Effects of Abattoir Effluent on Microbial Degradation of Diesel Oil in Tropical Agricultural Soil.

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ABSTRACT

The effects of non-sterile and sterile abattoir effluent on microbial degradation of diesel oil in soil were evaluated. Samples collected were analyzed chemically and microbiologically. The residual diesel oil recovered in treatments (TA, TB, TC, and TD) at week 10 were 0.013, 0.033, 0.004 and 0.015 g/g soil respectively from initial corresponding concentrations of 0.169, 0.169, 0.169 and 0.17 g/g soil, representing percent degradations of 92.31, 80.47, 97.63, and 91.12%, respectively, at which time the corresponding value obtained for control was 35.50%.

(Keywords: soil contamination, residual diesel oil, hydrocarbon utilizers, biodegradation, aerobic bacteria and fungi)

INTRODUCTION

The efficiency of diesel engines as prime movers and their ability to generate electricity more economically than any other device in their size range has placed high demand on domestic and commercial uses of diesel engines. However, the diesel oil used to fuel these engines is an important contributor to environmental pollution problem globally.

Emissions from diesel not only pollute air, water, and soil, but also results in the development of cancer, heart, and respiratory problems (Lloyd and Cackette, 2001). Diesel oil is a complex mixture of normal, branched, and cyclic alkanes, and aromatic compounds. Their linkages from underground storage tank, distribution facilities and various industrial operations as well as accidental spill from oil tanker due to bad roads represent an important source of soil and water contamination (Nwaogu *et al.*, 2008).

The problem of environmental pollution from diesel oil is further compounded with Nigeria being a major producer of crude oil in the world, yet with a very unstable electricity supply. This epileptic power supply has compelled all companies and most individuals in Nigeria to the use of diesel engine plants and generators to generate electricity for their businesses and homes, resulting in an increased pollution of the environment with diesel.

Soil is a reservoir of myriad of microorganisms and other biotic factors. Contamination of the soil with diesel oil limits the available soil nitrogen and phosphorus, and also causes C-N ratio imbalance in the affected soil. The overall consequence is reduced plant growth resulting from direct toxic effect on plants and unsatisfactory soil condition due to insufficient aeration of the soil (Nwaogu *et al.*, 2008; Das and Chandran, 2011).

Among several clean-up techniques available to remove diesel oil pollutant from the soil, biodegradation by intrinsic populations of microorganisms is one of the primary mechanisms by which petroleum hydrocarbon pollutant can be removed from the environment since it is cheaper than other remediation technologies. These processes rely on the innate ability of microorganisms to mineralize hydrocarbons, leading to formation of CO₂, H₂O and cell biomass (Alexander, 1994; Das and Chandran, 2011).

Of all strategies to accelerate the biological breakdown of hydrocarbons in soil, biostimulation of the intrinsic microorganisms by addition of nutrients is the most frequently used bioremediation technique as the introduction of enormous amount of carbon sources as contaminant tends to result in quick reduction or depletion of the available nitrogen and

phosphorus which are essential for microbial growth (Margesin and Schinner, 2001).

The use of organic manures such as cow dung, poultry droppings, household refuse and effluents for crop production has been a usual practice amongst the subsistence farmers in West Africa since the prospect of obtaining adequate chemical fertilizers to meet the requirement of the large farming population is challenging (Lombin et al., 1991; Osemwota, 2010). Abattoir effluent comprises blood, urine, feces and water of the slaughtered animals. It is usually obtained as residual material from the abattoir after the slaughter of animals like cattle, sheep, goats, etc. (Adesemoye et al., 2006; Osemwota, 2010). Municipal effluent, apart from serving as organic manure, has also been used to enhance bioremediation of oil based drilling mud (Umanu and Nwachukwu, 2010).

Osemwota (2010) revealed that abattoir effluent increased the pH and available phosphorus of the soil. The increase in pH value coupled with that of the available phosphorus could help in stimulating bacterial growth since most aerobic bacteria involved in hydrocarbon degradation grow best at pH range of near neutral to slightly alkaline. Although large volume of wastewater has been reported to reduce or deplete the available soil nitrogen for crop production (Rabah et al., 2010), the quantity of abattoir effluent (100 and 125ml/kg soil) used in this study was not enough to deplete the available soil nitrogen as compared with the study by Osemwota (2010). Thus, this present study investigated the effects of abattoir effluent in bioremediation of diesel oil contaminated soils with a view to enhance microbial degradation (exsitu treatments) of diesel oil in soil using abattoir effluent and also to reduce environmental pollution caused by abattoir effluent.

