Original Research Article

BIOREMEDIATION OF THREE BRAZILIAN SOILS CONTAMINATED WITH USED LUBRICATING OIL

Authors Contribution

AJ-Adeyemo carried out the field study, soil and plant analysis, statistical analysis and helped to draft the manuscript. JWV-de Mello and SO-Agele conceived the study, performed the statistical analysis, participated in its design and coordination and helped to draft the manuscript.

All authors read and approved the final manuscript.

ABSTRACT

Objectives: This study aimed at bioremediation potentials of organic pollutants, in particular, used lubricating oil contaminated soils, using commercial microbial nutrient. Other objectives were the evaluation of kinetic model to determine the rate of biodegradation of petroleum hydrocarbon in soil and to subsequently determine the half-life of the oil degradation.

Materials and Methods: The patterns of biodegradation of used motor oil were studied for a period of 90 days under laboratory condition. The model soil (300 g) was contaminated with 1.5 % (w/w) of used motor oil at room temperature in the laboratory using microcosm of 1 L. The microcosm was used to simulate the comparative effect of used lubricating oil addition and bioremediation using a commercially available hydrocarbon degrading microbial consortium - Amnite P1300 as bioaugmentation (T1), nutrients amendments - $(NH_4)_2SO_4$ and K_2HPO_4 (NPK) as biostimulation (T2), unammended soil - natural attenuation as (T3) and the control soil treated with sodium azide (NaN_3) as (T4).

Results: Treatment effects were evaluated on microbial community using three soil types (S1, S2 and S3). Hydrocarbon-utilizing bacterial counts were obtained in the amended soils under treatments T1, T2, and T3 ranging from 3.47×10^6 to 3.27×10^8 cfu/g compared to T4 throughout the 90 days of study. Soils amended with Amnite p1300 showed highest reduction in total petroleum hydrocarbon with net loss of 36.17 % throughout the period of experiment compared to other treatments. The changes (decline and recovery) in population of microbial community are a useful and sensitive way of monitoring the impact and recovery of used motor oil-contaminated soils.

Conclusion: The results suggest that different soils have different inherent microbial potential to degrade hydrocarbons of soils contaminated with used lubricating oil.

Key words: Bioremediation, used lubricating oil, hydrocarbons, microbial consortium, soil types.

1. Introduction

There is rise in consumption of automotive lubricating oil worldwide, this increases had consequences for ecosystem health in terms of disposal of used engine/lubricating oil. In Brazil, the problems tend to worsen with economic and population growth, and rapid industrialization without concern thus disregard for environmental health, particularly in relation to used motor oil. The consumption of lubricating oil in Brazil is around 10^6 m^3/year (ConselhoemRevista, 2005; Magalhães et al.,2007). Approximately 6.5 x 10^5 m^3/year are consumed in the lubrication process, and from 3.5 x 10^5 m^3/year remainder, only 20% are treated or recycled; therefore, significant volumes of used motor oil are continually discharged into the ecosystems (local environment). Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger et al., 1997). These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their natural environment. Despite efforts in some countries to recover and recycle used motor oils, significant amounts of lubricants are input into the environment, particularly in environmentally sensitive applications such as forestry and mining, or through engine losses (Battersby, 2000). Consequently, considerable attention has been given to lubricant biodegradability and persistence in the environment. Therefore, there is a need for effective and environmentally safe cleanup treatments of oil spills (crude or used petroleum hydrocarbon compounds). The United State Environmental Protection Agency (40 CFR Part 279) defined “used oil’’ as “any oil that has been refined from crude oil or any synthetic oil that has been used and, as a result of such use is contaminated by physical or chemical impurities.’’ Used motor oil contains metals and heavy polycyclic aromatic hydrocarbons derived from engine oil - a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Butler and Mason, 1997) that is used to lubricate parts of an automobile engine, in order to smooth engine operation (Hagwell et al., 1992; Boonchan et al., 2000).The persistent hydrocarbon components are known to have carcinogenic and neurotoxic activities (Reddy and Matthew, 2001; Das and Chandran, 2011). One gallon of used motor oil, improperly disposed of, may contaminate 1 million gallons of fresh water, which is enough to supply 50 people with drinking water for one year. One pint (4 gills or 568.26 cubic centimetres) of used motor oil improperly disposed of can create a one-acre slick on the surface of a body of water and kill floating aquatic organisms (USEPA, 1996).

