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# When is a soil remediated? Comparison of biopiled and windrowed soils contaminated with bunker-fuel in a full-scale trial

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**Abstract**

A six month field scale study was carried out to compare windrow turning and biopile techniques for the remediation of soil contaminated with bunker C fuel oil. End-point clean-up targets were defined by human risk assessment and ecotoxicological hazard assessment approaches. Replicate windrows and biopiles were amended with either nutrients and inocula, nutrients alone or no amendment. In addition to fractionated hydrocarbon analysis, culturable microbial characterisation and soil ecotoxicological assays were performed. This particular soil, heavy in texture and historically contaminated with bunker fuel was more effectively remediated by windrowing, but coarser textures may be more amendable to biopiling. This trial reveals the benefit of developing risk and hazard based approaches in defining end-point bioremediation of heavy hydrocarbons when engineered biopile or windrow are proposed as treatment option.

Capsule: Windrows outperform biopiles in the bioremediation of bunker oil contaminated soils

***Keywords*:** Bunker fuel, windrows, biopiles, bioremediation, soil ecotoxicology

## 1. Introduction

On site ex situ techniques such as windrow turning and biopiling rely on the management of the biodegradative potential of microorganisms to render hydrocarbons less toxic through mineralisation, biotransformation and assimilation (Barr, 2002). A benefit of bioremediation is the broad scope of petroleum fuel compounds amenable to the process, including branched and unbranched chain aliphatic and aromatic compounds (Chatterjee et al., 2008). Using windrow turning, a site contaminated with high concentrations of diesel range organics (DRO, $C_6$-$C_{24}$) was remediated from an initial TPH concentration of 10,000 mg $kg^{-1}$ to less than 1,000 mg $kg^{-1}$ within six months (Barr, 2002).

In this study the petroleum hydrocarbon was No. 6 fuel oil (referred to as bunker C fuel oil) a grade of residual fuel for marine engines. Bunker C has been reported to be the least susceptible to biodegradation in a study carried out by Walker *et al.*, (1976) where the biodegradation of two crude and two fuel oils were compared. However in a large-scale study, Comeau *et al*. (1991) reported the successful biological treatment of 21,475 $m^3$ of bunker fuel contaminated soil within 12 to 14 weeks under active management. Initial TPH concentrations of 6,000 mg $kg^{-1}$ decreased to less than 1,000 mg $kg^{-1}$ during that study. This observation is in contrast with Song *et al*., (1990) who reported that bioremediation of bunker C was very slow and incomplete and that the initial steady rate of degradation ceased after 8 weeks. It was concluded that some bunker C constituents were structurally resistant to biodegradation.

Windrows require the frequent turning of soil to enhance aeration, homogenise pollutant sources, nutrients and degraders and to accelerate bioremediation. Biopiles require forced aeration and often use complementary suction of soil gas to feed air to the microbial community. Both are managed under similar regulatory frameworks and can be conducted on site but they require careful water management to optimise the degradation rate. Continuous monitoring of soil hydrocarbon concentration, oxygen level and qualitative and quantitative microbial community changes commonly complement management strategies (Chatterjee et al., 2008). To assess bioremediation as an active process, a measure of the number of hydrocarbon degraders present in a sample is required. Despite moving towards molecular approaches, there is still a great reliance upon culture based techniques including most probable number (MPN) and simple colony forming unit enumeration (CFU) (Coulon et al., 2004; Masak *et al*., 2003). Larger microbial populations have been correlated with enhanced rates of bioremediation (Lindstrom *et al*., 1991).

Soil ecotoxicity assays are based upon direct measurements of soil attributes and enable a rapid and real-time assessment of changes in the soil processes as a function of remedial treatment adopted (Paton *et al*., 2006). Microbial assays commonly used as indicators of hydrocarbon contamination include microbial biomass measurements (CFU, MPN, PLFA), microbial community diversity, function, size species response, and soil respiration (Delistraty, 1984; Van Beelen and Doelman, 1997; Phillips *et al*., 2000; Coulon et al., 2004). Such measurements serve to complement the enumeration of hydrocarbon degraders.

To monitor changes in the hazard of remediating soils, plant bioassays have been commonly adopted either for plant growth or seed germination (Molina-Barahona *et al*., 2005). When considering soil organisms, earthworms are used because they are sentinel soil test organisms (Callahan *et al*., 1991; Dorn and Salanitro, 2000). Ecological measurements enable an empirical evaluation of hazard assessment and the interpreted response indicates the cumulative impact of potential harm in the soil (Dawson *et al*., 2007). Dawson et al (2007) acknowledged the complexity of interpreting the response of a battery of assays and proposed a ranked approach to selecting the most suitable method. In the UK, and increasingly across the world the end-point of remedial activity is defined not by the total concentration of the chemical of concern but by the concentration likely to pose significant risk. The use of human derived risk-derived approaches can be related to the intended end use of a given site and the target concentration to protect the target receptor is adopted accordingly (Khan and Hussain, 2001).

For this study, risk assessment of each hydrocarbon fraction was carried out using the risk based corrective action model: RISC4. The default parameters used were for a toddler (residential) and adult (workplace) receptors and criteria were defined as pass or fail for each fraction. There are three approaches that are often adopted in defining the remedial targets for a site. These are: general action concentrations (GAC) soil clean-up target levels (SCTL) and site specific assessment criteria (SSAC). GAC are derived using largely generic assumptions about the characteristics and behaviour of contaminants, pathways and receptors and apply to a range of sites. SCTL are back calculated risk

acceptable concentrations, but often generic. Where circumstances relevant to a specific site scenario and receptor behaviour define the SSAC.

The aim of this work was to compare the performance of two bioremediation techniques (windrows and biopiling) on bunker C Fuel oil impacted soils at the field scale. Individual treatments were compared and assessed using hazard and risk criteria associated with the soil. Furthermore the aim was to consider at what point soil may be deemed to be remediated and how different clean-up treatments compared in meeting these end-points.

## 2. Material and Methods

### *2.1. Regularly applied techniques*

#### *2.1.1. Site set up, sampling and sample preparation*

Soil (40 tonnes) was collected from a contaminated Bunker fuel site located at a former dockyard in eastern Scotland. Soil was pre-screened through a >20mm sieve and field screening was conducted to ensure that there was no co-contamination with heavy metals and metalloids (with a Niton 700 XRF multi-element analyser). Basic soil characterisation was performed (pH, bulk density loss on ignition, extractable N and P concentration) as Guicharnaud et al., 2009. For the experiment, duplicate 3 tonne masses of soil were set up as either windrows or biopiles. These were constructed to have a 2.5 $m^2$ base and were tapered in a pyramidal shape to a peak ogf 1.4 m. Bulked amendments were applied (nutrients and inocula, nutrients alone or no enhancement) using a six tonne capacity hybrid agricultural silage mixer hydraulically powered by a tractor. Nutrients

were added in the form of ammonium nitrate and potassium orthophosphate to obtain a C: N: P ratio of 100: 20: 2. The inocula, supplied by Remedios Limited (Aberdeen), was an attenuated enrichment culture from No.6 oil impacted soil and the cell concentration added was such as to give $5x10^7$ CFU $g^{-1}$ soil. After further mixing by a Bobcat excavator, the treatments were split into two replicates. A control soil was set up which was left fallow with no enhancement or physical remedial action. The windrows were turned twice a week. All treatments and the fallow soil were maintained at between 70 % and 80 % of their maximum water holding capacity. Air was pumped into the biopiles in a 48 hour cycle to prevent oxygen limitation. Samples were collected from each windrow treatment every two weeks for a period of 28 weeks. Microbial counts were made after 0, 4, 10, 16, 22 and 28 weeks. A strict sampling protocol was adhered to: five replicate samples were taken from across each soil treatment, each of these individual replicates being composed of homogenised composite bulked 500 g samples. Prior to analysis samples were stored at 4 $^{\circ}$C and analysis were performed on representative soils after the bulked replicates had been coned and quartered. Analysis was always carried out within eight days of sampling. For other analysis, the samples were collected and bulked at the start, mid-point (12 weeks) and end of the experiment (28 weeks). All biological and chemical analysis was performed on the five independent replicates for each biopile or windrow at each sample time described.

