthalene

Soil (5 g) were thoroughly mixed with 5 g of anhydrous sodium sulphate and then placed in cellulose extraction thimbles. Extraction was carried out in 500 ml PTFE lined Duran bottles containing 90 ml of dichloromethane with 10 ml acetone. The samples were agitated for 4 h on an orbital shaker at 200 rpm and sonicated each hour for 5 min in a sonic bath. The solvent was then poured into round bottom flasks to which 2.5 g of Cu turnings were added. The sample was evaporated down to 10 ml and passed through a silica gel glass column, prior to sample concentration (through rotary evaporation), removal of DCM and transfer into 4.9 ml of an *n*-butanol phase. Naphthalene was analysed by reverse phase high performance liquid chromatography (HPLC) (Thermo Separation product, FL, USA). The HPLC system consists of a binary pump (P2000), an autosampler (AS3000), UV–vis detector (UV1000) and an integrator (SN4000). Naphthalene was separated on an ODS-IK5 column (15 cm × 4.6 mm id., ODS2-interpak material packing, 5 μm particle diameter) by elution with 70% acetonitrile and 30% deionised water. Flow rate was 1 ml min^{-1}. Detector wavelength was set at 254 nm.

2.9. Statistical analysis

After normality testing, analyses of variance (ANOVA) were carried out on the data using the statistical package Minitab for Windows, release 15.1 (State College, PA, USA). Significant differences between treatment were assessed using least significance difference (LSD) values and are reported throughout at $P \leq 0.05$. Regression analyses were performed using SigmaPlot for Windows version 9 (Jandel Corporation, CA, USA).

3. Results

3.1. Biosensor response to naphthalene

The growth characteristics for *P. fluorescens* HK44 pUTK21 were the same as reported by Burlage et al. (1993). At naphthalene

concentrations between 0 and 15 mg l^{-1}, there was a linear response to bioluminescence (data not shown). As the concentration rose, the bioluminescence response increased greatly before peaking at 18.1 mg l^{-1}; declining thereafter. Accordingly, for the assays conducted, at concentrations exceeding 15 mg l^{-1}, a dilution step was carried out.

3.2. Soil amendment experiment: chemical and biosensor response

Two soils (Boyndie Series (a loamy sand cultivated Podzol of pH 5.2 with 2.1% organic matter) and Insch Series (a sandy loam cultivated Dystric Cambisol of pH 5.9 with 3.4% organic matter)) were used in this study. After amendment with naphthalene, the soils were extracted using a range of NEETs: water, β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPCD), 10% methanol (MeOH) and combination of cyclodextrins and methanol (Table 1). Extracting solutions were chemically analysed and tested against the biosensor *P. fluorescens* HK44. At the zero naphthalene concentration control it was observed that the NEETs alone had no stimulatory or inhibitory impact on the biosensor response with respect to the water only control.

For all of the NEETs, there was a rise in the concentration of naphthalene extracted with increased soil naphthalene concentration. For each soil amendment concentration, both of the cyclodextrin NEETs extracted more than twice that of the water extractions (Table 1). However, there was no difference in the performance of βCD and HPCD for concentrations of naphthalene up to and including 400 mg kg^{-1} in both of the soils tested. At concentrations above this, the HPCD extracted significantly more ($P \leq 0.05$) than the βCD. The order of extraction efficiency and corresponding luminescence induction, for the two soils was MeOH > HPCD > HPCD/MeOH > βCD> βCD/MeOH > water. Water consistently extracted the lowest concentration of naphthalene. For Insch soil, the MeOH/βCD extracted significantly more ($P \leq 0.05$) than the βCD at all concentrations tested. For both soils the βCD consistently extracted less naphthalene than the HPCD and this was most apparent at concentration greater than 400 mg kg^{-1} where the solution concentration reached a plateau. When soils were extracted with cyclodextrin in the presence of methanol (10%), there was no significant increase in the extraction efficiency for both soils. The 10% MeOH extraction removed significantly more naphthalene than any of the other procedures for all of the more elevated concentrations (those exceeding 200 mg kg^{-1}).

