IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 3 Ver. II (March. 2018), PP 01-10
www.iosrjournals.org

Biostimulation of Soil Contaminated With Spent Motor Oil Using Cow Dung and Poultry Litter in Land Farming Microcosm

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Abstract: *The unintended discharge of hydrocarbons into the environment can negatively have impact on characteristics of soils, human and animal health. Biostimulation of hydrocarbon contaminated soil using organic stimulants are usually based on trial and error method rather than the concentration of the contaminants (digital approach) and most studies in this area were not geared towards development of process parameters that could lead to the development of realistic large scale treatment technology. In view of the aforementioned, a study was conducted on the biostimulation of soil contaminated with spent motor oil using blends of cow dung and poultry litter employing Box-Behnken design of experiment. The oil and grease content (O&G) was used to assess the extent of bioremediation. After eight (8) weeks of bioremediation, the efficiency of degradation in all microcosms (S1 to S14) varied from 67.74 – 89.58% except the water amended control (S14) with 35.29%. S1 showed the highest response to bioremediation of 89.58% and bio-stimulant efficiency of 60.40%. The microbial counts increased with the duration of study (3.69×10^6 – 1.19×10^9) cfu/g. The digital approach employed showed remarkable potential for the bioremediation of spent motor oil contaminated soil at a contaminant load of 1.0 – 13.0% (w/w) and 15:1 – 10:1 carbon –nitrogen molar ratio (C: N). The microcosm (S2) recorded the highest germination index (GI) (77.76%) corresponding to 86.67% biodegradation, the water amended controlled microcosm (S14) recorded GI of 0% corresponding to 35.29 % biodegradation while the uncontaminated controlled soil recorded 100% germination. To this end, the digital approach employed in the study is a more effective way of application of animal dung as stimulants for bioremediation of hydrocarbon contaminated soil.*

Keywords*: Biodegradation, bioremediation, Land farming, microcosm, spent motor oil, cow dung and poultry litter*

Date of Submission: 05-02-2018 Date of acceptance: 23-03-2018

I. Introduction

Petroleum derived products are the major source of energy for our vehicles, industry and daily life. Due to its importance as energy source, it is prone to accidental spill regularly during the exploration, production, refining, transport and storage. The contamination of soil by crude oil and petroleum derived product has become a serious issue of global concern because of the potential consequences on ecosystem and human health [1]. Global production of crude oil is estimated at more than twelve million metric tons annually, it has been reported that 1.7 – 1.8 million metric tons [2] of petroleum contamination are treated via chemical and physical technologies which are expensive compared to the bioremediation [2]. Effective and economically attractive remediation techniques continue to elude scientist and engineers; the use of microbes to degrade or detoxify hazardous wastes is now recognized as not only a viable alternative but a desirable alternative and addition to the traditional remediation technologies [3].

Bioremediation has been described as "a treatability technology that uses biological activity to reduce the concentration or toxicity of a pollutant, it uses microorganisms to transforms or degrades chemicals in the environment [1]. Bioremediation technology which is based on the application of living organisms to decontaminate environmental contaminants has proven to be a promising and effectives means of getting rid of petroleum derived products from the environment microorganisms contributed a lot to the successes of cleaning the nature from contamination [4]. This technology can be applied either by stimulating microorganisms present at site called biostimulation which is believed to be more economically viable or by the addition of genetically grown microorganisms known as bioaugmentation [4]. During the process of bioremediation, environmental parameters such as temperature, pH, oxygen and moisture content, are optimized to achieve accelerated biodegradation [5]. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site [6]. Although these methodologies not technically complex, considerable experience and expertise may be required to design and

implement a successful bioremediation program due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.

For bioremediation to be effective the right type and amount of microorganisms must be present at site or genetically grown microorganisms must be added to the site. There are array of existing microorganisms that can detoxify a vast array of contaminants; some compounds are more easily degraded than others. In general, the compounds most easily degraded in the subsurface are petroleum hydrocarbons, but technologies for stimulating the growth of organisms to degrade a wide range of other contaminants are emerging and have been successfully field tested [7]. When deciding whether a site is suitable for bioremediation, it is important to realize that no single set of site characteristics will favor bioremediation of all contaminants.

However, many research works have been carried out on spent motor oil contaminated soil using biostimulation approach employing either organic or inorganic stimulants but the former has received more patronage in recent time because of its relatively low cost and environmental safety [8]. Spent motor oil is the brown-to black oily liquid removed from a motor vehicle when the oil is changed and is similar to unused oil except that it contains additional chemicals that are produced or build up in the oil when it is used as an engine lubricant at high temperatures and pressures, inside an engine as it runs due to engine wears and intrusion of dirt [9] and in addition, there is loss in viscosity of the oil. In addition, the characteristics of the hydrocarbon content of the petroleum mixture influences the degradability of individual hydrocarbon components; the simpler the hydrocarbon structure the easier its biodegradability and the complex the hydrocarbon structure the harder its biodegradability [10].