*2.1.2. Total hydrocarbon determination*

Homogenised soil (5 g) was ground with anhydrous $Na_2SO_4$ and the sample placed in a 50 ml glass centrifuge tube. Dichloromethane (DCM)/acetone solution (1:1, 20 ml) was

added to the sample and sonicated for 30 minutes (applied energy on soil suspension=1200 J $ml^{-1}$). Samples were then placed on an end-over-end shaker for 16 hours at 60 rpm then centrifuged at 1750 x g at 4 °C for 20 min. After phase separation with water to remove the polar fraction, a 10 ml aliquot of DCM/acetone extract was collected and added to 40 ml glass Wheaton vials and the sample concentrated under $N_2$ gas. The sample was then suspended in 4.9 ml of hexane and sonicated for 2 minutes. After the addition of copper turning (0.5 g), a squalane standard was added as an internal standard. Analysis was performed by GC-FID as described by Dawson *et al*. (2007).

*2.1.3. Basal respiration*

Basal respiration was determined as described by Paton *et al*. (2006). Briefly, 2 g of homogenised soil were weighed into gas vacuettes and allowed to stand for 6 hours in an incubator at 25 °C. A sample of headspace from the vial was injected into the GC injection loop (250 µl) system with a nitrogen carrier gas (20 ml $min^{-1}$) onto a 80/100 mesh Poropak Q column (2 m × 1/8″OD×2 mm). The oven temperature (Chrompack CP 9001) remained constant at 100 °C and the FID temperature was set at 250 °C. Respiration values were determined on a mg $CO_2$ g $soil^{-1}day^{-1}$ basis following subtraction of a blank vial containing atmospheric $CO_2$ only.

*2.1.4. Determination of culturable heterotrophs and hydrocarbon-degraders*

Homogenised soil (2 g) was weighed into a glass Universal bottle and 20 ml of ¼ strength Ringer's solution was added. Samples were then vortexed for 30 seconds and sonicated for 1 minute and allowed to stand for a further 2 minutes. A 100 µl aliquot of

soil suspension was removed and serially diluted in ¼ strength Ringer's solution to the appropriate dilution factor ($10^{-5}$ or $10^{-4}$ dilution factor). An aliquot of 10 µl of each dilution series was added in triplicate to ¼ strength Luria Bertani medium to determine heterotrophs and Bushnell-Hass with 1 % diesel as the sole carbon source for hydrocarbon-degraders. No 6 Fuel oil was unsuited as a substrate due to the viscous nature of the material. Samples were incubated at 25 $^{\circ}$C for 24 - 48 hours thereafter and colony-forming units (CFUs) enumerated. Results are expressed as CFU $g^{-1}$ of dry soil.

## *2.2. Techniques conducted at the start, mid-point and termination of the experiment*

### *2.2.1. Fractionated hydrocarbon (H-C) analysis*

The extraction procedure and GC-FID analysis settings for fractionated hydrocarbon fractions have been previously described by Risdon *et al.* (2008). Briefly, 5 g of soil was dried with 5g of anhydrous sodium sulphate ($Na_2SO_4$) and spiked with 1 mL of a solution containing *o*-terphenyl (oTP), squalane (Sq), heptamethylnonane (HMN) and 2-fluororbiphenyl (2-Fb) at a concentration of 200 µg $ml^{-1}$ each in acetone. Soil samples were extracted with 4 ml of acetone and sonicated for 2 minutes at 20˚C. Hexane and acetone were added to the samples to achieve a 1:1 ratio. The samples were sonicated for a further 10 minutes followed by manual shaking (twice) then followed by centrifugation for 5 minutes at 1000 x g. After passing the supernatant through a filter column, a sequential step series (including resuspension of samples in 10 ml of acetone: hexane (1:1), sonication for 15 minutes at 20 ˚C, centrifugation for 5 minutes at 1000 x g and then decanted into a filter column, this was performed and repeated twice). The final extract volume was adjusted to 40 ml with a mixture of acetone: hexane (1:1) before analysis. A silica gel column was used to separate the aliphatic and aromatic fractions. Approximately 80 ml of RO water and a spatula of sodium chloride (baked at 400˚C for 4 hours) were added to the extracts partitioning out any acetone into the water and ensuring the removal of the non-polar content. The split of the aliphatic/aromatic fractions was achieved by eluting with 3 ml of hexane followed by 3 ml of DCM, respectively.

H-C content was quantified using a gas chromatograph fitted with a flame ionization detector (GC-FID Agilent 7890). Quantification of H-C and sub-ranges of hydrocarbons

(Table 1) was made by integrating peak areas using Agilent Chemstation Software Revision B.01.01 (164) SR1 (2001-2005), and by comparison against refined mineral oil standards. External multilevel calibrations were carried out for both diesel/mineral oil fractions and surrogates, quantification ranging from 0.5 to 2500 μg $ml^{-1}$ and from 1 to 5 μg $ml^{-1}$, respectively.

*2.2.2. Bioluminescence microbial biosensor assay*

A methanol extract was used to extract bioavailable H–C for use in the bacterial biosensor assays; 2 g of soil (dry weight) was extracted with 4 ml methanol (Bundy *et al.*, 2004). The biosensor *Escherichia coli* HMS174 (Selifonova & Eaton, 1996) was used to measure the change in concentration of inducible hydrocarbon (as reflected by isopropylbenzene, (Selifonova and Eaton, 1996). The toxicity of the soil extracts was determined using 2 constitutively marked biosensors *Pseudomonas putida* F1 Tn5 and *Escherichia coli* HB101 pUCD607 (Paton *et al.*, 2006).

Cells for each of the assays were previously freeze-dried using standard procedures (Bundy *et al.*, 2001) and stored at -20 °C. All standards and extracts were diluted to 5 % methanol prior to analysis (Paton *et al*, 2009). Cells were resuscitated in 10 mL 0.1 M KCl between 30-60 minutes on an orbital shaker at 200 rpm and 25 °C before being sequentially aliquotted (0.1 ml cell suspension) into 0.9 ml of test sample. All procedures were carried out in 1.5 ml covered glass cuvettes and time exposure for the constitutive promoted sensors and inducible sensor was 30 and 120 minutes, respectively. A reference control for *Escherichia coli* HMS174 was prepared by adding 2.8 μl of isopropylbenzene

IPB) to 100 ml deionised water containing 0.4 ml methanol. All bioassays were carried out in triplicate, with a total volume of 1 ml and light output was measured using a Jade (Bio orbit 1251) portable luminometer. Results were expressed as a percentage of this postitve reagent control.

*2.2.3. Seed germination assay*

For this assay, 10 mustard seeds (*Brassica alba*) were added separately in 5 replicates to 120 ml wide mouth glass jars containing 20 g of soil re-wetted to 75% WHC. Lids were loosely screwed on to reduce evaporation but allow aeration and the seeds were left to germinate at 25 $^{\circ}$C, 80 % humidity and no light for 4 days. The number of seeds germinated was recorded after 4 days. A non-contaminated control soil (Boyndie), maintained at 44% (v/w), was used as the baseline to obtain a 100 % recovery in the assays.