Unsafe disposal of petroleum hydrocarbon products increase soil contamination, and this has constituted major environmental problems. Therefore, the development of research and technologies to remediate soils contaminated with used motor oils, in particular bioremediation, provides an effective and efficient strategy to speed up the clean-up processes (Mandri and Lin, 2007). Various factors including lack of essential nutrients such as nitrogen and phosphorus may limit the rate of petroleum hydrocarbon degradation from contaminated soil. Addition of inorganic nutrients (biostimulation) is therefore needed as an effective approach to enhance the bioremediation process

(Semple et al., 2006; Walworth et al., 2007). Also, many microbial strains, each capable of degrading a specific compound, are available commercially for bioremediation (Bragg et al., 1994; Korda et al., 1997; Song et al., 1990; Safi et al., 2014).

Remediation of hydrocarbons contaminated soil is necessary in order to preserve the safety and health of the ecosystem with consequences on environmental and human health. Biological remediation of hydrocarbon contaminated soil offers a better and more environmentally friendly technique that should be properly due to its enormous advantages over other methods of remediation. However, despite these enormous advantages of bioremediation, its potential is yet to be fully utilized in restoration of contaminated soil. This is possibly due to the fact that it takes a long period of time for the complete restoration of contaminated soil. This limitation can however be overcome through nutrient addition and introduction of microbes with biodegradative capability on petroleum hydrocarbon contaminated soils. This study aimed at bioremediation potentials of organic pollutants, in particular, spent motor oil contaminated soils, using commercial microbial consortium. Other objectives were the evaluation of kinetic model to determine the rate of biodegradation of petroleum hydrocarbon in soil and to subsequently determine the half-life of the oil degradation.

2. MATERIAL AND METHODS

2.1. Collection of samples. Soil samples (0–20 cm) were collected in 2011 in four sampling points using soil samplerfrom three locations (Sete Lagoas, Cachoeira Dourada and Tres Marias)(Lat. 19°28′ S: Long. 44° 15′ W, Lat.18°48′ S: Long. 49°62′ W and Lat.18°20′ S: Long. 45° 46′ W), and (732, 429 and 921 m) above sea level in Minas Gerais StateBrazil.The study site were characterised by annual rainfall of (1272,1328, and1226 mm) and average temperature of (22.0,24.9 and 23.2°C) in each locations respectively.Soils samples were collected in hermetic bags and transported to the laboratory for analysis. Used lubricating oil was collected from a gasoline and car service station close to the Federal University of Viçosa, Brazil.Amnite P1300 consisted of special bacterial strains (Amnite P1300) specially made to degrade used lubricating oil was obtained from Cleveland Biotech Ltd., UK.

2.2. Experimental Design and set-up of microcosm. Exactly 300 g each of the model soils was contaminated with 1.5 % (w/w) or (15000 mg/kg) of used motor oil at room temperature (25 ± 1 ^{0}C) under laboratory conditions using 1 litre capacity microcosm. The microcosms were used to simulate the biodegradation of effect of used lubricating oil polluted soil using a commercially available hydrocarbon degrading microbial consortium (Amnite P1300). Aminte consist of a mixture of *Bacillus subtilis, Bacillus megaterium*, *Pseudomonas putida, Pseudomonas fluorescens, Phanerochaetechrysosporium, Rhodococcusrhodocrous*on a cereal (bran) as the bioaugmentation treatment. The microorganisms were conditioned to degrade heavy hydrocarbons. The total population of microbes in Amnite P1300 was approximately 5 x 10^{8} cfu/g of bran. Also, the polluted soils were

amended with $(NH_4)_2SO_4$ and K_2HPO_4) to simulatebiostimulation. The C:N:P ratio of the nutrient compound was adjusted to 100:7.5:1 (optimum conditions). The same conditions provided in the biostimulation treatment were used in the bioaugmentation treatment in which the $(NH_4)_2SO_4$and K_2HPO4 were combined withP1300. The unammended soil (natural attenuation), in which nutrients were not added while microbial inoculum was included to indicate hydrocarbon degradation capability of microorganisms naturally present in the contaminated soils (i.e. the autochthonous microbes). There was a control soil in which most of the indigenous bacteria were killed by the addition of a biocide, sodium azide (NaN_3) (0.3% ww $^{-1}$) to inhibit soil microorganisms and to monitor abiotic hydrocarbon losses on the microbial community in three different soil types. There were six sampling dates (15, 30, 45, 60 75 and 90); hence 36 microcosms in total were used. Microcosms were arranged in a random order, and rearranged every 2 weeks ± 2 days throughout the duration of the experiment The treatments were replicated 3 times, while the content of each container was tilled every week for aeration, moisture content was maintained at 70% (Pramer and Bartha, 1972), and water holding capacity by the addition of sterile distilled water every week until the end of the experiment.

