

## Bioremediation of Used Motor Oil Contaminated Soil Using Elephant and Horse Dung as Stimulants

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**Abstract:** In view of the pollution of the underground water, economic loss, decreased in agricultural productivity of soil, poor animal and human health caused by contamination of soil with used and unused motor oil, a study was conducted for bioremediation of used motor oil contaminated soil by using elephant and horse dung as stimulants in five microcosms labeled  $M_1$  to  $M_5$ , at room temperature. In this study, the bioremediation potentials of these animal wastes were evaluated for six (6) weeks by taking samples from each of the microcosms for weekly analysis of Oil and Grease Content (O&G) and Total Heterotrophic Bacteria Count (THBC). After six (6) weeks, the results showed that  $M_4$ ,  $M_3$ ,  $M_5$ ,  $M_2$  and  $M_1$  exhibited 84%, 79%, 75%, 45% and 38% used oil degradation, respectively. Hence, all the biostimulants showed the ability to stimulate the growth of microorganisms for used motor oil degradation. The biodegradation data were fitted to first and second order kinetic equations and the biodegradation constant ( $k$ ) and half-life ( $t_{1/2}$ ) values obtained from all the kinetics showed that the order in which the biostimulants biodegrade the used motor oil contaminated soil was  $M_4 > M_3 > M_5 > M_2 > M_1$ . From this work, it can be concluded that the application of elephant dung ( $M_4$ ), horse dung ( $M_3$ ) and the combination of the two ( $M_5$ ) stimulate the growth of microorganisms that enhanced the degradation of the pollutant in the polluted soil with elephant dung ( $M_4$ ) being the most effective. Thus, can be applied to develop an environmentally safe and robust treatment strategy for used motor oil contaminated soil.

**Keywords:** Biodegradation, bioremediation, elephant dung, horse dung, kinetics, microcosm, used motor oil.

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### I. Introduction

The increase in the consumption of petroleum fractions has led to the rapid increase in the pollution of soil by used motor oil (UMO). The environment (soil and water) is highly contaminated with hydrocarbons by the disposal of used oils (engine oil, diesel or jet fuels). The disposal of used and unused lubricating oil into gutters, water drains, and land are common practice in Nigeria, especially by motor mechanics. This discharge contributes to soil pollution in Nigeria and makes up a larger percentage of polluted ground in the world [1],[2],[3]. Pollution of soils with petroleum hydrocarbons is a widespread environmental problem and a growing concern in many countries, especially in Asia and African continents especially in industrialised and oil producing countries [4],[3]. In the developing countries with poor regulatory policies on the environment, the problem is very severe [4],[3],[5],[6].

In today's world, oil spills at auto-mechanic workshops have been left uncared for over the years in many countries, and continuous accumulation of the oil is of high environmental concern as a result of hazard associated with it[2]. The attention of researchers have shifted towards the remediation of the environment (soil and water) polluted with hydrocarbons especially the polycyclic aromatic hydrocarbons (PAHs) due to the fact that most of the PAHs causes cancer, gene mutation and are very toxic [7].

The Release of persistent, bioaccumulative and toxic chemicals (benzene, toluene, ethylbenzene, xylene and polycyclic aromatic hydrocarbon) cause health and environmental hazards. These pollutants find their way into plant tissues, animals and human beings by the movement of hazardous constituents in the environment Ebenezer, 2013. Soil polluted with spent and fresh motor oil also create a serious effect on plant tissues, soil components, and its microorganisms, human and other animal health [3],[1][8]. Excess spillage of the oil causes fire hazards which lead to loss of lives and properties.

Since deficiency of nutrients (nitrogen and phosphorous) in the polluted environment limits the biodegradation of hydrocarbons by the indigenous microorganisms[9]. Hence, the need for the addition of organic biostimulants to the polluted soil in order to stimulate the microbial proliferation so as to enhanced the biodegradation process [10],[11],[12].

The aim of this research is to evaluate and compare the bioremediation potentials of animal wastes (elephant dung, horse dung and the combination of the two) as stimulants. The data obtained were subjected to first and second order kinetic modelling so as to determine the biodegradation rate constant and half-life of the degradation process.