*2.2.4. Earthworm assays*

*Lumbricus terrestris* were obtained from worms direct. Five replicate earthworms were exposed to 50 g of soil sample in 120 ml wide mouth glass bottles for 14 days (Dawson *et al.*, 2007; Shakir Hanna & Weaver, 2002). Before and after exposure, the earthworms were washed in tap water and placed in a plastic container lined with moist tissue paper to depurate overnight. Individual earthworms were then rinsed, dried, weighed and placed in a glass jar containing 50 g of soil re-wetted to 80% WHC. Earthworms were incubated individually for 14 days at 15 $^{\circ}$C, 80 % humidity and no light. Lids were fitted loosely to minimise evaporation but allow aeration. The control soil was Boyndie. Earthworms were

examined on days 1, 2, 3, 4, 7, 10 and 14 for lethality. Sublethal effects were assessed by measuring the mass of the earthworms after exposure and then depuriating using standard techniques as per Callahan et al (1991.)

### *2.3. Risk assessment derived remedial targets*

Fractionated H-C measurements were interpreted in a risk derived framework using the risk based corrective action model: RISC4 to compare the estimated and the obtained values (RISC4 Manual). Default parameters were used for toddler (residential) and adult (workplace) receptors.

### *2.4. Data analysis*

Data analysis was performed using the five independent replicates for each biopile or windrow at the stated timepoints described. Analysis of the treatments however were only assessed by considering the duplicate treatment data which represents the replication at the field scale. The H-C concentration, culturable heterotroph and degrader numbers were analysed by one-way ANOVA (following where appropriate log transformation of data) a non-parametric Mood median test was used as the alternative. Pearson correlation coefficients and where appropriate multiple linear regression equations were calculated to determine which parameters explained the most variation in the chemical and biological responses (i.e. H-C concentration, heterotroph number, degrader number and ecotoxicity tests). Results in this study represent mean and standard error of two independent replicates (for treatments) but for individual determinants and corresponding bioassays five replicates for comparative evaluation of techniques was performed. A multiple linear

regression was performed to assess the relationship between hydrocarbon fractions, time, treatments and toxicity response. Significance is expressed at $p \leq 0.05$. All analyses were conducted using MINITAB (Release 15).

## 3. Results

### *3.1 General soil attributes*

The pH range of the soil remained between 6.0 and 7.0 for (optimal range for H-C degradation (Vidali, 2001) the duration of the experiment regardless of the treatment (data not shown) and this is amenable to an active hydrocarbon degrading microbial population (Atlas, 1981). The bulk density of the biopile treatments remained constant throughout the duration of the study (0.93 g $cm^{-3}$) but was significant reduced for the windrow treatments (0.78 g $cm^{-3}$) (further data not shown).

### *3.2 Total hydrocarbon determination*

After 30 weeks, the total extractable petroleum hydrocarbon content in windrow treatments, including windrow, windrow with nutrients and windrow with nutrients and inocula, were reduced to 4%, 3% and 2%, respectively of their initial value (Figure 1). In contrast, extractable TPH in biopile treatments were only reduced to 22% of their initial values (Figure 2). The results reveal that biostimulation and bioaugmentation enhanced the decrease in TPH concentration throughout the duration of the experiment. The control values as represented by the fallow treatment decreased by only 19% during the field scale experiment.

*3.3 Basal Respiration*

Respiration values collected throughout the experiment for the windrow and biopile remediation study are shown in Figure 3 and Figure 4, respectively. Relative to the other treatments, the control had a significantly lower respiration value throughout the experiment. However, both the nutrient alone and nutrient and inocula treatments with both windrow and biopile management resulted in a significant increase ($p < 0.05$) in respiration. The highest $CO_2$ production was reached during the first the first 5 and 12 days in windrow and biopile treatments, respectively. Throughout the study the windrow treatments had significantly higher respiration values (up to 0.8 mg $CO_2$ $g^{-1}$ soil) than the biopile ones (up to 0.4 mg $CO_2$ $g^{-1}$ soil).

*3.4 Total culturable heterotroph and degrader numbers*

The number of culturable heterotrophs in both windrows and biopile treatments generally decreased by one order of magnitude with time (Table 1). In contrast, the number of hydrocarbon degraders increased at least by one order of magnitude regardless the type of treatments (Table 2).

*3.5 Fractionated hydrocarbons analysis*

Fractionation of the oil residues showed that the most prominent aliphatic and aromatic fractions were $C_{12}$ - $C_{35}$ and $C_{16}$ - $C_{35,}$ respectively (Tables 3). Overall, windrow treatments out competed biopile treatments. Degradation of the aliphatic and aromatic fractions in windrows was 6 and 4 times higher respectively than the biopile ones after 6 week of treatment. Nutrient addition and bioaugmentation of the biostimulated windrows

led to a 2 fold higher degradation rate of the aliphatic fraction whereas the aromatic one was barely enhanced by the treatments (Table 3). Higher degradation rates were registered for the aliphatic fraction followed by the aromatics. The mass fraction of aromatics relative to aliphatic hydrocarbons increased by more than 20% in windrows whereas in biopile the mass fraction remained nearly the same.

*3.6 Bioluminescence microbial biosensor assay*

Response of the constitutive biosensor *Escherichia coli* HB101 pUCD607 in both windrow and biopile treatments increased by more than 40% and 55%, respectively during the experiment (Table 4a). This trend was also observed for *Pseudomonas putida* F1 Tn5 and the controls. In contrast, response of the inducible sensor, *Escherichia coli* HMS174 (Table 4b), showed that the luminescence decreased over time, indicating a reduction in the concentration of the target analyte or metabolites to this biosensor. The results are expressed as the percentage of the maximum inducible luminescence from a saturated sample of isopropyl benzene. It has been shown that this biosensor is responsive to a wide range of analogues of this compound and these results do not reflect a reduction in the concentration of this specific analyte. In general, the results ranged between 15 – 20% luminescence at the start of the experiment to 1.4% or less luminescence at the end of the experimental time.

*3.7 Seed germination assays*

The control sample germination rate at the start of the experiment increased to double that rate at the end of the experimental time (Table 5). Windrow control and windrow

with nutrients had a similar increased germination rate as the control, while windrow with nutrients and inocula had about triple the increase in germination rate of the control. For the biopile treatments, a lower increase in seed germination rate was observed for both biopile and biopile with nutrients. Biopile with nutrients and inocula had tripled in germination rate at the end of the experiment.

### *3.8 Earthworm assays*

At the end of the experiment, there was significantly greater survival than at the start of the treatment or the mid point (Table 6). The windrow treatments resulted in a lower survival counts compared with the control with the windrow with nutrients and inocula having the lowest results. For the biopile treatments, the survival rate was different from the windrow treatments compared with the control. Biopile and biopile with nutrients had a significant increase in survival counts. The biopile with nutrients and inocula also had a small increase in survival at the end of the experiment.

### *3.9 Risk assessment derived remedial targets*

By the end of the experiment, the results obtained from the fractionated analysis in the soil (Table 3) were compared to that in the RISC 4 criteria and a pass / fail mark was given to each fraction and treatment. An overall risk assessment (RA) mark was awarded to indicate if the site had passed the criteria at that time point (Table 7).

## **4. Discussion**

Song *et al*. (1990) in a study of the biodegradation of bunker oil reported that it took 48 weeks of incubation to degrade fifty percent of TPH even under optimal nutrient conditions. It was reported that most of the bunker oil components were structurally resistant to biodegradation and they concluded that "bioremediation has only very limited beneficial effects on bunker oil elimination from soil".

In this study, the amendment of treatments with nutrients increased significantly the hydrocarbon degradation at the initial stages of the experiment. This was further increased at the initial stages by the addition of the inocula. It may be inferred, as many papers report, that the microbial population in the control soils was nutrient limited but that there was a capable microbial population present (Atlas, 1981; Coulon et al. 2004; Bamforth and Singleton, 2005; Delille and Coulon, 2008).

