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Comparison of landfarming amendments to improve bioremediation of petroleum hydrocarbons in Niger Delta soils

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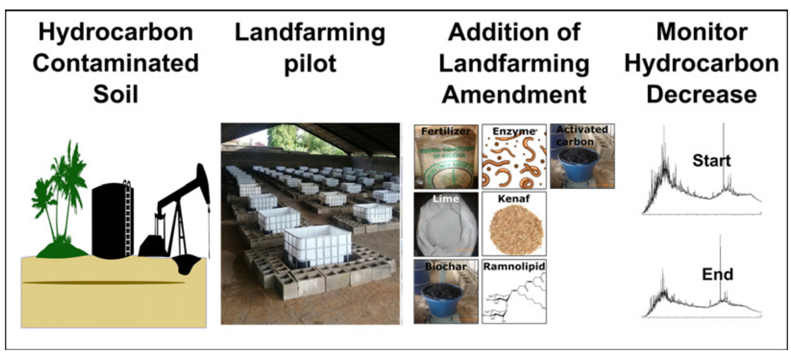
HIGHLIGHTS

- Seven amendments to improve landfarming of hydrocarbons in soils were tested.
- Most were no better than NPK nutrients except biochar combined with rhamnolipid.
- Attenuation rate and hydrocarbon carbon number inversely related.
- Heavier hydrocarbons contributed more to removal due to higher initial concentration.
- Aromatics attenuated faster than aliphatics for all hydrocarbon fractions.

GRAPHICAL ABSTRACT



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ABSTRACT

Large scale landfarming experiments, using an extensive range of treatments, were conducted in the Niger-Delta, Nigeria to study the degradation of oil in contaminated soils. In this work the effect of nutrient addition, biosurfactant, *Eisenia fetida* (earthworm) enzyme extract, bulking and sorption agents and soil neutralization were tested. It was found that these treatments were successful in removing up to 53% of the total petroleum hydrocarbon in the soil within 16 weeks. A comparison between treatments demonstrated that most were no more effective than agricultural fertilizer addition alone. One strategy that did show better performance was a combination of nutrients, biochar and biosurfactant, which was found to remove 23% more Total Petroleum Hydrocarbons (TPH) than fertilizer alone. However, when performance normalized costs were considered, this treatment became less attractive as a remedial option. Based on this same analysis it was concluded that fertilizer only was the most cost effective treatment. As a consequence, it is recommended that fertilizer is used to enhance the landfarming of hydrocarbon contaminated soils in the Niger Delta.

The attenuation rates of both bulk TPH and Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) fractions are also provided. These values represent one of the first large scale and scientifically tested datasets for treatment of contaminated soil in the Niger Delta region. An inverse correlation between attenuation rates

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and hydrocarbon molecular weight was observed with heavy fractions showing much slower degradation rates than lighter fractions. Despite this difference, the bioremediation process resulted in significant removal of all TPH compounds independent of carbon number.

1. Introduction

The Niger Delta (Nigeria) is a region with a substantial history of oil and gas extraction going back to the 1950s. Oil spills have occurred in the region as a result of sabotage crude oil theft, operational spills, and refining under very primitive conditions (Lindén and Pålsson, 2013; Moffat and Linden, 1995; UNEP, 2011). In 2012, the International Union for Conservation of Nature Niger Delta Panel (IUCN-NDP) reviewed the biodiversity recovery of selected impacted areas within the Niger Delta and provided recommendations on remediation and rehabilitation processes for oil impacted sites in the Niger Delta (IUCN-NDP, 2013). One of the main features of the work was focused on reviewing and recommending techniques to improve the effectiveness of landfarming as a method to enhance the degradation of oil in contaminated soil (IUCN-NDP, 2013).

Landfarming is a well-practiced technique used in the Delta, and elsewhere, to bioremediate crude oil contamination in soils to reduce the oil concentration and the associated risks to human health and the environment (Maila and Cloete, 2004). It reduces the concentration of petroleum hydrocarbon constituents principally by bacterially-mediated biodegradation, although volatilization, abiotic processes and fungal-mediated processes may also play a part (Maila and Cloete, 2004). It typically involves the spreading of excavated contaminated soils in a thin layer on the ground surface of a treatment site and stimulating aerobic microbial activity within the soils to accelerate naturally occurring biodegradation processes (Brown et al., 2017; Khan et al., 2004; Vidali, 2001). The removal of petroleum hydrocarbons using landfarming has been applied at large scale with good rates of success (Brown et al., 2017; Heitzer and Sayler, 1993). In addition, field experiments after real oil spills have also shown that the degradation rates can be enhanced with similar methods as those presented in this study (Zabbey et al., 2017; Atlas and Bragg, 2013). The technology is attractive for use in the Niger Delta because it is simple to implement, requires little in terms of infrastructure or equipment, is effective in reducing hydrocarbon concentrations in the environmental conditions founds in the Delta (a warm, wet tropical forest), has less detrimental impacts to surrounding communities than certain more aggressive remedial options, and provides employment opportunities for local communities. As landfarming is most effective when environmental conditions permit microbial growth and activity, its application often involves the enhancement of certain environmental parameters including moisture content, pH and availability of oxygen and nutrients (Brown et al., 2017; Liu et al., 2017).

Weathering of petroleum hydrocarbon contamination in soils is a process characterized by the attenuation of lighter, more volatile and water soluble hydrocarbons and a concomitant increase in the proportion of heavier and more structurally complex components in the residual oil. A number of studies have shown weathered hydrocarbons to be more difficult to biodegrade (Atlas and Bragg, 2013; Björklöf et al., 2008). This is caused by a change in the physical and chemical characteristics of the oil and an increased tendency to bind to soils (Björklöf et al., 2008; Brassington et al., 2010; Huesemann et al., 2004; Liu et al., 2012; Semple et al., 2003). As a result, weathered hydrocarbons are thought to show reduced bioavailability for biodegradation processes. Most remediation of onshore oil spills in the Niger Delta is performed on soils that have undergone some degree of weathering. Therefore, understanding and optimizing landfarming conditions to improve the removal of weathered hydrocarbons is an important step to improving remediation strategies of contaminated soils. The aim of this study was to assess various strategies used to improve the effectiveness of landfarming on oil contaminated soils.