MATERIALS AND METHODS

Source of Material

The soil samples used in this study were randomly collected from the upper 25 cm of a plot of agricultural land (measuring 30 x 25 m) beside Bells University of Technology, Ota, Nigeria, using sterile hand auger. The plot of the land from which the soils were obtained has no known history of contamination with crude oil or petroleum related products. The soils collected from different spots on the plot were put together,

mixed thoroughly and sieved before use. The diesel oil used was bought from a petrol filling station in Ota, while the abattoir effluent used was randomly collected from Agege abattoir in Lagos using sterile sampling bottles. The bottles were used to aseptically draw part of the effluent running off the drainage system just as it was leaving slaughter pavement.

Experimental Design

The experiment was set up in the laboratory using a completely randomized design. The soil samples collected were mixed thoroughly and sieved before use. The abattoir effluent collected from different points were combined and mixed thoroughly, half of the quantity collected was sterilized by tyndallisation while the other half was left unsterile. Two kilogram of the soil contained in five open pans, 29.0 cm \times 18.0 cm \times 10.0 (internal dimension) were separately contaminated with 200 ml of diesel oil, to give approximately 10% (v/w) pollution. Four of the setups designated Treatments (TA, TB, TC and TD) were treated with abattoir effluent, while the fifth setup without abattoir effluent treatment was designated Control. While TA and TB were supplemented with 200 ml of non-sterile and sterile abattoir effluent respectively, TC and TD were supplemented with 250 ml of non-sterile and sterile abattoir effluent respectively.

Setups TA and TB were designed to determine the effects of non-sterile and sterile abattoir effluent in bioremediation of diesel contaminated soil, while setups TC and TD were designed to determine the effects of abattoir effluent concentrations in bioremediation of diesel oil contaminated soil when compared with the setups TA and TB respectively. However, the control was designed to determine contribution made by microorganisms indigenous to the soil. While treatments TA and TB were flooded with 50 ml of sterile distilled water, the control setup was flooded with 250 ml sterile distilled water to maintain relatively similar moisture level with treatments TC and TD. The four treatments and the control designs were setup in three replicates and kept in the laboratory at room temperature (29 ± 2°C) throughout the investigation periods (10 weeks). They were watered weekly with 200 ml sterile distilled water. Samples were taken at 2-week interval for analysis.

Population Densities and Characterization of Soil and Abattoir Effluent Aerobic Microorganisms

Bacterial, fungal, and hydrocarbon utilizer counts of the soil and abattoir effluent 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 g/l), and incubation was carried out at 30°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, diesel oil served as the sole carbon and energy source and was made available through vapor 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 soil and abattoir effluent chemical properties such as available nitrate, phosphate and sulphate were evaluated using standard analytical protocols described by APHA (1998). Moisture content was determined using moisture analyzer and the pH by a pH meter (Jenway) according to Nwachukwu (2000).

Analysis of Diesel Oil

The residual diesel oil was extracted twice from the contaminated soil sample (10 g) using n-hexane: dichloromethane solvent system (1:1) and quantified gravimetrically as described by Nwachukwu (2001). To do this, 10 g of soil sample was randomly taken from each replicate at surface, middle, and bottom and mixed thoroughly before analysis. The oil was extracted by mixing the soil 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 80°C. The residual diesel 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

Preliminary studies conducted on the abattoir effluent used for this study revealed an appreciable quantity of hydrocarbon-utilizers $(3.07\pm0.01\times10^{5} \text{cfu/ml}).$ Representative hydrocarbon-utilizers isolated from the abattoir effluent were Candida sp., Rhodotorula sp., Penicillium Fusarium sp., chrysogenum, Aspergillus niger, Pseudomonas aeruginosa, Bacillus sp., Alcaligenes faecalis, and Serratia sp. The physicochemical properties and the microbial loads of the abattoir effluent and the soil samples are presented in Table 1. The abattoir effluent contained a relatively significant amount of nutrient sources such as nitrate, phosphate and sulphate needed for microbial growth.

Diesel oil biodegradation of the abattoir effluent treated soil ecosystems were compared with that of control containing similar materials in the without treatments but abattoir effluent amendment. Changes in mean microbial population densities and reduction in the diesel oil pollutant analyzed gravimetrically monitored periodically as indices of biodegradation.

