

## **Effect of fertiliser formulation and bioaugmentation on biodegradation and leaching of crude oils and refined products in soils**

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## **Abstract**

The effects of soil characteristics and oil types as well as the efficacy of two fertiliser formulations and three bioaugmentation packages in improving the bioremediation of oil-contaminated soils were assessed as a means of ex-situ treatment selection and optimisation through seven laboratory microcosm studies. The influence of bioremediation on leaching of oil from the soil was also investigated. The studies demonstrated the benefits of biostimulation to overcome nutrient limitation, as most of the soils were nutrient depleted. The application of both liquid and pelleted slow-release N and P fertilizers increased both the hydrocarbon biodegradation rates (by a factor of 1.4 to 2.9) and the percentage of hydrocarbon mass degraded (by > 30% after 12 weeks and 80% after 37 weeks), when compared to the unamended soils. Slow release fertilisers can be particularly useful when multiple liquid applications are not practical or cost effective. Bioaugmentation products containing inoculum plus fertilizer also increased biodegradation by 20% to 37% compared to unamended biotic controls; however there was no clear evidence of additional benefits due to the inocula compared to fertiliser alone. Therefore biostimulation is seen as the most cost effective bioremediation strategy for contaminated soils with the levels of crude oil and refined products used in this study. However, site-specific considerations remain essential for establishing the treatability for oil-contaminated soils.

**Keywords:** bioremediation, fertiliser, bioaugmentation, crude oil, refined petroleum products, microcosm.

## 1. Introduction

It has been estimated that there are around 292,000 hectares of land affected by contamination in England and Wales, associated with a wide range of former contaminative uses [1]. Some of these sites may present an unacceptable risk to human health and/or the environment, for which there is a requirement for remediation [2]. Civil engineering-based remediation methods have commonly been used within the UK. However, the implementation of the Landfill Directive [3], which includes requirements for the pre-treatment of wastes and separation of 'hazardous' and 'non-hazardous' waste streams, together with a desire for more sustainable remediation [4], is influencing remediation practice.

Petroleum hydrocarbons continue to be a widely utilised global resource. Their use has resulted in accidental spillage and leakage [2,5]. Bioremediation has a long and broadly successful history of application in the UK [6,7,8] for hydrocarbons and other biodegradable contaminants. Regulatory approvals for on-site bioremediation are now facilitated by the provision of well-organised and compelling data (and other accompanying evidence) from pilot trials to support professional regulatory judgements on the capacity of an oil impacted soil to respond to engineered bioremediation [9].

Judgement is required, for example, on (i) the compositional characteristics of the oil and thus the behaviour (e.g., solubility, volatility, sorption) or recalcitrance of the source term; (ii) the feasibility of bioremediation, given initial concentrations of hydrocarbon compounds in the soil (the source term); (iii) the likely rate of biotransformation, given the proposed engineering process and any novel amendments and/or bulking agents claimed to quicken biotransformation; (iv) the possible residual hydrocarbon composition and concentration following treatment, in the context of acceptable residual concentrations of each component; (v) the time available for bioremediation in the context of the site operations and/or redevelopment timescale; (vi) the likely release of volatile biotransformation products, such as odours or hazardous vapours (e.g., [10]), during bioremediation particularly in residential areas; and (vii) the sustainability of bioremediation in comparison to other remediation options [4,11].

This paper describes a series of seven laboratory microcosm bioremediation studies that were performed over several years to investigate controls and influences on bioremediation effectiveness. They are

evaluated to extract information relating to issues of feasibility and optimisation of bioremediation strategies for soils contaminated with crude oil and refined oil products. Such an analysis serves as a valuable guide to those responsible for selecting and approving bioremediation options. Our discussion therefore seeks to highlight the types of judgements required to evaluate bioremediation strategies for oil-contaminated soils.

## **2. Materials and Methods**

An overview of the experimental setup of the seven microcosm experiments is provided in Table 1.

### ***2.1 Tests done prior to bioremediation studies***

#### ***2.1.1 Soil characteristics***

For all soils, moisture content, pH, water holding capacity (WHC), particle size, organic carbon, nitrate, phosphate, and ammonium content were determined. The methods used were as follows. Moisture content was measured by reweighing duplicate samples of 20 g soil wet weight after drying at 105 °C for 24 hours [12]. Soil pH was measured in a distilled water slurry (1 part soil: 2 parts water) after a 30 minute equilibration period [12]. Maximum water holding capacity (WHC) was determined in duplicate by flooding the wet weight equivalent of 100 g dry soil in a filter funnel and allowing it to drain overnight [12]. Particle size analysis was performed by combination of wet sieving (sand) and sedimentation (silt and clay) as described by Gee and Bauder [13]. Organic carbon content was analysed by potassium dichromate oxidation as described by Schnitzer [14]. Soil (10 g) was extracted in 0.5 M potassium bicarbonate (adjusted to pH 8.5) and analysed by high performance ion chromatography (HPIC) for nitrate [15] and phosphate [16] and colourimetrically for ammonium [17].

### ***2.1.2 Rate and extent of degradation of nine types of oils in soil microcosms (Experiment A)***

The rate and extent of degradation of nine different oils was assessed in soil microcosms under equivalent conditions (Table 2). The oils selected represent the broad range of oils encountered at integrated refineries and were typical of contaminant-source terms considered for bioremediation.

The soil was collected from an uncontaminated site and was air dried and homogenised by screening through a 2-mm sieve. The soil was mixed in a 1:1 ratio with sharp building sand to improve drainage. Triplicate microcosms were then established in sterile 50 ml serum bottles containing 10 g of soil aliquots mixed with 100 mg of the respective oil. Microcosms were established for each time point so that they could be destructively sampled. The water content of the soil was adjusted to 50 % WHC using a fertiliser solution containing  $25.7 \text{ g l}^{-1} \text{ NH}_4\text{NO}_3$  and  $21.4 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$  dissolved in sterile water, applied to achieve a C:N:P ratio of 100:10:1. Triplicate controls were also established using 1 ml of mercuric chloride ( $\text{HgCl}_2$ ) solution at 2% w/w (equivalent to  $2 \text{ mg HgCl}_2 \text{ g}^{-1} \text{ soil}$ ) to re-hydrate the soil instead of fertiliser solution (to assess the abiotic losses). All microcosms were incubated aerobically at  $20^\circ\text{C}$  in darkness at high humidity, for a total of 30 weeks and sampled at 0, 2, 4, 6, 8, 12, 20 and 30 weeks for total petroleum hydrocarbons (TPH) analysis as described in the section 2.4.

### ***2.1.3 Determining the levels of mercuric chloride required for effective soil sterilisation and the non-inhibitory levels of fertiliser required for bioremediation (Experiment B)***

Bioremediation experiments often include use of killed control to assess non-biological losses. Typical soil sterilising agents used in laboratory microcosms are sodium azide ( $\text{NaN}_3$ ) and  $\text{HgCl}_2$  [18,19]. The latter is preferred for long-term (months/years) experiments because the former breaks down and loses efficacy over a period of weeks [20]. However, the choice of sterilising agent concentration is often arbitrarily taken from values in the literature and may fail to completely eliminate microbial activity, thereby rendering the killed control invalid [19]. In addition the efficacy of the killed controls is rarely assessed. In the same manner, fertiliser application is usually based on the theoretical C:N:P ratio 100:10:1 of a microbial cell [21,22] but rarely really takes into account the fact that excessive fertiliser

application can be inhibitory due to toxic or osmotic effects. Thus there is a need for a rapid measurement method to optimise the application of both mercuric chloride and fertiliser. Feasibility of using soil oxygen uptake as a rapid measurement tool was tested on three different soils collected from the shallow soil layers (30 cm depth) of three petroleum sites. Stones and large pieces of plant debris were removed through a 2 mm sieve. The soil samples were stored at 4 °C and at the water content occurring in the field to keep the losses of microbial biomass to a minimum until conducting oxygen uptake measurement. The physicochemical characteristics of the soils are presented in Table 3.

The effect of HgCl<sub>2</sub> concentration and volume application was initially investigated using a loam soil (10 g) amended with a glucose solution (5 mg g<sup>-1</sup> dry soil) 24 hours prior to the addition of 1.7 ml HgCl<sub>2</sub> solution (HgCl<sub>2</sub> concentration ranging from 0.01 % w/v to 5 % w/v). Glucose was chosen as a rapidly degradable carbon source to enable the efficacy of HgCl<sub>2</sub> to be assessed quickly (in hours/days as opposed to days/weeks for oil). Further it is reasonable to assume that if HgCl<sub>2</sub> inhibits glucose degradation, oil degradation will be inhibited too.

Subsequent tests on the contaminated soils were carried out on duplicate soil samples (10 g) without glucose amendment. Samples were dosed with 5% w/v HgCl<sub>2</sub> at inputs of 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3 and 0.4 ml g<sup>-1</sup> dry soil which were equivalent to 0.5, 1, 1.5, 3, 5, 10, 15 and 20 mg HgCl<sub>2</sub> g<sup>-1</sup> dry soil, respectively. The goal was to determine the dose of HgCl<sub>2</sub> solution at 5% (w/v) required for inhibiting microbial activity in the three soils investigated.

A second set of duplicate soil samples (10 g) was amended with nine fertiliser solution concentrations to assess the soil tolerance to an excessive fertiliser application (Table 4). Each soil sample was then incubated overnight at room temperature. The oxygen uptake rate was then monitored over several hours by differential manometry on a Gilson respirometer.

