GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

EFFECTS OF COW DUNG ON MICROBIAL DEGRADATION OF MOTOR OIL IN LAGOON WATER

G. Umanu[1]*, S. C. U. Nwachukwu[2] & O. K. Olasode[1]
[1]Department of Biological Sciences, Bells University of Technology, Km 8, Idiroko Road, Benja Village, P.M.B. 1015, Ota, Ogun State, Nigeria.
[2]Department of Microbiology, University of Lagos, Akoka, Nigeria
*Correspondence author E-mail: goddeysu@yahoo.com

ABSTRACT
Accidental and indiscriminate discharges of petroleum products are of global environmental concern. The effectiveness of cow dung as a bioremediation agent was evaluated with a view to develop an alternative low cost strategy for bioremediation of motor oil contaminated water. Lagoon water artificially contaminated with motor oil, treated with 30gm non-sterile and sterile cow dung were designated TA1 and TA2 respectively. Similar polluted lagoon water treated with 40gm non-sterile and sterile cow dung were designated TB1 and TB2 respectively, while motor oil contaminated lagoon water without cow dung amendment served as control (CON). Samples collected were analysed chemically and microbiologically. The mean counts of microflora revealed an initial decrease between week 0 and 2 before assuming an increasing trend. The concentrations of motor oil recovered gravimetrically in TA1, TA2, TB1 and TB2 at week 10 were 0.01, 0.026, 0.014 and 0.031 gm/ml water respectively from initial corresponding concentrations of 0.086, 0.086, 0.085 and 0.085 gm/ml water, representing percent degradations of 88.37, 69.77, 83.53 and 63.53% respectively at which time the corresponding value obtained for control was 22.99%. These results suggest that application of cow dung in appropriate concentration could be very useful in bioremediation of motor oil contaminated lagoon water.

KEYWORDS: Biodegradation, cow dung, motor oil, indigenous microorganisms, residual oil, lagoon water.

INTRODUCTION

Motor oil is a complex mixture of hydrocarbons and other organic compounds derived from petroleum-based and non-petroleum-synthesized chemical compounds. It is a lubricant used in internal combustion engines of cars, motorcycles, buses, commercial vehicles, large agricultural and construction equipment, locomotives, aircraft and static engines such as electrical generators. Its main function is to provide a separating film between surfaces of adjacent moving parts thereby minimizing direct contact between them, decreasing heat caused by friction and reducing wear and tear, thus protecting the engine and improving its efficiency (Nwoko *et al*., 2007; Butler and Mason, 1997). However, fresh motor oil is more of environmental concern as it contains high percentage of volatile and water soluble hydrocarbons that could be acutely toxic to organisms (Mandri and Lin, 2007). The discharge of motor oil from refineries, oil pipes, packaging companies, mechanic workshops, petrol stations and many industrial plants in Nigeria is one of the main sources of oil pollution in the environment. This environmental pollution problem is more complex in Nigeria where siting of mechanic workshops is under-regulated and where most motor mechanics are illiterate who knows little or nothing about the environmental implications of indiscriminate discharge of either fresh or used motor oil. Irrespective of the sources of pollution, motor oil may find its way to surface water, groundwater reserves, lakes and water courses serving as source of potable water for community consumption (Adebusoye *et al*., 2007). In addition to the fact that the polycyclicaromatic hydrocarbons (PAHs) present in this oil may biomagnify through the food chain and cause cancer, skin problems and life threatening effects on the aquatic lives, such contamination also produces objectionable odour and taste (Hilyard *et al*., 2008; Adebusoye *et al*., 2007; Plohl *et al*., 2002). In Nigeria, areas adversely affected are the city of Lagos and other cities, towns and villages within the Niger Delta states which rely mainly on groundwater and rivers as sources of drinking water. The microbial communities are not left out as some of their beneficial activities such as their involvement in biogeochemical cycles of that ecosystem could be inhibited by the presence of oil pollutant and this affects the productivity of such ecosystems (Rhodes and Hendricks, 1990). The process of bioremediation is an emerging method for the removal of many environmental pollutants. It involves the use of microorganisms with diverse metabolic capabilities to detoxify or remove pollutants from specific environments (Adenipekun and Isikhuemhen, 2008; Medina-Bellver *et al*., 2005). Apart from the presence of microorganisms with the appropriate metabolic capabilities, the ability of a bioremediation scientist to establish and sustain conditions necessary for enhanced petroleum or petroleum product degradation rate is a determining factor for the success of bioremediation of oil contaminated environments. Bioremediation technology is relatively cheap compared to the conventional methods (April *et al*., 2000; Leahy and

Colwell, 1990). Elimination of crude oil pollutant by indigenous microorganisms is one of the primary mechanisms by which crude oil and other hydrocarbon pollutants can be removed from the environment (Ulrici, 2000).