In the control soil, the indigenous population was capable of degrading the available hydrocarbon without any further treatment, but the rate was slower and hence there is a requirement for the remediation management team to relate the need for speed of degradation with the cost of amendment (Khan et al, 2004). Throughout the study the degrader numbers were higher than the total heterotrophic numbers. This is contrary to the observation of Adako and Orugbani (2007) who reported a corresponding increase in heterotrophic and petroleum degrading bacteria during hydrocarbon degradation. However, the effective remediation management (aeration, sieving and nutrients) could have liberated additional substrates (mainly hydrocarbons) and resulted in an increase in the total bacterial population (Townsend *et al*., 2000). It has been documented that culturable heterotrophic numbers can decrease in fuel oil amended soils (Turco *et al*.,

1995) as a response to physical and chemical changes to the soil which may affect aeration and nutrient diffusion.

Respiration has been identified as a key indicator of bioremediative activity (Diplock et al., 2009). In this study, by week 28, the nutrient treatment for both the windrow and biopile techniques was observed to have the highest respiration rate corresponding with an increased number of hydrocarbon degraders. This could be a result of a shift in the assimilation efficiency of the cells due to the addition of nutrients and hence the change of the metabolic quotient. Alternatively, it could be simply that a higher cell number correlates with increased degradation (Coulon et al., 2004; Margesin et al., 2007).

A measure of the luminescence of biosensors is a surrogate for metabolic activity of soil bacteria (Steinberg et al., 1985; Bundy et al., 2004). This may be used to assess the burden of pollution on the potential metabolic activity of key soil organisms and this may in turn aid in decision making for bioremediation (Trott et al., 2006; Dawson et al., 2007). Dawson et al. (2008) compared the response of a constitutive and monoaromatic hydrocarbons induced biosensor in a site contaminated with benzene, toluene, ethyl benzene and xylenes. The results are similar to those in this study, in that the decline in hydrocarbon concentration caused a reduction in bioluminescence of the induced sensor while the constitutive sensor increased in bioluminescence. Bundy *et al.* (2001) also noted that at the start of the remediation activity, the presence of more water soluble intermediates caused an increase in toxicity and this was also evident in this study.

The other ecotoxicity assays suggest recovery as the total hydrocarbon concentration declines. This finding is supported by previous studies including Dawson *et al.,* (2007) who reported the indicators that differentiated the extent of soil remediation were respiration, earthworm toxicity and mustard seed germination. Salanitro *et al.* (1997) reported that bioremediated soils were neither toxic to earthworms, inhibitory in the Microtox assay, nor deleterious to seed germination after months of treatment.

Although the response of the biosensor correlated both with that of the earthworms and seed germination there was little linkage to the human risk assessment criteria. One of the key problems is determining the point at which recovery has been met or where hazard is deemed acceptable and then relating this to risk derived criteria. While $LD_{50}$ and $EC_{50}$ values are often used, these may be insensitive to predicting ecological protection. Furthermore NOEC and LOEC values require considerable data sets for accurate estimations and in light of the nature of these experiments this may not be practicable (Dawson et al., 2007). In the case of the most effective treatments (as assessed by degradation) there was a considerable improvement on the recovery of the ecotoxicity assays (Dawson *et al.,* 2007; Salanitro *et al.,* 1997). This could be interpreted, in the context of this study, to mean that the hazard assessment criteria were such that the soils were all acceptable (regardless of treatment) at the end time point.

There is no doubt that a measure of the TPH could be misleading as it is the component fractions of the hydrocarbon that will pose a hazard or potential risk to defined receptors. In the case of this study, despite exhaustive regression analysis no single fraction could

be deemed as the causal agent for the measured ecotoxicological response (TPHCWG. 1998). In part this could be because the adopted banding may not reflect the relative sensitivity of the receptors under investigation (TPHCWG. 1998). These fractions are not individual compounds and there is very limited dose response characterisation of these receptors used to the fractions measured. Furthermore, these fractions do not occur in isolation and it is difficult to factor out the relative impact of these grouped compounds. The discipline as a whole will benefit from consideration of fractions and their dose dependency responses to relevant soil ecotoxicity assays. It should also be remembered that polar metabolites that may be more bioassimilable and potentially more toxic than the non polar hydrocarbons particular are likely to remain undetected in this study (TPHCWG. 1998).

Hydrocarbon fractions are however, widely used in human risk assessment. Placed in the context of human risk assessment, fractionation quantification reveals an interesting pattern relative to treatment adopted. At the end-point for GAC only WN passes while for SSAC values W, WN and WNI all pass the criteria (Table 7). This means perhaps that depending on the selection of an ecotoxicity acceptable dose factor, the risk to a toddler from ingesting soil is greater than the hazard derived from earthworm, seed germination and biosensor applications to the soil itself. Each receptor has a different response to the substance of interest.

In the future, comparative evaluations of defined receptors will become more important, because although the risk assessment models exist to protect humans and water courses

under a range of different conditions, this is not the case with ecological receptors. There is currently a need to carry out empirical assays to quantify the response and then consider using these data to derive ecologically protective doses.

Hazard and risk approaches are key in defining the status of soil in the context of being "fit for purpose". Such approaches are of more meaning than a total hydrocarbon measurement and may also place the relative potency of fractions in a suitable context.

## 5. Conclusion

Active management enhanced the biodegradation of bunker fuel oil in soil in a controlled field-scale trial. The addition of nutrient and inocula accelerated the degradation rate for the period of the study. Microbial measurements used in conjunction with chemical analysis increased our understanding of field-based bioremediation. For this heavily textured soil, windrow turning was more effective than biopiling because the resultant soil was more friable. For coarser textured soils, biopiling may perform better. The likely decision as to which technique to adopt would be determined by remediation managers. The end-point of remediation needs to be defined relative to the receptor that requires protection. In this study, soil that showed a significant ecological recovery was still impaired with respect to human risk criteria and *visa versa*. There is a need to carry out more comparative studies to better assess the relationship and relative sensitivity of receptor-based end-points.

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**Table 1 Culturable heterotroph numbers (CFUs $g^{-1}$ dry soil) for windrow and biopile treatments (observations annotated with the same letter are not significantly different $p \leq 0.05$)**

**Windrow treatments**

| Time | C | W | WN | WNI |
|---|---|---|---|---|
| **0** | 3.11E+07[a] | 2.58E+07[a] | 3.71E+08[b] | 3.78E+07[b] |
| **4** | 2.17E+08[b] | 2.95E+07[a] | 3.43E+08[b] | 2.78E+08[b] |
| **10** | 4.05E+07[a] | 2.10E+07[a] | 1.25E+07[a] | 1.48E+08[b] |
| **16** | 1.26E+08[b] | 5.38E+07[a] | 1.46E+08[b] | 1.01E+08[b] |
| **22** | 1.85E+06[a] | 2.39E+06[a] | 8.02E+06[a] | 6.66E+06[a] |
| **28** | 3.54E+06[a] | 4.46E+06[a] | 1.74E+07[a] | 5.64E+07[a] |

**Biopile Treatments**

| Time | C | B | BN | BNI |
|---|---|---|---|---|
| **0** | 3.11E+07[a] | 2.56E+08[b] | 2.75E+08[b] | 2.91E+08[b] |
| **4** | 2.84E+08[b] | 2.39E+08[b] | 2.78E+08[b] | 2.61E+08[b] |
| **10** | 4.88E+07[a] | 4.95E+06[a] | 7.60E+07[a] | 2.04E+08[b] |
| **16** | 1.86E+08[b] | 1.07E+08[b] | 1.30E+08[b] | 9.61E+07[a] |
| **22** | 1.59E+06[a] | 6.92E+06[a] | 2.79E+07[a] | 1.75E+07[a] |
| **28** | 2.31E+06[a] | 9.98E+06[a] | 1.29E+07[a] | 4.12E+07[a] |

| | | | |
|---|---|---|---|
| C | Control | | |
| W | Windrow | B | Biopile |
| W+N | Windrow + Nutrient | B+N | Biopile + Nutrient |
| W+N+I | Windrow + Nutrient + Inoculum | B+N+I | Biopile + Nutrient + Inoculum |

**Table 2 Culturable hydrocarbon degrader numbers (CFUs $g^{-1}$ dry soil) for windrows and biopile treatments (observations annotated with the same letter are not significantly different $p \leq 0.05$)**