#### ***2.1.4 Determining the effect of bulking agents on bioremediation (Experiment C)***

Bulking agents such as straw, bark chips, and compost are frequently employed to improve aeration, drainage and the physical handleability of contaminated soils that are deemed candidates for

bioremediation. Here a sandy clay loam soil was homogenised and mixed with sharp sand as previously described in section 2.1. Duplicate microcosms were established in shallow trays using 2.5 kg of soil, into which Brent Crude oil was mixed to achieve a final concentration of 9,400 mg-TPH kg<sup>-1</sup>. Four microcosm conditions were established as follows: (i) soil mixture + 225 ml of 5% (w/v) HgCl<sub>2</sub> equivalent to 4.5 mg HgCl<sub>2</sub> g<sup>-1</sup> dry soil (to assess abiotic losses); (ii) un-amended soil mixture (to assess intrinsic biodegradation); (iii) soil mixture + sterilised wet chopped straw (250 g) and fertiliser powder (1 g) (to assess the effect of the bulking agent), and; (iv) soil mixture + sterilised wet pine bark (250 g) and fertiliser powder (1 g) (to assess the effect of the bulking agent). The fertiliser powder used consisted of NH<sub>4</sub>NO<sub>3</sub> and NaH<sub>2</sub>PO<sub>4</sub> in a 4:1 ratio and was applied to achieve a C: N: P ratio of 100:10:1 (please note that we have not reported the studies in chronological order and this one was done before Experiment B where we became aware of the inhibitory effect of high levels of fertiliser). All the trays were adjusted to 50 % WHC using 225 ml sterile water and incubated aerobically in darkness at 25 °C, for 84 weeks with sampling at week 0, 4, 8, 12, 24, 48, and 84 for soil physicochemical and hydrocarbon analyses.

## **2.2 Bioremediation trial studies**

### ***2.2.1 Biostimulation trials: determining the efficacy of two fertiliser formulations (liquid and pelleted slow release fertiliser) in improving the biodegradation of two oily wastes and the extent of leaching of the oily wastes (Experiment D and E)***

The efficacy of two different types of fertiliser formulations in improving the biodegradation of two oily wastes was assessed using duplicate microcosm experiments. The oily-waste samples were oil based mud (OBM) and tank bottom sludge (TBS) collected from an oil storage tank. Before use, both oily wastes were mixed with dry wood chips (1:1 ratio) to aid oxygen transfer and drainage. The microcosms were then established in sterile shallow glass trays using 1 kg of the OBM or TBS mixture. Four experimental treatments were set up as follows: (i) oiled mixture + 91 ml of 5 % w/v HgCl<sub>2</sub> equivalent to 4.5 mg HgCl<sub>2</sub> g<sup>-1</sup> soil (killed abiotic control); (ii) oiled mixture + 91 ml sterile distilled water (intrinsic biodegradation); (iii) oiled mixture + liquid fertiliser, and; (iv) oiled mixture + pelleted slow-release fertiliser and sterile water. The liquid fertiliser solution used contained 1.5 g l<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 0.25 g l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> and, 0.25 g l<sup>-1</sup>

K<sub>2</sub>HPO<sub>4</sub> which was applied at a C:N:P ratio of 100:1:0.1 and adjusted to a pH 7. This ratio was used as preliminary experiments showed that the oil degradation rate declined at C:N:P ratios greater than the range 100:1:0.1 to 100:1:0.2 (data not shown). Pelleted slow-release fertiliser was applied to achieve a C:N:P ratio of 100:2:0.2. This ratio was the closest equivalent to the liquid fertiliser application rate that could be achieved due to the size of the pellets and the scale of the experiment. The microcosms were incubated aerobically at 25 °C in darkness for a total of 12 weeks and sampled at 0, 2, 4, 8 and 12 weeks for soil physicochemical and hydrocarbon analyses.

Leaching tests on the two oily wastes were also performed to evaluate the amount of benzene, toluene, ethyl benzene and xylenes (BTEX) that may leach from the oily wastes, both prior to and after 12 weeks of bioremediation (Experiment E). Tests were performed in duplicate by mixing and shaking the oily wastes with ultra pure water containing 5 % w/w HgCl<sub>2</sub> in completely filled (no headspace) 50 ml Teflon centrifuge tubes for 72 hours followed by centrifugation at 3000 rpm for 15 minutes. The leachate was analysed for BTEX by purge and trap gas chromatography. Measurement was performed in duplicate.

### ***2.2.2 Bioaugmentation trials: the efficacy of three commercial packages to promote the bioremediation of hydrocarbon-contaminated soils (Experiment F)***

The efficacy of three commercial bioaugmentation packages ( $\alpha$ ,  $\beta$  and  $\gamma$ ) was assessed for their ability to promote the bioremediation of hydrocarbon-contaminated soils in microcosm experiments. The bioaugmentation package characteristics were as follows: ( $\alpha$ ) microbial inoculum + mineral nutrient solution + natural surfactant of plant origin; ( $\beta$ ) microbial inoculum + mixture of biodegradable surfactants (nonylphenol ethoxylates and alkoxylated linear alcohols) and ( $\gamma$ ) microbial inoculum + mineral nutrient solution + biocatalyst solution. Each of the bioaugmentation packages were used according to the manufacturer's instruction. Duplicate microcosms were established in 500 ml sterile glass jar containing 350 g (dry weight) of either a sandy soil freshly contaminated with diesel (+ package  $\alpha$ ) or a sandy soil contaminated with weathered crude oil (+ packages  $\beta$  and  $\gamma$ ). The WHC of the soils was adjusted to 65 %. The following duplicate controls using the same conditions were also established: (i)



killed control (soil + 31.5 ml of 5 % (w/v)  $\text{HgCl}_2$  which equalled to  $4.5 \text{ mg HgCl}_2 \text{ g}^{-1}$  soil, to determine whether any abiotic degradation was occurring), (ii) soil + 31.5 ml distilled sterile water (to determine intrinsic biodegradation) and (iii) soil + fertiliser solution (to compare with biostimulation approach). The liquid fertiliser consisted of  $23.8 \text{ g l}^{-1} \text{ NH}_4\text{NO}_3$ ,  $2.3 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$  and  $2.1 \text{ g l}^{-1} \text{ K}_2\text{HPO}_4$ , applied at a C:N:P ratio 100:1:0.1. Microcosms were incubated aerobically at  $15^\circ\text{C}$  in the dark for 52, 64 and 150 weeks for packages  $\alpha$ ,  $\beta$  and  $\gamma$ , respectively. Microcosms were sampled for soil physicochemical and hydrocarbon analyses at 0, 3, 5, 10, 14, 23, 34 and 52 weeks for package  $\alpha$ ; 0, 3, 6, 10, 27, 42, 52 and 64 for package  $\beta$ ; and 0, 3, 6, 12, 18, 29, 32, 40, 50, 56, 62, 79, 90, 100, 112, 131 and 150 weeks for package  $\gamma$ .

### ***2.2.3 Bioremediation studies on soil contaminated with crude oil (Experiment G)***

The extent to which crude oil contaminated soil could be cleaned by bioremediation was investigated in microcosms established in 500 ml sterile glass jars containing 350 g of a contaminated sandy soil with crude oil ( $44\,000 \pm 932 \text{ mg-TPH kg}^{-1}$  dry soil) amended with 21 ml of either 5% w/v  $\text{HgCl}_2$  which equalled to  $3 \text{ mg HgCl}_2 \text{ g}^{-1}$  dry soil (killed control), sterile distilled water (intrinsic bioremediation), or fertiliser solution composed of  $\text{NH}_4\text{NO}_3$  ( $23.8 \text{ g l}^{-1}$ ),  $\text{KH}_2\text{PO}_4$  ( $2.1 \text{ g l}^{-1}$ ) and  $\text{K}_2\text{HPO}_4$  ( $2.1 \text{ g l}^{-1}$ ) (biostimulation) equivalent to a C:N:P ratio of 100:1.7:0.17. This ratio was used as we observed that the oil degradation rate declined at C:N:P ratios greater than 100:1:0.2 (data not shown).

The volume added of 21 ml corresponded to the amount of water required to bring the soil from its content on collection to 50% of field capacity. Since all three treatments must have the same soil water content, this figure also determined the volume of liquid in which any fertiliser or  $\text{HgCl}_2$  addition could be made.

The soil pH and water saturation were adjusted to 6.8 and 64%. Each treatment was set up in triplicate. TPH concentration, pH and microbial activity (respiration) were determined for each treatment on a regular basis up to 37 weeks of bioremediation. Further samplings were made at week 50, 69, 84, 95, 116, 157, 175 and 196 where only chemical analysis was carried out to assess the extent of biodegradation.

## 2.4 Chemical analyses

The studies reported here were performed over an extended time-scale (3 months to 4 years; Table 1). As a result, several different extraction and analysis techniques were used (Table 5). Generally the samples were extracted either by Soxhlet (acetone: hexane, 1:1) or ultrasonic extraction (Freon™-113).

Depending on the experiment, the extracts were then used to gravimetrically determine the content of total petroleum hydrocarbons (TPH), aliphatic, aromatics and 'oil and grease' fractions. TPH content was analysed using infra-red spectroscopy (IR) and/or gas chromatography with flame ionisation detection (GC-FID). GC-FID was used to characterise the oil at the start and end of the experiment and IR used throughout to monitor degradation.