Table 2 depicts the mean population densities of bacteria enumerated in treatments (TA, TB, TC and TD) and control. As indicated in Table 2, there was an initial decrease in bacterial count from $3.23\pm0.01~\times~10^{10}$ to $2.53\pm0.01~\times~10^{10}$, $2.93\pm0.00~\times~10^{10}$ to $1.81\pm0.01~\times~10^{10}$, $3.64\pm0.01~\times~10^{10}$ to $2.730.01~\times~10^{10}$, $2.88\pm0.00~\times~10^{10}$ to $1.79\pm0.01~\times~10^{10}$ and $2.94\pm0.01~\times~10^{10}$ to $1.82\pm0.00~\times~10^{10}$ respectively for TA, TB, TC, TD and control between weeks 0 and 2, thus, revealing the toxicity of diesel oil to the intrinsic microorganisms.

Table 1: Physicochemical Properties and Microbial Load of Soil and Abattoir Effluent.

Parameter	Soil	Abattoir Effluent	
Moisture content (%)	2.20±0.03	-	
рН	5.35	7.41	
Temperature(°c)	31	29	
Nitrate (ppm)	4.25± 0.20	22.60±0.23	
Phosphate	1.81± 0.02 ppm	0.03±0.05 %	
Sulphate	1.58± 0.12 ppm	0.03±0.15 %	
THM	3.70 ±0.01×10 ¹⁰ cfu/g	2.32 ±0.01×10 ¹⁰ cfu/ml	
THCU	4.42 ±0.01×10 ⁵ cfu/g	3.07±0.01×10 ⁵ cfu/ml	

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

Table 2: Mean Bacterial Count for Treatments and Control.

Sampling	Mean Bacterial Count (cfu/g ± SD) ×10 ¹⁰				
Time	TREATMENTS				CONTROL
(Week)	TA	TB	TC	TD	CON
0	3.23±0.01 ^r	2.93±0.00 ⁿ	3.64±0.01 ^w	2.88±0.00 ^l	2.94±0.01 ⁿ
2	2.53±0.01 ^h	1.81±0.01 ^b	2.730.01 ^j	1.79±0.01 ^a	1.82±0.00 ^b
4	2.84±0.02 ^k	2.21±0.01 ^e	3.01±0.01°	2.20±0.01 ^e	1.94±0.02 ^c
6	3.10±0.01 ^q	2.63±0.01 ⁱ	3.25±0.01 ^s	2.62±0.01 ⁱ	2.12±0.01 ^d
8	3.40±0.01 ^t	2.91±0.01 ^m	3.55±0.00 ^u	2.88±0.01 ¹	2.29±0.01 ^f
10	3.61±0.00°	3.03±0.02 ^p	3.94±0.01 ^x	3.01±0.01°	2.45±0.01 ^g

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

However, there was a subsequent increase in bacterial count which was much more remarkable in treatments (TA, TB, TC, and TD) supplemented with abattoir effluent than the control not supplemented with abattoir effluent.

Table 3 presented the mean population densities of fungi enumerated in treatments (TA, TB, TC, and TD) and control. Although there was an initial decrease in mean fungal count apparently due to toxic effect of diesel oil, the fungal population density later assumed an increasing trend which was more pronounced in treatments (TA, TB, TC, and TD) fortified with abattoir effluent. In Table 4,

the mean counts of hydrocarbon-utilizers were found to be higher in treatments (TA, TB, TC, and TD), especially those treated with non-sterile abattoir effluent (TA and TC). At week 10, the mean population densities of hydrocarbon-utilizers enumerated in TA, TB, TC and TD were $7.01\pm0.01 \times 10^6$, $5.21\pm0.00 \times 10^6$, $7.23\pm0.01 \times 10^6$ and $5.32\pm0.01 \times 10^6$, respectively, while the corresponding mean count of hydrocarbon-utilizers enumerated in control not fortified with abattoir effluent was $2.31\pm0.01 \times 10^6$, hence the higher diesel oil degradation observed in the treatments.

Table 3: Mean Fungal Count for Treatments and Control.

Sampling	Mean Fungal Count (cfu/g ± SD) ×10 ⁷				
Time	TREATMENTS				CONTROL
(Week)	TA	TB	TC	TD	CON
0	3.21±0.00 ^u	2.94±0.01 ^r	3.35±0.02 ^v	2.92±0.01 ^q	2.95±0.00 ^r
2	2.31±0.01 ^h	1.86±0.01 ^b	2.41±0.01 ^j	1.83±0.01 ^a	1.88±0.01 ^c
4	2.65±0.01 ¹	2.14±0.00 ^e	2.27±0.01 ^m	2.13±0.00 ^e	2.01±0.01 ^d
6	2.82±0.02°	2.32±0.01 ^h	2.88±0.00 ^p	2.31±0.00 ^h	2.20±0.01 ^f
8	2.98±0.01 ^s	2.51±0.01 ^k	3.09±0.01 ^t	2.51±0.01 ^k	2.26±0.01 ^g
10	3.21±0.01 ^u	2.78±0.01 ⁿ	3.35±0.01 ^v	2.77±0.01 ⁿ	2.37±0.01 ⁱ

SD, standard deviation; cfu, colony forming unit; CON, control.