**Windrow treatments**

| Time | C | W | WN | WNI |
|---|---|---|---|---|
| **0** | 2.44E+07[a] | 2.67E+07[a] | 2.71E+07[a] | 2.88E+07[a] |
| **4** | 2.84E+08[b] | 6.37E+07[a] | 5.99E+08[b] | 4.82E+08[b] |
| **10** | 4.34E+08[b] | 2.15E+07[a] | 2.15E+07[a] | 2.15E+07[a] |
| **16** | 3.05E+08[b] | 3.98E+08[b] | 1.69E+08[b] | 1.67E+08[b] |
| **22** | 4.42E+08[b] | 1.39E+07[a] | 4.96E+08[b] | 1.95E+09[c] |
| **28** | 1.32E+08[b] | 2.31E+08[b] | 1.42E+09[c] | 2.18E+09[c] |

**Biopile Treatment**

| Time | C | B | BN | BNI |
|---|---|---|---|---|
| **0** | 2.15E+07[a] | 2.80E+07[a] | 3.41E+07[a] | 4.31E+07[a] |
| **4** | 3.16E+08[b] | 4.91E+07[a] | 4.67E+08[b] | 1.91E+08[b] |
| **10** | 5.67E+08[b] | 1.67E+07[a] | 1.08E+07[a] | 1.51E+07[a] |
| **16** | 1.09E+08[b] | 1.09E+08[b] | 1.18E+08[b] | 2.76E+08[b] |
| **22** | 5.19E+08[b] | 2.09E+07[a] | 2.91E+08[b] | 6.22E+08[b] |
| **28** | 1.32E+08[b] | 1.63E+08[b] | 1.42E+08[b] | 3.54E+08[b] |

| | | | |
|---|---|---|---|
| C | Control | | |
| W | Windrow | B | Biopile |
| W+N | Windrow + Nutrient | B+N | Biopile + Nutrient |
| W+N+I | Windrow + Nutrient + Inoculum | B+N+I | Biopile + Nutrient + Inoculum |

**Table 3: Quantification of TPH and sub-ranges of hydrocarbons in the windrow and biopile treatments during the time course of the remediation study (with standard error of the mean)**

| | | | | Week 14 | | | | | | | | Week 28 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hydrocarbon Fraction | | Start | SE | C | SE | W | SE | W+N | SE | W+N+I | SE | C | SE | W | SE | W+N | SE | W+N+I | SE |
| | **Total** | 13,009 | 152 | 11844 | 982 | 3691 | 381 | 2208 | 147 | 1474 | 129 | 10639 | 1034 | 539 | 47 | 419 | 30 | 361 | 38 |
| **Al** | **C10-12** | 71 | 21 | 64 | 8 | 21 | 3 | 12 | 2 | 10 | 1 | 43 | 4 | 10 | 1 | 0 | 0 | 10 | 0 |
| **Al** | **C12-16** | 1432 | 161 | 1123 | 98 | 453 | 18 | 421 | 28 | 350 | 20 | 1311 | 155 | 21 | 1 | 23 | 1 | 10 | 1 |
| **Al** | **C16-21** | 3124 | 209 | 2314 | 87 | 521 | 12 | 411 | 12 | 434 | 21 | 2671 | 31 | 110 | 21 | 24 | 10 | 31 | 3 |
| **Al** | **C21-35** | 2100 | 981 | 1876 | 121 | 629 | 31 | 349 | 21 | 219 | 12 | 1932 | 63 | 43 | 2 | 49 | 8 | 38 | 4 |
| **Ar** | **C10-12** | 21 | 11 | 34 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Ar** | **C12-16** | 671 | 54 | 576 | 34 | 130 | 14 | 54 | 2 | 10 | 2 | 422 | 23 | 21 | 1 | 12 | 3 | 17 | 1 |
| **Ar** | **C16-21** | 2376 | 329 | 3021 | 176 | 631 | 32 | 480 | 17 | 210 | 16 | 2398 | 101 | 103 | 13 | 67 | 5 | 71 | 5 |
| **Ar** | **C21-35** | 3214 | 210 | 2876 | 109 | 1306 | 121 | 481 | 21 | 241 | 21 | 1862 | 12 | 231 | 19 | 244 | 10 | 184 | 21 |

| | | Start | SE | C | SE | B | SE | B+N | SE | B+N+I | SE | C | SE | B | SE | B+N | SE | B+N+I | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | 13,009 | 152 | 11844 | 982 | 8561 | 522 | 9649 | 637 | 8884 | 1091 | 10639 | 1034 | 2655 | 320 | 2989 | 308 | 2604 | 319 |
| **Al** | **C10-12** | 71 | 21 | 64 | 8 | 55 | 4 | 70 | 4 | 23 | 1 | 43 | 4 | 25 | 3 | 32 | 4 | 42 | 3 |
| **Al** | **C12-16** | 1432 | 161 | 1123 | 98 | 981 | 74 | 1129 | 76 | 870 | 108 | 1311 | 155 | 421 | 54 | 548 | 72 | 461 | 62 |
| **Al** | **C16-21** | 3124 | 209 | 2314 | 87 | 1850 | 109 | 2131 | 101 | 2231 | 39 | 2671 | 43 | 254 | 12 | 162 | 41 | 238 | 18 |
| **Al** | **C21-35** | 2100 | 981 | 1876 | 121 | 1293 | 87 | 1082 | 24 | 879 | 31 | 1932 | 61 | 549 | 21 | 487 | 30 | 504 | 42 |
| **Ar** | **C10-12** | 21 | 11 | 34 | 2 | 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Ar** | **C12-16** | 671 | 54 | 576 | 34 | 290 | 3 | 310 | 13 | 281 | 19 | 422 | 23 | 132 | 16 | 213 | 17 | 376 | 28 |
| **Ar** | **C16-21** | 2376 | 329 | 3021 | 176 | 2101 | 76 | 2876 | 18 | 2845 | 101 | 2398 | 59 | 421 | 31 | 545 | 105 | 419 | 34 |
| **Ar** | **C21-35** | 3214 | 210 | 2876 | 109 | 1981 | 81 | 2051 | 23 | 1755 | 65 | 1862 | 102 | 853 | 42 | 911 | 53 | 564 | 31 |

| | | | |
|---|---|---|---|
| Al | Aliphatic | Ar | Aromatic |
| C | Control | SE | Standard Error |
| W | Windrow | B | Biopile |
| W+N | Windrow + Nutrient | B+N | Biopile + Nutrient |
| W+N+I | Windrow + Nutrient + Inoculum | B+N+I | Biopile + Nutrient + Inoculum |

**Table 4a Bioluminescence as expressed as a percentage of control (non-hydrocarbon impacted soil) obtained for the constitutive biosensors used in the experiment (wd ith standard error of the mean)**

*Escherichia coli* HB101

| Week | C | SE | W | SE | WN | SE | WNI | SE | B | SE | BN | SE | BNI | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 44.20 | 2.1 | 43.20 | 1.2 | 40.20 | 2.1 | 38.90 | 3.2 | 39.40 | 1.7 | 41.30 | 1.8 | 40.20 | 2.0 |
| **2** | 32.40 | 3.2 | 68.70 | 2.0 | 70.20 | 2.7 | 66.80 | 2.0 | 33.40 | 2.3 | 37.60 | 1.2 | 36.50 | 2.3 |
| **6** | 45.10 | 1.7 | 71.20 | 1.6 | 73.80 | 1.3 | 69.10 | 1.8 | 49.50 | 3.0 | 51.30 | 1.4 | 40.40 | 3.4 |
| **12** | 52.50 | 1.2 | 68.90 | 2.4 | 75.20 | 1.1 | 76.40 | 1.7 | 56.70 | 2.9 | 58.70 | 2.0 | 61.20 | 2.9 |
| **18** | 60.32 | 1.5 | 86.20 | 3.0 | 93.20 | 1.9 | 88.10 | 1.5 | 62.70 | 2.5 | 60.30 | 2.1 | 63.80 | 1.8 |
| **26** | 57.80 | 2.7 | 91.10 | 1.0 | 94.60 | 1.3 | 90.12 | 2.1 | 66.80 | 2.2 | 65.70 | 2.0 | 66.90 | 1.8 |
| **28** | 62.30 | 2.9 | 88.20 | 2.1 | 91.30 | 2.1 | 94.70 | 1.9 | 69.10 | 2.0 | 71.60 | 1.0 | 74.30 | 2.2 |