Data is presented from a large scale mesocosm trial performed in Nigeria using an extensive range of treatment variations. To our knowledge, this is the first time a large number of landfarming enhancement technologies have been compared together in a scientifically robust manner to identify the best remedial options available to enhance removal of hydrocarbons from soils using landfarming.

2. Experimental methods

2.1. Test soils and crude oils

The effectiveness of various landfarming treatments were tested on clean, sieved (5 mm mesh) Niger Delta agricultural top soil, sourced from Port Harcourt, combined with fresh Bonny Light Crude oil. The soil used in this work originated from the “low land forest” eco-zone in the Niger Delta. The lowland rain forests areas in the Niger Delta are the most habituated of the ecozones. They are typically cleared of native woodland vegetation and most of the former lowland rainforest is now derived savannah. The land does not flood during the wet season, apart from the areas adjacent to surface watercourses or within surface water flood plains. Soils are typically red clayey silt or silty clay.

2.2. Methods for hydrocarbon analyses in soils

Solvent extractable material (SEM) was measured gravimetrically following Soxhlet extraction with dichloromethane according to Standard Method 5520 E (Clesceri et al., 1998). Total petroleum hydrocarbon (TPH), C_8 to C_{40}, was determined by Gas Chromatography (GC) after extraction with acetone and hexane (1:1) according to a USEPA 8015B method (USEPA, 1996a). TPHCWG of hydrocarbon fractions was measured using USEPA 8015B after fractionation into aliphatic and aromatic fractions using a Rapid Trace Solid Phase Extraction Column (Biotage, Uppsala, Sweden). Hydrocarbons measured by the TPHCWG method were grouped into 14 fractions based on equivalent carbon (EC) numbers (TPHCWG, 1997). For determination of Gasoline Range Organics (GRO) in the carbon chain range of C_5–C_{10} by was performed by headspace GC-FID using the extraction method as described in US EPA method 5021 (USEPA, 2014). Volatile hydrocarbons were quantified using headspace GC–MS according to USEPA methods 8260 (USEPA, 1996b). Semi-volatile hydrocarbons were determined by GC–MS according to USEPA 8270 (USEPA, 1998) after dichloromethane extraction. All work described above was performed by Jones Environmental Laboratories, Flintshire, UK.

2.3. Methods for non-hydrocarbon analysis in soils

pH was determined using Metrohm automated probe analyser after extraction of soil using one part solid to 2.5 parts deionised water. Soil metals were determined by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) using US EPA Method 200.7 (Martin et al., 1994) following acid digestion of dried and ground solid samples using Aqua Regia refluxed at 112.5 °C. Organic carbon was quantified by combustion in an Eltra Total Organic Carbon furnace/analyser following method USEPA 415.1 (USEPA, 1974). Total nitrogen was determined using the Kjeldahl method (Bradstreet, 1954). Anion and cation concentrations in soil pore-water were determined by a Thermo Aquakem Photometric Automatic Analyser. The experimental procedures described above were performed by Jones Environmental Laboratories, Flintshire, UK.

2.4. Crude oil characteristics

Heavy metal content of Bonny Light crude oil (API gravity 28.5) was below detection limit (1 mg/kg) for As, Cd, Mn, Mo and Hg. For Co and V the concentrations were 1.9 mg/kg and 1.3 mg/kg respectively. Total benzene, toluene, ethylbenzene and xylene (BTEX) of crude oil prior to combining with soil was 1666 mg/kg and predominant polyaromatic hydrocarbons, 2-methylnaphthalene and naphthalene, were 97 and 57 mg/kg respectively. All other USEPA priority PAHs in the oil were below detection limit of 10 mg/kg. Distribution of TPHCWG fractions is presented in Fig. 4.

2.5. Soil characteristics

The soil was classified as silty loam, with an organic carbon content of 1.2% (w/w) and pH 5.9. Total nitrogen content was 1300 mg/kg and ortho-phosphate, as PO_4, was <0.3 mg/kg. Initial moisture content of soil was 10.3% (w/w) and total dissolved solid (TDS) concentration was 87 mg/L. Dissolved anion and cation concentrations in soil pore-water were Ca, 2.7; Mg, 0.9; K, 2.3; Na, 0.8; SO_4, 7.1; Cl, 0.4; NO_2, <0.02; NO_3,7.6; PO_4, 0.18 (all in mg/L).

2.6. Spiking of soil with oil and weathering process

Approximately 350 kg of Bonny light crude was combined with 25,000 kg (23,750 kg dry weight) of previously sieved soil in small 100 kg batches and hand mixed using a shovel. After thorough mixing, oil-spiked soil was transferred to clean plastic bulk containers (1 m × 1 m × 0.7 m) and left for 115 days under static conditions to artificially weather the oil. During this period, an average of 22% of the original hydrocarbon mass was lost from the soils (data not shown). After this weathering period, all BTEX components in soil were ≤5 μg/kg, and all 16 USEPA priority PAHs were below the detection limit of 0.01 μg/kg. Heavy metal concentrations were: As, 0.1; Ca, 0.1; Cr, 27.4; Co, 7.5; Hg, 0.3; Pb, 1.4; Ni, 4.4; Zn, 23 (mg/kg dry soil).

2.7. Landfarming control and amendment treatments

The following controls were used in the study to gauge the natural capacity of the soil to biodegrade the oil and benchmark the effectiveness of the various treatments:

- Soil only without oil.
- Soil and oil only.