The Soxhlet extracted samples were extracted in standard Soxhlet apparatus using 300 ml acetone: hexane in a 1:1 ratio for 16 hours. The ultrasonically extracted samples were chemically pre-dried with 10 g of anhydrous sodium sulphate (thermally treated overnight at 120 °C and stored in a desiccator prior to use) and ultrasonically extracted using Freon™-113 (20 ml) for 30 minutes in a Decon™ FS200 sonication bath. Samples were thoroughly mixed and allowed to settle overnight in the dark. The extracts were then shaken for 1 hour and left to settle before the solvent was decanted. This process was then repeated a further two to four times depending upon the sample. Polar compounds were removed using Florisil™ (30-60 mesh, previously heat treated at 500 °C stored in a desiccator and activated prior to use by the addition of 6 % w/w distilled water). The aliphatic and aromatic fractions were separated using column chromatography after the drying and re-dissolving of the Freon extract in hexane. Where required samples were blown down to dryness under a gentle stream of nitrogen prior to gravimetric analysis or re-dissolving into an appropriate solvent for further analysis.

The TPH content was analysed in all samples by Fourier transform infrared (FTIR) spectroscopy on Freon extracts using a Perkin Elmer 881 IR scanning spectrophotometer. Samples were scanned at wavenumbers between 3200 and 2700  $\text{cm}^{-1}$  against a solvent blank. The peak heights at wavenumbers 2960, 2930 and 2860  $\text{cm}^{-1}$  were summed and the concentration of the TPH was determined by reference to a standard curve prepared with diesel ranging from 0 to 500  $\mu\text{g ml}^{-1}$ .

The TPH, aliphatic and aromatic extracts were analysed by gas chromatography using a HP 5890A GC-FID fitted with a HP 7673 autosampler. The column used was a Chrompack™ fused silica capillary column (30 m x 0.32 mm x 0.12µm), onto which 1µl of sample was injected in split ratio mode (17:1) at 250 °C. Different temperature programmes were used for the aliphatic and aromatic fractions. The initial oven temperature for the aliphatic programme was 80 °C held for 5 minutes before being raised to 300 °C at a rate of 5 °C per minute then held for 15 minutes. The initial oven temperature for the aromatic programme was 90 °C held for 5 minutes before being raised at a rate of 5 °C per minute to 160 °C, then raised to 300 °C at a rate of 10 °C per minute, and held for 17 minutes. Mixed n-alkanes and polycyclic aromatic hydrocarbons (PAHs) were analysed concomitantly for calibration purposes.

## **2.5 Monitoring**

During the experimental period of all the microcosms, the soil water evaporation was adjusted by the addition of sterile water. The pH, ammonium, nitrate, phosphate and moisture content were determined as described in section 2.1. Soil respiration was monitored at 25 °C by differential manometry on a Gilson respirometer.

## **3. Results and Discussion**

### ***3.1 Rate and extent of degradation of various oils in soil microcosms***

After 30 weeks 70-82% of the crude, diesel and gas oils, 57% of the lubricating oil and 45% of the Brightstock had disappeared (Table 2). There was negligible disappearance of the heavy dewaxed base oil and Ondina 68 marine oil (9%) (Table 2). The fact that some disappearance was also observed in the killed controls for some of the oils which would not have been expected to volatilise much under the incubation conditions (diesel, the gas oils and lube oil), indicates that the killed controls were not 100% effective and that some biodegradation had taken place in the abiotic controls. It is therefore reasonable to conclude that most of the disappearance observed for these light-medium, medium and heavier oils was due to biodegradation. It is likely however that some of the losses from the light crudes were due to

volatilisation, but how much cannot be ascertained, because the losses from the ineffective killed controls would have been due to a combination of biodegradation and volatilisation. However, it is well-known that low to medium molecular weight petroleum hydrocarbons are readily biodegradable [23,24], so it is reasonable to consider the losses from the two light crudes to be a good indication of their biodegradability when compared to the other oils in this study.

These results confirm those of others, namely that lighter oils degrade faster and more extensively than heavier oils [2,23,25,26]. Such studies have indicated that the extent of oil biodegradation is affected most by the oil type rather than aging processes (biotic or abiotic weathering and incorporation of contaminants deep into soil pores) of the source term [27]. Thus even weathered light oil (e.g. gasoline or diesel) is likely to be relatively easier to bioremediate than fresh (unweathered) heavy oil (e.g. lube oil).

### ***3.2 Determining the levels of mercuric chloride for soil sterilisation and the fertiliser tolerance of soils prior to bioremediation studies***

As a result of the uncertainty over the efficacy of the killed controls and hence the interpretation of the results in the Experiment A, the effect of  $\text{HgCl}_2$  concentrations and volume application for soil sterilisation was investigated. Similarly the effect of fertiliser application was investigated as information on excessive fertiliser application is rarely discussed in bioremediation studies.

In both cases optimising concentration and volume application is likely to be one of the key factors in the efficacy of any addition, since the larger the volume the easier it will be to uniformly distribute the added chemicals. However, balance between adding enough volume to ensure homogenous distribution without over wetting a soil will vary from soil to soil and may even vary between different batches collected at different times of the year. Therefore measurement of oxygen uptake as a rapid measurement method for estimating microbial activity in soil has been used to optimise the levels of  $\text{HgCl}_2$  and N and P fertilisers used in bioremediation experiments.

The preliminary experiment using glucose amended loamy soil showed that oxygen consumption was completely inhibited at  $\text{HgCl}_2$  concentration  $\geq 1\%$  w/v equivalent to  $1.7 \text{ mg HgCl}_2 \text{ g}^{-1}$  dry soil (Figure

1a). Therefore all subsequent experiments were conducted using a 5% w/v  $\text{HgCl}_2$  solution (close to saturation) to ensure excess inhibitor. While  $\text{HgCl}_2$  solubility is reported as 7.4% w/v in water at 20 °C [28], it is worthy to note that we found  $\text{HgCl}_2$  was often falling out in solution at 7% w/v and therefore not practical for use in the killed control.

The dose of  $\text{HgCl}_2$  solution at 5% (w/v) required to completely inhibit oxygen consumption in the amended loamy soil was  $\geq 0.6$  ml which equals to 3 mg  $\text{HgCl}_2 \text{ g}^{-1}$  dry soil (Figure 1b). The same approach taken with the three unamended soils showed that oxygen consumption of the sandy soil was easily inhibited with a volume application of 0.3 ml of 5%  $\text{HgCl}_2$  solution equivalent to 1.5 mg  $\text{HgCl}_2 \text{ g}^{-1}$  dry soil (Figure 1b). In contrast, it was not possible to completely inhibit the oxygen consumption in the loam and peat soil (Figure 1b). The discrepancy between the glucose amended and unamended loam results suggests that the use of glucose amendment to speed up the test cannot be recommended.

The peaty soil which had the highest organic carbon content (12%) proved to be the most resistant to  $\text{HgCl}_2$  with 10 % of the oxygen uptake activity remaining after treatment (Figure 1b). Long-term bioremediation experiments (20 weeks) indicated that no satisfactory killed controls could be obtained with soil having a high native organic carbon content (data not shown). Previous studies demonstrated that  $\text{HgCl}_2$  sterilisation generally results in minimal changes in soil properties compared with other techniques [18,19] and may maintain a sterile environment for a long time [20,29]. Our results further demonstrated that the concentration of  $\text{HgCl}_2$  into the soil should be as high as possible especially when the soil had appreciable native soil organic carbon content (SOC). Since large amount of  $\text{HgCl}_2$  couldn't be added, the highest concentration of  $\text{HgCl}_2$  solution (here 5% w/v) was used and the volume added was as much as 50% of the soil's water holding capacity allowed. In addition it is strongly recommended that verification of the efficacy of the killed controls should be carried out for the duration of the experiment by subjecting sub-samples of the killed controls to respirometry testing. Shall it be not the case, there is not much that can be done but in our experience this approach is effective for most soils from industrial sites, as they have inherently low native SOC levels. While the peaty soils are problematic they are rarely encountered at industrial sites. This approach was used for all the subsequent studies reported in this paper.

The response of the three soils to fertiliser application rates was similar but their sensitivity to higher application rates varied (Figure 2). Oxygen consumption was stimulated in all soils when fertiliser was applied at low concentration indicating that the addition of nutrients (N/P) was effective in stimulating microbial activity and alleviating the N and/or P supply limitations. The maximum fertiliser loadings tolerated by the three soils (based on a C:N:P ratio of 100:10:1) before the oxygen consumption started to decline were 0.2, 0.8 and 3 mg g<sup>-1</sup> dry soil, equivalent to soil water concentrations of 1.2, 2.1 and 5 g l<sup>-1</sup> respectively for the sandy, loamy and peaty soil (Figure 2 and Table 3). Since all soils were brought to 60% of their field capacity, they all contained different amounts of water per gram dry weight basis. The field capacity of the peaty soil was 1.7 and 4.8 times greater than of the loamy and sandy soil, respectively (Table 3). Consequently for a given fertiliser application rate, the concentration of N and P in the sandy soil was approximately 3 and 5 times greater than in the loamy and peaty soils, respectively (Table 4). This explains why the peaty soil was the least sensitive and the sandy soil was the most sensitive to high fertiliser application. This finding further suggests that the optimisation of fertiliser application should be assessed based on the resulting concentration in soil solution rather than the theoretical N & P demand based on the typical 100:10:1 C:N:P ratio of a microbial cell grown in laboratory culture. The latter assumes that hydrocarbon degradation results in cell growth, but this needs not be the case.