2.3. LaboratorySampling. Periodic sampling from each microcosm was carried out at 15-day intervals for 90 days. Composite samples were obtained by mixing 10 g of soil collected from different areas of the microcosm for bacteria enumeration and determination of total petroleum hydrocarbon.

2.4. Determination of the physicochemical property of the soil. Table 1 shows the origin and selected physical and chemical characteristics of the non-contaminated soil samples used for the bioremediation studies. Particle size analysis was done using hydrometermethod (Sheldrick and Hand Wang, 1993).Total nitrogen content of the soil was determined using the micro-Kjeldahl method(Bremner, 1996), the available phosphorus was determined by colometry after Mehlich 1 extraction and Organic Carbon content was determinedby the procedure of Walkley and Black using the dichromate wet oxidation method (Nelson and Sommer, 1996). The pH was determined using 1:2.5 ratio by weight with distilled water (w/v) after 30-min equilibration using a pH meter and electrode calibrated with pH 4.0 and 7.0 standards(Defelipo& Ribeiro, 1981). Determinations were made in triplicate.

Table 1: Selected physical and chemical characteristics of the non-contaminated soil samples

Parameters	Soil 1 (S_1)	Soil 2 (S_2)	Soil 3 (S_3)
pH (H_20)	5.20	5.91	4.92
Total Nitrogen (%)	0.43	0.24	0.11
Avail. P (mg/dm^3)	1.00	1.8	0.40
Organic C (dag/kg)	3.50	1.54	0.81
C:N ratio	8.14	6.42	7.56
ECEC ($cmol_c/dm^3$)	3.19	2.29	0.78
Moisture Content (%)	33.80	28.3	11.30
Sand (dag/kg)	11.00	10.00	68.00
Silt (dag/kg)	9.00	22	4.00
Clay (dag/kg)	80.00	68	28.00
Texture	Clayey	Clayey	Clay loamy sand
Soil Type	Red Latosol	Red Lotosol	Red Yellowish Latosol
Parent Material	Sete Lagoas – MG	Cachoeira Dourada - MG	Tres Marias - MG

2.5. Microbial monitoring and enumeration of total aerobic heterotrophic and hydrocarbon-degrading bacteria. Triplicates samples were collected fortnightly (0, 15, 30, 45, 60, 75, 90 days) over the entire period of study of the variously amended soils (S_1, S_2 and S_3). In order to monitor cell numbers and biodegradation, 1 g of soil was removed from each microcosm at the set times and suspended in 9 mL of saline solution in sterile centrifuge tubes. The mixture was vigorously shaken on a vortex mixer for 3 minutes and then the soil particulates were allowed to settle for 1 min before 0.1 mL of the supernatant was sampled for CFU counts. The number of colony-forming total aerobic heterotrophic bacteria (AHB) was determined by plating three replicate samples from each treatment withdrawn every 15 days. Serially diluted samples (0.1 mL) were plated on nutrient agar medium (Oxoid) supplemented with 10 mg/mL solution of cycloheximide in which 1 mL/L was drawn to suppress the growth of fungi. The oil agar plates were incubated at 30℃ for 24 hours, and the colonies were counted. Also, enumeration of hydrocarbon-degrading bacteria (HDB) was attempted on a mineral medium containing motor oil as the sole carbon source. The mineral medium contained 1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4.7H_2O$, 0.1 g NaCl, 20 g agar, one percent (1%) used engine oil in 1,000 mL distilled water, and the medium was adjusted to

pH 7.4 (Zajic and Supplission, 1972). The oil agar plates were incubated at 30℃ for 7 days before the colonies were counted.