A total of 16 different landfarming treatments, using combinations of seven different amendments, were assessed for their effectiveness in treating weathered hydrocarbon in Niger-Delta soils (Table 1). The seven amendments assessed were:

- 20:10:10 NPK agricultural fertilizer (Springfield Agro ltd, Nigeria) containing mainly ammonium nitrate, potassium sulphate and diammonium phosphate.
- Dried and homogenized kenaf (*Hibiscus cannabinus*, particle size 10–100 mm) supplied by Inkas Environmental Protection Ltd., Port Harcourt Nigera.
- Agricultural lime (calcium carbonate) supplied by Ronnuel Ltd., Port Harcourt Nigeria.
- R-90 mono (rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate) and di (L-rhamnosyl-l-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate) rhamnolipid mixture from *Pseudomonas aeruginosa* (AGAE Technologies, Oregon, USA).
- Biochar with a surface area of 10–12 m^2/g, a pore volume of 3.2 cm^3/g and a particle size of <3 mm provided by C-Cure Solutions (Surrey, UK).
- Activated carbon (HC200) with a surface area of 600 m^2/g and a pore volume of 0.45 cm^3/g supplied in a powdered form (<1 mm particle size) by C-Cure. It was produced in a self-activating system which did not involve the addition of activating agents.
- 0.1% w/w 'Hydrocarbonase' enzyme mixture purified from *Eisenia fetida* supplied by BOOS TRADE Inc. (Ottawa, Canada).

The designated names for each treatment are provided in Table 1. All treatments, except "Oil only" and "Oil only$^{pH\ 7}$", included equal amounts

Table 1
Description of landfarming treatments performed in study.
Treatments for landfarming hydrocarbons in soils were based on variations of fertilizer use, pH amendment (Lime), biomass bulking agents, sorption agents, enzymes and rhamnolipid biosurfactants.

Amendment	Sample names	Dose or application details	Proposed mechanism of action
Agricultural fertilizer	All mesocosms except Oil only Oil only$^{pH\ 7}$	C:N:P:K ratio of 100:1:0.5:0.5	Provides nutrients necessary for effective biodegradation (Coulon et al., 2012).
Agricultural lime	Fertilizer$^{pH\ 7}$ Oil only $^{pH\ 7}$	Soil neutralized to pH 7	Neutralized pH improves biodegradation effectiveness.
Kenaf	1% Kenaf 3% Kenaf 5% Kenaf	1, 3 or 5% w/w dose	Bulking agent used to increase soil porosity and water retention, lower soil bulk density to increase oxygen access (Lang et al., 2000; Marín et al., 2006; Rhykerd et al., 1999).
Biochar	Biochar	5% w/w dose.	Reduces the hydrophobicity and increase the holding water capacity of the soil. Provides safe harbor for microbial colonization.
Biochar^R	Biochar^R	5% w/w dose. Contains 0.5 g/kg Rhamnolipid	Same mechanism as Biochar. Rhamnolipid increases bioavailability of hydrocarbons.
Activated carbon	Activated carbon	5% w/w	Rapid and irreversible immobilization of hydrocarbons.
Eisenia fetida Enzyme	Enzyme^S Enzyme^M Enzyme^W	Enzyme and rhamnolipid added either as a single dose (Enzyme^S) or several smaller doses (Enzyme^M). Enzyme^W was performed in excess water.	*In vitro* hydrocarbon biodegradation in aqueous phase. Requires surfactant to 'solubilize' hydrocarbon and improve bioavailability.
Rhamnolipid	Rhamnolipid$_{1dose}$ Rhamnolipid$_{5\ doses}$ Rhamnolipid$_{10\ doses}$	Total of 0.5 g/kg added in varying amounts and frequencies.	Surface active agent that improves bioavailability of hydrocarbon in aqueous phase (Whang et al., 2008).

of NPK fertilizer to ensure parity across the experiment. A standard agricultural fertilizer was used in the study because it is widely available and easily sourced in the Niger Delta. In-depth details on the use of amendments and setup of the mesocosms can be found in Supplementary data S1.

2.8. Landfarm mesocosm biodegradation experiments

Mesocosm experiments were performed at the SPDC Remediation Centre of Excellence, Rivers State, Nigeria. Approximately 425 kg of oil contaminated soil, with an initial moisture content of 15% w/w (~65% water holding capacity of soil), was added to each container with a range of amendments (Supplementary data S1). Soils were placed into the containers at a depth of 30–50 cm. A total of 18 different experimental conditions were tested in triplicate. Initial oil dosing varied from 10,000 mg/kg to 12,000 mg/kg for TPH (Table 2) and 11,000 mg/kg to 14,000 mg/kg for solvent extractable material (Supplementary data S2). The arrangement of test plots was assigned randomly within the test area. The mesocosms were kept in a large open ended hangar to reduce the negative effects of heavy rain occurrences that are common in the region. Soils were manually mixed on a weekly basis and moisture content was checked and re-adjusted to 15% (w/w) if necessary every 1–2 weeks. Daytime temperatures during the test ranged from 28 to 36 °C. The sampling of mesocosms was performed immediately after turning using a composite sample consisting of five randomly chosen samples of 200 g. This sample was subsampled and submitted for various analytical analyses.