Pseudomonas putida F1 Tn5

| Week | C | SE | W | SE | WN | SE | WNI | SE | B | SE | BN | SE | BNI | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 37.10 | 1.2 | 38.20 | 1.1 | 35.40 | 1.9 | 33.20 | 2.2 | 33.60 | 1.8 | 32.60 | 1.9 | 32.50 | 1.2 |
| **2** | 23.40 | 1.6 | 63.20 | 1.8 | 64.60 | 2.1 | 62.60 | 1.9 | 22.90 | 2.0 | 34.50 | 0.3 | 32.87 | 2.1 |
| **6** | 40.60 | 1.6 | 66.20 | 1.6 | 70.60 | 1.2 | 65.80 | 1.4 | 44.70 | 1.4 | 44.70 | 2.3 | 35.70 | 2.7 |
| **12** | 56.30 | 1.4 | 63.90 | 2.1 | 73.80 | 1.1 | 71.90 | 1.4 | 49.90 | 2.1 | 50.60 | 1.6 | 55.80 | 3.0 |
| **18** | 58.80 | 1.1 | 82.70 | 1.9 | 90.30 | 2.1 | 83.70 | 1.3 | 56.30 | 1.3 | 57.10 | 1.8 | 60.30 | 1.1 |
| **26** | 60.20 | 1.2 | 87.40 | 1.2 | 92.70 | 1.2 | 85.60 | 1.1 | 61.90 | 1.9 | 61.00 | 1.6 | 63.80 | 1.6 |
| **28** | 59.70 | 2.0 | 91.30 | 2.1 | 88.50 | 2.2 | 92.80 | 1.2 | 63.70 | 2.1 | 67.30 | 1.5 | 71.70 | 2.2 |

**Table 4b Bioluminescence as expressed as a percentage of a positive control sample (containing 2.8 µ/ 100ml$^{-1}$) obtained for *Escherichia coli* HMS174 (with standard error of the mean)**

*Escherichia coli* HMS174

| Week | C | SE | W | SE | WN | SE | WNI | SE | B | SE | BN | SE | BNI | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 16.40 | 0.2 | 18.31 | 0.5 | 17.66 | 0.3 | 16.43 | 0.5 | 15.72 | 0.7 | 14.99 | 0.9 | 15.12 | 1.0 |
| **2** | 12.34 | 1.0 | 4.23 | 0.4 | 2.13 | 0.7 | 3.54 | 0.4 | 11.54 | 0.6 | 9.32 | 1.0 | 8.76 | 0.8 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **6** | 8.31 | 0.2 | 1.29 | 0.7 | 2.22 | 0.3 | 3.02 | 0.4 | 6.76 | 0.9 | 7.49 | 1.0 | 6.81 | 1.0 |
| **12** | 6.22 | 0.4 | 0.23 | 0.1 | 0.03 | 0.0 | 0.14 | 0.0 | 2.17 | 0.1 | 2.01 | 0.1 | 3.01 | 0.1 |
| **18** | 3.21 | 0.4 | 0.43 | 0.0 | 0.12 | 0.0 | 0.11 | 0.0 | 1.32 | 0.4 | 1.29 | 0.3 | 1.04 | 0.1 |
| **26** | 1.29 | 0.2 | 0.59 | 0.0 | 0.19 | 0.0 | 0.09 | 0.0 | 0.41 | 0.0 | 0.54 | 0.1 | 0.38 | 0.0 |
| **28** | 1.39 | 0.1 | 0.39 | 0.1 | 0.13 | 0.1 | 0.18 | 0.0 | 0.12 | 0.1 | 0.19 | 0.1 | 0.11 | 0.1 |

| | | | |
|---|---|---|---|
| C | Control | SE | Standard Error |
| W | Windrow | B | Biopile |
| WN | Windrow + Nutrients | BN | Biopile + Nutrients |
| WNI | Windrow + Nutrients + Inoculum | BNI | Biopile + Nutrients + Inoculum |

1

**Table 5 Percentage of survival of mustard seeds (with standard error of the mean)**

| Week | C | SE | W | SE | WN | SE | WNI | SE | B | SE | BN | SE | BNI | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | **22** | **0.4** | **24** | **0.6** | **28** | **0.5** | **18** | **0.4** | **21** | **0.6** | **17** | **0.6** | **19** | **0.5** |
| **12** | 36 | 0.4 | 32 | 0.4 | 38 | 0.4 | 22 | 0.3 | 38 | 0.4 | 44 | 0.5 | 30 | 0.5 |
| **28** | 92 | 0.2 | 74 | 0.4 | 94 | 0.2 | 76 | 0.3 | 88 | 0.3 | 90 | 0.2 | 84 | 0.5 |

**Table 6 Percentage of earthworm survival after the 14 day duration assay (with standard error of the mean)**

| Week | C | SE | W | SE | WN | SE | WNI | SE | B | SE | BN | SE | BNI | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 10 | 0 | 0 | 0 | 10 | 10 | 20 | 10 | 10 | 0 | 0 | 0 | 0 | 0 |
| **12** | 40 | 0 | 50 | 20 | 50 | 10 | 100 | 0 | 20 | 20 | 10 | 10 | 80 | 0 |
| **28** | 100 | 0 | 80 | 20 | 80 | 0 | 90 | 10 | 70 | 10 | 90 | 10 | 90 | 10 |

| | | | |
|---|---|---|---|
| C | Control | SE | Standard Error |
| W | Windrow | B | Biopile |
| W+N | Windrow + Nutrient | B+N | Biopile + Nutrient |
| W+N+I | Windrow + Nutrient + Inoculum | B+N+I | Biopile + Nutrient + Inoculum |

## Table 7 Treatments that pass the remedial target criteria

| Three Months | GAC | | SSAC | | SCTL | |
|---|---|---|---|---|---|---|
| **Fractions** | **Residential without plant** | **Industrial / Commercial** | **Residential without plant** | **Industrial / Commercial** | **Residential without plant** | **Industrial / Commercial** |
| **EC10-C12** | None Pass | C, W, WN, WNI, B, BN, & BNI | WN & WNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC12-C16** | None Pass | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC16-C35** | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC35-C44** | C, W ,WN ,WNI, B, BN,& BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC10-C12 Aro** | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC12-C16 Aro** | WN & WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC16-C21 Aro** | WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI |
| **EC21-C35 Aro** | WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **RA Mark** | **Fail** | **Pass** | **Fail** | **Pass** | **Fail** | **Pass** |

| Six Months | GAC | | SSAC | | SCTL | |
|---|---|---|---|---|---|---|
| **Fractions** | **Residential without plant** | **Industrial / Commercial** | **Residential without plant** | **Industrial / Commercial** | **Residential without plant** | **Industrial / Commercial** |
| **EC10-C12** | WN | C, W, WN, WNI, B, BN, & BNI | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC12-C16** | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC16-C35** | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | - | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC35-C44** | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | - | - | - |
| **EC10-C12 Aro** | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC12-C16 Aro** | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, & B | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC16-C21 Aro** | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **EC21-C35 Aro** | W, WN, & WNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI | C, W, WN, WNI, B, BN, & BNI |
| **RA Mark** | **Fail** | **Pass** | **Fail** | **Pass** | **Pass*** | **Pass** |

| | | | |
|---|---|---|---|
| **RA** | Risk Assessment | | |
| **GAC** | General Action Concentration | **SSAC** | Site Specific Action Concentration |
| **SCTL** | Soil Clean-up Target Level | **Ali** | Aliphatic Fraction |
| **C** | Control | **Aro** | Aromatic Fraction |
| **W** | Windrow | **B** | Biopile |
| **WN** | Windrow + Nutrient | **BN** | Biopile + Nutrient |
| **WNI** | Windrow + Nutrient + Inoculum | **BNI** | Biopile + Nutrient + Inoculum |