When the extracts (βCD, HPCD and 10% MeOH) were analysed with *P. fluorescens* HK44, there was a dose-dependant increase in bioluminescence with naphthalene concentration (Fig. 1a–d.). The *x*-axis has been expressed as mg kg^{-1} extractable fraction to reflect the performance of the different NEET systems (Table 1). The naphthalene was more readily extractable from the Boyndie soil (a loamy sand with 2.1% organic matter) than the Insch soil (a sandy loam with 3.4% organic matter) which had a less sandy texture and a higher organic matter content. As a consequence, the bioluminescence levels associated with Boyndie were greater than those for Insch at corresponding naphthalene amendments.

3.3. Historical soils: chemical and biosensor analysis

For the historically contaminated soils, the βCD and the MeOH–cyclodextrin combinations were not used. The concentrations of naphthalene in the ten contaminated soils ranged from 25.4 to 189.6 mg kg^{-1} and there was a dose-dependant response for *P. fluorescens* HK44 (Fig. 2a–c.). Pre-screening of the soils with metabolic biosensors showed that there were no other co-contaminants present that would have impacted on the biosensor response (data not shown). The extraction efficiency by the different NEETs was the same as observed by soil amendment experiment. Due to the low concentrations of naphthalene in the water extraction, levels of bioluminescence from the biosensor were only significantly different from the control value at concentrations exceeding 20 mg kg^{-1}, rendering this as an insensitive extraction technique (Fig. 2a). However, the HPCD and 10% MeOH methods were more suited to the soil concentrations tested in these environmental samples (Fig. 2b and c). The luminescence response to the HPCD extract had a more defined linear response (and hence easier to calibrate) than that for the MeOH, which appeared biphasic, with a steeper gradient at lower concentrations.

3.4. Historical soils: biodegradation experiment

Biodegradation is in part defined by the catabolism of the microbial population and by the bioavailability of the chemical of concern. An inoculum capable of degrading the naphthalene (*P. fluorescens* TTC1) was added to the soil and the extent of biodegradation was chemically measured (Fig. 3). The amount of naphthalene removed by the water extraction was significantly lower ($P \geq 0.05$) than the portion that was degraded, while the portion extracted by methanol was greater than the biodegradable fraction at the lower concentrations (up to 50 mg kg^{-1}). In general both the HPCD and MeOH extracted naphthalene correlated with the biodegradable fraction (Fig. 3). The measured concentration of naphthalene degraded was plotted against the bioluminescence response and this revealed a similar profile. Across the concentrations tested the luminescence response was least in the water extractions, followed by HPCD and then MeOH (Fig. 4).

Table 1
Mean concentration values (mg l^{-1}) of naphthalene in soil solutions.

Soil	Naphthalene (mg kg^{-1})	Water + NEET			Methanol + NEET		
		H_2O	βCD	HPCD	10% MeOH	10% MeOH + βCD	10% MeOH + HPCD
Boyndie	100	0.97	3.78	3.78	7.18	6.11	4.96
	200	2.30	7.89	7.95	15.24	11.12	9.69
	400	6.07	16.15	16.89	21.37	15.49	18.28
	600	8.95	14.94	26.51	35.55	13.67	30.23
	800	12.97	14.35	36.28	49.33	13.38	39.74
	LSD	***0.41***	***0.34***	***0.33***	***2.12***	***0.49***	***1.89***
Insch	100	0.54	2.31	2.61	5.74	3.49	3.41
	200	1.70	5.52	6.24	9.44	7.48	7.27
	400	4.49	11.51	13.91	18.66	15.18	13.33
	600	7.59	12.95	19.71	28.71	13.98	22.75
	800	10.95	12.00	25.19	38.12	14.27	29.59
	LSD	***0.09***	***0.26***	***5.88***	***1.59***	***0.58***	***1.04***