## II. Materials And Methods

### 2.1 Sample Collection

Surface soil contaminated naturally with used motor oil (0 – 10 cm) was collected from old Dan Gombe Auto-Mechanics Workshop situated along Jos Road in Bauchi, Bauchi State – Nigeria in a black polythene bag and transported to Abubakar Tafawa Balewa University Bauchi Chemical Engineering Reaction Laboratory. The soil awaiting microbial analysis was stored at 4°C in a refrigerator. The elephant dung was collected from Yankari Games Reserve Bauchi State, Nigeria and the horse manure was obtained from horse stable in Kobi Street Bauchi State, Nigeria

### 2.2 Preliminary Analysis of the Soil Sample

The contaminated soil sample was subjected to the following physicochemical and microbial analysis. The pH was determined according to [2], moisture content was determined according to [13], the organic carbon was determined according to [14]. The particle density was determined according to [15], bulk density was determined according to [16] while the porosity was calculated from bulk and particle density [2], available phosphorus in soil samples was determined using the spectrophotometer, and total nitrogen content by the Kjeldahl method [2]. Pure bacterial isolates will be characterized and identified using the standard procedure based on Bergey's manual [17], the total heterotrophic bacterial count was determined by inoculating 0.1 ml of serially diluted sample on a nutrient agar plate using the spread plate method [18], the oil grease content was determined using Soxhlet extraction method [6]. The physicochemical and microbiological analyses of the soil and different animal manures were done in duplicate.

### 2.3 Experimental Design and Treatment

One thousand five hundred grams (1500 g) of sieved (2mm) soil was mixed with 10% w/w [19],[20],[21],[22] of different animal dungs (elephant dung, horse dung and the combination of the two) in a plastic containers (microcosms). Control vessels consisting of heated and non-heated contaminated soil without amendment were set up. The moisture content was adjusted by adding water one day before sampling and was maintained at 20% water holding capacity [23] by the addition of distilled water. The incubation was done at room temperature (28 ± 2°C) and the content of each vessel was pulverised twice a week for aeration. Periodic sampling from each of the microcosms was done weekly for six weeks to determine the residual oil and grease content and microbial count. The design of the experimental setup is as shown in Table 1

**Table 1:** Summary of the experimental design

Microcosms	Treatment
M <sub>1</sub>	1500 g Contaminated soil + Heat Sterilization + Water
M <sub>2</sub>	1500 g Contaminated + Water
M <sub>3</sub>	1500 g Contaminated + 10% Horse dung + Water
M <sub>4</sub>	1500 g Contaminated + 10% Elephant dung + water
M <sub>5</sub>	1 500 g Contaminated + 10% Horse and elephant dungs + water

### 2.4 Determination of Oil and Grease Content

The oil and grease content of the soil was extracted using Soxhlet extraction method. Sodium sulphate was purified by drying overnight in an oven at 105°C. Round Soxhlet flask was dried at 105°C for 30 min. After cooling, the weight of the round flask and boiling chip was recorded (w<sub>1</sub>). 3.0 grammes of contaminated soil was mixed with 3.0 grammes anhydrous Na<sub>2</sub>SO<sub>4</sub> and placed in a cellulose extraction thimble. 60 ml of n-hexane was added to the flask, and the oil was extracted for 1 hours. The residual oil was determined by evaporating the n-hexane in a hot water bath; the round bottom flask was allowed to cool and weighed again (w<sub>2</sub>). Residual oil/grease content in the soil was calculated using (1) and (2).

$$\text{Gain in weight of flask (mg)} = w_1 - w_2 \quad (1)$$

$$\text{Oil and grease fraction} \left( \frac{\text{mg}}{\text{kg}} \right) = \frac{w_1 - w_2}{W} \times 100 \quad (2)$$

W<sub>1</sub> = weight of flask, boiling chips and residue after evaporation of hexane

W<sub>2</sub> = weight of round flask and boiling chips

W = the weight of the contaminated soil in gramme

The percentage degradation (D) of the oil was determined using (3)

$$D = \frac{C_{O\&Gi} - C_{O\&Gr}}{C_{O\&Gi}} \times 100 \quad (3)$$

Where C<sub>O&Gr</sub> and C<sub>O&Gi</sub> are the residual and initial oil and grease concentrations, respectively.

### 2.5 Determination of Total Heterotrophic Bacterial Count

The enumeration of the total heterotrophic bacterial count present in the microcosms was determined by spread plate techniques. The samples were subjected to serial dilution which was plated on nutrient agar

(NA) oxid and incubated at (28±2°C) for 24 h and plate that yielded count between 30 – 300 colonies were counted[19].