Inhibition of oxygen consumption ranged from 40-70%, indicating that some microbial activity and therefore hydrocarbon degradation was possible at these higher fertiliser application rates, albeit at a lower rate than the lower fertiliser concentrations.

Overall the effects of HgCl<sub>2</sub> and fertiliser on microbial activity in soil can be rapidly assessed (within 24 hours) by comparing the oxygen consumption rates of the soil in presence or absence of the chemicals prior to setting up long-term bioremediation experiments. This approach therefore enabled an informed selection of inhibitor and fertiliser levels for the subsequent bioremediation experiments and increased the chances of running successful long-term bioremediation.

### ***3.3 Influence of bulking agent type on bioremediation efficiency***

Bulking agents have frequently been used during bioremediation to improve soil structure, break up and mix the soil, lower the bulk density and increase porosity to facilitate better transfer of oxygen, water and nutrients into the soil and enhance removal of oily products [30-31]. Bulking agents also increase the surface area of contaminated soil exposed to microbial processes by increasing the surface to volume ratio [31,32].

Here the use of bulking agents was expected to enhance bioremediation, as others have demonstrated. However, no additional beneficial effects attributable to the addition of straw or pine bark was observed compared to biostimulation alone after 84 weeks (Figure 3). This result may be due to the fact that the soil was initially mixed with sharp building sand which was sufficient to improve drainage and oxygen transfer. Another possible explanation for the lack of effect of the bulking agents may be that the oil became bound to the bulking agents and consequently less available for biodegradation. Pine bark was the least effective at enhancing degradation of the aromatic fraction (Figure 3). Presumably pine bark had a high lignin content, which being an aromatic polymer of random phenylpropane subunits can strongly absorb aromatic compounds and make them unavailable for bioremediation. Namkoong *et al.* [34] also reported that bulking agents can represent a preferential carbon source and can be degraded in preference to target compounds, which may account for the reduced degradation seen here.

### ***3.4 Biostimulation treatments: comparison of liquid and pelleted slow release fertilisers efficacy***

Oil-contaminated soils naturally contain a high level of carbon as an energy source for microbial growth, but often require the addition of N and P containing fertilisers to alleviate N and P limitation [23]. Here, the application of nitrogen and phosphorous contained in the two types of fertilisers rapidly stimulated the microbial activity of all oily waste microcosms (data not shown). This was evidenced by the increase of oxygen consumption which peaked in the region of 80-100 mg oxygen kg<sup>-1</sup> OBM h<sup>-1</sup> after 4 days and thereafter slowly declined to 40 mg kg<sup>-1</sup> OBM h<sup>-1</sup> as the readily degradable carbon sources in the wood chips were degraded and possibly other factors became limiting. In contrast, the respiration rates in the

TBS fertilised microcosms increased rapidly and stabilised in the region of 120-160 mg oxygen kg<sup>-1</sup> TBS h<sup>-1</sup> after 10 days.

Gas chromatography analysis further confirmed that TPH reduction in all fertilised treatments was due to biodegradation as considerable reduction in the numbers and concentration of the resolvable hydrocarbon peaks was observed as well as a dramatic reduction in the C17:pristane ( $T_0 = 0.71$  and  $T_{8\text{weeks}} = 0.04$ ; 95% degradation) and C18:phytane ( $T_0 = 3.4$  and  $T_{8\text{weeks}} = 0.14$ ; 96% degradation) ratios. In contrast, TPH reduction in the killed controls and unfertilised microcosms (Figure 4) was primarily due to volatilisation and sorption as no changes in the n-alkanes/isoprenoid ratios were observed.

In the first two weeks, the TPH content of TBS and OBM were reduced by 50% (28 000 to 14 000 mg kg<sup>-1</sup>) and 34% (90 000 to 60 000 mg kg<sup>-1</sup>), respectively in all fertilised treatments (Figure 4). However the TPH degradation rate of TBS and OBM was 1.3 and 2 times higher respectively when the pelleted slow release fertiliser was used instead of the liquid fertiliser. Subsequently biodegradation appeared to cease in the liquid fertiliser treated microcosms (week 8) but still continued at a much slower rate in the pelleted slow release fertiliser treated microcosms such that the TPH concentration of TBS and OBM declined to 7500 and 26 000 mg kg<sup>-1</sup>, respectively after 12 weeks. This result suggests that the pelleted slow release fertiliser can be advantageous over the liquid fertiliser as all required nutrients can be added at the start of bioremediation and be effective several weeks compared to just few weeks for liquid formulation. In addition liquid fertiliser has to be added at lower doses and more frequently to prevent fertiliser toxicity to the oil degrading microorganism, soil over wetting and leaching losses which can potentially result in groundwater contamination with nitrate.

As the degradation halted in the liquid fertiliser treated microcosms, more fertiliser was added at week 8. However, no major increase in TPH biodegradation compared to the slow release fertiliser microcosms was observed between week 8 and 12 (Figure 4), indicating that the slow TPH decline after the first month represents the degradation of the less readily biodegradable hydrocarbons in TBS and OBM, rather than a N or P limitation. The extent of hydrocarbon biodegradation is strongly dependent on the nature of the contaminant, the soil chemistry and the partitioning of specific compounds into the aqueous phase where the biological uptake occurs readily [5,31,35,36,37]. In some cases, bioavailability is relatively



unimportant, while in others it may be critical. Therefore the influence of site-specific compound bioavailability on bioremediation of hydrocarbon products must be considered.

### ***3.5 Influence of bioremediation on leaching***

Although the water solubility of most petroleum hydrocarbons is very low, a small number (e.g. BTEX) do possess sufficient solubility in water to potentially cause a risk to groundwater and surface water as a result of leaching [38,39]. BTEX compounds are readily biodegradable, so bioremediation can be a very effective means of managing the risk to groundwater and surface waters, by removing these leachable hydrocarbons from the contaminated matrix.

Prior to bioremediation, the leachate of the OBM contained 20 - 100  $\mu\text{g l}^{-1}$  of individual BTEX compounds and the leachate of the TBS contained 90 - 240  $\mu\text{g l}^{-1}$  of individual BTEX compounds (Figure 5). Mixing the OBM and TBS with wood chips had no significant effect on leachable toluene, ethylbenzene and xylenes, but benzene in both leachates was reduced in the presence of wood chips. This reduction could either have been due to volatilisation of the benzene while the OBM and TBS were mixed with the wood chips or sorption of the benzene by the wood chips. The fact that benzene is somewhat more volatile than TEX, but is less prone to sorption to organic matter [40] suggests that volatilisation is the most likely explanation. The apparent lack of significant sorption of BTEX compounds to the wood chips is contrary to what other workers have reported [32] and may reflect the lack of sufficient contact time between adding the wood chips and mixing them with the OBM and TBS prior to solvent extraction which took less than 15 minutes.

After 12 weeks of bioremediation BTEX concentrations in the leachate of the fertilised treatments were < 1  $\mu\text{g l}^{-1}$  with one exception where toluene in the TBS leachate was 5  $\mu\text{g l}^{-1}$  (Figure 6). Reduction of BTEX in the leachate was also observed in the killed controls (although not to the same extent as in the fertilised live treatments). Since respiration measurements demonstrated that the killed controls were effectively sterilised, the reductions in leachable BTEX in the killed controls were due to volatilisation, sorption or a

combination of the two over the 12 weeks incubation period. These results confirm those of Chaîneau *et al.* [41], namely that bioremediation is very effective at managing the risk of leaching to groundwater posed by soils containing petroleum hydrocarbons with appreciable solubility in water.

### ***3.6 Bioaugmentation trials: benefits and limitations***

Some workers have claimed that in some situations an indigenous microbial population may not be sufficient for bioremediation of a soil and as such it may be necessary to add cultured hydrocarbon degrader organisms [42]. Proprietary inocula either immobilised on a carrier with or without an accompanying surfactant, or used on their own can be used to augment the indigenous microbial community in contaminated soils. Claims have been made for shifting the microbial community structure to a consortium more able to transform the hydrocarbon substrate, or able to degrade hydrocarbons more quickly [29,43,44].

In the present study the bioaugmentation packages improved bioremediation performance compared to natural attenuation processes alone, but gave no additional benefit compared to application of a fertiliser solution alone (Figure 7). Indeed, the fertiliser treatment (biostimulation) performed even better than the bioaugmentation packages  $\beta$  and  $\gamma$  used with a weathered oil-contaminated sandy soil. In the case of bioaugmentation package  $\alpha$ , it was similarly effective as fertiliser alone on the sandy soil freshly contaminated with diesel. Therefore, any claimed benefit of bioaugmentation package is debateable as the positive effect seems to be due to the fertiliser component of the package rather than the microbial inoculum. In addition, no adjustment of the concentration and rate of mineral nutrients applied with the bioaugmentation packages can be made and consequently be optimised according to the initial soil conditions.