2.6. Extraction of residual oil and analysis of total petroleum hydrocarbons. Total Petroleum Hydrocarbons (TPHs) were extracted according to EPA method 3546 (US EPA, 2007) using the Microwave Automated Reaction System from CEM (Matthews, NC). Briefly, Sodium sulfate (Na_2SO_4) was purified by drying overnight in an oven at 150℃ and quickly transferred into a desiccators. Five grams (5 g) of homogenised contaminated soil was weighed out, mixed with 5 g dry anhydrous Na_2SO_4 and ground to less than 1 mm particle sizes, extracted in GreenChem vessels with 25 mL of a 1:1 hexane:acetone mixture according to manufacturer's protocol at 100 ℃ for 20 minutes. The n-hexane and acetone was filtered throughwhatman No 1 filter paper to separate the extract from the soil particles, and transferred into 100 mL amber vials through separatory funnel and sequentially rinsed with equal volume of solvent mixture. The solvent were evaporated topartial dryness with a rotary evaporator (FizatomRotavapor 801), transferred into 2 mL vials and then dried completely using nitrogen gas. Dried samples were dissolved in 600 µL dichloromethane for gas chromatography analysis. The residual oil was analyzed on Shimadzu GC-17A Chromatograph equipped with a Flame-Ionization Detector (FID) by using fused silica capillary column DB-5 (30 x 0.25 mm), and AOC-17 Shimadzu auto injector complying with Environmental Protection Agency (EPA) standard method 8015 (US EPA 2007). The flow rate of the helium carrier gas was 1.81 mL/min with linear velocity of 38.49 cm/s. The initial temperature was programmed at 40^{o}C and held for 15 min. The temperature was then increased to 280^{o}C at a rate of 10^{o}C /min. The final temperature was held for 31 min. The injector was set in the split mode, the split ratio was set to 1:10; the injection volume was 1 µL and the injector and the detector temperature for GC were maintained at 260 and 280℃, respectively, and the oven temperature was programmed to rise from 40 to 280℃ in 10℃/min increments and to hold at 280℃ for 31 min. The dry weight of the soil samples was determined following baking of 10 g of wet soil at > 80 ℃ for at least 48 hours. Before analyzing the s ample extract, a mixture of standards including *n*-alkanes (*n*-decane*n*-C_{10}, *n*-dodecane*n*-C_{12}, *n*-tetradecane*n*-C_{14}, *n*-hexadecane *n*-C_{16}, *n*-octadecane*n*-C_{18}, *n*-eicosane n-C_{20}, *n*-docosane*n*-C_{22}, *n*-tetracosane*n*-C_{24}, *n*-hexacosane*n*-C_{26}, *n*-octacosane*n*-C_{28} and a pure standards containing *n*-triacontane*n*-C_{30}, *n*-dotriacontane*n*-C_{32}, *n*-tetratriacontane*n*-C34, and *n*-hexatriacontane*n*-C_{36}, and a mixture of polycyclic aromatic hydrocarbon consisting of acenaphthene, acenaphthylene, anthracene, benzo (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (g, h, i) perylene, benzo (k) fluoranthene, chrysene, dibenz (a, h) anthracene, fluoranthene,fluorine, indeno (1, 2, 3-cd) pyrene, naphthalene, phenanthrene, pyrene, 1-methylnaphthalene and 2-methylnaphthalene, Supelco) were used for calibration. Five points calibration curves using peak areas were obtained and the response factors were used to determine the concentrations of various hydrocarbons in the sample extract. The total petroleum hydrocarbons were identified and quantified by comparing the peak area of samples with that of the standard of the

TPH mixture with reference to the curve derived from standards. Percentage of degradation was calculated by the following expression:

$$\%\ biodegradation\ = \left[\frac{TPHcontrol - TPHtreatment}{TPHcontrol}\right] \times 100 \qquad (1)$$

TPH data were fitted to the first-order kinetics model (Yeung et al., 1997):

$$y = ae^{-kt} \qquad (2)$$

where *y* is the residual hydrocarbon content in soil (mg/kg), a is the initial hydrocarbon content in soil (mg/kg), *k* is the biodegradation rate constant (day^{-1}) and *t* is time (days). The biodegradation rate constant *(k),* and half-life *ln(2)/k* of the hydrocarbons in soil during the bioremediation process were calculated from the model using Statistical ® software (Stat Soft. Inc. (2007). The model was used to estimate the rate of biodegradation and half-life of hydrocarbons in soil under each treatment and the model was based on the assumption that the degradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in the soil.

2.7. Statistical Analysis. Statistical analysis of data obtained was carried out using analysis of variance. Means of different treatments were also compared statistically using a General Linear Model (ANOVA) (Tukey test, P>0.05) using statistical 8.0 software (Stat Soft, 2007).