### III. Results And Discussions

#### 3.1 Physicochemical Properties of Soil and Organic Wastes

The physicochemical properties of the used motor oil contaminated soil and the animal dung employed for the studies are presented in Table 2. The high level of percentage total organic carbon (24.74 %) in the polluted soil was as a result of the used motor oil in the soil whose oil and grease content (112 734 mg/kg) was above the safe limit of 500 mg/kg set by the Nigeria Ministry of Environment[24], hence the need for the remediation of the polluted soil. The soil pH (6.9) was within the acceptable limit of 5.5 – 8.5 for effective bioremediation according to Vidali (2001). The soil moisture content (2.57 %) fell out of the limit of 12 – 25 % required for optimum growth and proliferation of microbes [8] hence the need for the moisture content adjustment.

The nitrogen content (0.42%) of the polluted soil was low, hence the need for amendment with organic wastes (elephant and horse dung). The nitrogen content of the elephant and horse dung was found to be 1.96% and 0.98% respectively which is one of the limiting nutrient required for effective bioremediation.

**Table2:** Physicochemical properties of the sample

Parameter	Samples		
	Soil	Elephant	Horse
pH	6.9	6.9	6.8
Nitrogen (%)	0.42	1.96	0.98
Organic C (%)	24.74	4.79	10.51
Phosphorus (%)	0.67	0.21	0.46
Moisture (%)	2.57	ND	ND
Oil & Grease (ppm)	112734	ND	ND

#### 3.2 The heterotrophic bacterial count in the contaminated soil

The total heterotrophic bacteria count (THBC) is presented in Table 3. From the table, THBC in the used motor oil-contaminated soil was found to be 9.10E+08. The density of the indigenous bacteria in the contaminated soil was enough for effective bioremediation since it exceeded the minimal value of 1.00E+5 required.

**Table 3:** Heterotrophic Bacteria count in the contaminated soil

Sample	Total Heterotrophic Bacteria Count (CFU g <sup>-1</sup> soil)
Contaminated Soil	9.10 x 10 <sup>8</sup>

#### 3.3 Oil and grease content (O&G) for the Microcosms

According to [2], the oil and grease content is a better bioremediation index for studying the extent of degradation of pollutant in used motor oil contamination since the concentration of total petroleum hydrocarbon is low due to the decrease in C-H bond in used motor oil. The residual oil and grease content with bioremediation time are shown in Fig 1. As shown in the Figure the percentage degradation of O&G was noticed to increase with the bioremediation time, which is an normal trend for a typical oil degradation process.

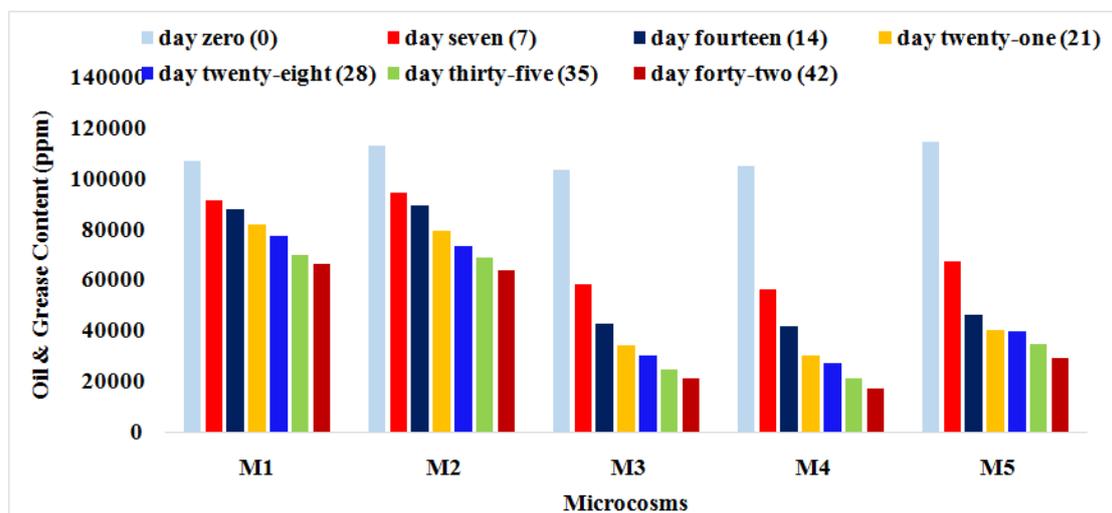
From the results (Fig.1), it was observed that the reduction of oil and grease content of the contaminated soil was relatively fast for the first 14 days of the process in all the soil microcosms amended, with horse dung (M<sub>3</sub>), elephant dung (M<sub>4</sub>) and combination of elephant + horse dung (M<sub>5</sub>) when compared to that of the two unamended microcosms (M<sub>1</sub> and M<sub>2</sub>). After 14 days, there was reduction of O&G in microcosms M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> from 106 809, 112 734, 103 149, 104 642 and 114 568 mg kg<sup>-1</sup> to 87 941, 89 450, 42 427, 41 756 and 46 101 mg kg<sup>-1</sup> respectively which corresponded to 23.3, 29.5, 66.9, 71.4 and 64.8 % reduction in O&G contents.