The use of conventional remediation methods, such as dredging, incineration, use of sorbent materials, sinking and dispersion, can be costly and may further destroy the environment by making toxic hydrocarbons more bioavailable (Hilyard *et al*., 2008). Thus, the biological alternative is a more attractive method and indispensible as the most natural technique to remove the bulk of crude oil pollutant from oil polluted sites, where the addition of specific microorganisms with appropriate metabolic capabilities, or stimulation of microorganisms already present can improve biodegradation efficiency in both *in-situ* and/or *ex-situ* processes (Cookson Jr, 1995; Freeman and Harris, 1995). Cow dung is a vast reservoir of nutrients and energy capable of supporting microbial growth, thereby enhancing microbial degradation of various pollutants (Akinde and Obire, 2008). Apart from improving soil fertility for crop production, it also contributed diverse species of microorganisms such as *Acinetobacter* spp, *Bacillus* spp, *Pseudomonas* spp, *Serratia* spp, and *Alcaligenes* spp which are important for natural biogeochemical processes (Akinde and Obire, 2008; Adebusoye *et al*., 2007). Efficiency of biodegradation is dependent on microorganisms, capable of producing enzymes that will degrade the target pollutant. As reported by many scientists, mixed population of microorganisms with broad enzymatic capacity are needed to eliminate complex mixtures of hydrocarbons in soil, fresh water, or marine environments (Adebusoye *et al*., 2007). Animal manure amendments have over time been used for bioremediation of petroleum hydrocarbon polluted soil. In this study, we investigated the effects of cow dung on biodegradation of motor oil in lagoon water.

MATERIALS AND METHODS

Source of materials

The water sample used for this study was collected from Lagos Lagoon. Five surface water samples (200 ml each), 5 subsurface water samples (200 ml each) and 5 bottom water samples (200 ml each) were randomly collected from 5 different points at a distant of 100 metres apart using sterile wide-mouth 500 ml Winchester reagent bottles. The cow dung used was collected from Sifor cattle ranch in Ota, while the motor oil was that of Mobil HD SAE 40 High performance monograde motor oil.

Bioremediation protocols

The surface, subsurface and bottom lagoon water samples collected were mixed thoroughly before used. The cow dung collected was mashed and mixed thoroughly, half of the quantity collected was sterilised by tyndallisation while the other half was left unsterile. Two hundred and seventy millilitres each of lagoon water contained in five 500 ml Erlenmeyer flasks were separately contaminated with 30 ml of motor oil to give 10% (v/v) pollution. Four of the setups designated Treatments (TA1, TA2, TB1 and TB2) were treated with cow dung, while the fifth setup without cow dung treatment was designated Control. While TA1 and TA2 were supplemented with 30gm of non-sterile and sterile cow dung respectively, TB1 and TB2 were supplemented with 40gm of non-sterile and sterile cow dung respectively. Setups TA1 and TA2 were designed to determine the effects of non-sterile and sterile cow dung in bioremediation of motor oil contaminated lagoon water, while setups TB1 and TB2 were designed to determine the effects of cow dung concentrations in bioremediation of motor oil contaminated lagoon water when compared with the setups TA1 and TA2 respectively. However, the control was designed to determine the contribution made by microorganisms indigenous to the lagoon water. The four treatments and the control designs were setup in three replicates and incubated aerobically at 30 ^{o}C and 120 rpm in a shaker incubator throughout the investigation periods (10 weeks). Samples were taken at 2-week interval for analysis.

Enumeration and characterization of lagoon water and cow dung aerobic microorganisms

The population densities of the lagoon water and cow dung microorganisms were determined by standard plate count techniques. Total viable counts of bacteria were performed on nutrient agar plates while that of fungi was evaluated on potato dextrose agar (PDA) plates fortified with streptomycin (0.125 gm/l), and incubation was carried out at 30 ^{0}C for 1–3 days. The population densities of hydrocarbon-utilizing organisms were determined by plating on minimal salt agar (MSA) previously described by Nwachukwu (2001). For hydrocarbon-utilizing bacteria, the medium was adjusted to pH 7.2 while for fungi, it was adjusted to pH 5.6 and further fortified with streptomycin to inhibit bacterial growth. In both cases, motor oil served as the sole carbon and energy source and was made available through vapour phase transfer previously described by Raymond *et al*. (1976). Microbial colonies were counted, screened, and pure cultures obtained by replica plating. Identification was based on the taxonomic schemes and descriptions of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994), Barnett and Pankhurst (1974) and O'Donnell (1979).