**Figure legends:**

**Fig. 1** TPH degradation from Windrows field time course of remediation.

**Fig. 2** TPH degradation from Biopile field time course of remediation.

**Fig. 3** Respiration values from the Windrow field time course remediation study

**Fig. 4** Respiration values from the Biopile field time course remediation study

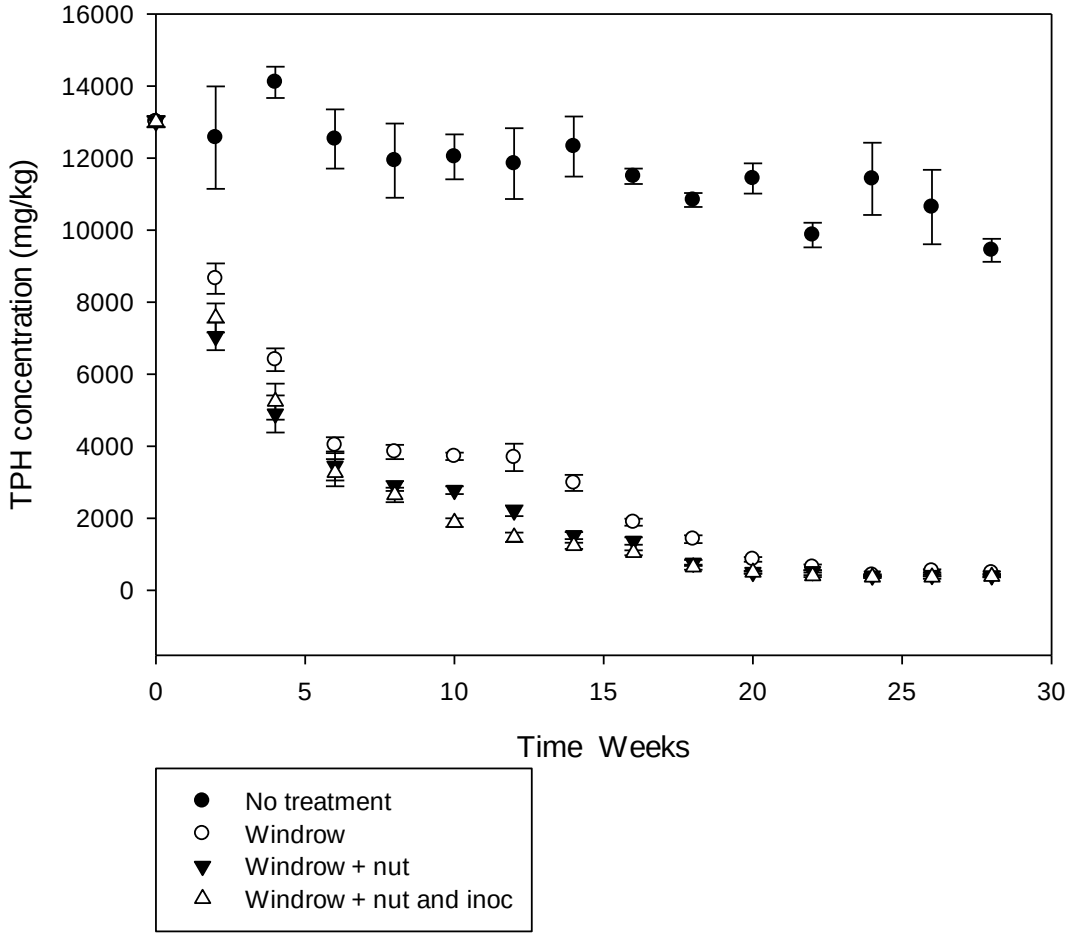

16000
14000
12000
10000
8000
6000
4000
2000
0
TPH concentration (mg/kg)
0
5
10
15
20
25
30
Time Weeks
No treatment
Windrow
Windrow + nut
Windrow + nut and inoc

**Figure 1**

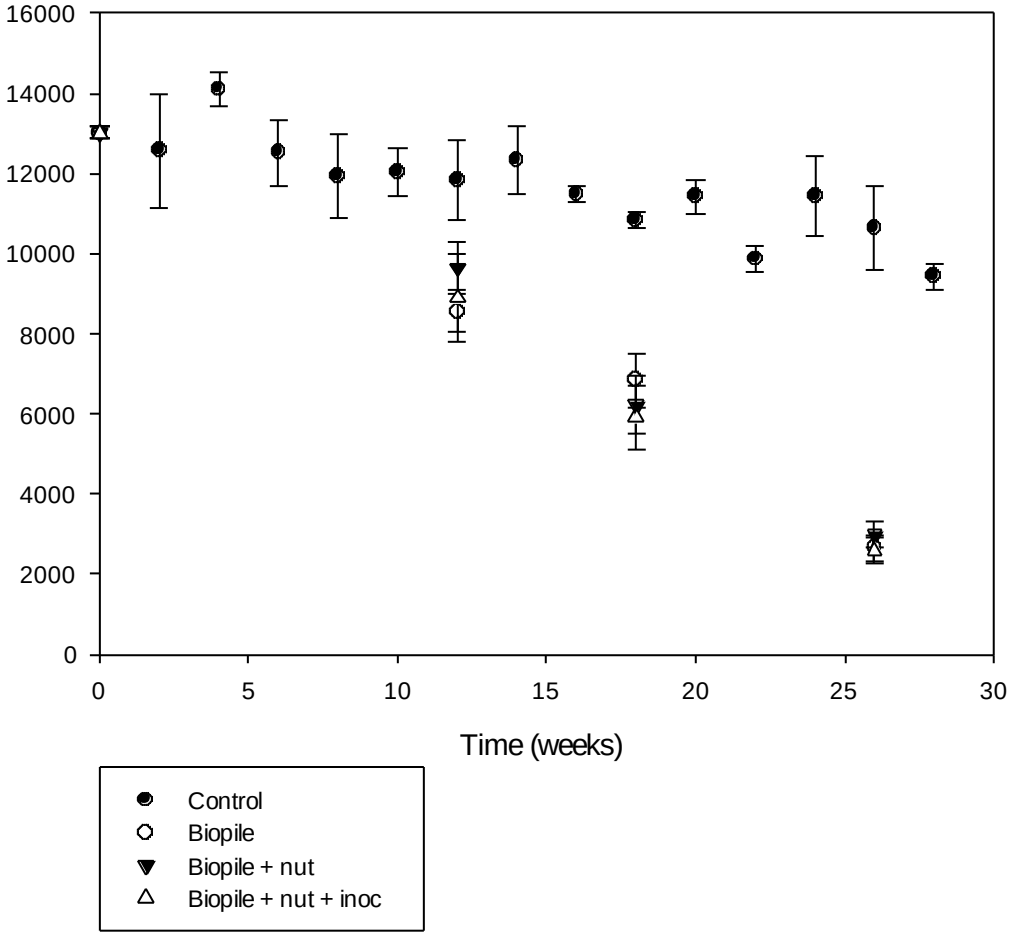

16000
14000
12000
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
Time (weeks)
Control
Biopile
Biopile + nut
Biopile + nut + inoc