After 42 days of remediation process, the concentration of the used motor oil reduced to 66 444, 63 655, 21 265, 17 025 and 28 952 mg kg<sup>-1</sup> and correspondingly 37.8, 43.5, 79.4, 83.7 and 74.7 % reduction in O&G contents for microcosms M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> respectively. It was observed that the degradation of used oil in M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> resulted in effective bioremediation response with M<sub>4</sub> having the highest bioremediation response (83.7 % loss in O&G contents).

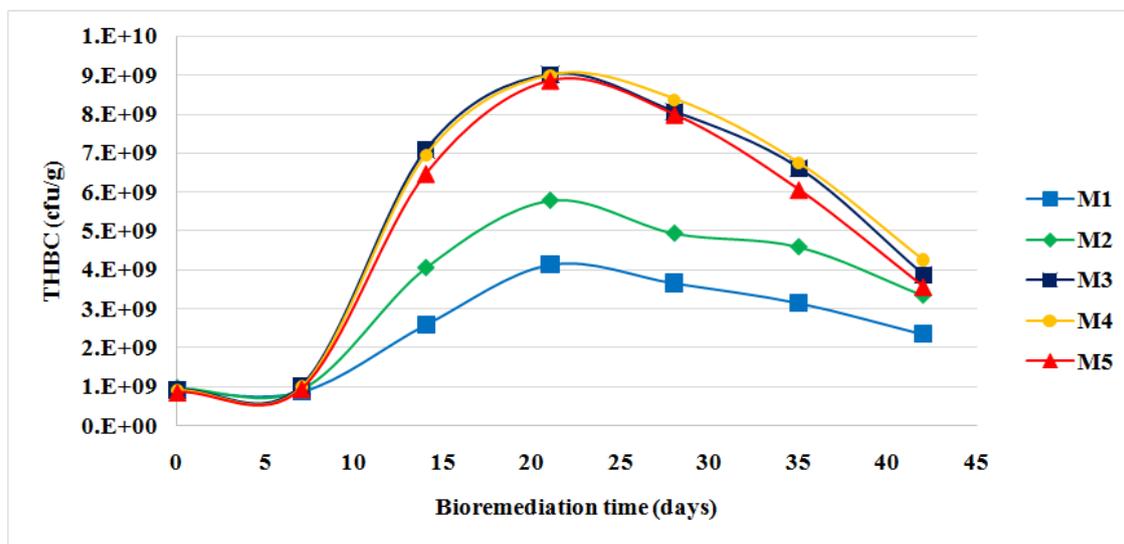
#### 3.4 Microbial analysis

The results of total heterotrophic bacteria count (THBC) throughout the remediation process are presented in Fig. 2. It was observed that microbial growth profile followed a typical microorganisms' growth

pattern with lag phase, exponential phase, stationary phase and death phase. All the microcosms showed a similar trend of lag phase which is the period of adaptation. This period lasted for seven (7) days. Between days 7 and 21, the microcosms followed a similar pattern of exponential phase which is the period of maximum oil degradation. After 42 days of incubation, the THBC in microcosms M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, and M<sub>5</sub> were found to be 2.36E+09, 3.35E+09, 3.88E+09, 4.26E+09 and 3.57E+09 respectively. The trend observed in the microbial growth corresponds with the percentage degradation of the oil and grease. Hence, it is clear that the bacteria utilised the used motor oil as their carbon and energy source.