Pysico-chemical Properties

The lagoon water and cow dung physico-chemistry was evaluated using standard analytical protocols described by APHA (1998). Dissolved oxygen (DO) was monitored by dissolved oxygen meter (Jenway) and pH by a pH meter (Jenway) according to Nwachukwu (2000). Biochemical oxygen demand (BOD) was determined using standard method described elsewhere (Nwachukwu, 2000; APHA, 1998).

Analysis of motor oil

The residual motor oil was extracted twice from the lagoon water sample (10 ml) using n-hexane: dichloromethane solvent system (1:1) and quantified gravimetrically as described by Nwachukwu (2001). To do this, 10 ml of water sample was randomly taken from each replicate at surface, middle, and bottom and mixed thoroughly before analysis. The oil was extracted by mixing the water with 40 ml volume of the solvent system, stirred for 5 min and filtered through whatman No 1 filter paper. The procedure was repeated twice and

extracts pooled and dried in an oven at 100^0C. The residual motor oil was then obtained by mass difference.

Statistical analysis

Statistical analysis including mean, standard deviation, analysis of variance (ANOVA), as well as the significant evaluation were performed using the SPSS 17.0 statistics.

RESULTS

Initial studies conducted on the cow dung used for this study revealed that the proportion of hydrocarbon-utilizers within the heterotrophic community was approximately 0.07%. Representative hydrocarbon-utilizers isolated from the cow dung were *Penicillium chrysogenum*, *Aspergillus* sp, *Pseudomonas aeruginosa*, *Bacillus* sp, *Alcaligenes faecalis*, *Morganella* sp and *Serratia* sp. The physicochemical properties and the microbial loads of the cow dung and the lagoon water samples are illustrated in Table 1.

TABLE 1: Physicochemical Properties and Microbial load of Cow dung obtained from Sifor cattle ranch in Ota and Lagoon water obtained from Lagos Lagoon.

Parameter	Cow dung	Lagoon water
Moisture (%)	42.2±0.1	-
pH	8.41	6.87
Temperature (^{0}C)	32	27
Nitrate (ppm)	7.59±0.1	52.40±0.02
Phosphate (ppm)	2.24±0.01	22.41±0.01
Sulphate (ppm)	2.25±0.2	94.67±0.02
THM	6.61± 0.1x 10^8 cfu/gm	3.51±0.1 x 10^7 cfu/ml
HCUM	4.31 ±0.01x 10^5 cfu/gm	2.49±0.01^4 cfu/ml

THM, total heterotrophic microorganisms; HCUM, hydrocarbon utilizing microorganisms; cfu, colony forming unit; -, not determined

TABLE 2: Mean Changes in Dissolved Oxygen of Various Treatments and Control

Sampling Time	Mean Changes in Dissolved Oxygen (mg/l) ±SD				
(Week)	TA1	TA2	TB1	TB2	CONTROL
0	4.51±0.01^n	4.50±0.00^n	4.49±0.00^n	4.51±0.01^n	4.49±0.01^n
2	3.40±0.01^j	3.61±0.01^k	3.39±0.01^j	3.59±0.01^k	4.19±0.01^m
4	3.21±0.01^i	3.40±0.01^j	3.21±0.01^i	3.39±0.01^j	3.97±0.01^l
6	2.60±0.01^d	3.05±0.06^g	2.70±0.00^e	3.11±0.01^h	3.94±0.00^l
8	2.40±0.00^b	2.70±0.01^e	2.41±0.01^b	2.79±0.01^f	3.94±0.01^l
10	2.30±0.01^a	2.50±0.00^c	2.28±0.01^a	2.53±0.01^c	3.95±0.01^l

SD, standard deviation; TA1, treatment A1; TA2, treatment A2; TB1, treatment B1; TB2, treatment B2. Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

As indicated in Table 1, the cow dung contained a relatively significant amount of nutrient sources such as nitrate, phosphate and sulphate needed for microbial growth. To investigate the effectiveness of cow dung as an agent for bioremediation, an artificially contaminated lagoon water ecosystem simulated in the laboratory was treated with the cow dung. Motor oil biodegradation of this ecosystem was compared with that of control containing similar materials in the treatments but without cow dung fortification. Changes in mean microbial population densities, reduction in the motor oil pollutant analysed gravimetrically, dissolved oxygen as well as the biochemical oxygen demand were monitored periodically as indicators of biodegradation. The mean changes in dissolved oxygen and biochemical oxygen demand of the treatments and control setups are presented in Tables 2 and 3 respectively.