3. Results and discussion

3.1. An assessment of land-farming amendments for removal of hydrocarbons from soil

The effectiveness of various amendments to bioremediate hydrocarbon contamination from soils was determined through several soil TPH measurements during the experiment (Fig. 1). After 110 days of landfarming a reduction in oil concentration was observed under all conditions. Overall, hydrocarbon removal appeared quasi-biphasic in nature, with the largest decreases occurring within the first 37 days of testing compared to the remainder of the experiment. This pattern is typical for soils undergoing biodegradation (Grace Liu et al., 2011; Tomei and Daugulis, 2013). The decrease in soil hydrocarbon ranged between 15% and 53% (Table 2). Most treatments demonstrated larger TPH decreases compared to the no fertilizer control (Oil only) suggesting an enhancement of the landfarming process (Fig. 1; Table 2). NPK fertilizer (Fertilizer) resulted in a 43% reduction in TPH concentration. This compared favorably against the "Oil only" control mesocosms (32% reduction) and is consistent with other studies that have demonstrated additional nitrogen and phosphate can enhance biodegradation in oil contaminated soils (Coulon et al., 2012). These nutrients are often limited in hydrocarbon contaminated soils but are essential for microbial growth which is metabolically linked to the biodegradation process. Neutralization of the soil with lime (Oil only$^{pH\ 7}$ and Fertilizer$^{pH\ 7}$) caused a small incremental improvement in TPH removal (36% and 45% reduction respectively) compared to the corresponding treatments of soils at native pH (Oil only and Fertilizer) in line with the concept that neutral pH is optimal for microbial processes such as biodegradation (Maier and Gentry, 2015).

In most cases the use of supplementary treatments, in addition to NPK fertilizer, did not stimulate further removal of TPH. Landfarming with kenaf, or *Eisenia fetida* enzyme were no better than NPK treatment alone (Table 2). Previous studies have demonstrated the benefits of using plant biomass, such as kenaf, as bulking agents to improve landfarming treatments under a range of environmental conditions (Hamdi et al., 2007; Souza et al., 2009; Vasudevan and Rajaram, 2001; Wang et al., 2016). In this study, however, adding kenaf to the soil did not stimulate additional hydrocarbon biodegradation. It is possible that the characteristics of the soil used in the experiment (sieved and easily friable), combined with the high soil aeration negated any benefits provided by the kenaf. Interestingly, temperature and soil gas measurements for kenaf showed observable differences compared to other treatments. Higher temperatures, lower soil O_2 and higher CO_2 soil concentrations were observed suggesting enhanced microbial respiration (data not shown), but the increase in microbial activity did not translate into greater rates of hydrocarbon biodegradation which suggests it was associated to the degradation of the kenaf itself.

The *Eisenia fetida* enzyme product is a purified hydrolase enzyme mixture produced from earthworms (Tomečková et al., 2012). Treatment of soils with live earthworms has been shown to improve TPH removal (Dendooven et al., 2011; Ekperusi and Aigbodion, 2015). A major mechanism considered important in this process is the removal of hydrocarbons from soils ingested by the worms (Dendooven et al.,

Table 2
Initial and final concentrations of TPH, percent remaining and calculated attenuation rate constants and half-lives for various landfarming treatments.
Final TPH values generated after 110 days of landfarming. All values represent the average of three individual replicates. Standard errors (SE) for all measurements except for attenuation rate constant data which is the coefficient of determination (r^2) value. k was calculated from equation: $-1n[\Delta TPH] = kt + b$. Attenuation half-life calculated from equation: $0.693 / (2\ k)$. * Indicates statistically different to Fertilizer treatment by paired student t-test ($p < 0.05$).

Treatment	Initial TPH		Final TPH		TPH remaining		Attenuation rate constant		Attenuation half-life	
	mg/kg dry soil	SE	mg/kg dry soil	SE	%	SE	k (d^{-1})	r^2	days	SE
Soil only control	0	n/a	427	95	n/a	n/a	n/a	n/a	n/a	n/a
Oil only	12,133	616	8277	448	68	1.1	0.0031	0.90	226	23
Oil only^{pH7}	10,420	491	6632	180	64	1.8	0.0041	0.99	170	10
Fertilizer	11,474	315	6534	278	57	2.7	0.0048	0.95	144	4
Fertilizer^{pH7}	11,387	378	6268	244	55	0.3	0.0050	0.93	142	15
Fertilizer^{NT}	10,674	257	6797	278	64	3.5	0.0036	0.92	196	26
1% Kenaf	11,329	448	6730	268	60	4.5	0.0042	0.88	173	30
3% Kenaf	11,275	374	6270	709	55	4.6	0.0043	0.82	162	15
5% Kenaf	12,214	157	6709	274	55	1.6	0.0050	0.89	138	6
Biochar	10,803	574	5992	53	56	2.4	0.0049	0.90	145	14
Biochar^{R}	10,896	245	5161	405	47	1.7	0.0067	0.93	103*	5
Activated carbon	11,105	212	7628	737	71	3.9	0.0028	0.54	267	143
Enzyme^{M}	11,449	98	6549	102	60	0.3	0.0048	0.99	148	0.5
Enzyme^{S}	11,748	457	6931	284	59	3.2	0.0051	0.94	134	13
Enzyme^{W}	10,242	513	8722	426	85	3.6	0.0014	0.90	518	112
Rhamnolipid^{1dose}	9957	507	6589	325	67	6.2	0.0038	0.97	186	43
Rhamnolipid^{5doses}	10,218	383	6468	133	64	3.6	0.0039	0.98	193	43
Rhamnolipid^{10doses}	11,736	462	7431	330	63	0.5	0.0034	0.86	209	21

