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The Role of Natural Weed Species from Soil Seed Bank in the Natural Attenuation of a Petroleum Hydrocarbon Polluted Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GOA and BI designed the study. All authors performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the analyses of the study. Author CCCO managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The present study investigated the effect of the presence of natural weeds in the remediation of oil polluted soils that have not been disturbed or modified anthropogenically.
Study Design: The experimental design chosen was the completely randomized design (CRD) following assumption of homogeneity of the experimental plot in use.
Place and Duration of Study: Botanic Garden, University of Benin, Benin City. Study period spanned from April 2011 through December 2012.
Methodology: Top soil was collected from a marked plot and thoroughly mixed with waste engine oil on weight basis to obtain 2.5%w/w oil-in-soil concentrations. Ten kilograms of the contaminated soil were measured each into experiment bowls. The entire set up was divided into 4 sets. The natural weeds that eventually emerged in the first set (W_{wds}) were left undisturbed throughout the duration of the experiment. Those in the second set (W_{4mw}) were removed once every four months. Those in the third set were manually removed as soon as they appeared, leaving none on the soil surface (W_{non}).

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The soils in the fourth set were sterilized before amendment with waste engine oil. The entire set up was exposed to the various treatment conditions for 20 months.
Results: There were significant reductions in composition of soil heavy metals and polyaromatic hydrocarbon contents in the all the treatments. The treatment showing most enhanced remediation was W_{wds}, followed by W_{4mw}, W_{non}, and $W_{ctr/ste}$ in that order. Seven out of the nineteen weed species that originally made up the soil seed bank, were identified in the polluted soil. These included *Euphorbia hirta, Fluerya aestuans, Panicum maximum, Phyllanthus amarus, Spigelia anthelmia,* and *Tridax procumbens.* The predominant weed species was *Euphorbia heterophylla.* These weed species are likely oil tolerant species.
Conclusion: The study further affirms that weed composition of any soil is to be reckoned with as an important factor in the natural attenuation of a petroleum hydrocarbon-polluted soil.

Keywords: Attenuation; pollution; petroleum; soil seed bank.

1. INTRODUCTION

The petroleum industry holds a prime position in the modern world economy, and it is mainly centered on crude oil. The economic importance of crude oil is to be found in the numerous possible products obtainable from crude oil through refining – the uses of these products in the modern industrialized world justify the resources committed to its exploration and production. Motor fuels, domestic fuels, industrial fuels for heating and power generation as well as lubricants are among the products derivable from crude oil. The industry is however responsible for the generation of large amounts of organic residues, as well as for the pollution of soils, rivers and seas.

In Nigeria, most of the terrestrial ecosystem and shorelines in oil-producing communities are important agricultural land under continuous cultivation. However, in none oil-producing States, most of the agricultural lands are predisposed to waste engine oil (WEO) from nearby workshops and even homes that use engine oil and dispose of WEO. Any contact with WEO results in damage to soil condition of these agricultural lands, microorganisms and plants, and the resultant effects in the economy and the lives of the populace may be very devastating.

Twelve million gallons of crude oil are spilled into the environment annually as against 20 million gallons of WEO that are generated annually from mechanic workshops and discharged carelessly into the environment [1,2]. The impact of oil pollution is very devastating particularly in agricultural soil. The need, therefore, for remediation, cannot be over emphasized. When an oil polluted soil is left to lie fallow for a long period of time, the level of pollutants inherent in the soil eventually reduces significantly until no pollutant is left. This ability for the soil to remediate the soil without any artificial disturbance or amendment is referred to as natural attenuation. Most of the successfully attenuated soils are found to have diverse inherent plant populations. It is the assumption of the researchers that the plant population significantly enhances remediation of such soils. The determination of this assumption formed the basis for this study.