Furthermore, the order of biodegradability of hydrocarbon is alkanes > alkenes > alkynes > aromatics. Many works has been performed and reported on biostimulation of hydrocarbon contaminated soils using organic wastes as stimulants but applications of these stimulants are usually based on proportion of contaminated soil rather than the concentration of contaminants in the soil digital method. In addition, biostimulation of spent motor oil contaminated soil was carried out using the blends of cow dung and poultry litter in land farming microcosm through digital method, isolation and enumeration of hydrocarbon degrading bacteria and conducting bioremediation experiment via Box-Behnken design of experiment were among the objectives of this study. The study clearly show that in bioremediation of hydrocarbon contaminated soil, the exact approach employed via Box-Behnken design of experiment is a better option for optimization of process variables.

II. Materials and Method

2.1 Sample Collection

Soil (0 – 18 cm) contaminated with spent motor oil was collected from Bappah master auto-mechanic workshop located along Railway New market Bauchi, Bauchi State in a polythene bags and kept at the Biochemical Engineering Laboratory, Department of Chemical Engineering, Abubakar Tafawa Balewa University, Bauchi. The soil prior to microbiological analysis was kept at 5°C in a refrigerator. The organic stimulants; cow dung and poultry litter were collected from cattle settlement along Gida dubu Bauchi and Biodun poultry farm Bauchi, Bauchi State Nigeria respectively.

2.2 Isolation of Heterotrophic Bacteria

Pure cultures of the isolates were obtained by repeated sub-culturing on selective media used for primary isolation, Centrimide agar was used for *pseudomonas spp*, MacConkey agar (with salt) was used for *Klebsiella and proteus spp*, MacConkey agar (without salt) was used for *Bacillus spp* and MacConkey agar (without salt) was used for *Micrococcus spp*. The pure isolates were maintained on agar slants for further characterization and identification [11]. The following biochemical characteristic were performed to confirm the bacterial isolates namely Gram Staining [12], Citrate utilization test[13], Coagulase test [12], Catalase test [12], Indole test [12], Motility test [12], Spore staining and Triple sugar iron (TSI) test [12].

2.3 Preliminary Analysis of the Contaminated Soil Sample

The contaminated soil was subjected to physicochemical and microbiological analyses. The texture was determined according to Day [14], pH was determined according to Bates [15], organic carbon was determined according to Walkley [16], bulk density, particle density and porosity was determined according to Brandy [17], oil and Grease (O&G) was determined according to Chang [18], total organic content was determined according to Camobreco [19] and total heterotrophic bacteria counts was determined according to John [20]. All analyses were carried out in triplicate.

2.4 Experimental Design and Soil Treatment

The values and levels of the independent variables were presented in Table 1 and coded Box Behnken design for the three independent variables and their responses are presented in Table 2.

Table 1: Experimental Factors and Levels of Variables

Factor	Level		
	Low (-1)	Average (0)	High (+1)
Cow dung (C: N)	15:1	12.5:1	10:1
Poultry litter (C: N)	15:1	12.5:1	10:1
Moisture content (%)	20	25	30

The amounts of the independent variables in Table 2 were calculated based on C: N molar ratio as described in Table 1 using 5 kg of contaminated soil sample as basis for calculation.

Table 2: Coded Box Behnken Design for the Three Independent Variables

Run	Cow Dung (kg)	Poultry Litter (kg)	Moisture (kg
1	-1 (1.95)	-1 (0.98)	0 (1.98)
2	+1 (2.93)	-1 (0.98)	0 (2.23)
3	-1 (1.95)	+1 (1.47)	0 (2.11)
4	+1 (2.93)	+1 (1.47)	0 (2.35)
5	-1 (1.95)	0 (1.18)	-1 (1.63)
6	+1 (2.93)	0 (1.18)	-1 (1.82)
7	-1 (1.95)	0 (1.18)	+1 (2.44)
8	+1 (2.93)	0 (1.18)	+1 (2.73)
9	0 (2.36)	-1 (0.98)	-1 (1.66)
10	0 (2.36)	+1 (1.47)	-1 (1.77)
11	0 (2.36)	-1 (0.98)	+1 (2.50)
12	0 (2.36)	+1 (1.47)	+1 (2.65)
13	0 (2.36)	0 (1.18)	0 (2.14)
14	-	-	-

2.5 Bioremediation Experiment

Cow dung and poultry litter (stimulants) were sun dried, grinded and homogenised. These stimulants were package in polythene bags prior to their application for bioremediation experiment. Contaminated soil was excavated base on its long time exposure to the environment. Debris were removed from the contaminated soil and then homogenised and sieved using 2 mm diameter mesh size. Physicochemical and microbiological analyses were performed on the soil and the two organic stimulants (cow dung and poultry litter). Five kilograms (5kg) of soil contaminated with spent motor oil and different quantities of stimulants were mixed and introduced to the treatment microcosms base on the digital approach employed in the design of the experiment. Initial quantity of water in kilograms was apply to all the treatment microcosms base on the calculation of the coded Box-Behnken design of experiment, in the subsequent weeks percentage moistures were determined and used to adjust the water lost and what need to be added. The samples in the microcosms were tilled and aerated daily, periodic sampling was made after every 7-days to determine the oil and grease (O&G), total heterotrophic bacteria counts (THBC) and pH. The bioremediation experiment lasted for 8 weeks under laboratory condition, Figure 1 shows some of the microcosms stacked with various treatments as presented in Table 2.