3. RESULTS AND DISCUSSIONS

3.1. Microbial Counts.The aerobic heterotrophic bacterial (AHB) counts in T1 ranged between 1.01 x 10^8 and 2.4 x 10^9 CFU/g while T2 and T3 ranged from 1.03 x 10^8 to 1.7 x 10^9 and 1.0 x 10^8 to 3.8 x 10^8 CFU/g respectively (Fig. 1)across soil types. The treatment T4 had AHB counts ranging from 1.27 x 10^3 to 6.03 x 10^5 CFU/g. Hydrocarbon Degrading Bacterial (HDB) counts were also higher inused lubricating oil contaminated soil under T1, T2 and T3 (Fig.1). The count of HDB in soil amended with Amnite P1300 (T1) was about 2% higher than those amended with $(NH_4)_2SO_4$ and K_2HPO_4 (T2) andunamended – natural attenuation (T3). HDB count in soil amended with T1 ranged from 3.6 x 10^6 to 3.3 x 10^8 CFU/g, while those amended with T2 and T3 ranged from 3.7 x 10^6 to 2.6 x 10^8 and 3.5 x 10^6 to 5.41 x 10^7CFU/g, respectively. However, the HDB counts in T4 lower than T1, T2 and T3 ranged from 1.07 x 10^3 to 7.07 x 10^4 CFU/g. These results were similar to that obtained by Antai and Mgbomo (1989) whose counts of HDB in hydrocarbon-contaminated soil was 10^8 CFU/g, but higher than that of Ghazali et al. (2004), who obtained 10^7CFU/g; from hydrocarbons degradation in diesel oil polluted soil. The discrepancies in the results may be due to thecharacteristics from different ecologies of the different soil types used for the experiments. The microbial counts of the high clayey soil (S1) and low clay soil (S2)were similar in HDB. Counts in soils amended with T1 were highest followed by T2 and T3. Whereas, microbial counts in Clay loamy sand (S3) showed different pattern compared

with S1 and S2. Sodium azide (NaN_3) treated soil (T4) has the least results in all the soils used for the experiment. This result clearly demonstrates the benefit of bioaugmentation, biostimulation and indigenous microorganisms from used lubricating oil polluted soil. The different responses of the investigated are shown in Fig.1, T4 is a control system where most of the indigenous bacteria were killed with a biocide (NaN_3).