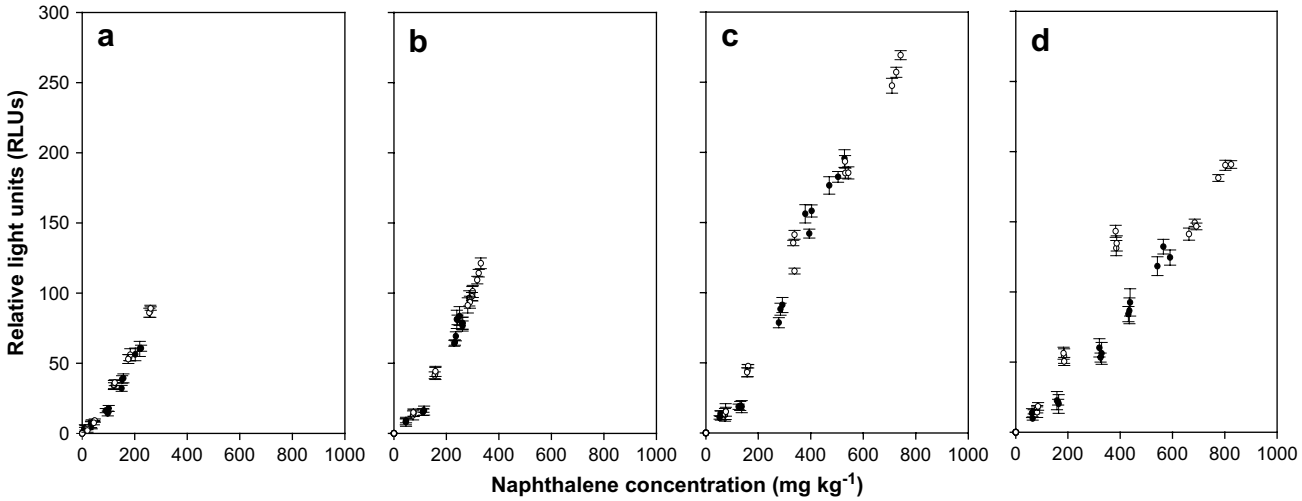

Fig. 1. The bioluminescence response (as relative light units) of *Pseudomonas fluorescens* HK44 pUTK21 to (a) water, (b) βCD, (c) HPCD and (d) methanol extracts from two soils (●) Insch and (○) Boyndie amended with a range of naphthalene concentrations. Naphthalene concentrations have been calculated as mg kg^{-1} to enable a direct comparison between the techniques used ($n = 3$, error bars represent the standard error of the mean).

4. Discussion

Over the last decade, a great deal of interest has focused on the description and measurement of the bioavailability of contaminants in soils and sediments. From an analytical perspective, it is widely acknowledged that exhaustive organic solvent extractions overestimate the bioavailable fraction and have a poor correlation with microbial degradation. This is because microorganisms can only generally assimilate a hydrophobic organic contaminant which is extractable via the aqueous phase or rapidly desorbable (Miller and Alexander, 1991) and this may be limited by mass transfer kinetics from the soil solid phase (Bosma et al., 1997). A number of studies have investigated the use of aqueous extractions as a determinant for bioavailability; however, these methods have proven unsuitable for sparingly soluble compounds, such as PAHs. Dissolution may be limited by the low solubility of the compound and, thus, an under estimation of availability will be obtained (Semple et al., 2003).

In this study, the strict use of analytical controls combined with detailed knowledge of the samples and the response of the biosensor confirmed that naphthalene was the sole agent causing the measured bioluminescence response. The study progressed from soil amendments with naphthalene to assess the bioavailability of naphthalene in historically contaminated MGP soils (before and after biodegradation). This enabled a unique appraisal of the performance of coupling biosensors with NEETs, by investigating the linkage between chemically extractable concentration of naphthalene and the induction of catabolism within the biosensor.