**Fig. 1:** Variation of oil and grease content (ppm) with bioremediation time (week)



**Fig. 2:** Variation of total heterotrophic bacteria count (THBC) with bioremediation time.

### 3.5 Bioremediation kinetic studies

To investigate the biodegradation process of the hydrocarbon present in the microcosms, first, second and shift order kinetic models were used to estimate the biodegradation constant and half-life so as to compare the effectiveness of the stimulants in enhancing the degradation of hydrocarbon in the microcosms. (4) and (5) gives the first and second order kinetic model expressions while (6) and (7) gives the expressions for calculating the half-life ( $t_{1/2}$ ) in days, of the biodegradation process

$$\ln C_{O\&Gr} = -k_1 t + \ln C_{O\&G0} \quad (4)$$

$$\frac{1}{C_{O\&Gr}} = k_2 t + \frac{1}{C_{O\&G0}} \quad (5)$$

$$t_{1/2} = \frac{\ln 2}{k_1} = \frac{0.693}{k_1} \quad (6)$$

$$t_{1/2} = \frac{1}{k_2 C_{TPH0}} \quad (7)$$

Where  $C_{O\&Gr}$  is the residual concentration of the oil and grease (mg/kg),  $C_{O\&G0}$  is the initial concentration of the oil and grease (mg/kg) at day zero,  $t$  is the bioremediation time (day),  $k_1$  ( $\text{day}^{-1}$ ) and  $k_2$  ( $\text{kg/mg}\cdot\text{day}$ ) are the first and second order biodegradation constants, respectively.

The parameters obtained from the two kinetic models are shown in Table 4. The best fit was first expressed by the linear regression coefficient of determination  $R^2$ , the  $R^2$  values gotten from the plots of all the studied microcosms ranges from 0.8718 to 0.9907 for the first order and 0.9485 to 0.9958 for second order kinetic models. The model with relatively high  $R^2$  value best described the degradation of hydrocarbon in the microcosms.

Results from Table 4 shows that the biodegradation of used oil in soil amended with elephant dung ( $M_4$ ) had a higher rate constant and lower half-life for first order ( $k_1 = 0.0399 \text{ d}^{-1}$  and  $t_{1/2} = 17.37$  days), second order ( $k_2 = 1\text{E-}06 \text{ kg mg}^{-1}\text{d}^{-1}$ ) than that in soil amended with horse dung ( $M_3$ ), soil amended with horse and elephant dung ( $M_5$ ), unamended control soil ( $M_2$ ) and heat sterilized unamended soil ( $M_1$ ).

**Table 4:** Summary of the samples rate constants, half-lives and correlation coefficients for first and second order

sample	First Order			Second Order		
	$k_1$ ( $\text{d}^{-1}$ )	$t_{1/2}$ (d)	$R^2$	$k_2$ ( $\text{kg mg}^{-1}\text{d}^{-1}$ )	$t_{1/2}$ (d)	$R^2$
M1	0.0106	65	0.9745	1E-07	94	0.9827
M2	0.0130	53	0.9827	2E-07	44	0.9920
M3	0.0346	20	0.9287	9E-07	11	0.9958
M4	0.0399	17	0.9463	1E-06	10	0.9863
M6	0.0285	24	0.8609	6E-07	15	0.9485

#### IV. Conclusions

In this work, the biostimulation potentials of elephant dung, horse dung and the combination of the two were investigated in microcosms to remediate used motor oil contaminated soil. The residual used motor oil reduction as well as the microbial data after 42 days of incubation, revealed the occurrence of biodegradation of used motor oil and increase in density of the indigenous heterotrophic bacteria population in all the microcosms. The result showed that, the application of horse dung ( $M_4$ ), elephant dung ( $M_3$ ) and combination of the two dung ( $M_5$ ) to stimulate the indigenous microbes in the contaminated soil gave percentage degradation of 84 %, 79 % and 75 % respectively with elephant dung being more effective than the horse dung the combination of the two dung.

The rate constants and half-life of the studied kinetics (first and second order) calculated showed that the elephant dung was more effective than the horse dung and their combination as biostimulant since it had the least half-life value for both first order (17 days) and second order (10 days), and highest rate constant value for both first order ( $0.0399 \text{ day}^{-1}$ ) and second order ( $1\text{E-}06 \text{ kg mg}^{-1}\text{d}^{-1}$ ).

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