Rows and Columns with the same superscript are not significantly different (p≤ 0.05).

Table 4: Mean Population Densities of Hydrocarbon Utilizing Microorganisms (HCUM) for Treatments and Control.

Sampling	Mean HCUM Count (cfu/g ± SD) ×10 ⁶				
Time	TREATMENTS				CONTROL
(Week)	TA	ТВ	TC	TD	CON
0	2.41±0.01 ^h	1.84±0.01 ^a	2.51±0.00 ⁱ	1.83±0.01 ^a	1.86±0.01 ^b
2	2.77±0.01 ^j	2.01±0.01 ^d	2.82±0.00 ^k	2.01±0.01 ^d	1.91±0.00 ^c
4	4.01±0.01°	3.04±0.01 ^m	3.13±0.01 ⁿ	3.02±0.00 ^l	2.01±0.01 ^d
6	5.92±0.02 ^u	4.01±0.01°	5.98±0.01 ^v	4.03±0.01 ^p	2.18±0.01 ^e
8	6.88±0.001 ^w	4.71±0.01 ^q	7.01±0.01 ^x	4.81±0.02 ^r	2.22±0.00 ^f
10	7.01±0.01 ^x	5.21±0.00 ^s	7.23±0.01 ^y	5.32±0.01 ^t	2.31±0.01 ^g

SD, standard deviation; cfu, colony forming unit; CON, control.

Rows and Columns with the same superscript are not significantly different (p≤ 0.05).

Figure 1 shows the mean residual diesel oil recovered in treatments (TA and TB) and control, while Figure 2 represents the mean residual diesel oil recovered in treatments (TC and TD) and control. In Figure 1, it was obvious that the disappearance of residual diesel oil was much more rapid in TA supplemented with nonsterile abattoir effluent compared with TB fortified with sterile abattoir effluent. Similar trend was observed in Figure 2 where the magnitude of loss in residual diesel oil was much more remarkable in TC supplemented with non-sterile abattoir effluent and differed significantly at 5% probability level when compared with TD fortified with sterile abattoir effluent and control not fortified with abattoir effluent. Expectedly, the non-sterile abattoir effluent has added additional hydrocarbon-utilizers, hence the much more rapid diesel oil disappearance observed.

The concentrations of residual diesel oil recovered in TA, TB, TC and TD at week 10 were 0.013, 0.033, 0.004 and 0.015 g/g soil respectively from initial corresponding concentrations of 0.169, 0.169, 0.169 and 0.17 g/g soil, representing percent diesel oil degradations of 92.31, 80.47, 97.63 and 91.12% respectively at which time the corresponding value obtained for control was 35.50%.

Although it may be possible to expect negative effect on microbial degradation of diesel oil when very high volume of abattoir effluent is applied, results obtained for the moderate quantities applied in this study revealed that microbial degradation of diesel oil increases as the concentration of abattoir effluent applied increases. However, these differences in diesel oil degradations were 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 diesel oil as the sole source of carbon and energy were species of *Penicillium*, *Aspergillus*, *Candida*, *Fusarium*, *Rhodotorula Pseudomonas*, *Bacillus*, *Alcaligenes*,and *Serratia*.

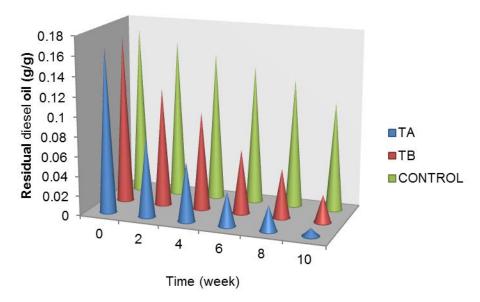


Figure 1: Residual Diesel Oil Recovered in TA, TB, and Control within a Period of Ten Weeks.

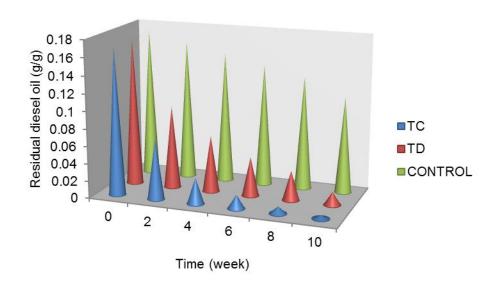


Figure 2: Residual Diesel Oil Recovered in TC, TD, and Control within a Period of Ten Weeks.