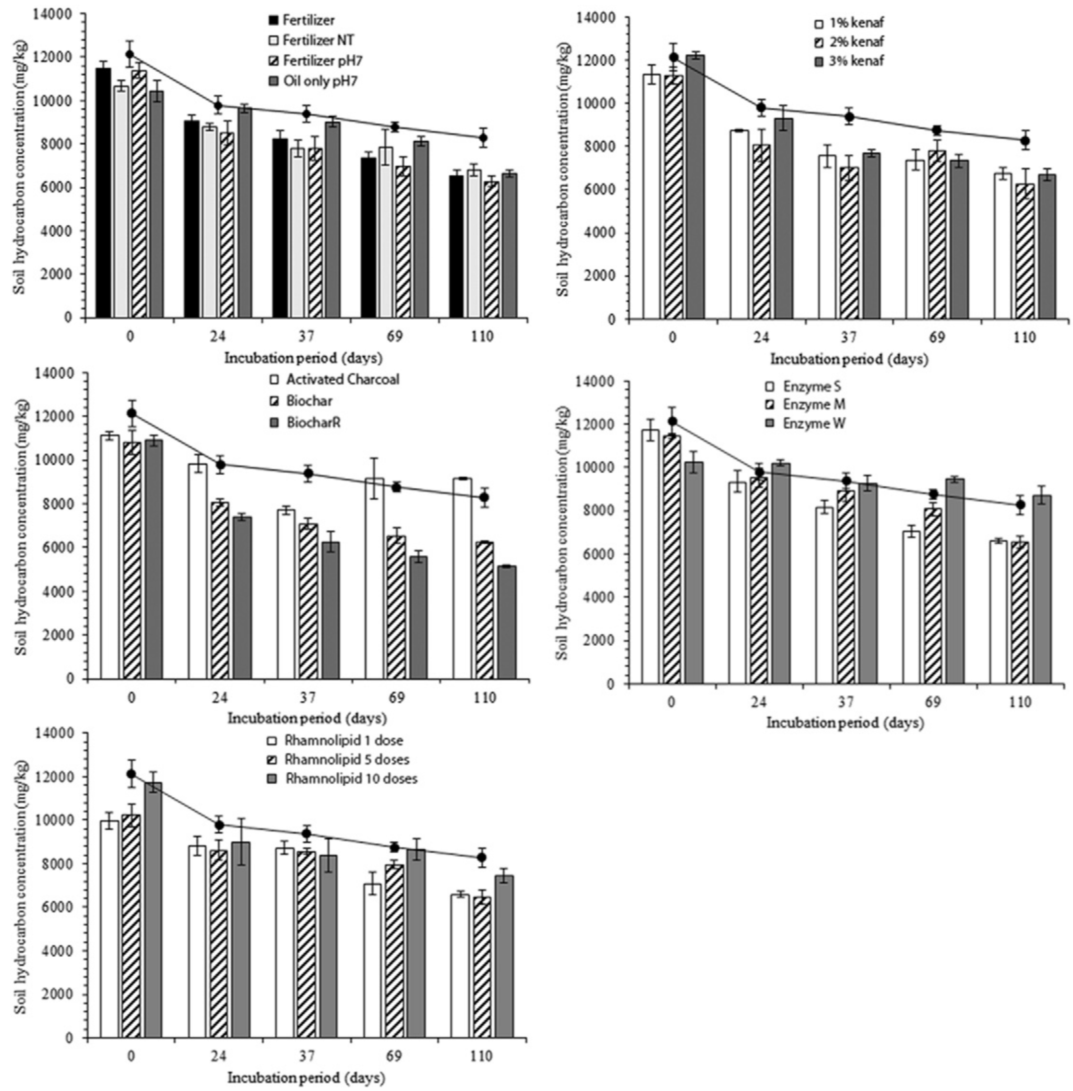

Fig. 1. The effect of amendments on TPH removal from soils during landarming Niger Delta soils. Data is the average of three replicates and based on soil TPH concentrations (mg/kg dry soil) measured at day 0, 24, 37, 69 and 110. The line graph in each figure represents the "Oil only" control data. Error bars show the standard error.

2011). Intracellular enzymes, produced by the worm or microbes living symbiotically in the gut of the worm, are considered the main protagonists of this process (Azadeh and Zarabi, 2014). *In vitro* use of purified *Eisenia fetida* enzymes requires hydrocarbons to be pseudo-solubilized using a surfactant. Therefore, rhamnolipid was used together with the enzyme. Three different treatment procedures were undertaken to assess *Eisenia fetida* enzyme performance. Two were performed under moisture and aeration conditions consistent with other treatments, using either a single large dose at the beginning of the landfarming procedure or smaller multiple doses of enzyme (Enzyme^S and Enzyme^M respectively) throughout. The third procedure (Enzyme^W), recommended by the supplier, used the same overall enzyme dosage but required excess water in the mesocosm to assist with hydrocarbon solubilization (Supplementary data S1). Despite reductions in TPH the use of *Eisenia fetida* enzyme did not enhance TPH removal from soils when compared to fertilizer only treatments (Table 2). Although the ineffectiveness of the *Eisenia fetida* enzyme treatment was not further elucidated in this study, the result does highlight some of the potential difficulties associated with using purified intracellular enzymes in field conditions. These products generally require a narrow set of conditions (pH, moisture level, temperature) to work effectively, lack stability and may require additional co-factors (Eibes et al., 2015). There are examples of free enzyme products working successfully to remove hydrocarbons from soils (Vinson and Garret, 2000). Therefore, further work may be warranted to develop this technology to work effectively under *ex situ* conditions present in landfarms.

Rhamnolipids are surface active compounds that promote wetting, solubilization, and emulsification of hydrocarbons to increase bioavailability for microbial biodegradation processes (Lai et al., 2009). Their impact on bioremediation reported in previous studies has been inconsistent (Chrzanowski et al., 2012; Santa Anna et al., 2007; Whang et al., 2008), presumably due to the specificity of the interactions between target organic compounds, the specific microbial communities and surfactants (Volkering et al., 1997). In this study, performed at single or multiple doses of between 50 and 500 mg/kg dry soil (Supplementary data S1), rhamnolipids did not enhance hydrocarbon biodegradation (Table 2). Furthermore, these treatments were less effective than fertilizer only controls. This suggests that rhamnolipids caused some form of inhibition on biodegradation in these mesocosms. Evidence for direct inhibition of microbial processes by rhamnolipids has been demonstrated. Shin et al. (2005) reported that they are toxic to microorganisms, whilst Bondarenko et al. (2010) noted that several strains of gram negative bacteria are impacted when measured using a bioluminescence assay. In-direct toxicity from pseudo-solubilized hydrocarbons may also cause toxicity of microorganisms and contribute to reduced biodegradation. Previous work has shown increased toxicity to bacteria of

Table 3
Estimated amendment cost of landfarming treatments.
The cost of each amendment was calculated on a performance basis from data provided in Table 2 and using the doses described in Supplementary data S1. Details of calculation are provided in Supplementary data S5.