The breakdown of contaminants in soil resulting from microbial activity that is enhanced in the presence of the plant root zone (rhizosphere) has been termed *rhizoremediation.* Most remediation occurring in plant zones have been found to occur in many natural weed

species [3]. This formed the basis for a study conducted by Anoliefo et al. [3] on the ecotaxonomic distribution of weed species that were resident in auto-mechanic workshops in Edo and Delta States of Nigeria, where they suggested that such weeds, based on their frequency of abundance, were likeable candidates for phytoremediation strategies. Identification of plants that is prevalent in polluted sites is an important criterion for determination of capacity for tolerance of such plants, and their potential for phytoremediation of such polluted sites could be assessed. In the study by Anoliefo et al. [3], plant species and their families that were present in auto mechanic workshops in Benin City and Asaba were surveyed. The frequency of occurrence of plants in the sites visited was used to determine prevalence. The high rate of occurrence of a particular plant species in the frequency table, suggests that such plants are tolerant and may be introduced as a possible phytoremediating agent. The use of native plants offers an economically feasible and environmentally sustainable cleanup option for the rehabilitation and restoration of hydrocarbon contaminated sites in Nigeria. The aim of the study therefore was to determine the place of weed species in the remediation of oil polluted soils that have not been disturbed or modified anthropogenically, thereby evaluating the potential of native weed species for the rhizoremediation of hydrocarbon contaminated soil. The present study is a follow up to the previous study conducted by Anoliefo et al. [3].

2. MATERIALS AND METHODS

An area, located at the main campus of the University of Benin, Benin City that had not been cultivated in the last 5 years was selected for the study.

All plant species inherent in the area were identified in order to determine soil seed bank. Ten kilograms of top soil (0 – 10 cm) was measured into large bowls (50 cm diameter). The soil in each basin was mixed thoroughly with 250g WEO to obtain 2.5% w/w oil-in-soil. The set up was divided into 4 sets. The bowls in the first set (W_{wds}) were left undisturbed. The weeds that eventually emerged in these bowls were allowed to remain throughout the study. There was partial clearing of weeds in the second set every 4 months (W_{4mw}). In the third set, there was total clearing of weeds (W_{non}). Weeding was done by carefully removing any weed that appeared above surface as soon as they were visible, without having to disturb or turn the soil. The soils in the fourth set were initially sterilized by oven drying at 130 ^{o}C for 3 days before amendment with WEO. The aim was to eliminate the inherent soil seeds bank. This was the control experiment ($W_{ctr/ste}$). Each set comprised of 10 bowls as replicates, making up a total of 40 bowls. The entire set up was left for a period of 20 months.

2.1 Soil Microbial Analyses

Isolation and characterization of heterotrophic and hydrocarbon degrading microorganisms was carried out using the methods of Cheesebrough [4] and Taiwo and Oso [5]. The soil samples were air-dried and sieved through a 2 mm mesh to remove undesirable material. The dilution series for the soil sample was done by transferring 1 gram of the soil to nine (9 ml) millimetres of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 minutes and was taken as $l0^{-1}$ dilution factor, 10 ml were then transferred from the 10^{-1} dilution into another 9 ml blank to obtain a 10^{-2} dilution and same process of transfer was repeated twice to obtain a dilution factor of 10^{-4}.

2.1.1 Heterotrophic bacterial and fungal counts

The spread plate method was employed in taking the heterotrophic bacteria counts. One (1) ml of the serially diluted portion of 10^{-4} of each soil sample was inoculated onto nutrient agar plates for bacteria and Potato dextrose agar plates for fungal counts. The plates were inoculated at room temperature for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation colonies were then counted and the colony forming unit (cfu/g) of the soil samples determined.

2.1.2 Isolation of bacterial and fungal oil degraders

Bushnell- Haas (BH) medium ($MgSO_4$, 0.20 g/l; $CaCl_2$, 0.02 g/l; $K_2H,P0_4$, 1 g/l; NH_4NO_3, 1 g/l; $FeCl_3$, 0.05 g/l; KH_2PO_4, 1 g/l; pH 7.0, was used as the enrichment medium with 8 % (v/v) filter sterilized oil as the sole carbon source. The medium was dispensed into in 100 ml Erlenmeyer flasks and autoc1aved at 121 ^{0}C for 15 minutes. Thereafter, 5 g of each soil sample was inoculated into each flask of the medium and incubated at 130 rpm at room temperature in a HY-4 multifunctional shaker (B. Bran Scientific and Instrument Company, England). After 10 days, 1 ml of enriched media was transferred into freshly prepared enrichment media and incubated under the same conditions as described above. Serial dilutions from the third enrichment process were inoculated onto nutrient agar plates and potato dextrose agar plates for oil-degrading bacterial and fungal counts respectively.

2.1.3 Characterization and identification of bacterial oil-degrading isolates

The bacterial isolates that were predominantly isolated were identified to their species level using conventional microbiological and biochemical tests as described by Cheesebrough [4] and Taiwo and Oso [5].