Bacterial biosensors have been promoted as tools in the study of bioavailability (van der Meer et al., 2004) and yet their greatest constraint is the ability to interface with the environmental matrix (Harms et al., 2006). There is no doubt that the molecular techniques exist to develop a suite of sensitive and ecologically relevant biosensors but the commonly used water extract fails to remove an adequate or meaningful environmental sample from the target matrix. For example, Tecon et al. (2006) used crystals of PAHs to assess the induction of a GFP marked bacterial biosensor. The sparingly soluble nature of phenanthrene made the assay difficult to transfer to more realistic scenarios. These authors also acknowledged that in laboratory prepared samples, *lux*-marked

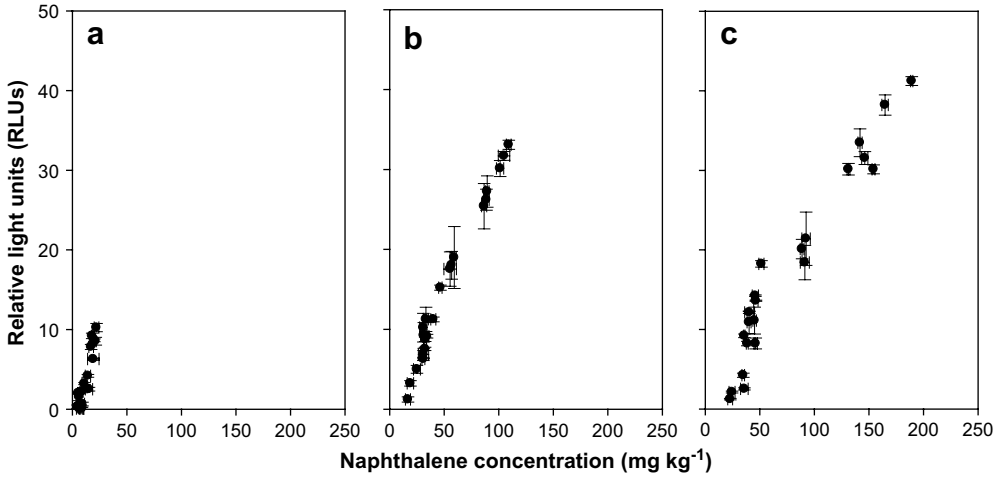

Fig. 2. The bioluminescence response (as relative light units) of *Pseudomonas fluorescens* HK44 pUTK21 to (a) water, (b) HPCD and (c) methanol extracts from a range of soils historically contaminated with naphthalene. Naphthalene concentrations have been calculated as mg kg^{-1} to enable a direct comparison between the techniques used ($n = 3$, error bars represent the standard error of the mean).

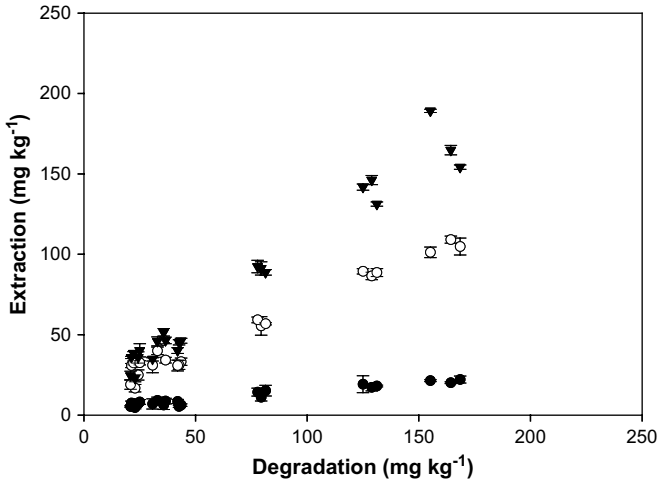

Fig. 3. The biodegradation of naphthalene (mg kg^{-1}) following inoculation with *Pseudomonas fluorescens* TTC1 in historically contaminated soils after one month plotted against extracts of (●) water, (○) HPCD and (▲) methanol from historically contaminated soils prior to degradation of naphthalene ($n = 3$, error bars represent the standard error of the mean).