Treatment	Total amendment cost to remediate soil to 50% of initial TPH concentration ($\$/m^3$ soil)
Fertilizer	0.5
Fertilizer^{NT}	0.7
Oil only$^{pH\ 7}$	0.7
Fertilizer$^{pH\ 7}$	1
1% Kenaf	2
3% Kenaf	4
5% Kenaf	6
Biochar	12
Activated Carbon	44
Biochar^{R}	165
Rhamnolipid^{10doses}	223
Rhamnolipid^{5doses}	229
Rhamnolipid^{1dose}	250
Enzyme^{M}	262
Enzyme^{S}	268
Enzyme^{W}	715

diesel contaminated soils in the presence of rhamnolipids (Marecik et al., 2012). These studies contrast with others that show a positive impact on removal of hydrocarbons during biodegradation (Cameotra and Singh, 2009; Inakollu et al., 2004; Whang et al., 2008). This disparity highlights the unpredictability of effects of rhamnolipids when used in landfarming experiments. Interestingly, our preliminary small-scale laboratory based studies using the same rhamnolipids demonstrated enhanced hydrocarbon biodegradation and cell growth compared to controls (Supplementary data S3). These differences emphasize the importance of testing treatment performance under appropriate field conditions.

Biochar has been reported to improve soil quality by raising soil pH, increasing moisture holding capacity and retaining nutrients in the soil (Atkinson et al., 2010; Laird et al., 2010). Because of its high surface area and porosity, biochar may be a good substrate for colonization of soil-indigenous fungi and bacteria (Quilliam et al., 2013). Biochar can adsorb organic compounds, such as hydrocarbons, onto its surface in a mechanism that is dependent on type of biochar (Domene et al., 2015). Reports on biodegradation of organic compounds in the presence of biochars have suggested that sorption of both the organics and microbes to biochar surfaces may give rise to a greater concentration of organics close to the colonizing microbes and therefore increase the rate of biodegradation of these compounds (Anyika et al., 2015). The use of biochar (Biochar) in this study was found to be no better than fertilizer only treatment at the removal of hydrocarbons from soils (Table 2). To expand the assessment of biochar further, it was evaluated together with rhamnolipid (Biochar^{R}). Combining the two products was found to be synergistic and enhance hydrocarbon removal from soils (Table 2). Based on TPH measurements, 53% of hydrocarbons in soils were removed during the remediation process compared to 43% with fertilizer only (Fertilizer). To our knowledge, this is the first report demonstrating enhanced oil biodegradation in soils through the synergistic action of biochar and biosurfactant. The exact mechanism of action responsible for this improvement is unclear. Biochar has been shown to reduce mobility of rhamnolipids in soils through adsorption (Vu et al., 2015). Slow diffusion of the rhamnolipid into the soil pore water may result in a long term yet low concentration source that negates possible toxicity effects observed in this study. Alternatively, a complex mobilization/adsorption mechanism of oil from the soil onto the biochar may be considered. Enhanced biodegradation would be achieved by microbes in the biochar as described earlier in this work (Anyika et al., 2015).

Assessment of activated carbon was also performed. This product has extremely high surface area (600 m^2/g) and consequently has a high capacity to bind hydrocarbons and other organic contaminants (Zhang et al., 2016). Previous studies have shown that strong sorption of hydrocarbons to activated carbon reduces bioavailability and subsequent biodegradation (Meynet et al., 2012). As a consequence, it may promote the stabilization of oil in soil through immobilization rather than enhanced biodegradation. The results generated in this work support that position. Activated carbon treatment was found to hinder hydrocarbon removal from soils (Table 2). Extraction tests, with methanol as a polar solvent, demonstrated activated carbon had a greater capacity to adsorb hydrocarbons compared to other treatments (Supplementary data S4). It was calculated that 77% of the residual hydrocarbon was resistant to methanol extraction in soils containing activated carbon compared to 18% in fertilizer only control soils (Fertilizer). Although this treatment may not be suitable for landfarming it may be beneficial in cases were rapid immobilization of oil contamination is necessary.

From an implementation perspective, the use of various amendments to improve landfarming performance needs to be balanced with financial costs and the practical considerations related to treatment application. The use of a combined biochar and rhamnolipid treatment (Biochar^{R}) may be challenged when considering cost and performance together. From performance-normalised cost calculations of each amendment (Table 3 and Supplementary data S5), it can be seen that Biochar^{R} performance is reduced relative to other treatments. Based on this analysis the best performing amendment was the use of fertilizer alone. Other factors, such as adequate soil moisture, a soil pH of between 6 and 8, and good aeration are known to be important in stimulating biodegradation in soils (Brown et al., 2017). Therefore, it is important to recognize that the use of fertilizer during landfarming of hydrocarbon contaminated soils also requires good control of soil moisture and pH and frequent aeration, through tilling, to be most effective. In this work soils were tilled on a weekly basis and soil moisture was measured and adjusted every 1–2 weeks using a portable moisture sensor (Delta-T Devices, Cambridge, UK). Applying this regime during the bioremediation of real sites should not be challenging.