2.1.4 Characterization and identification of fungal oil-degrading isolates

The fungal isolates that were predominantly isolated were identified to their species level by colonial characteristic and microscopic examination of hyphal morphology as well as by structure and nature of the fruiting body.

2.2 Soil Physicochemical Analyses

In the laboratory, soils were dried at ambient temperature (22-25ºC), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2mm samples were stored in polythene bags for subsequent analysis. The <2mm fraction was used for the determination of the heavy metal fractions (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni, V) by AAS according to the methods of APHA [6]. Polyaromatic hydrocarbon contents were determined by gas chromatography (GC) using the by methods of ASTM [7] and ERI [8].

2.3 Statistics

The experimental design chosen was the completely randomized design (CRD) following assumption of homogeneity of the experimental plot in use. As a result, the treatments were randomized over the whole plot. Each treatment consisted of 10 replicates. There were 4 treatments, each designated as $W_{ctr/ste}$, W_{non}, W_{4mw}, and W_{wds}. In order to avoid bias and misidentification, treatment bowls were properly labeled according to a given treatment

name and replicate number. Mean of data as well as analysis of variance in the study was done using the *SPSS-15* statistical software, and means were separated by using the Least Significant Difference.

3. RESULTS AND DISCUSSION

Table 1 presents a list of weeds species that were previously identified in the area from which soil was collected for the present study. The list is therefore a composition of the soil seed bank. There were 19 different weed species comprised of the families Convolvulaceae, Euphorbiaceae, Lamiaceae, Loganiaceae, Urticaceae and predominantly Amaranthaceae, Asteraceae, and Poaceae.

Table 1. Weeds identified in the area where soil was collected for the experiment

Name	Family
Acalypha ciliata Forsk.	Euphorbiaceae
Acanthospermum hispidum DC.	Asteraceae
Ageratum conyzoides Linn.	Asteraceae
Brachiaria deflexa (Schumach.)C.E Hubbard ex	Poaceae
Echinochloa stagina Beauv.	Poaceae
Eragrostis tenella (Linn.) P. Beauv. ex Roem & Schult	Poaceae
Erigeron floribundus (H.B.&k) Sch. Bip	Asteraceae
Euphorbia heterophylla Linn.	Euphorbiaceae
Euphorbia hirta Linn.	Euphorbiaceae
Fluerya aestuans (Linn.)ex Miq.	Urticaceae
Gomphrena celosioides Mart.	Amaranthaceae
Ipomoea involucrate P. Beauv.	Convolvulaceae
Panicum maximum Jacq.	Poaceae
Paspalum polystachyrm R.Br.	Poaceae
Phyllanthus amarus Schum,et Thonn.	Euphorbiaceae
Platosfonca africanum P. Beauv.	Lamiaceae
Spigelia anthelmia Linn.	Loganiaceae
Synedrella nodiflora Gaertn.	Asteraceae
Tridax procumbens Linn.	Asteraceae

Originally, the total heterotrophic bacteria was 2.3 x 10^6 cfu/g as against 11.3 x 10^5 cfu/g hydrocarbon degrading bacteria and 2.8 x 10^4 cfu/g hydrocarbon degrading fungi (Table 2).

Physiochemical properties of soil used in the study, before and one week after soil amendment with waste engine oil (WEO) are presented on Table 3. Polluted soil composed of 1601mg/kg of Fe, 54.2 mg/kg of Zn, 6.8 mg/kg of Ni and 6843 mg/kg as total hydrocarbon content.

Table 2. Total heterotrophic and oil-degrading microorganism counts in soil originally used for this experiment

Bacteria	Het (x10^6 cfu/g)	2.30
	Hyd (x10^5 cfu/g)	11.30
	% Hyd	49.00
Fungi	Het (x10^5 cfu/g)	7.00
	Hyd (x10^4 cfu/g)	2.80
	% Hyd	40.00
Actinomycetes	Het (x10^4 cfu/g)	4.00
	Hyd (x10^3 cfu/g)	6.00
	% Hyd	15.00

Het Heterotrophic micro organism, Hyd Hydrocarbon degraders, %Hyd Percentage hydrocarbon degraders

Table 3. Physiochemical properties of soil used in the study, before and one week after soil amendment with waste engine oil (WEO)