sensors were more sensitive than their GFP constructs because the luciferase is highly responsive at low doses and is also sensitively quantified against background values. Biosensors must be integrated into a bioassay with an extraction step that is of ecological relevance; this is the current limitation of this application. Ho and Quinn (1993) compared a range of solvent extraction techniques for toxicity measurements using *Vibrio fischeri*. They judged acetone as the most suitable technique because those extracts had the most pronounced response to the sampled tested and there was an inferred correlation with the total chemical burden measured, but to no other biological parameter. Kelsey et al. (1997) took a more pragmatic approach by relating the extraction technique to a measured biological response, mainly mineralization or earthworm toxicity. They reported that for atrazine the best extraction predictor was butanol while for phenanthrene, it was methanol. However, at the concentrations used in their study, the extractions would require considerable dilution before testing with the biosensor. Indeed, a considerable constraint of these alcohol extractions is that they require routine quantification and a biological assay would be difficult to perform. For example, Barriuso et al. (2004) assessed atrazine bioavailability and reported that the methanol extraction closely correlated with the mineralised fraction. In this case, the design of the degradation experiment was similar to that used in this study, but unlike Kelsey et al. (1997) no other comparative solvent was considered. Bundy et al. (2001a,b) developed the use of MeOH for a range of biosensors because water was inadequate for hydrocarbon extraction, while Ho and Quinn (1993) recommended both acetone and methanol extracts be used. Indeed, Bundy et al. (2003) showed that DMSO (also considered by Ho and Quinn, 1993) had a significant impact on the chemical form of the test analytes that to some extent devalued the use of the biosensor. As a consequence of these findings, numerous authors have decided to use methanol as an extraction, not least because it interacts well with biosensors after a dilution step. Despite this, it remains apparent that this type of analysis firstly requires an extraction using an organic solvent, followed by a dilution step and, as a result, questions the linkage between the extractable concentration and bioavailability.

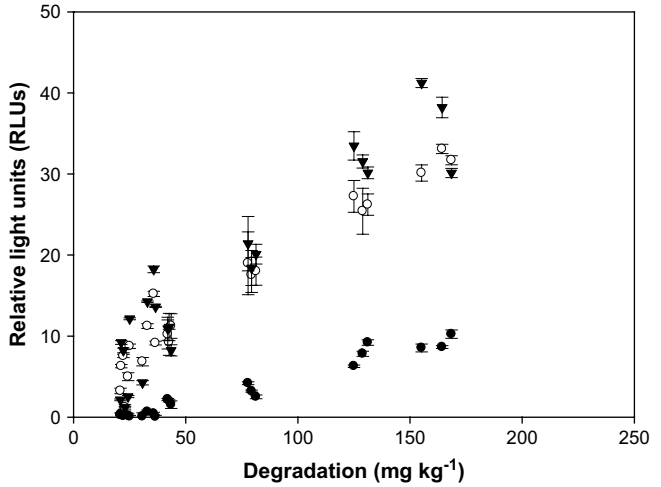

Fig. 4. The bioluminescence response (as relative light units) of *Pseudomonas fluorescens* HK44 pUTK21 in extracts of (●) water, (○) HPCD and (▲) methanol from historically contaminated soils prior to degradation plotted against the net degradation of naphthalene (mg kg^{-1}) in those soils after one month ($n = 3$, error bars represent the standard error of the mean).