**Figure 2**

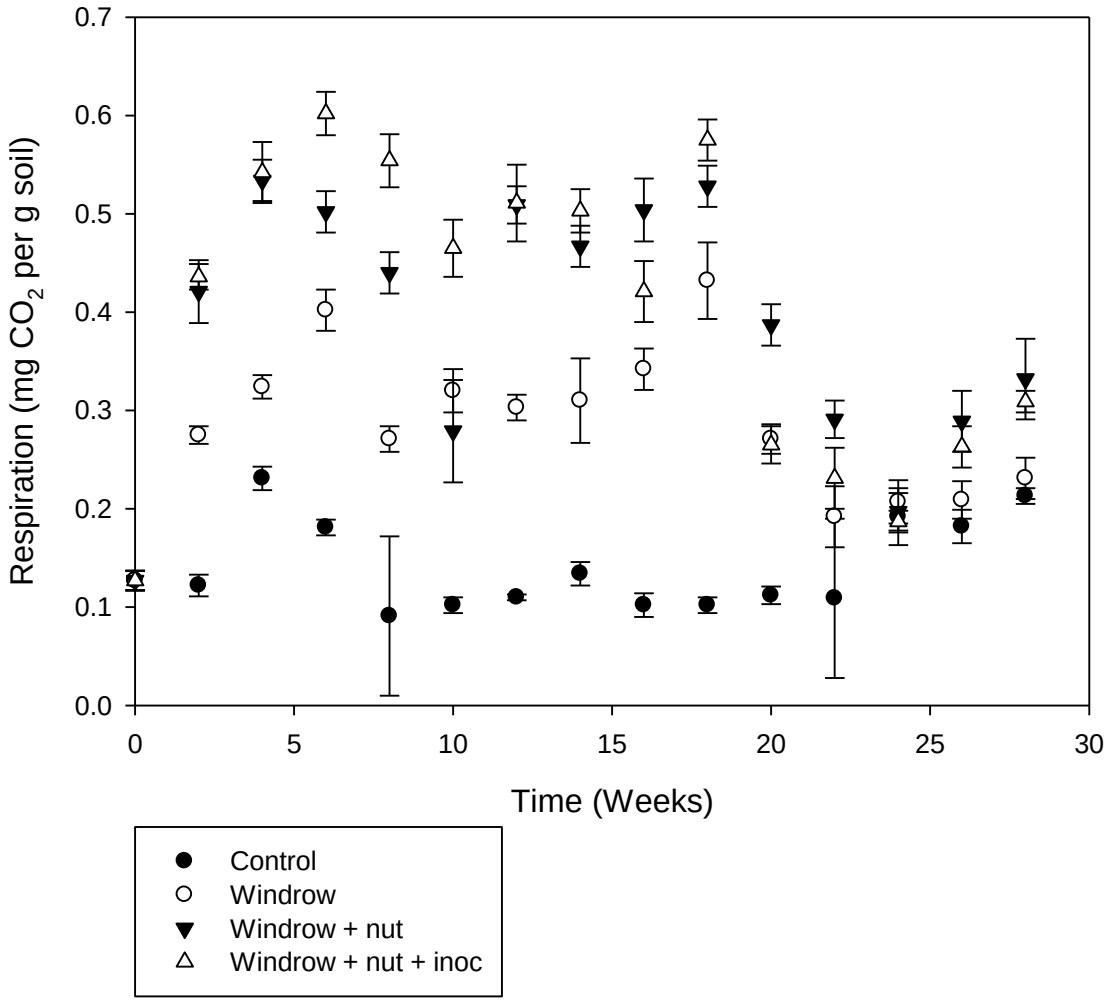

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Respiration (mg $CO_2$ per g soil)
0
5
10
15
20
25
30
Time (Weeks)
Control
Windrow
Windrow + nut
Windrow + nut + inoc

**Figure 3**

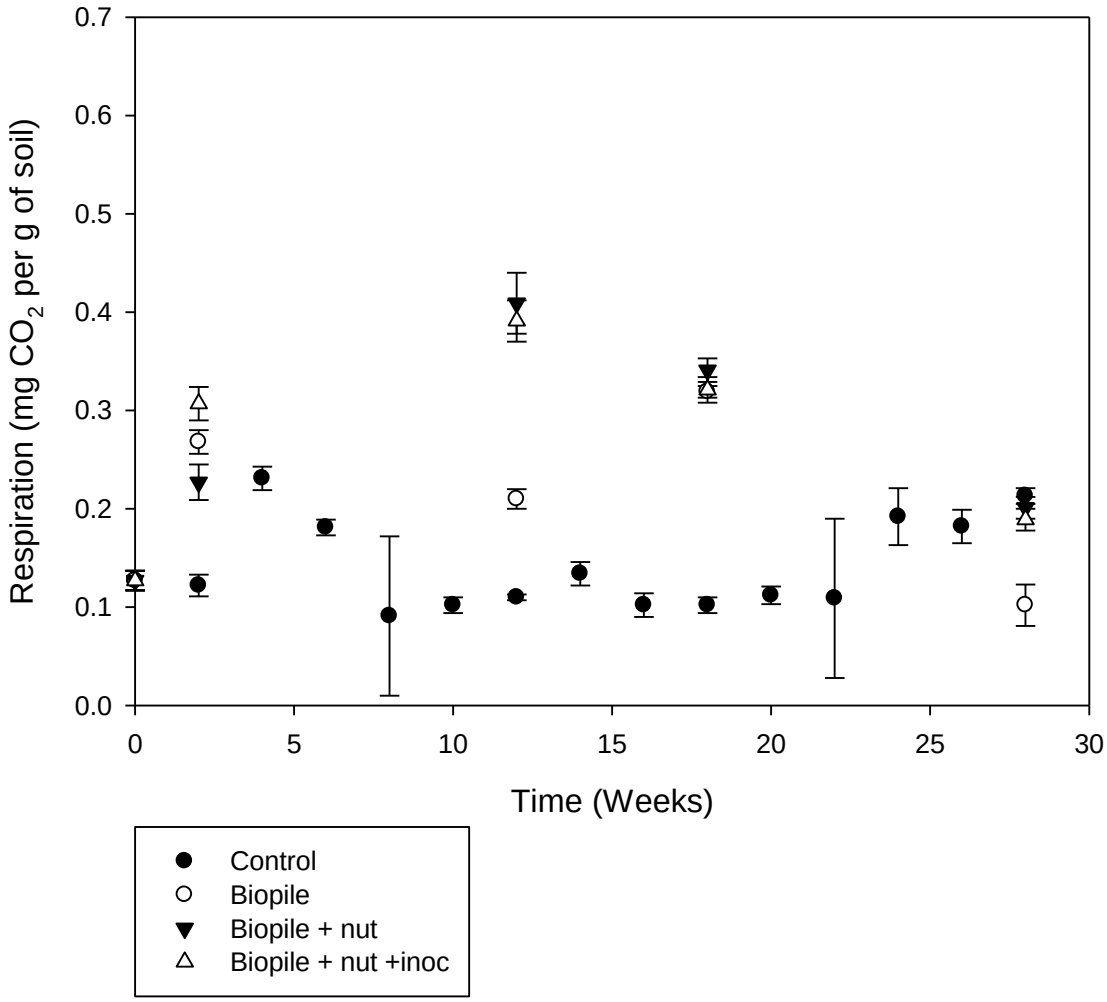

Respiration (mg $CO_2$ per g of soil)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
5
10
15
20
25
30
Time (Weeks)
Control
Biopile
Biopile + nut
Biopile + nut +inoc

**Figure 4**



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# When is a soil remediated? Comparison of biopiled and windrowed soils contaminated with bunker-fuel in a full-scale trial

**Coulon, Frederic; Al Awadi, Mohammed; Cowie, William; Mardlin, David; Pollard, Simon J. T. ...**

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Coulon F, Al Awadi M, Cowie W, et al., (2010) When is a soil remediated? Comparison of biopiled and windrowed soils contaminated with bunker-fuel in a full-scale trial. Environmental Pollution, Volume 158, Issue 10, October 2010, pp. 3032-3040

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