DISCUSSION

Diesel oil discharged into the environment either by motor mechanics (Odjegba and Sadiq, 2002) or linkages from underground storage tank, distribution facilities and various industrial operations (Nwaogu et al., 2008) as well as accidental spill from oil tanker due to bad roads represent major contributors to environmental pollution problem globally. Contaminants such as diesel oil and heavy metals are capable of altering microbial properties in soil (Osubor and Anoliefo, 2003; Odejegba and Sadiq, 2002; Atunaya, 1987).

Persistence of these pollutants in the environment for a long period is partly due to low number of hydrocarbon utilizers and the toxicity of oil pollutant on natural flora (Nwachukwu, 2000; Atlas, 1991). Among all strategies to speed up the biological breakdown of hydrocarbons in soil, biostimulation of the intrinsic microorganisms by addition of nutrients is the most frequently used bioremediation technique as the contaminant introduces enormous amount of carbon source which tends to result in rapid depletion of the available nitrogen and phosphorus which are essential for microbial growth (Margesin and Schinner, 2001). In view of this, we enhanced microbial degradation of diesel oil in soil using abattoir effluent, in order to develop alternative bioremediation of crude method for oil and contaminated soil also to reduce environmental pollution caused by abattoir effluent.

The physico-chemical properties and microbial loads of soil and abattoir effluent as shown in Table 1, revealed that there are essential nutrient in abattoir effluent especially nitrate and phosphate which are needed for microbial growth. In addition, microorganisms capable of hydrocarbon utilization are also present. This is in line with the previous report by Osemwota (2010).

Bacteria capable of diesel oil degradation isolated from soil and abattoir effluent in this study are *Pseudomonas aeuruginosa*, *Bacillus* spp, *Alcaligens feacalis*, and *serratia* spp, while the fungi isolated are *Aspergillus niger*, *Penicillium chrysogenum*. *Fusarium* sp, *Rhodotorula* sp, *Saccharomyces* sp and *Candida* sp. Previous works also reported the presence of these bacteria and fungi in Kitchen effluent, animal dung and poultry droppings (Umanu and Nwachukwu, 2010; Facundo *et al.*, 2001; Obire *et al.*, 2008).

The initial decrease in both bacterial and fungal count (Tables 2 and 3, respectively) observed at week two in this study revealed the toxic effect of diesel oil and probably prove that some of the microorganisms present in the soil and the abattoir effluent cannot survive in the oil polluted environment. This is in accordance with the reports by Umanu and Nwachukwu (2010) and Atlas (1981). However, Table 4, revealed a continuous increase in hydrocarbon utilizing microorganisms especially in the treatments, hence the higher reduction in residual diesel oil observed in the treatments especially those fortified with non-sterile abattoir effluent as compared to the control (Figures 1 and 2) and differed significantly at 5 % probability level. Also, the population densities of hydrocarbon utilizers present in treatments supplemented with nonsterile abattoir effluent (TA and TC) were higher and significantly different when compared with the treatments fortified with sterile abattoir effluent (TB and TD) and the control not supplemented with abattoir effluent (p≤0.05). This finding is in accordance with the reports by Facundo et al. (2001). Obire et al. (2008) and Umanu and Nwachukwu (2010).

Although it may be possible to expect negative effect on microbial degradation of diesel oil when very high volume of abattoir effluent is applied, results obtained for the moderate quantities applied in this study revealed that microbial degradation of diesel oil increases as the concentration of abattoir effluent applied increases. These differences in diesel oil degradations were however, not significant when subjected to variance analysis. A similar trend was reported in the use of kitchen effluent for bioremediation of oil based drilling mud (Umanu and Nwachukwu, 2010).

In conclusion, it was obvious that the introduction of diesel oil into the soil ecosystem changed the environment, resulting in the death of many biotic factors, hence the initial drop in both bacterial and fungal counts. Abattoir effluent contains a wide array of microorganisms with potential hydrocarbon degrading capacity. In addition, it could also serves as source of nutrients such as nitrate and phosphate which are essential for microbial growth and metabolism, hence the higher residual diesel oil degradation observed in the treatments compared with the control. Therefore, addition of abattoir effluent in moderate quantity to diesel oil contaminated soil could enhance the bioremediation of the diesel oil

polluted soil and also reduce environmental pollution caused by abattoir effluent.

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