In this study, the biosensor response reflected the available fraction of naphthalene in the soil, which in turn was related to the physicochemical attributes of the soil types used. Patterson et al. (2004) reported that HPCD was an ideal choice of NEET when considering coupling the analysis to a biosensor because other methods, such as Tenax TA and XAD-4, would require the application of an aqueously soluble solvent elution followed by a dilution stage. Furthermore, Patterson et al. (2004) reported poor correlation between XAD biosensor response and the fraction available for mineralization for a range of soils tested.

The response of the diluted methanol extracts in this study was similar to those of the cyclodextrin. While the methanol procedure required the use of a dilution step, the cyclodextrin extraction technique was entirely compatible with the biosensor providing a strong correlation between the amount of naphthalene extracted from the soils and the bioluminescent response. Additionally this was able to define a relationship with the biodegradable fraction. Previous and recent work (Reid et al., 2000; Doick et al., 2005, 2006; Hickman and Reid, 2005; Allan et al., 2006; Semple et al., 2006) found that the HPCD extractable fraction of phenanthrene correlated with bacterial mineralization of the PAH. Hence, HPCD represented not only the aqueous fraction of the PAH, but the fraction of the PAH that was loosely partitioned to the soil matrix yet still available for microbial degradation. Biodegradation represents a clear functional process within soil, and the direct relationship between HPCD and this process has distinct advantages. For example, Cuypers et al. (2001) showed a strong relationship between PAH degradation and HPCD extractability in PAH-contaminated sediments and, more recently, Doick et al. (2005) and Stokes et al. (2005) described the use of the HPCD extraction technique to directly predict the biodegradable fraction of PAHS in field-contaminated soils.

For the application of biosensors in environmental sampling, NEETs are an essential part of the assay. While both methanol and cyclodextrin performed well in this study, with the biosensor response relating to the concentration, each method had advantages and shortcomings. Both procedures enable significant advantages to the biosensor application and are rapid, of low cost and offer high reproducibility. The importance of this study is that this is the first report of the coupling of HPCD extraction with a bioluminescence-based biosensor, linking the chemically determined microbially available fraction with catabolic induction in the biosensor. This could well prove to be useful in the assessment of contaminated land, when deciding upon the applicability of microbial degradative strategies for remediation.

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References

Allan, I.J., Semple, K.T., Arya, R., Reid, B.J., 2006. Prediction of mono- and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction. Environmental Pollution 44, 562–571.

Allan, I.J., Semple, K.T., Arya, R., Reid, B.J., 2007. Cyclodextrin enhanced bioremediation of polycyclic aromatic hydrocarbons and phenolic compounds. Environmental Science and Technology 41, 5498–5504.

Barriuso, E., Koskinen, W.C., Sadowsky, M.J., 2004. Solvent extraction characterization of bioavailability of atrazine residues in soils. Journal of Agricultural and Food Chemistry 52, 6552–6556.

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

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

Bundy, J., Durham, D.G., Morriss, A., Campbell, C.D., Paton, G.I., 2001a. Development of QSARs to investigate the bacterial toxicity and biotransformation potential of aromatic heterocylic compounds. Chemosphere 42, 885–892.

Bundy, J.B., Campbell, C.D., Paton, G.I., 2001b. Comparison of the response of six different luminescent bacterial bioassays to bioremediation of five contrasting oils. Journal of Environmental Monitoring 3, 404–410.

Bundy, J.G., Maciel, H., Cronin, M.T.D., Paton, G.I., 2003. Limitations of a cosolvent for ecotoxicity testing of hydrophobic compounds. Bulletin of Environmental Contamination and Toxicology 70, 1–8.

Burlage, R.S., Palumbo, A.V., Heitzer, A., Sayler, G.S., 1993. Bioluminescent reporter bacteria detect contaminants in soil samples. Applied Biochemistry and Biotechnology 45-6, 731–740.

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

Dardenne, F., Smolders, R., De Coen, W., Blust, R., 2007. Prokaryotic gene profiling assays to detect sediment toxicity: evaluating the ecotoxicological relevance of a cell-based assay. Environmental Science & Technology 41, 1790–1796.