TABLE 3: Mean Changes in Biochemical Oxygen Demand of Various Treatments and Control

Sampling Time	Mean Changes in Biochemical Oxygen Demand (mg/l) ±SD				
(Week)	TA1	TA2	TB1	TB2	CONTROL
0	11.08±0.01^c	11.04±0.06^b	11.10±0.01^c	11.00±0.00^{ab}	10.96±0.02^a
2	33.10±0.01^i	29.89±0.01^g	32.85±0.07^h	29.85±0.01^g	19.02±0.01^d
4	46.03±0.01^x	41.50±0.01^o	45.78±0.01^v	41.30±0.01^n	19.09±0.01^e
6	46.12±0.00^y	42.02±0.01^q	45.85±0.07^w	41.90±0.01^p	19.13±0.01^{ef}
8	44.30±0.01^s	40.10±0.00^k	44.22±0.01^r	40.01±0.01^j	19.13±0.00^{ef}
10	45.46±0.01^u	40.90±0.01^m	44.76±0.01^t	40.50±0.01^l	19.17±0.01^f

SD, standard deviation; TA1, treatment A1; TA2, treatment A2; TB1, treatment B1; TB2, treatment B2. Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

As indicated in both tables, the trends observed for these variables were much more remarkable in treatments (TA1, TA2, TB1 and TB2) compare with control. Table 2 revealed that the magnitude of loss in dissolved oxygen (DO) levels was much more pronounced in treatments (TA1, TA2, TB1 and TB2) than the control. While the mean dissolved oxygen values obtained at week 10 for TA1, TA2, TB1 and TB2 were 2.30, 2.50, 2.28 and 2.53 mg/l water respectively from initial corresponding dissolved oxygen values of 4.51, 4.50, 4.49 and 4.51 mg/l water, representing percent decreases of 49.0, 44.4, 49.2 and 43.9% respectively, the corresponding percent decrease for control was 12.0%. Table 3 indicated that the increasing trend in biochemical oxygen demand (BOD) was much more remarkable in treatments (TA1, TA2, TB1 and TB2) with BOD values ranging significantly ($P \leq 0.05$) from 11.08 to 45.46, 11.04 to 40.90, 11.10 to 44.76 and 11.00 to 40.50 mg/l water respectively for TA1, TA2, TB1 and TB2 at which time the corresponding BOD value for control ranged from 10.96 to 19.17 mg/l water. Table 4 depicts the mean population densities of bacteria enumerated in treatments (TA1, TA2, TB1 and TB2) and control. As indicated in Table 4, there was an initial decrease in population densities of bacteria from 6.44 x 10^8 to 4.99 x 10^8, 4.23 x 10^8 to 2.10 x 10^8, 6.62 x 10^8 to 5.0 x 10^8, 4.22 x 10^8 to 2.09 x 10^8 and 4.23 x 10^8 to 2.07 x 10^8 cuf/ml respectively for TA1, TA2, TB1, TB2 and control between weeks 0 and 2, thus, suggesting the toxic effect of motor oil to the indigenous microorganisms. However, there was a subsequent increase in bacterial population which was much more remarkable in treatments (TA1, TA2, TB1 and TB2) supplemented with cow dung than the control not supplemented with cow dung. Table 5 presented the mean population densities of fungi enumerated in treatments (TA1, TA2, TB1 and TB2) and control. Although there was an initial decrease in mean population densities of fungi due to oil toxicity, the fungal population later assumed an increasing trend which was more pronounced in treatments (TA1, TA2, TB1 and TB2) fortified with cow dung. At week 8 when the mean population densities of fungi in TA1 treated with non-sterile cow dung was 3.55 x 10^6 cfu/ml, the corresponding population of fungi for control was 1.56 x 10^6 cfu/ml. In Table 6, the mean population densities of hydrocarbon-utilizers were found to be higher in treatments (TA1, TA2, TB1 and TB2), especially those treated with non-sterile cow dung (TA1 and TB1). At week 10, the mean population densities of hydrocarbon-utilizers enumerated in TA1, TA2, TB1 and TB2 were 1.46 x 10^6, 8.13 x 10^5, 1.31 x 10^6 and 7.30 x 10^5 cfu/ml respectively, while the corresponding mean population density of hydrocarbon-utilizers enumerated in control not fortified with cow dung was 3.05 x 10^5 cfu/ml, hence the higher motor oil degradation recorded in the treatment. The % hydrocarbon-utilizers calculated for the treatments and control relative to the total heterotrophic organisms was significantly different at 5% level of probability. Figures 1 and 2 summarized the mean residual oil content in treatments (TA1, TA2, TB1 and TB2) and control. In Figure 1, it was clear that the disappearance of residual oil content was much more rapid in TA1 supplemented with non-sterile cow dung compared with TA2 fortified with sterile cow dung. Similar observation was made in Figure 2 where the magnitude of loss in residual motor oil was much more remarkable in TB1 supplemented with non-sterile cow dung and differed significantly at 5% probability level when compared with TB2 fortified with sterile cow dung and control not fortified with cow dung. Not surprisingly, the non-sterile cow dung has added additional hydrocarbon-utilizers, hence the much more rapid oil disappearance observed.