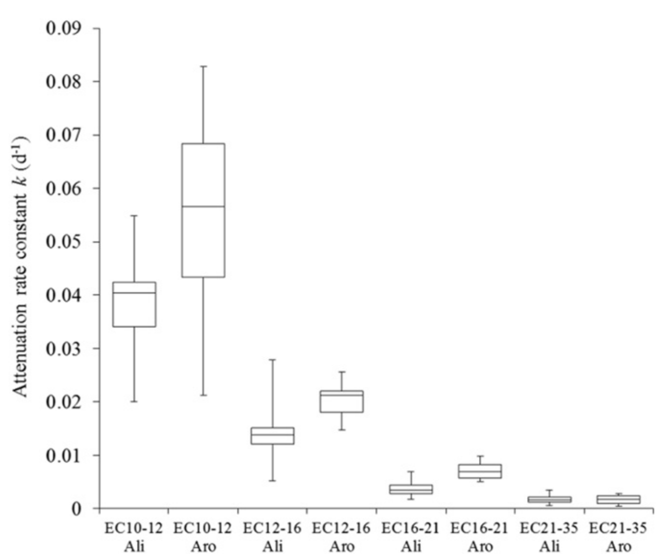

Fig. 2. First order attenuation rate constants k of TPHCWG hydrocarbon fractions. First order attenuations rate constants (k) of TPHCWG fractions were calculated from equation: $-1n[\Delta TPH] = kt + b$. Hydrocarbon fraction designation based on TPHCWG assignment up to EC35 (TPHCWG, 1997). EC is equivalent carbon number, Ali is aliphatic hydrocarbon and Aro is aromatic hydrocarbon. EC10–12 is EC > 10–12; EC12–16 is EC > 12–16; EC16–21 is EC > 16–21; EC21–35 is EC > 21–35. There Rates calculated from four separate measurements of TPHCWG fractions of landfarming treatments on days 0, 39, 69 and 110. Data presented is a boxplot of all landfarming treatments except activated carbon, Enzyme^{W} and soil only control (15 treatments in total). The minimum and maximum extents of the boxes represent the first and third quartiles respectively, whilst the centre line shows the median value. The bars mark the minimum and maximum extent of the data. Complete dataset is provided in Supplementary data S5.

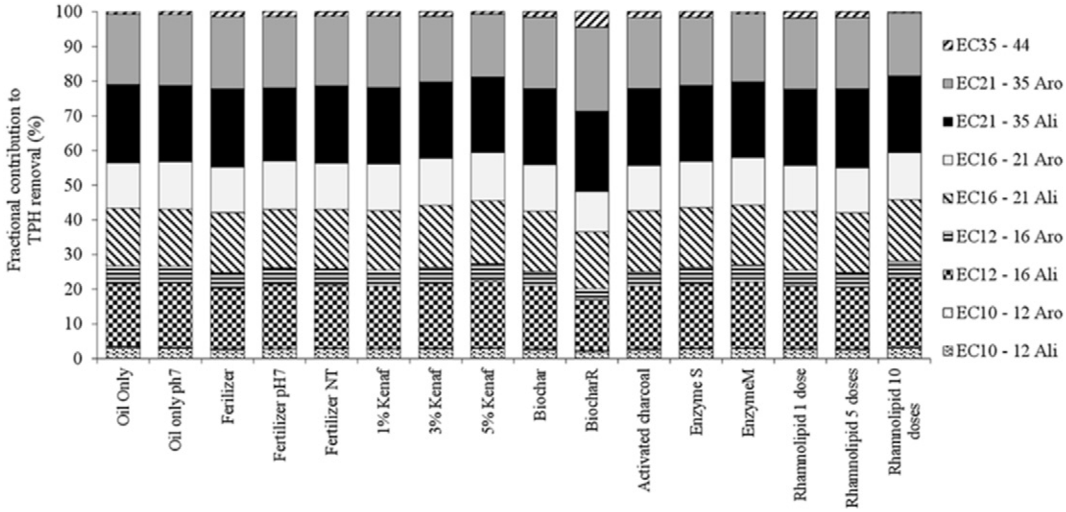

Fig. 3. Contribution of individual TPHCWG fractions to bulk TPH removal during landfarming. Data is based on initial and final TPHCWG fraction concentrations in relation to total TPH reduction. Average of three data values shown. Bars represent individual TPHCWG fractions (TPHCWG, 1997) and are described in the legend. EC is the equivalent carbon number, Ali is aliphatics and Aro is aromatics. EC10–12 is EC > 10–12; EC12–16 is EC > 12–16; EC16–21 is EC > 16–21; EC21–35 is EC > 21–35; EC35–44 is EC > 35–44.

3.2. Rates of hydrocarbon attenuation

First order attenuation rate constants and half-lives, useful for calculating remediation timespans (Liu et al., 2016), were determined using bulk TPH measurements (Table 2 and Supplementary data S6). The attenuation rate constants for most treatments were calculated to be between 0.003 and 0.005 d^{-1}, with biochar plus rhamnolipid demonstrating a faster rate of 0.007 d^{-1}. A similar range of rates (0.001–0.008 d^{-1}) have been described in a number of other worldwide landfarming reports demonstrating the current study to be within expectations for TPH removal from soils (Gallego et al., 2010; Genouw et al., 1994; Wang et al., 2016). Translated into half-lives, the attenuation rate values represent 103 to 226 days. Student *t*-test comparisons of attenuation half-lives against the fertilizer only treatment (Fertilizer) were performed (data not shown). This showed that of all the treatments investigated only biochar plus rhamnolipid (Biochar^R) was statistically different ($p < 0.05$) (103 days) than fertilizer only treatment (144 days, Table 2). From a practical perspective, the reduction in half-life may be useful in circumstances were remediation time is limited.