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

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

Harms, H., Wells, M.C., van der Meer, J.R., 2006. Whole-cell living biosensors - are they ready for environmental application? Applied Microbiology and Biotechnology 70, 273–280.

Heitzer, A., Webb, O.F., Thonnard, J.E., Sayler, G.S., 1992. Specific and quantitative assessment of naphthalene and salicylate bioavailability by using a bioluminescent catabolic reporter bacterium. Applied and Environmental Microbiology 58, 1839–1846.

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

Ho, K.T.Y., Quinn, J.G., 1993. Physical and chemical parameters of sediment extraction and fractionation that influence toxicity, as evaluated by Microtox. Environmental Toxicology and Chemistry 12, 615–625.

Huang, W.E., Wang, H., Zheng, H.J., Huang, L.F., Singer, A.C., Thompson, I., Whiteley, A.S., 2005. Chromosomally located gene fusions constructed in *Acinetobacter* sp. ADP1 for the detection of salicylate. Environmental Microbiology 7, 1339–1348.

Kelsey, J.W., Alexander, M., 1997. Declining bioavailability and inappropriate estimation of risk of persistent compounds. Environmental Toxicology and Chemistry 16, 582–585.

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

Kohlmeier, S., Mancuso, M., Tecon, R., Harms, H., van der Meer, J.R., Wells, M., 2007. Bioreporters: *gfp* versus *lux* revisited and single-cell response. Biosensors & Bioelectronics 22, 1578–1585.

Miller, M.E., Alexander, M., 1991. Kinetics of bacterial-degradation of benzylamine in a montmorillonite suspension. Environmental Science 25, 240–245.

van der Meer, J.R., Tropel, D., Jaspers, M., 2004. Illuminating the detection chain of bacterial bioreporters. Environmental Microbiology 6, 1005–1020.

Paton, G.I., Weitz, H., Killham, K., Semple, K.T., 2005. Biological tools for the assessment of contaminated land. Soil Use and Management 21, 487–499.

Patterson, C.J., Semple, K.T., Paton, G.I., 2004. Non-exhaustive extraction techniques (NEETs) for the prediction of naphthalene mineralisation in soil. FEMS Microbiology Letters 241, 215–220.

Reid, B.J., Northcott, G.L., Jones, K.C., Semple, K.T., 1998. Evaluation of spiking procedures for the introduction of poorly water-soluble contaminants into soil. Environmental Science & Technology 32, 3224–3227.

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

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

Semple, K.T., Dew, N.M., Doick, K.J., Rhodes, A., 2006. Does mineralisation predict the microbial availability of organic chemicals in soil? Environmental Pollution 140, 164–172.

Shaw, L.J., Beaton, Y., Glover, L.A., Killham, K., Osborn, D., Meharg, A.A., 2000. Bioavailability of 2,4-dichlorophenol associated with soil water-soluble humic material. Environmental Science & Technology 34, 4721–4726.

Stokes, J.D., Paton, G.I., Semple, K.T., 2005. Behaviour and assessment of the bioavailability of organic contaminants in soil: relevance for risk assessment and remediation. Soil Use and Management 21, 475–486.

Trang, P.T.K., Berg, M., Viet, P.H., Van Mui, N., Van Der Meer, J.R., 2005. Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples. Environmental Science & Technology 39, 7625–7630.

Tecon, R., Wells, M., van der Meer, J.R., 2006. A new green fluorescent protein-based bacterial biosensor for analysing phenanthrene fluxes. Environmental Microbiology 8, 698–708.

Werlen, C., Jaspers, M.C.M., van der Meer, J.R., 2004. Measurement of biologically available naphthalene in gas and aqueous phases by use of a *Pseudomonas putida* biosensor. Applied and Environmental Microbiology 70, 43–51.