Parameters	**Before amendment with WEO**	**1 Week after amendment with WEO**
pH	6.11	5.49
Electrical Conductivity (µs/cm)	301.00	398.00
Total Org. Matter (%)	0.61	0.72
Total Nitrogen (%)	1.12	0.58
Exchangeable Acidity (meq/100 g soil)	0.22	0.48
K (meq/100 g soil)	1.43	1.68
Ca (meq/100 g soil)	15.26	28.03
Mg (meq/100 g soil)	10.97	45.42
P (mg/kg)	153.00	493.21
Fe (mg/kg)	998.80	1601.00
Mn (mg/kg)	16.71	40.10
Zn (mg/kg)	12.12	54.20
Cu (mg/kg)	4.98	6.00
Cr (mg/kg)	2.08	3.80
Cd (mg/kg)	N.D	0.09
Pb (mg/kg)	N.D	1.24
Ni (mg/kg)	3.60	6.80
V (mg/kg)	0.76	4.01
Total Hydrocarbon Content (mg/kg)	224.06	6843.00

ND: Not detected (≤0.001 mg/kg)

Polyaromatic hydrocarbon contents of soil included acenaphthene (2.46mg/kg), anthracene (10.42mg/kg), benzo[a]pyrene (8.18mg/kg), fluorine (1.35mg/kg), and pyrene (19.18mg/kg) (Table 4). Total PAH was 363.02mg/kg.

Table 4. PAH content of 1 week old WEO polluted soil

PAH (mg/kg)	
Acenaphthene	2.46
Acenaphthylene	2.42
Anthracene	10.42
Benzo[a]anthracene	4.95
Benzo[a]pyrene	8.18
Benzo[b]fluoranthene	1.64
Benzo[g,h,i]perylene	176.49
Benzo[k]fluoranthene	98.54
Chrysene	1.02
Dibenzo[a,h]anthracene	1.07
Fluoranthene	21.03
Fluorene	1.37
Indeno[1,2,3-c,d]pyrene	0.96
Naphthalene	1.06
Phenanthrene	12.25
Pyrene	19.18
Total PAH	**363.02**

After 20 months of exposure to various soil treatments, results of heavy metal and THC analyses are presented on Table 5. There was significant ($p<0.05$) changes in composition of soil heavy metals when compared with the control experiment. The sterilized (control) soil was highest in composition of all the heavy metals assayed, followed by the polluted soil that was weeded throughout the experiment. Soil composition of Fe was 1003.12mg/kg in the control, as against 901mg/kg in the unsterilized but weeded soil (W_{non}) and 596.12mg/kg in the unsterilized and the unweeded (W_{wds}) soil. Significant reduction in heavy metal and THC of soil was recorded in the soil that was left untouched throughout the experiment period with its incident/resident weed species. Significant reduction in THC from 3887.32mg/kg in the control compared to 757.65mg/kg in W_{wds}.

Table 5. Heavy metal and total hydrocarbon (THC) contents of a naturally attenuated 20 month old waste engine Oil polluted soil

mg/kg	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
W_{wds}	596.12^{c}	21.31^{b}	19.52^{b}	1.21^{b}	1.01^{b}	0.008^{b}	0.35^{c}	0.12^{c}	0.089^{b}	757.65^{c}
W_{4mw}	765.92^{bc}	26.86^{ab}	20.11^{b}	1.98^{b}	1.06^{b}	0.008^{b}	0.46^{c}	0.48^{b}	0.102^{b}	917.61^{bc}
W_{non}	901.33^{ab}	29.43^{ab}	29.43^{b}	2.98^{a}	1.11^{b}	0.010^{b}	0.71^{b}	0.58^{b}	0.098^{b}	1165.06^{b}
$W_{ctr/ste}$	1003.12^{a}	33.32^{a}	42.51^{a}	3.86^{a}	2.96^{a}	0.023^{a}	1.01^{a}	2.14^{a}	1.983^{a}	3887.32^{a}

Means on the same column with similar alphabet superscripts do not differ from each other significantly at 5% level of probability. W_{wds}= Soil undisturbed throughout expt. W_{4mw}= Partial clearing of weeds allowed every 4 monthly. W_{non}= Total removal of weeds. $W_{ctr/ste}$= Sterilized soil (Control).