Microbial consortium addition has also been shown to have little effect on increasing degradation of petroleum hydrocarbons [30,45] and in some cases may impede bioremediation efficiency [46]. Indeed, a view that is now being expressed by many as a general rule is that addition of microbial cultures is ineffective at enhancing biodegradation as in most cases the indigenous microbial population is better

adapted to the soil conditions and may perform better than a foreign consortium [45,47,48] which is supported by the results of the current investigation.

### ***3.7 The limits to bioremediation of soil contaminated with crude oil***

One of the key questions of remediation technologies for the clean-up of contaminated land is the final concentration of contaminants that can be reached. Here the performance of biostimulation as a means of cleaning soil contaminated with crude oil has been evaluated over an extended time period (196 weeks). Gas chromatography analysis of the hydrocarbons extracted by Soxhlet showed that the crude oil components ranged between C<sub>12</sub> and C<sub>26</sub>. The absence of heavier hydrocarbons and the presence of many resolvable hydrocarbons indicated that the oil contamination was amenable to treatment by bioremediation and a high degree of clean-up could be expected. After an initial rapid decline in TPH concentration during the first two weeks (from 43 000 to 29 000 mg kg<sup>-1</sup>) in the unamended and killed microcosms, there were little changes in the amount of TPH measured in the two treatments after 37 weeks (Figure 8). Microbial respiration confirmed the effectiveness of the killed controls thus the initial loss was mainly attributed to volatilisation. GC traces of the aliphatic and aromatic fractions of the unamended microcosms at week 37 also showed that bioremediation was minimal in the absence of fertiliser. This result clearly demonstrated that the soil was severely limited by a shortage of available N and P.

Following the addition of fertiliser the TPH declined from 43 000 to 4000 mg kg<sup>-1</sup> over 37 weeks (Figure 8a). As evidenced by the killed control, 72% of the TPH decrease was attributed to biodegradation. Bioremediation appeared to have come to a halt after 37 weeks despite GC traces indicating that there was still some potentially biodegradable material left in the soil. The soil analysis further indicated that it was unlikely that bioremediation was limited by adverse pH or by mineral nutrient limitations (data not shown). It was therefore possible that hydrocarbon biodegradation was still taking place in the soil but at a much lower rate, such that it would only be detected over a longer timescale. The regular measurements of TPH by gas chromatography confirmed this hypothesis as the residual TPH concentration was 520 mg

kg<sup>-1</sup> after 196 weeks (Figure 8b). By extrapolating the data, an average degradation rate of the oil in the fertilised soil was estimated to be 150 mg-TPH kg<sup>-1</sup> day<sup>-1</sup> until 37 weeks which was 1.7 times higher than in the unamended soil (88 mg-TPH kg<sup>-1</sup> day<sup>-1</sup>) and then decreased to 4 mg-TPH kg<sup>-1</sup> day<sup>-1</sup> until week 196. It is possible that some of the remaining 520 mg TPH kg<sup>-1</sup> contained a fraction recalcitrant to biodegradation due to reduced bioavailability [24]. The decrease of the bioavailability may result from: incorporation of the hydrocarbons into the soil natural organic material due to the chemical oxidation reactions; slow diffusion into very small pores; sorption onto natural organic matter; or formation of semi-rigid films around non aqueous-phase liquids causing a high resistance to the mass transfer [49]. Bioavailability can be a significant contributory factor determining the extent of mass reduction achievable by bioremediation. However, the objective of bioremediation should not be mass reduction *per se*, but risk reduction / management. Hydrocarbon residues in soil which are not bioavailable are unlikely to pose a significant risk to human health or the environment.

#### 4. Conclusions

Overall the studies suggest that the success of bioremediation, considering both biostimulation and bioaugmentation approaches, remains largely dependent on the form of oil present and the soil structure characteristics. The individual studies presented identified:

- (1) For the majority of the contaminated soils investigated addition of mineral nutrients played an essential role, without which significant bioremediation did not occur in a number of cases.
- (2) Pelleted slow-release fertilisers were shown to be an effective alternative to liquid fertilisers.
- (3) Stimulation of indigenous hydrocarbon-degrading populations by the addition of N and P containing fertilisers was a highly effective means of achieving bioremediation of oil-contaminated soils.
- (4) Bioaugmentation of soil with hydrocarbon-degrading microorganisms in the form of commercially available packages had no significant effect on the rate and extent of degradation of any of the crude oils or refined petroleum products used in this study.

- (5) Bioremediation is highly effective at degrading the more water soluble components in oil and thereby reducing leaching of water soluble hydrocarbons (*e.g.* BTEX) from the contaminated soils to groundwater.

Clearly, the design of an efficient bioremediation process always requires a careful site assessment taking into account the physical, chemical and biological properties of the contaminated sites in order to establish appropriate response and recovery methods.

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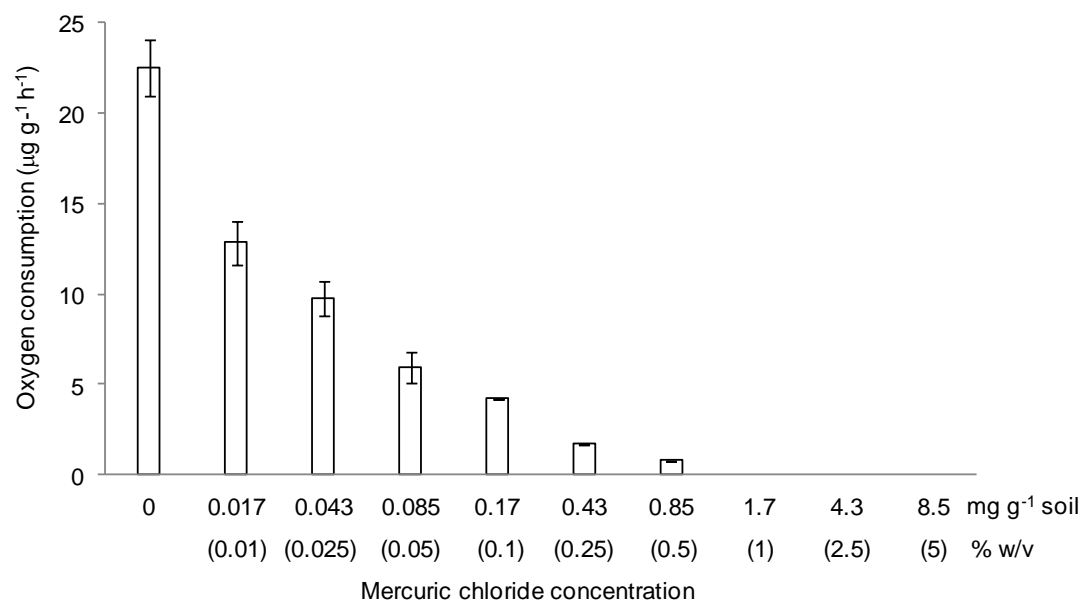
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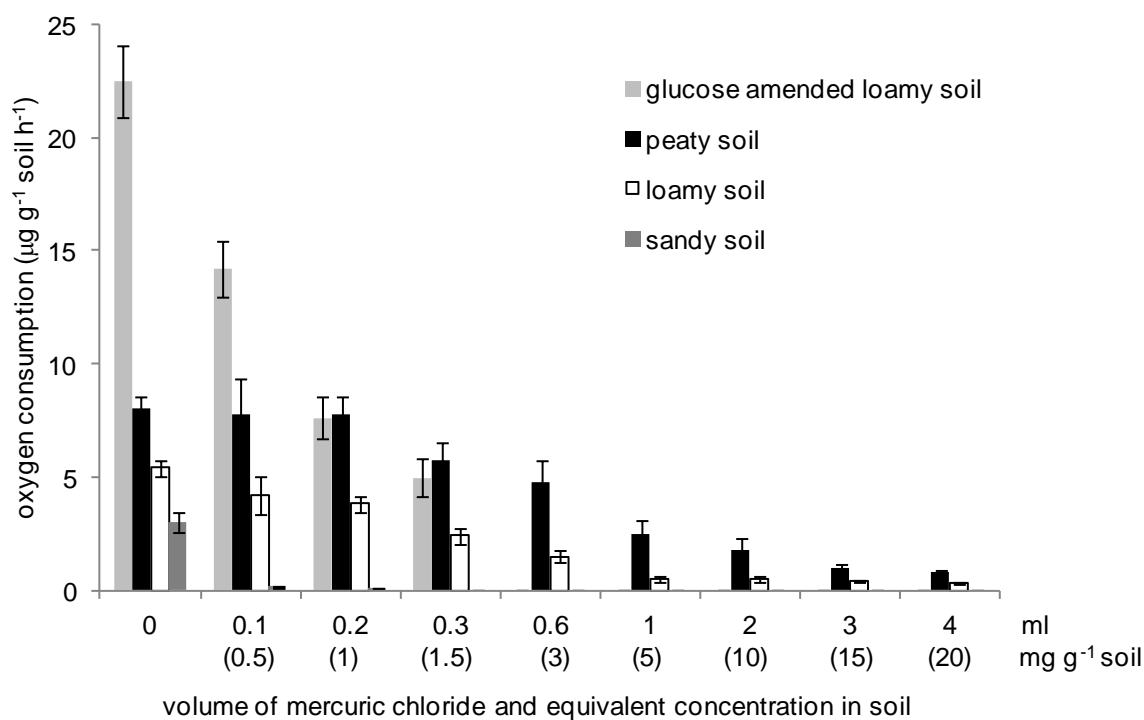