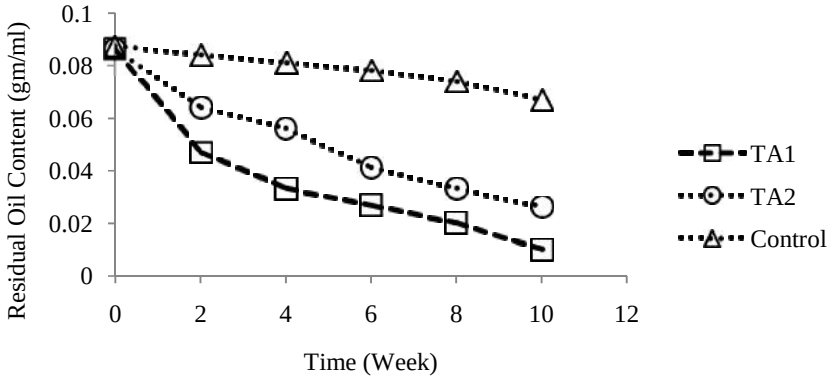

FIGURE 1: Residual motor oil recovered from treatments (TA1 and TA2) and control at two weeks interval for a period of ten weeks.

The concentrations of residual motor oil recovered in TA1, TA2, TB1 and TB2 at week 10 were 0.01, 0.026, 0.014 and 0.031 gm/ml water respectively from initial corresponding concentrations of 0.086, 0.086, 0.085 and 0.085 gm/ml water, thereby giving percent degradations of 88.37, 69.77, 83.53 and 63.53% respectively at which time the corresponding value obtained for control was 22.99%. It is important to note that, though our investigation on the possible effects of increasing concentration of cow dung on microbial degradation of motor oil in the studied ecosystem revealed that higher concentration of cow dung slowed down the degradation process, the effect was not significant when subjected to variance analysis.

The microorganisms which occurred frequently in the treatments and control, but with higher species diversities in the former and also grew well in minimal salt medium fortified with motor oil as the sole source of carbon and energy were species of *Penicillium*, *Aspergillus*, *Candida*,

Pseudomonas, Bacillus, Alcaligenes, Morganella, Serratia and *Klebsiella*.

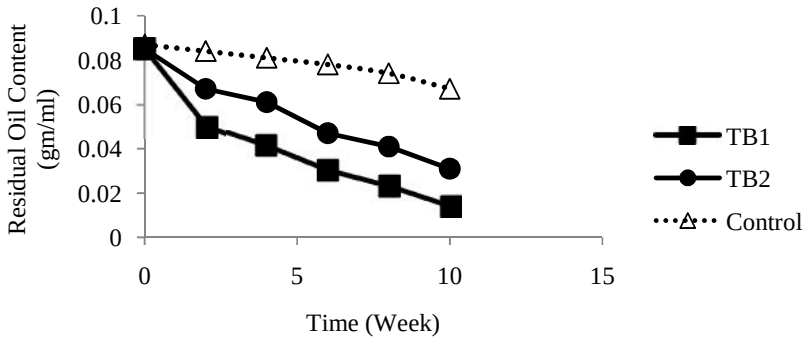

FIGURE 2: Residual motor oil recovered from treatments (TB1 and TB2) and control at two weeks interval for a period of ten weeks

TABLE 4: Mean Bacterial Count for Treatments and Control

sampling	Mean Bacterial Count (cfu/ml ± SD) ×10^8				
Time	TREATMENTS				CONTROL
(Week)	TA1	TA2	TB1	TB2	CON
0	6.44 ± 0.01^{r}	4.23 ± 0.02^{j}	6.62 ± 0.01^{s}	4.22 ± 0.00^{j}	4.23 ± 0.02^{j}
2	4.99 ± 0.02^{n}	2.10 ± 0.01^{bc}	5.0 ± 0.01^{n}	2.09 ± 0.01^{b}	2.07 ± 0.01^{a}
4	5.90 ± 0.01^{q}	3.23 ± 0.01^{h}	5.91 ± 0.00^{q}	2.76 ± 0.01^{f}	2.10 ± 0.01^{c}
6	7.34 ± 0.01^{t}	4.34 ± 0.00^{k}	7.55 ± 0.01^{u}	3.96 ± 0.00^{i}	2.19 ± 0.01^{d}
8	8.62 ± 0.02^{w}	4.95 ± 0.01^{m}	8.46 ± 0.01^{v}	4.48 ± 0.01^{l}	2.24 ± 0.00^{e}
10	10.21 ± 0.01^{y}	5.64 ± 0.01^{p}	8.94 ± 0.00^{x}	5.41 ± 0.01^{o}	2.90 ± 0.01^{g}

SD, standard deviation; cfu, colony forming unit; CON, control.
Rows and Columns with the same superscript are not significantly different ($p\leq 0.05$).