The polyaromatic hydrocarbon content of the soil at 20 months is presented on Table 6. There was total remediation of acenaphthene in W_{wds}-treatment compared to residual acenaphthene contents of 0.016mg/kg in W_{4mw}, 0.145mg/kg in W_{non} and 1.345mg/kg in the control experiment. Total remediation of anthracene, benzo[a]pyrene, benzo[b]fluoranthene, chrysene, fluorene, fluoranthene, pyrene and indeno[1,2,-c,d]pyrene in the unsterilized and undisturbed soil treatments (W_{wds}). Although total remediation of chrysene, fluorene, and

indeno[1,2,-c,d]pyrene was recorded in the unsterilized soil treatments (W_{wds}, W_{4mw}, and W_{non}), no total remediation of PAH fraction was recorded in the sterilized soil treatments (control). Comparatively, there was significant reduction in polyaromatic hydrocarbon contents from $W_{ctr/ste} < W_{non} < W_{4mw} < W_{wds}$. Similarly, the treatments with abundant weed species appeared to have the greatest influence on stimulation of hydrocarbon degrading microorganisms, followed by the treatment with a 4-monthly weed control strategy. The importance of weeds species in the bioremediation of oil polluted soil cannot be overemphasized, therefore.

Most of the soil's life exists beneath the surface of the soil where billions of organisms inhabit the upper layers of the soil, and break down dead organic matter as well as petroleum hydrocarbons, releasing the nutrients necessary for plant growth [9]. In an undisturbed soil, leaves and other organic debris accumulate on the surface, where they are broken down by the decomposers.

Table 6. Polyaromatic hydrocarbon content of a naturally attenuated 20-month old waste engine oil polluted soil

PAH (mg/kg)	W_{wds}	W_{4mw}	W_{non}	$W_{ctr/ste}$
Acenaphthene	0^{c}	0.02^{c}	0.15^{b}	1.35^{a}
Acenaphthylene	0.68^{b}	0.79^{ab}	0.91^{a}	1.97^{a}
Anthracene	0^{c}	0^{c}	0.24^{b}	5.65^{a}
Benzo[a]anthracene	1.79^{b}	1.99^{b}	2.01^{b}	3.01^{a}
Benzo[a]pyrene	0^{d}	0.12^{c}	1.87^{b}	6.99^{a}
Benzo[b]fluoranthene	0^{c}	0.01^{c}	0.06^{b}	1.05^{a}
Benzo[g,h,i]perylene	117.32^{a}	139.43^{a}	128.12^{a}	147.01^{a}
Benzo[k]fluoranthene	56.46^{b}	62.93^{b}	59.73^{b}	81.96^{a}
Chrysene	0^{b}	0^{b}	0^{b}	0.18^{a}
Dibenzo[a,h]anthracene	0.52^{b}	0.67^{ab}	0.76^{a}	0.89^{a}
Fluoranthene	0^{c}	0^{c}	0.19^{b}	2.54^{a}
Fluorene	0^{b}	0^{b}	0^{b}	0.13
Indeno[1,2,3-c,d]pyrene	0^{b}	0^{b}	0^{b}	0.77^{a}
Naphthalene	0.12^{c}	0.09^{c}	0.31^{b}	0.84^{a}
Phenanthrene	0.59^{b}	0.79^{b}	2.11^{b}	7.46^{a}
Pyrene	0^{c}	0.09^{c}	1.06^{b}	7.43^{a}
Total PAH	117.45	206.92	197.51	269.22

Means on the same row with similar alphabet superscripts do not differ from each other significantly at 5% level of probability. W_{wds}= Soil undisturbed throughout expt. W_{4mw}= Partial clearing of weeds allowed every 4 monthly. W_{non}= Total removal of weeds. $W_{ctr/ste}$= Sterilized soil (Control).

Most hydrocarbon degradation is believed to occur through microbial processes, and so the plant-associated microbial community was expected to thrive in the available tolerant species. Seven (7) out of the original 19 weeds species were identified in the polluted soil (compare Table 1), including *Euphorbia hirta, Fluerya aestuans, Panicum maximum, Phyllanthus amarus, Spigelia anthelmia, Tridax procumbens,* and predominantly *Euphorbia heterophylla.* (Table 7). There were however 5 unidentified weed species (< 5cm tall). These species are likely oil tolerant species as previously suggested by Anoliefo et al. [3]. The assessment of the influence of grass on the abundance and activity of microorganisms in the rhizosphere revealed species-specific plant-induced changes in the soil microbial community [9]. Selective enrichment of hydrocarbon degrading microorganisms was demonstrated in the soils with abundant and unaltered plants resources.