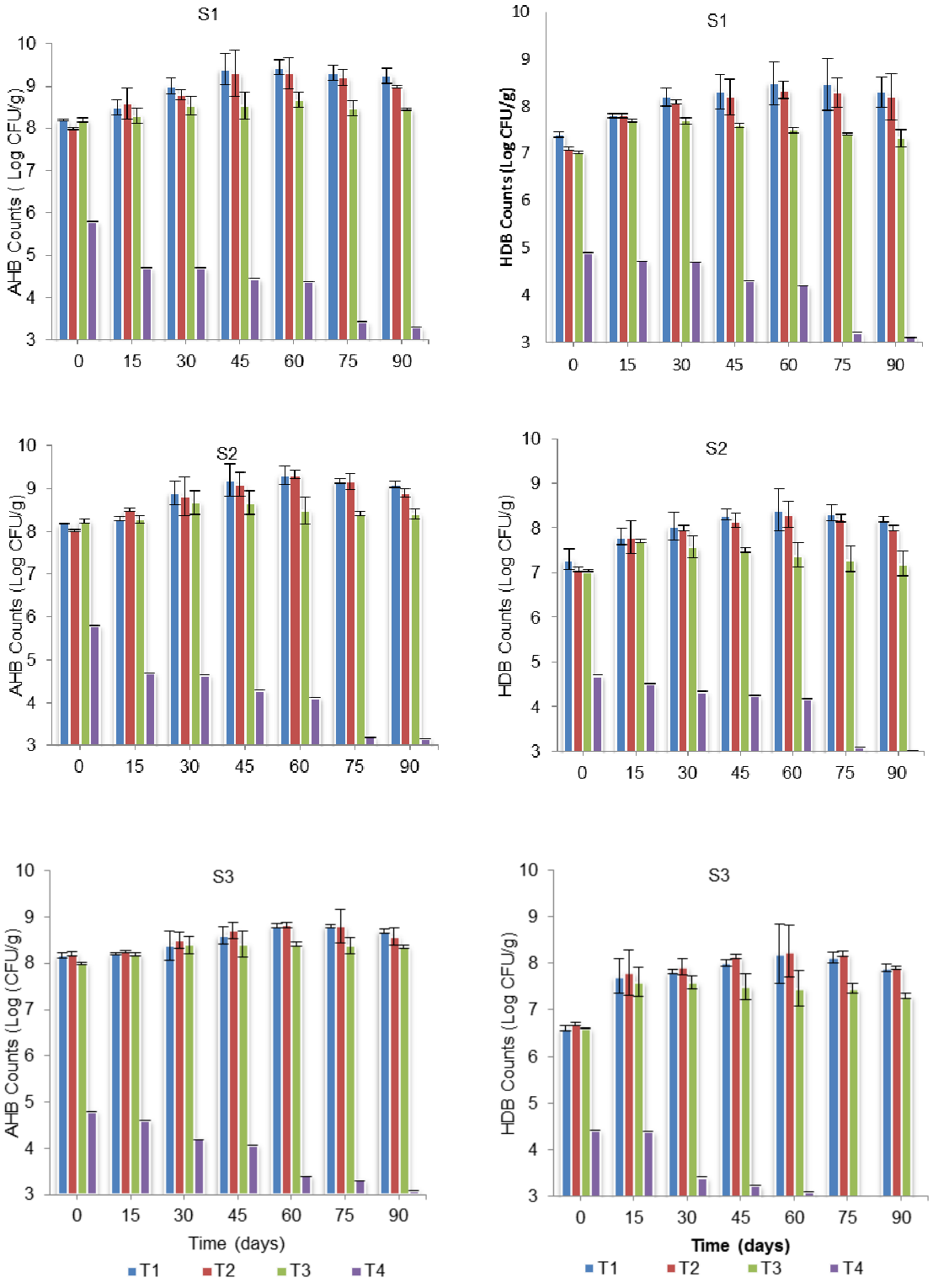


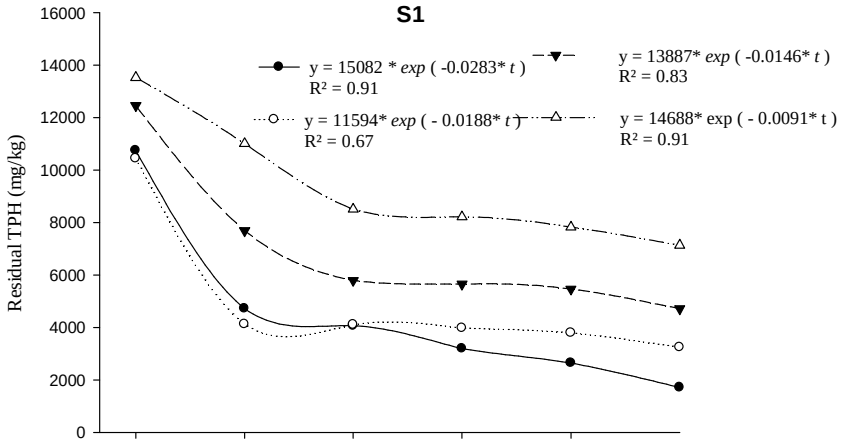
Fig 1: Counts of aerobic heterotrophic bacterial (AHB) and hydrocarbon degrading bacterial (HDB) population in oil- contaminated soils. Vertical bars indicate standard error of means SE (n=3)

3.2. Used engine oil hydrocarbon biodegradation. There was a noticeable reduction in the total petroleum hydrocarbon within the first 15 days in all the treatments, but higher reduction was observed at 30 days for T1, T2 and T3 compared to the control (T4). At the end of 30 days, 49, 69 and 73 % TPH reduction were obtained in T3, T1 and T2 respectively. About 7,306; 10,278 and 10,881 mg/ kg reduction in TPH was observed in these treatments compared to 27 % (3,991 mg/ kg) TPH reduction in the control soil (S1). Similar trend was noticed in soils S2 and S3 with T2 (NPK) having the highest TPH reduction (Fig. 2). Because, feeding nutrient solutions containing inorganic substances, such as nitrogen and phosphorus to natural soil bacteria population often enhances the ability of the microorganisms to degrade organic molecules into carbon dioxide and water (Stotzky and Norman, 1961a; and b). During this period, the added bacteria product acclimatized to their new source of carbon. At the end of (90 days), oil-contaminated soil amended with T1 (Soil + Oil + Amnite P1300) showed the highest reduction in soil concentration of used engine oil (89%), followed closely by soil amended with T2 (Soil + Oil + NPK) (78%), but no significant differences were observed between the treatment T1 and T2. Lower reduction in TPH obtained in soil type S3 compared to S1 and S2,may be due to high clay content in these soils which have been shown to offer greater capacity for physicochemical attenuation of contaminants than coarse sands (Pye and Patrick, 1983). However, highest reduction (68%) of TPH was observed in soil amended with T2 in soil S3 at the end of 90 days experiments. The net percentage loss of used oil in the contaminated soils could indicate the effectiveness of the treatments in biodegradation. The highest net percentage loss was observed at 30 days in T2 (45.93%), (40.33%) and (32.58%) followed by T1 (41.91%), (36.36%) and (28.83%) and T3 (22.10%), (22.10%) and (10.32%) in soils S1, S2 and S3, respectively (Table 2). However, the net percentage loss of used oil increased from45 days in T1 to the end of the experiment (90 days) compared with other treatments.