Table 5: Mean Fungal Count for Treatments and Control

Sampling	Mean Fungal Count (cfu/ml ± SD) ×10^6				
Time	TREATMENTS				CONTROL
(Week)	TA1	TA2	TB1	TB2	CON
0	3.65 ± 0.00^{y}	2.08 ± 0.01^{n}	3.75 ± 0.01^{z}	1.95 ± 0.01^{m}	2.09 ± 0.02^{n}
2	1.60 ± 0.01^{i}	1.02 ± 0.01^{a}	1.65 ± 0.00^{k}	1.22 ± 0.01^{d}	1.12 ± 0.01^{b}
4	2.69 ± 0.01^{r}	1.53 ± 0.00^{g}	2.54 ± 0.01^{q}	1.42 ± 0.02^{f}	1.20 ± 0.01^{c}
6	3.15 ± 0.02^{w}	2.33 ± 0.01^{o}	2.99 ± 0.01^{u}	1.63 ± 0.01^{j}	1.39 ± 0.00^{e}
8	3.55 ± 0.01^{x}	2.88 ± 0.01^{t}	3.55 ± 0.01^{x}	2.48 ± 0.01^{p}	1.56 ± 0.01^{h}
10	$4.50\pm0.01^{b'}$	3.04 ± 0.00^{v}	$4.05\pm0.02^{a'}$	2.84 ± 0.01^{s}	1.81 ± 0.01^{l}

SD, standard deviation; cfu, colony forming unit; CON, control
Rows and Columns with the same superscript are not significantly different except those with superscript bearing prime sign ($p\leq 0.05$).

TABLE 6. Mean population densities of hydrocarbon utilizing Microorganisms (HCUM) for Treatments and Control

Sampling	Mean HCUM Count (cfu/ml ± SD) ×10^5				
Time	TREATMENTS				CONTROL
(Week)	TA1	TA2	TB1	TB2	CON
0	3.67 ± 0.00^{l}	2.48 ± 0.01^{b}	3.71 ± 0.01^{m}	2.45 ± 0.02^{a}	2.48 ± 0.01^{b}
2	4.97 ± 0.01^{q}	2.86 ± 0.00^{g}	4.18 ± 0.02^{o}	2.63 ± 0.01^{e}	2.52 ± 0.01^{c}
4	7.33 ± 0.01^{v}	3.46 ± 0.01^{k}	6.60 ± 0.01^{t}	3.14 ± 0.01^{j}	2.57 ± 0.00^{d}
6	8.66 ± 0.01^{y}	4.88 ± 0.01^{p}	7.81 ± 0.01^{w}	4.01 ± 0.02^{n}	2.72 ± 0.01^{f}
8	$10.72\pm0.02^{a'}$	5.64 ± 0.01^{s}	10.01 ± 0.00^{z}	5.20 ± 0.01^{r}	2.95 ± 0.00^{h}
10	$14.58\pm0.01^{c'}$	8.13 ± 0.00^{x}	$13.07\pm0.01^{b'}$	7.30 ± 0.01^{u}	3.05 ± 0.01^{i}

SD, standard deviation; cfu, colony forming unit; CON, control.
Rows and Columns with the same superscript are not significantly different except those with superscript bearing prime sign ($p\leq 0.05$).

DISCUSSION

The indiscriminate disposal of motor oil from refineries, oil pipes, packaging companies (Plohl *et al.*, 2002), as well as the discharge of used motor oil into water drains, gutters, farmlands and open plots when motor oil is changed are common practices by oil servicing companies,

motor mechanics and static electrical generator mechanics (Odjegba and Sadiq, 2002). These practices are more complex in Nigeria where influx of these pollutants are not properly checked, the siting of mechanic workshops is under-regulated and where the majority of motor or generator mechanics are uneducated.

Contamination of the environment with these hazardous pollutants is a global problem. Microorganisms with diverse metabolic capabilities naturally degrade toxic hydrocarbons present in petroleum products (Kastner *et al*., 1994). Thus, biological approach has become an alternative way of cleaning oil polluted sites, where the addition of oil-adapted microorganisms or stimulation of indigenous oil-degraders can enhance the rate of microbial degradation of hydrocarbons in both *in-situ* and/or *ex-situ* procedures (Cookson Jr, 1995; Freeman and Harris, 1995). The success of these approaches largely depends not only on the number and catabolic diversity of indigenous hydrocarbon-degraders in such environments but also on established and sustained conditions favourable for enhanced biodegradation of hydrocarbons. Upon this background, the effects of cow dung on microbial degradation of motor oil in lagoon water were investigated in this study.