Table 7. Weeds distribution of a naturally attenuated waste engine oil polluted soil that was unsterilized and undisturbed for 20 months

Weeds	No. of weeds/50cm diameter basin
Euphorbia heterophylla	4
Euphorbia hirta	2
Fluerya aestuans	3
Panicum maximum	2
Phyllanthus amarus	3
Spigelia anthelmia	1
Tridax procumbens	2
Unidentified plants (<5 cm tall)	5
Total	22

Data was not presented for other treatments as it was not reliable. The growth of weeds was interrupted during the study in these treatments.

Microorganism counts after 20 months of exposure of polluted soil to the various soil treatments are presented on Table 8. Significant differences were recorded in heterotrophic bacterial counts ($p>0.05$). Heterotrophic bacteria counts were 567.01 $x10^2$ cfu/g in W_{wds}, 112.42 $x10^2$ cfu/g in W_{4mw}, 0.85 $x10^2$ cfu/g in W_{non}, and 0.03 $x10^2$ cfu/g in the control. Similar significant decreases were also recorded hydrocarbon degrading bacteria and fungi. Hydrocarbon degrading fungal counts was 158.24 $x10^2$ cfu/g in W_{wds}, 69.02 $x10^2$ cfu/g in W_{4mw}, and 0.03 $x10^2$ cfu/g in the control.

Table 8. Effects of weeds on micro organism counts ($x10^2$ cfu/g) in the remediation of a WEO-polluted soil

		W_{wds}	W_{4mw}	W_{non}	$W_{ctr/ste}$
Bacteria	Het	567.01^{a}	112.42^{b}	0.85^{c}	0.03^{d}
	Hyd	203.21^{a}	58.84^{b}	0.34^{bc}	0.02^{c}
	% Hyd	35.83	52.33	40.00	66.67
Fungi	Het	499.23^{a}	156.89^{b}	108.07^{b}	0.11^{c}
	Hyd	158.24^{a}	69.02^{b}	40.08^{b}	0.03^{c}
	% Hyd	31.69	43.99	37.09	27.27
Actinomycetes	Het	28.62^{a}	18.12^{a}	15.15^{ab}	0.02^{b}
	Hyd	1.68^{a}	2.51^{a}	2.09^{a}	0.01^{b}
	% Hyd	5.87	13.85	13.79	50.00

Means on the same row with similar alphabet superscripts do not differ from each other significantly at 5% level of probability. W_{wds}= Soil undisturbed throughout expt. W_{4mw}= Partial clearing of weeds allowed every 4 monthly. W_{non}= Total removal of weeds. $W_{ctr/ste}$= Sterilized soil (Control).

Table 9 shows distribution of microorganisms in the polluted soil exposed to the various soil treatments after 20 months. Microorganisms identified in W_{wds} treatments included *Achromobacter* sp, *C. perfringens , Sarcina* sp, M*. roseus, Bacillus pumilis , B. subtilis, P. aeruginosa , Aspergillus* sp, *A. niger, A. Flavus , Penicillium* sp, *F. solani* and *Nocardia* sp. In W_{non} treatment, microorganisms identified included *Niger, E. aerogenes, Pseudomonas* sp, and *Rhiszopus stolonifer. A. niger, A. flavus,* and *Nocardia* sp were the only species common in the control experiment. *A. niger* was predominantly common in all the treatments. *A. niger* was predominantly common in all the treatments. Mites, springtails, small insects, other arthropods and earthworms also assist the process of bioremediation by consuming, mixing and transporting materials. The rate of decomposition is affected by soil

temperature, moisture and food availability [9,10]. The main by-products of the decomposition process are soluble plant nutrients and microbial remains that bind the soil particles together, giving a stable crumb structure.

Table 9. Distribution of microorganisms in 20-month old waste engine oil polluted soil exposed to various soil treatments