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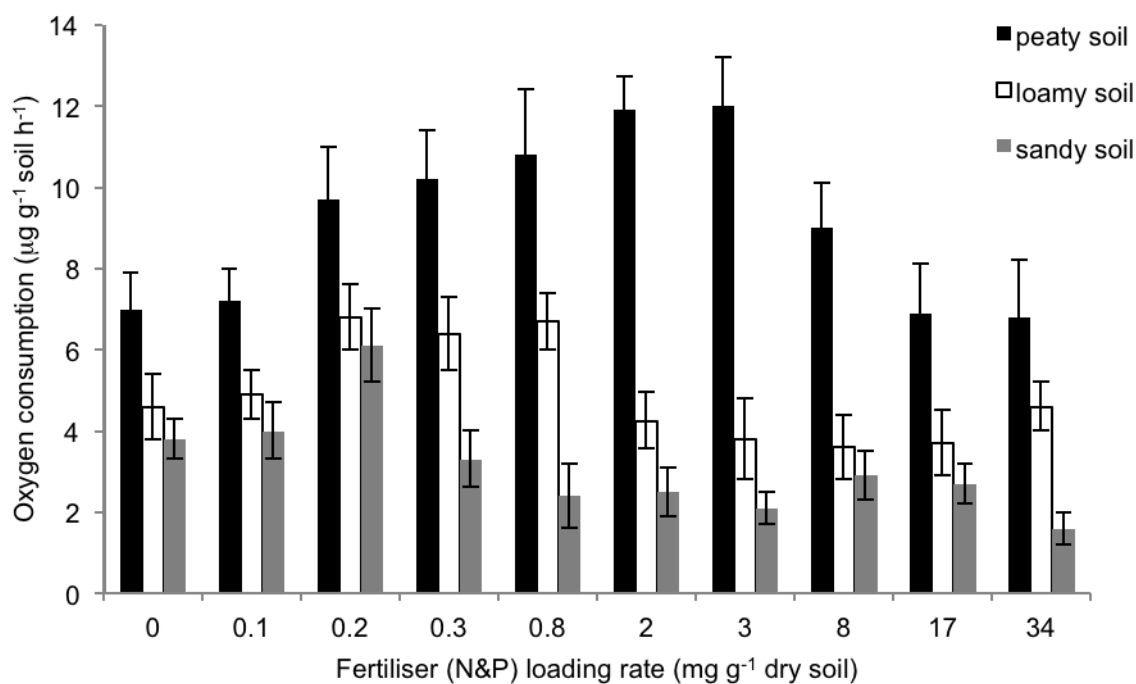
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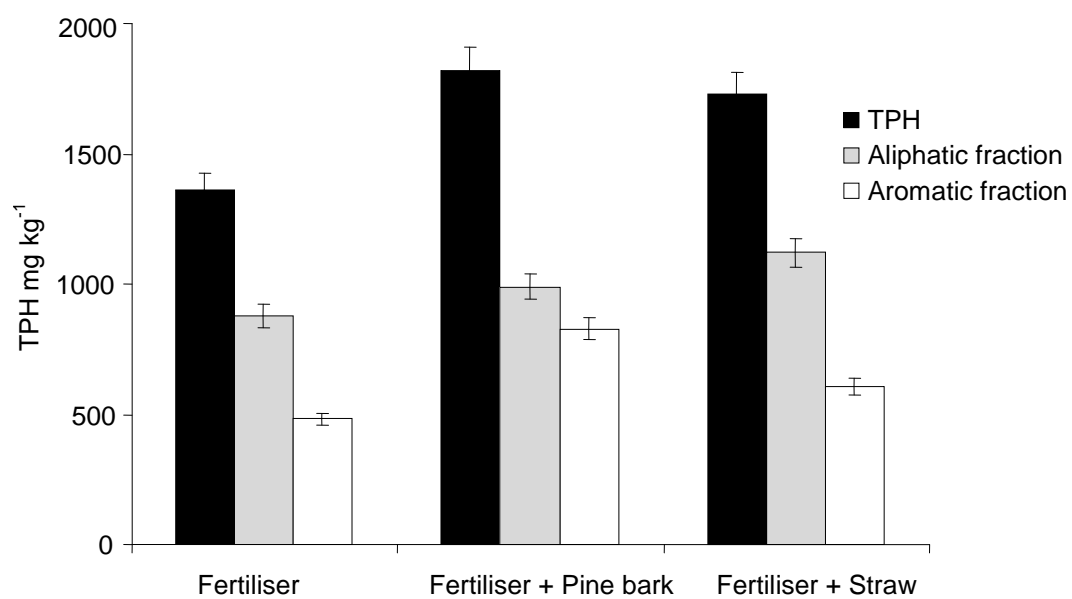
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**Figure 1:** Effect of mercuric chloride concentration on soil microbial respiration after 24 hours.(a. preliminary test on 10 g loamy soil amended with glucose to assess quickly HgCl<sub>2</sub> concentration required to inhibit microbial respiration; b. determination of the volume of 5% w/v HgCl<sub>2</sub> solution required to inhibit microbial respiration in oil contaminated soils)

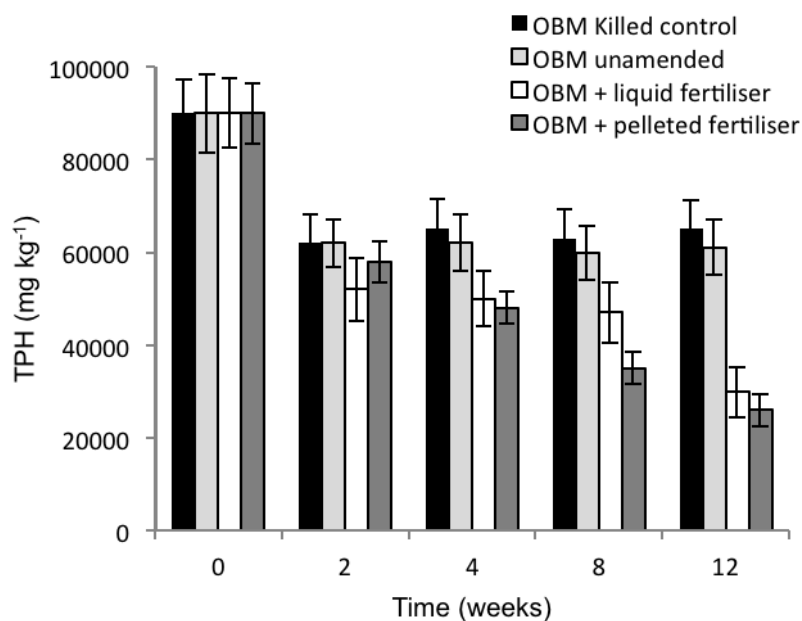


**Figure 2:** Effect of fertiliser application rates on the microbial respiration in three oil-contaminated soils after 24 hours. (The concentration of fertiliser in soil was the theoretical amount required for complete bioremediation of the oil assuming a C:N:P ratio of 100:10:1; Table 3).

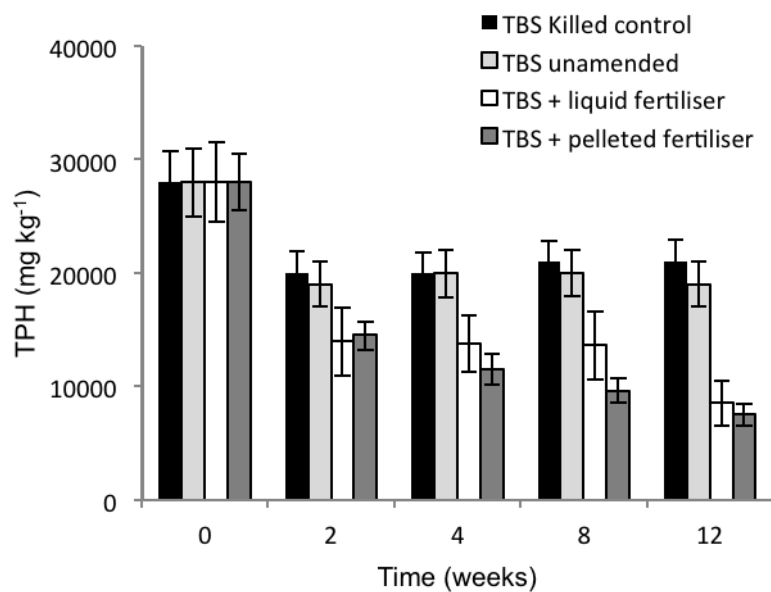


**Figure 3:** Influence of the bulking agents on TPH, aliphatic and aromatic fractions degradation after 84 weeks of bioremediation. (The initial concentration of TPH, aliphatic and aromatic fractions was 9400, 7140 and 1970 mg kg<sup>-1</sup>, respectively).