Microbial degradation of hydrocarbons is mostly an aerobic process and required a relatively high oxygen level (Adebusoye *et al*., 2010; Nwachukwu, 2000). Thus, the decrease in dissolved oxygen (Table 2) and the increase in biochemical oxygen demand (Table 3) which was much more remarkable in the treatments (TA1, TA2, TB1 and TB2) were probably due to the greater utilization of oxygen in breaking down motor oil resulting in higher values of biochemical oxygen demand when compared with the control. The high bacterial and fungal counts (Tables 4 and 5 respectively) in the treatments and the control, at week 0, as revealed in this study could be attributed to the presence of diverse species of microorganisms in the cow dung and the lagoon water. This is comparable with the findings by Amadi and Ue-Bari (1992) and Adebusoye *et al*. (2007). The relatively low bacterial and fungal counts (Tables 4 and 5 respectively) observed in both treatments and control at week 2 could be a confirmation of the toxic or unfavourable effect of oil contaminant. This result is in agreement with results obtained by many investigators (Nwachukwu, 2001; Atlas, 1991; Amund and Igiri, 1990). However, there was a continuous increase in the population densities of hydrocarbon utilizers (Table 6) throughout the period of study which indicated the presence of hydrocarbon utilizers and/or hydrocarbon tolerant microorganisms in both systems. This finding corroborates the work of Adebusoye *et al*. (2007), which reported the presence of hydrocarbon utilizing microorganisms in polluted tropical stream. It also agreed with the work of Akinde and Obire (2008), which revealed the presence of potential hydrocarbon degraders in cow dung. Notwithstanding, the population densities of hydrocarbon utilizers present in treatments supplemented with non-sterile cow dung (TA1 and TB1) were higher and significantly different when compared with the treatments fortified with sterile cow dung (TA2 and TB2) and the control not supplemented with cow dung ($p \leq 0.05$). As shown in Figures. 1 and 2, the residual motor oil of both systems analysed at the beginning of the study (week 0) were relatively equal. However, as the weeks went on, there were gradual reductions in residual motor oil of both systems. These reductions were much more higher in treatments (TA1, TA2, TB1 and TB2), especially in those with non-sterile cow dung amendments (TA1 and TB1) and differed significantly ($p \leq 0.05$) when compared with control. These results probably prove the efficacy of cow dung as an agent for bioremediation of oil polluted water. Although not significant when subjected to variance analysis, we also noticed that more oil degradations occurred in TA1 and TA_2 with lesser concentrations of cow dung compared to their corresponding TB1 and TB_2 with higher concentrations of cow dung, thus revealing that excess cow dung could slow down biodegradation of oil in water. This finding corroborates previous findings by Oudot *et al*. (1998), Chaineau *et al*. (2005) and Chaillan *et al*. (2006) who reported the negative effects of high nutrients on biodegradation of hydrocarbon. It is apparent that cow dung contains a wide array of microorganisms with potential hydrocarbon degrading capacity. In addition, it is also a good source of nutrients such as nitrate and phosphate which are essential for microbial growth and metabolism, hence the higher rate of motor oil degradation observed in the treatments compared with the control. However, this study also revealed that excess cow dung slowed down biodegradation of motor oil in water. Therefore, application of cow dung in appropriate concentration could serve as alternative source of nutrients as well as hydrocarbon utilizers for enhancement of bioremediation of oil polluted water.

REFERENCES

Adebusoye, S. A., Ilori, M. O., Amund, O. O., Teniola, O. D. and Olatope, S. O. (2007) Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World J. Microbiol. Biotechnol*. 23, 1149-1159.

Adebusoye, S. A., Ilori, M. O., Obayori, O. S., Oyetibo, G. O., Akindele A. A. and Amund, O.O. (2010) Efficiency of cassava steep liquor for bioremediation of diesel oil-contaminated tropical agricultural soil. Environmentalist 30, 24-34.

Adenipekun, C. O. and Isikhuemhen, O. S. (2008) Bioremediation of engine oil polluted soil by the tropical white rot fungus, *Lentinus squarrosulus* Mont (Singer). *Pak. J. Biol. Sci*. 11, 1634-1637.

Akinde, S. B. and Obire, O. (2008) Aerobic heterotrophic bacteria and petroleum utilizing-bacteria from cow dung and poultry manure. *World J. Microbiol. Biotechnol*. 24, 1999-2002.

Amadi, A. and Bari, Y. U. (1992) Use of poultry manure for amendment of oil-polluted soils in relation to growth of maize (*Zea mays L*). *Environ. Intern*. 18, 521-527.

Amund, O. O. and Igiri, C. O. (1990) Biodegradation of petroleum hydrocarbons under tropical estuarine conditions. *World J. Microbiol. Biotechnol*. 16, 255-262.

APHA (1998) Standard methods for the examination of water and waste water. 20th edition. APHA – AWWA – WPCF. Washington DC. pp. (4 – 114) – (4 – 179).