	Names of microorganisms	Soil treatments			
		W_{wds}	W_{4mw}	W_{non}	$W_{ctr/ste}$
1.	*Achromobacter* sp	+	+	-	-
2.	*Clostridium* sp	-	-	+	-
3.	*C. perfringens*	+	-	-	-
4.	*Sarcina* sp	+	+	-	-
5.	*Micrococcus* sp	-	-	-	-
6.	*M. luteus*	-	+	-	-
7.	M*. roseus*	+	-	-	-
8.	*Bacillus pumilis*	+	-	-	-
9.	*B. subtilis*	+	-	-	-
10.	*E. aerogenes*	-	+	+	-
11.	*Pseudomonas* sp	-	+	+	-
12.	*P. aeruginosa*	+	-	-	-
13.	*Aspergillus* sp	+	-	-	-
14.	*A. niger*	+	+	+	+
15.	*A. Flavus*	+	+	-	+
16.	*A. fumigatus*	-	-	-	-
17.	*Penicillium* sp	+	+	-	-
18.	*P. notatum*	-	-	-	-
19.	*Fusarium* sp	-	-	-	-
20.	*F. solani*	+	-	-	-
21.	*Rhiszopus stolonifer*	-	+	+	-
22.	*Mucor* sp	-	-	-	-
23.	*Geotrichum* sp	-	-	-	-
24.	*Trichoderma* sp	-	-	-	-
25.	S*accharomyces* sp	-	-	-	-
26.	*Streptomyces* sp	-	-	-	-
27.	*Nocardia* sp	+	-	-	+

The following microbial isolates (1 – 27) were originally isolated from the soil (unpolluted). W_{wds}= Soil undisturbed throughout expt. W_{4mw}= Partial clearing of weeds allowed every 4 monthly. W_{non}= Total removal of weeds. $W_{ctr/ste}$= Sterilized soil (Control).

Plant roots leak or exude a large number of organic substances and continually slough off root caps into the soil. These materials are food for the many micro-organisms living in a zone of intense biological activity near the roots called the rhizosphere. Bacteria benefit most from the food supplied in the rhizosphere and may form a continuous film around the root. Roots form the microbial highways of the soil. Other micro-organisms liberate nutrients from the clay and humus colloids.

In order for the necessary microbes to thrive, appropriate habitats must be created within the treatment systems. Plants and artificial media (gravel and sand) are used in natural treatment systems to create the media necessary to support rich microbial communities. Nitrification of ammonia to nitrates occurs in the biofilms, which attach to the media. The root

zones of the higher plants play a central role in this process and provide some of the endogenous sources of carbon that support the biological systems.

The extensive root network or rhizosphere in the planted ecologies provides the structure and nutrient support for diverse microbial communities. Materials from the plant roots are exuded into the surrounding rhizosphere. These materials include hormones, antibiotics, metal chelators, nutrients, humic compounds and polysaccharide glues [11].

Revegetation may occur naturally via vegetative growth and seedling emergence or through restoration efforts (e.g., planting of nursery stock). While many studies have focused on the direct impacts of oil on plant surfaces and on regrowth from rhizomes and roots [12,13,14, 15] few studies have examined the effect of oil on seedling emergence from the seed bank. Yet, emergence from the seed bank may provide the most rapid recovery of vegetation in terms of cover, diversity and community structure because wetland soils contain an abundance of seeds.

Oil may affect recovering vegetation, derived from a seed bank, by reducing the number of emerging seedlings. Oil can be detrimental to germination via several mechanisms including direct toxicity to the embryo [16], and the formation of anaerobic [17] and hydrophobic [16] soil conditions. Oil may also affect the composition of the recovering vegetation, if differential sensitivity to oil by species occurs. This impact of oil on organisms have also been implicated in microbial diversity of affected soils [5, 9, 12]. The composition and size of the microbial community depend, to a very large extent, on plant type as well as plant age [19]. Although plants and microorganisms can degrade petroleum hydrocarbons independently of one another but it is the interaction between them (i.e., the rhizosphere effect) which is the primary mechanism responsible for petrochemical degradation in phytoremediation efforts [20]. The capability for remediation of polluted soils resides with both organisms. However, a synergistic approach has been found to be a better strategy [19, 20]. Plants are able to provide root exudates of carbon, energy, oxygen, nutrients, as well as enzymes to microbial populations in the rhizosphere. This stimulates microbial activity within this region and hence enhanced degradation of the hydrocarbon mixture.

4. CONCLUSION

Restoration via seed banks offers an economical alternative to planting nursery stock or sowing commercially available seeds. Seed banks serve as an *in situ* source of colonizers. These colonizers are abundant, diverse, and will promote onset of ecological functions within the system. Colonization may also speed degradation of residual oil by enhancing microbial activity via oxygenation of soil and discharge of exudates from roots. Importantly, soil biodiversity reflects the mix of living organisms in the soil. These organisms interact with one another and with plants and animals forming a web of biological activity. It is the summation of these activities that have now been found to show prospects for decomposition contaminants and pollutants, as well as a key role in nutrient cycling.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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