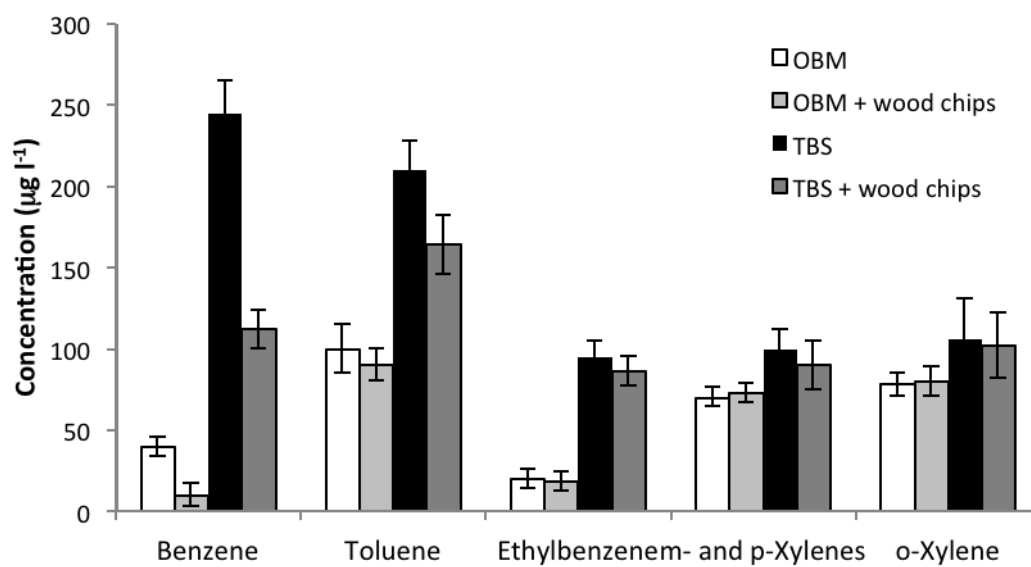
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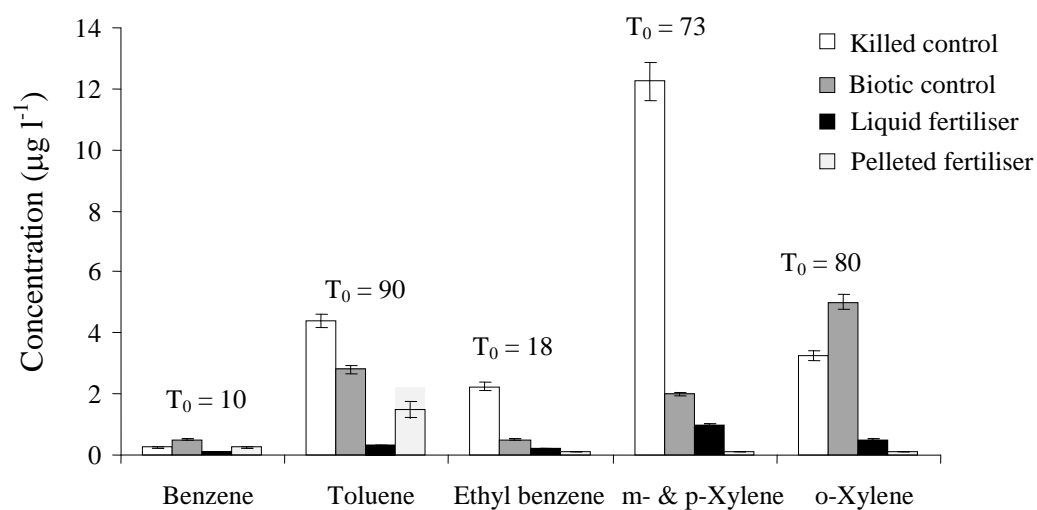
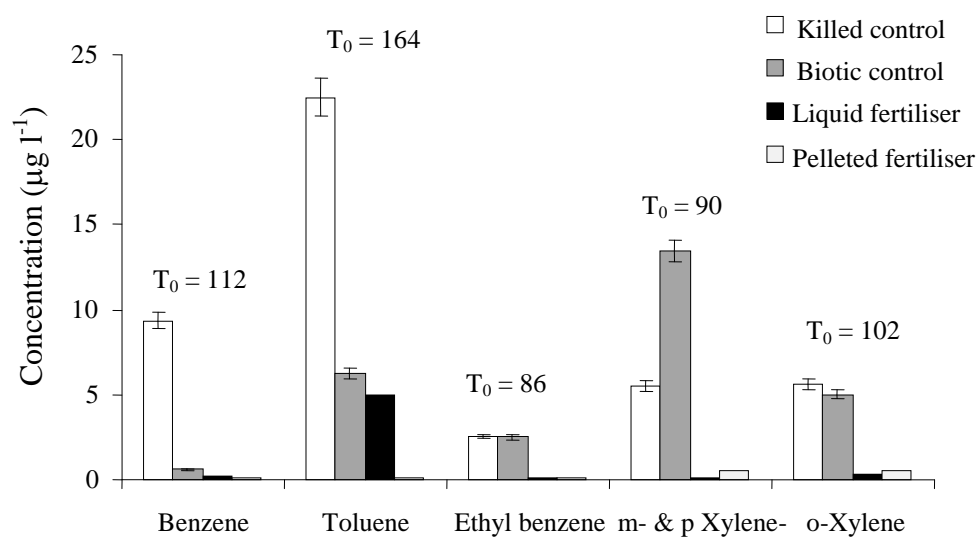
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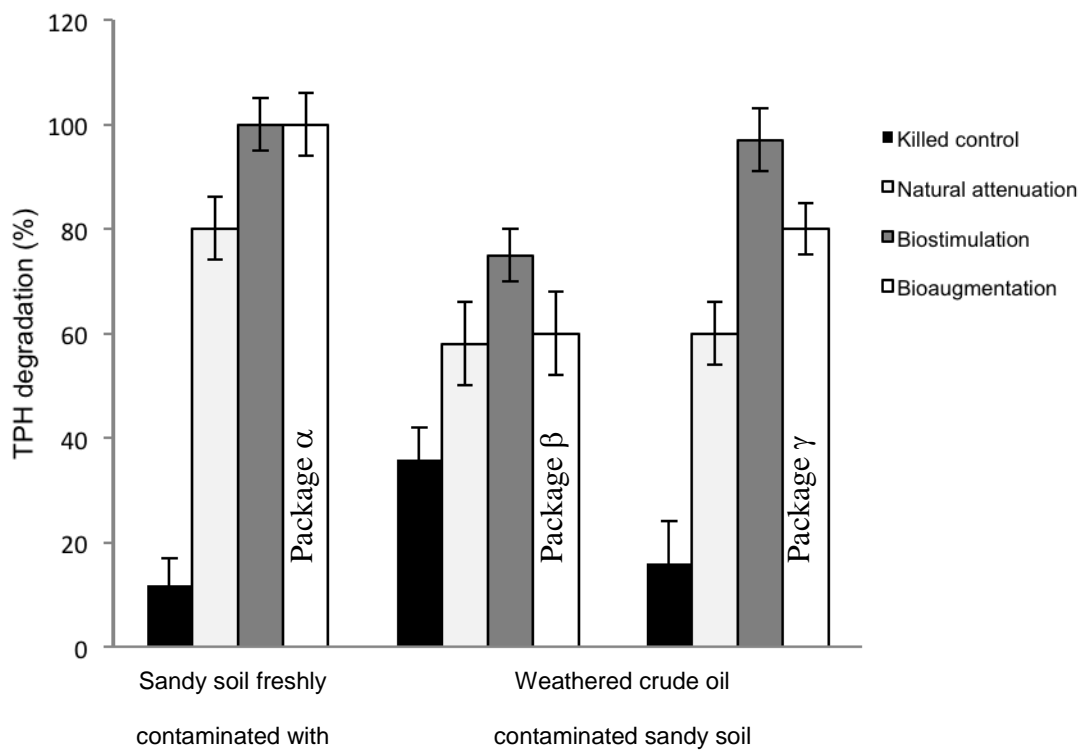
**Figure 4:** Comparison of the efficacy of the liquid fertiliser vs. the pelleted slow release fertiliser on TPH biodegradation from (a) oil based mud (OBM), and (b) tank bottom sludge (TBS) over a 12 week period. (The liquid fertiliser was reapplied after week 8 as the TPH degradation was observed to plateau).



**Figure 5:** Leaching of BTEX from OBM and TBS with and without wood chips prior remediation

**A****B**

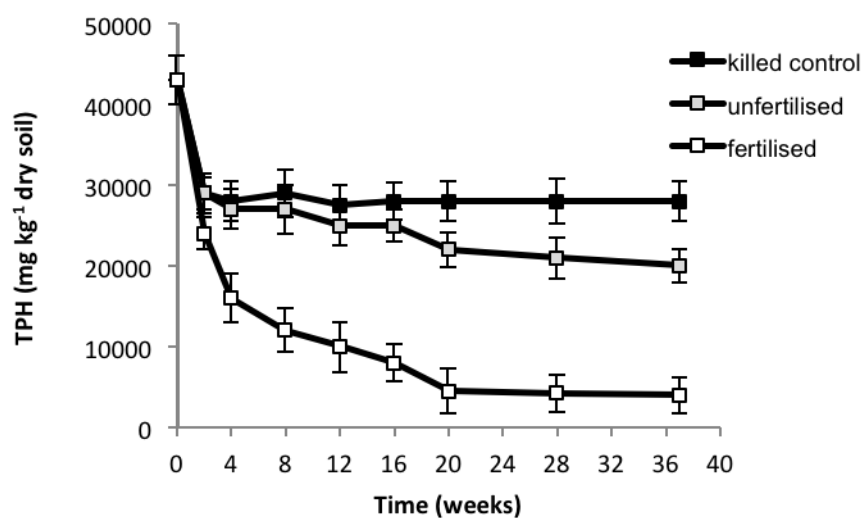
**Figure 6:** Leaching of BTEX from OBM (A) and TBS (B) after 12 weeks of bioremediation. (T<sub>0</sub> = concentration of individual BTEX compounds from the oily wastes mixed with wood chips before bioremediation).