Table 2: Net percentage loss of total petroleum hydrocarbon in soils during bioremediation

Soil types	Treatments	Time (days)					
		15	30	45	60	75	90
S1	T1	18.53±1.3	41.91±1.4	29.59±0.7	33.50±1.2	34.56±1.0	36.17±0.8
	T2	20.54±1.4	45.93±1.3	29.37±0.8	28.28±0.7	26.88±1.2	25.94±0.8
	T3	7.08±1.3	22.10±1.4	18.10±0.7	17.08±0.4	15.77±1.2	16.13±0.8
S2	T1	15.29±1.0	36.36±0.4	34.21±1.0	31.54±1.7	32.31±1.3	31.66±2.3
	T2	17.21±2.0	40.33±1.2	33.68±0.5	31.19±1.0	31.37±1.2	23.47±2.1
	T3	4.86±1.7	20.77±1.1	15.24±1.9	12.90±1.4	13.48±1.2	10.58±2.1
S3	T1	10.54±2.6	28.83±2.3	21.21±1.8	22.48±2.6	23.59±0.9	23.47±2.1
	T2	10.84±1.1	32.58±1.4	22.88±1.0	23.50±0.8	24.53±0.9	24.99±2.3
	T3	4.86±2.2	10.32±0.4	6.77±0.8	6.24±1.4	5.70±0.8	10.58±2.1

T1=soil+oil+Amnite P1300, T2=soil+oil+$(NH_4)_2SO_4$ and K_2HPO_4), T3=soil+oil alone; S1=Red Latosol, S2 = Red Latosol, S3= Red-Yellowish Latosol; Net % loss = % loss in TPH of oil-contaminated amended soils and oil-contaminated soil alone − % loss in TPH of unamended contaminated control soil with sodium azide.



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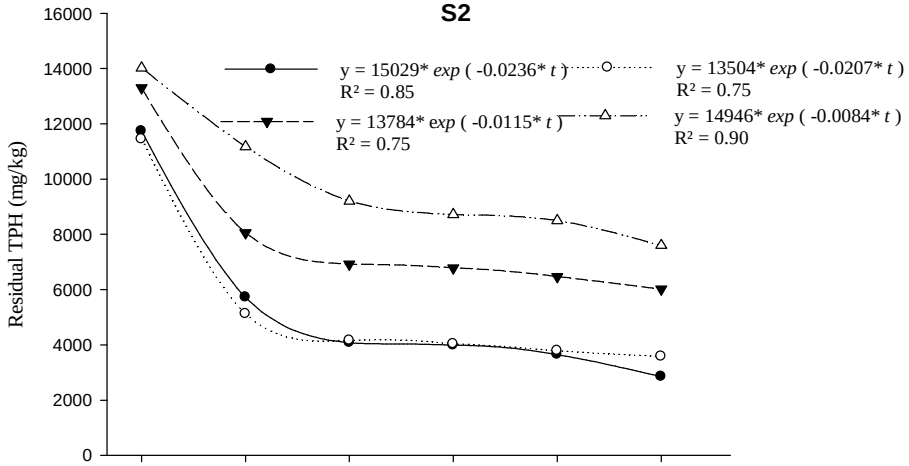

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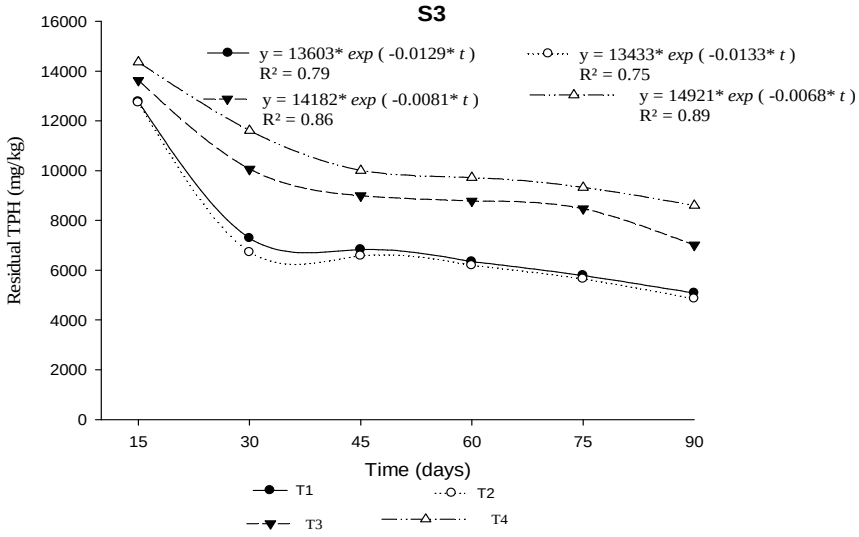