Attenuation rates for different Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) fractions are presented in Fig. 2 and Supplementary data S7. In agreement with previous studies an inverse correlation between attenuation rate and molecular weight was observed (Leahy and Colwell, 1990). Volatilization is expected to be a significant contributor to the attenuation rates of the EC > 10–12, whereas biodegradation will be the major force driving attenuation for the remaining fractions (Coulon et al., 2010; Pollard et al., 2008). Whilst this data shows the impact of hydrocarbon molecular weight it does not completely depict the impact the attenuation of each fraction has to the overall progression of hydrocarbon removal from soils. To fill this gap, the contribution of each fraction to the total removal of hydrocarbon was determined to provide clearer representation of attenuation in soils (Fig. 3). By representing data in this way it can be seen that despite comparatively slow rates of attenuation, the heaviest TPHCWG fractions show the greatest contribution to the overall removal of hydrocarbons soil. Up to 50% of total TPH removal was achieved *via* attenuation of hydrocarbons greater than >EC 21. This contribution is clearly brought about by the high concentration of these fractions in the starting oil relative to the lighter fractions which attenuate faster but

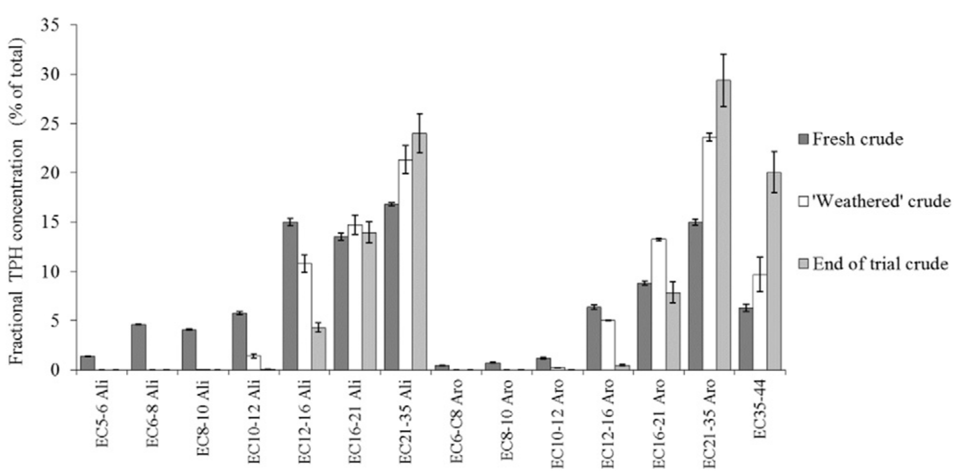

Fig. 4. Change in TPHCWG fraction distribution before and after 115-day weathering process and after 110 day landfarming trial. Data shows the change in crude oil composition for fertilizer only treatment, represented by TPHCWG fractions (TPHCWG, 1997). Data is average of three replicates and error bars show standard error. EC represents equivalent carbon number. Ali is aliphatic and Aro is aromatics. EC10–12 is EC > 10–12; EC12–16 is EC > 12–16; EC16–21 is EC > 16–21; EC21–35 is EC > 21–35; EC35–44 is EC > 35–44.

are present at lower amounts. The observation underlines the importance of heavier hydrocarbon biodegradation in overall TPH removal. It also agrees with previous studies that shows biodegradation is not a linear process and all hydrocarbons regardless of size or class are degraded simultaneously albeit at different rates (Grace Liu et al., 2011; Huesemann and Moore, 1993).

3.3. Crude oil compositional changes through landfarming and implications for human health and ecological risks

The present study resulted in a preferential removal of lighter fractions and a relative increase in heavier fractions (Fig. 4). TPHCWG data showed that the hydrocarbon composition changed from 16% to 5% for EC > 12–16; 27% to 22% for EC > 16–21; 51 to 53% for EC > 21–35; and 9% for 20% of EC > 35–44. Interestingly, our results show higher removal rates for aromatic fractions than aliphatic fractions, which contrasts with earlier studies (Oudot et al., 1998). It has been speculated that the preference for removal of certain hydrocarbon classes is related to the microbial community in the soil (Grace Liu et al., 2011). Unfortunately, this study did not assess microbial community structure during the landfarming process. TPHCWG fractions are defined by their EC number range which is based on boiling point rather than actual carbon number (TPHCWG, 1997). Therefore, it may be inappropriate to directly compare aliphatic and aromatic fractions using this system. Changes in oil composition resulting from landfarming activities may alter the risk profile of the residual oil (Brassington et al., 2010). Heavier hydrocarbons have a lower aqueous solubility, are less volatile and less mobile in the environment (TPHCWG, 1997) and are generally considered to be less toxic (Redman et al., 2014).

4. Conclusions

This work has demonstrated that landfarming can be an effective method for treatment of weathered oil contaminated soil. Assessment of a wide range of treatments showed that most resulted in no improvement compared with the fertilizer only treatments. Our experiments found that the remediation performance of fertilizer only treatment was only bettered by a combination of biochar and rhamnolipid. The cost of using biochar and rhamnolipid are high. As such, performance based cost calculations indicate this treatment to be less beneficial compared to a range of other amendments (Table 3). The best performing amendment, based on cost and performance, was agricultural fertilizer. It is recommended that this be used during the bioremediation of hydrocarbon contaminated soils in the Niger Delta in addition to maintaining adequate moisture, aeration and a pH between 6 and 8.

During the course of landfarming, hydrocarbon composition of the contaminating oil changed significantly. An observed relative increase in heavier TPHCWG fractions may indicate a potential reduction in human health and ecological risks based on changes in aqueous solubility, volatility and other physical and chemical properties. An alternative practical intervention strategy to reduce these risks is to reduce the solubility, mobility and bioavailability of the spilled hydrocarbon in the environment. As described previously, the trial results suggest that amendment of impacted soils with adsorbent materials, such as activated carbon, could have potential application in order to achieve this, though susceptibility for re-release of sorbed hydrocarbons would require further investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.04.072.

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