April, T. M., Foght, J. M. and Currah, R. S. (2000) Hydrocarbon-degrading filamentous fungi isolated from flare pit soil in northern and western Canada. *Canad. J. Microbiol*. 46(1), 38-49.

Atlas, R. M. (1991) Microbial hydrocarbon degradation: Bioremediation of oil spills. *J. Chem. Technol. Biotechnol*. 52, 149–156.

Barnett, J. A. and Pankhurst, R. J. (1974) A New Key to the Yeasts. North Holland Publishing, Amsterdam.

Butler, C. S. and Mason, J. R. (1997) Structure-function analysis of the bacterial aromatic ring-hydroxylating dioxygenases. *Adv. Microb. Physiol*. 38, 47-84.

Chaillan. F., Chaineau, C. H., Point, V., Saliot, A. and Oudot, J (2006) Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cutting. *Environ. Pollut*. 144(1), 255-265.

Chaineau, C. H., Rougeux, G., Yepremian, C. and Oudot, J. (2005) Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biol. Biochem.* 37(8), 1490-1497.

Cookson Jr, J. T (1995) Bioremediation engineering: Design and application. Mcgraw-Hill, New York.

Freeman, H. M. and Harris, E. F. (1995) Hazardous waste remediation: Innovation treatment technologies. Technomic Publ. Co. Inc., Lancaster, USA. pp. 342.

Hilyard, E. J., Jones-Meehan, J. M., Spargo, B. J. and Hill, R. T. (2008) Enrichment, isolation, and phylogenetic identification of polycyclic aromatic hydrocarbon-degrading bacteria from Elizabeth river sediments. *Appl. Environ. Microbiol*. 74(4), 1176-1182.

Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley J. T. and Williams. S. T. (1994) Bergey's Manual of Determinative Bacteriology. 9th Edn. Lippincott Williams and Wilkins. Baltimore, USA.

Kastner, M., Breuer-Jammali, M. and Mahro, B. (1994) Enumeration and characterization of soil micro flora from hydrocarbon-contaminated soil sites able to mineralise polycyclic aromatic hydrocarbon (PAH). *Appl. Microbiol. Biotechnol*. 41, 267-273.

Leahy, J. G. and Colwell, R. R (1990) Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev*. 54, 305-315.

Mandri, T. and Lin, J. (2007) Isolation and characterization of engine oil degradeng indigenous microorganisms in Kwazulu-Natal, South Africa. *Afr. J. Biotechnol.* 6(1), 023-027.

Medina-Bellver, J. I., Marin, P., Delgado, A., Rodriguez-Sanchez, A., Reyes, E., *et al*. (2005) Evidence for *in situ* crude oil biodegradation after the prestige oil spill. *Environ. Microbiol*. 7(6), 773-779.

Nwachukwu, S. C. U. (2000) Enhanced rehabilitation of tropical aquatic environments polluted with crude petroleum using *Candida subtilis. J. Environ. Biol*. 21(3), 241-250.

Nwachukwu, S. C. U. (2001) Bioremediation of sterile agricultural soils polluted with crude petroleum by application of the soil bacterium, *Pseudomonas putida*, with inorganic nutrient supplementations. *Curr. Microbiol*. 42, 231-236.

Nwoko, C. O., Okeke, P. N., Agwu, O. O. and Apkan, I. E. (2007) Performance of *Phaseolus vulgaris* L. in a soil contaminated with spent-engine oil. *Afr. J. Biotechnol.* 6(16), 1922–1925.

Odjegba, V. J. and Sadiq, A. O. (2002) Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. *The Environmentalist*. 22(1), 23-28.

O'Donnell, K. L. (1979) Zygomycetes in Culture. University of Georgia Press, Anthens.

Oudot, J., Merlin, F. X. and Pinvidic, P. (1998) Weathering rates of oil components in bioremediation experiment in estuarine sediments. *Marine Environ. Res*. 45(2), 113-125.

Plohl, K., Leskovsek, H. and Bricelj, M. (2002) Biological degradation of motor oil in Water. *Acta. Chim. Slov*. 49, 279-289.

Raymond, R. L., Hudson, J. O. and Jamison, V.W. (1976) Oil degradation in soil. *Appl. Environ. Microbiol*. 31(4), 522–535.

Rhodes, A. N. and Hendricks, C. W. (1990) A continuous-flow method for measuring effects of chemicals on soil nitrification. *Toxicity Assess*. 5, 77-89.

Ulrici, W. (2000) Contaminated soil areas, different countries and contaminant, monitoring of contaminants, in *Environmental processes II-Soil decontamination*, vol XI, edited by H. J. Rehm and G. Reed. Wiley-VCH, New York. pp. 5-42.