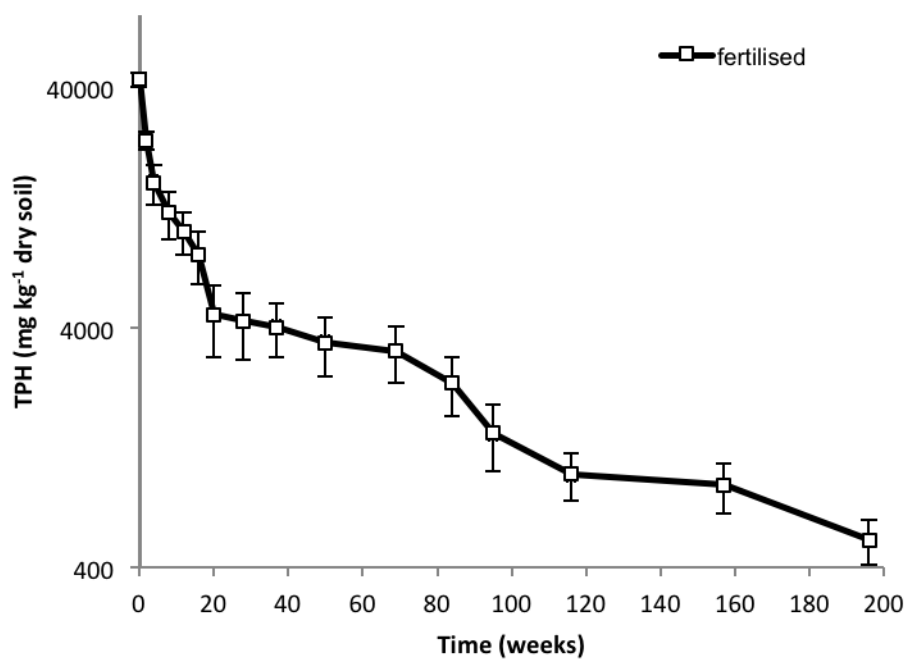
**Figure 7:** Effect of bioaugmentation vs. biostimulation on the extent of TPH degradation at the end of 52, 64 and 250 week trials for packages  $\alpha$ ,  $\beta$  and  $\gamma$ , respectively.



A



B



**Figure 8:** Long-term evaluation of the effect of biostimulation on TPH degradation (A) over the first 37 weeks and (B) until 196 weeks for the fertilised soil. (Panel B: Presentation of the TPH data on a logarithmic scale as the data cover a large range of values).

**Table 1:** Outline of the experimental set up of the seven microcosm experiments

Investigation			Duration (weeks)	Controls		Fertiliser		Treatments	
				<i>Abiotic</i>	<i>Biotic</i>	<i>Liquid</i>	<i>Pelleted</i>	Bulking agent	Augmentation
Oil type	A	Uncontaminated soil spiked with one of nine different oils	30	✓		✓		1:1 ratio with sharp building sand for all treatments	
Mercuric chloride and fertiliser levels	B	Sandy, loam and peaty soils	24 hours	✓	✓	✓			
Bulking agent	C	Sandy loam soil spiked with Brent crude oil	84	✓	✓	✓		Fertiliser treatment bulked with either sterilised straw or pine bark	
Fertiliser type	D	OBM & TBS	12	✓	✓	✓	✓	Dry wood chips in 1:1 ratio for all treatments	
Leaching	E	OBM & TBS	12	✓	✓	✓	✓	Dry wood chips in 1:1 ratio for all treatments	
Bioaugmentation	F	Two weathered and one freshly oil contaminated soils with crude oil and diesel, respectively	52*, 64** and 150***	✓	✓	✓			Three different commercial bioaugmentation packages ( $\alpha^*$ , $\beta^{**}$ and $\gamma^{***}$ )
Bioremediation end-point	G	Sandy soil contaminated with crude oil	37 (extended to 196 weeks for chemical analysis)	✓	✓	✓			

OBM: oil based mud; TBS: tank bottom sludge

**Table 2:** Characteristics, removal rates and percentage removal of nine different oil types (Experiment A)

Sample	Description	Oil Type	Removal rates (mg kg <sup>-1</sup> day <sup>-1</sup> )			Removal (%) <sup>#</sup>	
			2 weeks	6 weeks	20 weeks	20 weeks	30 weeks
<b>1. Diesel oil</b>	Commercial grade summer diesel available from retail filling station	Light - medium	295	107	39	61 [30]	82 [36]
<b>2. Brunei crude oil</b>	Light crude oil, suitable for production of gasoline and middle distillates	light	107	51	30	54 [22]	77 [34]
<b>3. Brent crude oil</b>	Light crude oil, suitable for production of gasoline and middle distillates	light	277	120	45	72 [29]	80 [32]
<b>4. Gas oil</b>	A Burmah-Castrol/CONCAWE standard, light oil for biodegradation testing method development (CAS 64741-90-8)	Light - medium	304	176	66	74 [35]	82 [40]
<b>5. XHVI gas oil</b>	Comparatively biodegradable, light oil for biodegradation testing method development (lube 2613)	Light - medium	223	113	50	68 [15]	70 [25]
<b>6 Lubricating oil</b>	Automotive multi grade engine oil (Castrol GTX)	medium	188	79	31	56 [12]	57 [30]
<b>7. 150 SN Dewaxed finished base oil</b>	A Burmah-Castrol/CONCAWE standard, middle range lubricant base oil	medium-heavy	<1	30	4	9 [<1]	9 [<1]
<b>8. 1200 SN Brightstock</b>	A Burmah-Castrol/CONCAWE standard heavy, de-asphalted distillation residue (CAS 64741-95-3)	heavy	68	110	32	42 [8]	45 [10]
<b>9. Ondina 68</b>	A marine oil of very low biodegradability (ST 85/128).	heavy	<1	15	4	9 [<1]	9 [<1]

<sup>#</sup> Numbers in brackets denotes % removal attributed to abiotic loss

**Table 3:** Physicochemical characterisation of the soils

	Sandy soil	Silty-clay-loam	Peaty soil
Sand (%)	93	20	29
Silt (%)	4	54	33
Clay (%)	3	26	38
Organic carbon (%)	0.3	3.4	22
Total nitrogen (%)	0.2	2.4	12
pH	8.2	6.2	7.3
Water content on collection (ml g <sup>-1</sup> )	0.07	0.17	0.54
Field capacity (ml g <sup>-1</sup> )	0.23	0.67	1.12
TPH (mg kg <sup>-1</sup> )	7042	7187	6550

**Table 4:** Concentration of fertiliser components in soils (w/w) and soil solution (w/v) based on a C:N:P ratio of 100:10:1

Oil concentration in soil (mg carbon equivalent g <sup>-1</sup> soil)	Fertiliser concentration mg g <sup>-1</sup> soil d.w.			Fertiliser concentration in soil solution (g l <sup>-1</sup> )								
				Sandy soil			Silty-clay-loam			Peaty soil		
	N	P	N+P	N	P	N+P	N	P	N+P	N	P	N+P
100	29	5	34	204.3	35.7	240	71.5	12.5	84	42.7	7.5	50
50	14	3	17	102.2	17.9	120	35.8	6.25	42	21.4	3.75	25
25	7	1	8	51.1	8.95	60	17.9	3.12	21	10.7	1.88	13
10	3	0.5	3	20.4	3.57	24	7.15	1.25	8	4.27	0.75	5
5	1.4	0.3	2	10.2	1.79	12	3.58	0.63	4	2.14	0.38	2.5
2.5	0.7	0.1	0.8	5.11	0.90	6	1.79	0.31	2	1.07	0.19	1.3
1	0.3	0.05	0.3	2.04	0.36	2.4	0.72	0.13	0.9	0.43	0.08	0.5
0.5	0.14	0.02	0.2	1.02	0.18	1.2	0.36	0.06	0.4	0.21	0.04	0.25
0.25	0.07	0.01	0.1	0.51	0.09	6	0.18	0.03	0.2	0.11	0.02	0.13

N: NH<sub>4</sub>NO<sub>3</sub>; P: KH<sub>2</sub>PO<sub>4</sub> plus K<sub>2</sub>HPO<sub>4</sub>

**Table 5:** Extraction and analysis techniques employed for each microcosm study

Experiment	Sample preparation				Analysis		
	<i>Extraction</i>		<i>Cleaning</i>	<i>Class Fractionation</i>	GC-FID*	IR*	P/T-GC
	Soxhlet	Ultrasonic	Florisil	Column chromatography			
<b>A</b>		✓	✓	✓	✓	✓	
<b>C</b>	✓	✓	✓	✓	✓	✓	
<b>D</b>		✓	✓	✓	✓	✓	
<b>E</b>							✓
<b>F</b>		✓	✓		✓	✓	
<b>G</b>	✓		✓	✓	✓		

GC-FID: gas chromatography coupled to flame ionisation detector; IR: Infra red spectroscopy; P/T GC: purge and trap coupled to gas chromatography

# Effect of fertilizer formulation and bioaugmentation on biodegradation and leaching of crude oils and refined products in soils

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