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362 Fig 2. Residual total petroleum hydrocarbons in soils during bioremediation

3.3. Biodegradation kinetics (rate constant and half-life). The highest biodegradation rates of 0.0283, 0.0236 and 0.0133 day^{-1} and half-lives of 24.49, 29.37 and 52.12 days were recorded under amniteP1300 in soil types S1 and S2 and nutrient amendment in soil S3, respectively. The control T4 showed the least biodegradation rate of 0.0091, 0.0084 and 0.0068 with highest half-lives of 76.17, 82.52 and 101.93 in soils S1, S2 and S3 respectively. The biodegradation rate obtained under amnite amendment of used oil T1 showed the best result for the kinetic parameters in this study, as a result of the added bacterial products, followed by T2 and T3, and this may be due to the bioavailability of the inorganic nutrients to the indigenous bacterial population present in the soils (Table 3).

Table 3: Biodegradation rate constant (K) and half-lives ($t_{0.5}$) of hydrocarbon in oil-contaminated soils

Treatments	K (day^{-1})			$t_{0.5}$ (days)		
	S1	S2	S3	S1	S2	S3
T1	0.0283 Dc	0.0236Cb	0.0129 Ca	24.49 Aa	29.37 Ab	53.73 Ac
T2	0.0188Cc	0.0207 Cb	0.0133Da	36.87 Aa	33.49 Ab	52.12 Ac
T3	0.0146 Bc	0.0115 Bb	0.0081 Ba	47.48 Ba	60.27 Bb	85.57 Bc
T4	0.0091Ac	0.0084 Ab	0.0068 Aa	76.17 Ca	82.52 Cb	101.93 Cc

T1=soil + oil + Amnite P1300, T2= soil + oil + $(NH_4)_2SO_4$ and K_2HPO_4), T3 = soil + oil alone, T4 = unamended contaminated control soil with sodium azide. S1 = Red Latosol, S2 = Red latosol, S3 = Red-Yellowish Latosol; K = Biodegradation constant (day^{-1}) and H = Half life (days). Values followed by the same capital or small letters are not significant difference between treatments (column) or soil types (row) respectively at the $P < 0.05$ level, while values followed by different capital or small letters indicate significant differences between treatments (column) or soil types (row) respectively at the $P < 0.05$ level.

4. CONCLUSIONS

Hydrocarbon-degrading bacteria counts were higher ranging from 3.47×10^6 to 3.27×10^8 CFU/g in the amended soils under treatment T1, T2 and T3 compared to T4 throughout the 90 days of study. Spent engine oil contaminated soil amended with amnite (T1) showed the highest reduction in total petroleum hydrocarbon with net loss of 36.17 % throughout the 90 days of the experiment compared to other treatments. The changes in population of microbial community (decline and recovery) are useful and sensitive means of monitoring the degradation and recovery of used lubricating oil-contaminated soils. Commercially available microbial-based bioremediation products appeared to be promising in the removal of petroleum hydrocarbons from contaminated clayey soil.

The tested kinetic model of biodegradation showed the highest biodegradation rate of 0.0283 day-1 and least half life of 24.49 days of the spent oil contaminated soil biodegradation washighest in high clayey and soil organic matter contents. This reveals the influence of organic matter in the degradation of petroleum hydrocarbons contaminated soils.

Remediation of hydrocarbons contaminated soil is necessary in order to preserve the safety and health of the ecosystem with consequences on environmental and human health. Biological remediation of hydrocarbon contaminated soil offers a better and more environmentally friendly technique that should be properly due to its enormous advantages over other methods of remediation. However, despite these enormous advantages of bioremediation,its potential is yet to be fully utilized in restoration of contaminated soil. This is possibly due to the fact that it takes a long period of time for the complete restoration of contaminated soil. This limitation can however be overcome through nutrient addition and introduction of microbes with biodegradative capability on petroleum hydrocarbons.

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