Figure 1: Some of the microcosms stacked with various treatments as presented in Table 2

2.6 Determination of Total Heterotrophic Bacterial Count

One gram (1 g) of soil contaminated with spent motor oil (SCSMO) was weighed on an electronic balance under aseptic conditions into a sterile paper and was transferred to a test tube containing 9 ml of sterile distilled water; the mixture was thoroughly agitated by vigorous hand shaking for uniform mixing and from this suspension, 0.1 ml was transferred into a test tube containing 9.9 ml of distilled water by means of a pipette, this mixture was thoroughly agitated by hand shaking (this is the 10 times dilution). Further dilutions was made by transferring 1 ml of this ten-fold dilution to 9 ml of sterile distilled water aseptically and by serial dilution stopping at 10^{-7}. The content of the tube was serially diluted from 10^{-0} to 10^{-6} for the first two weeks including the initial reading and the tube was then serially diluted from 10^{-0} to 10^{-7} for the remaining weeks. Aliquot (0.1 ml) from dilution (10^{-4}, 10^{-5}, and 10^{-6}) were used for the first two weeks and then changes to (10^{-5}, 10^{-6}, and 10^{-7}) for the subsequent weeks were plated in triplicates on to sterile nutrient agar (NA) for the enumeration of total aerobic heterotrophic bacteria. Nutrient agar medium was melted to 45℃ and poured in to Petri plates under aseptic conditions. The soil diluted pipette in to the petri plates and mixed with the medium by rotating clockwise and anticlockwise and subsequently, shaking crosswise horizontally several times for uniform mixing. The plates were allowed to cool in order to allow the agar to solidify. The Petri plates were then transferred to an incubator maintained at 35℃ and incubated under inverted position for 24 h. The petri plates were then examined for the growth of bacteria in colonies and plates was selected from the three dilutions in which the counts range between 30-300 colonies [20]. The total viable count was calculated using Eq. (1).

$$Total\ viable\ count(\frac{cfu}{g}) = \frac{number\ \ of\ colonies \times reciprocal\ \ of\ the\ dilution\ \ factor}{weig\,ht\ of\ the\ soil} \quad \ldots \quad (1)$$

2.7 Determination of Oil and Grease Content

The oil and grease content was determined using the gravimetric method as described below; 5g each of soil contaminated with spent motor oil was weighed on an electronic weighing balance and transferred in to a test tube ;5 ml of n-hexane was be added. The sample mixture was shaken vigorously for 5 minutes .After settling, the solvent and extract was decanted in to a pre-weighed 50 ml beaker .This procedure was repeated three times to bring the total solvent volume to 20 ml, the extract and the solvent obtained was evaporated on a heating mantle. The residue, which is the extract, was allowed to cool and weighed on a sensitive balance to four decimal places [18]. Results obtained were presented in mg/kg or ppm as follows:

$$O\&G(ppm\ or\ mgkg^{-1}) = (\frac{Weig\,ht\ of\ \ oil\ in\ soil\ \ sample\ \ (g)}{Weig\,ht\ of\ \ soil\ \ sample\ \ \ taken\ \ (g)} \times 10^{6}) \quad \ldots \quad (2)$$

Percentage (%) degradation (D) was calculated using Eq. (3) [1]

$$D = \frac{O\&G_i - O\&G_r}{O\&G_i} \times 100 \quad \ldots \quad (3)$$

where $O\&G_i$ and $O\&G_r$ are the initial and residual oil and grease (O&G) concentrations respectively.

The percentage (%) biostimulant efficiency (B.E) was calculated at the end of day-56 remediation period using Eq. (4) [1].

$$\%\ B.E = \frac{\%O\&G_S - \%O\&G_U}{\%O\&G_S} \times 100 \quad \ldots \quad (4)$$

where $\%\ O\&G_S$ is the removal of spent motor oil in the amended soil, and $O\&G_U$ is the removal of spent motor oil in the unamended soil.

2.8 Seed germination toxicity test

Fourty (40) viable seeds guinea corn (sorghum bicolor) were placed evenly on each fifteen (15) microcosms including the control and covered with dry sand for fourteen (14) days, all the microcosms were watered daily so as to control the soil moisture content. The numbers of seedlings that emerged from the surface of the sand was counted and recorded [21]. Germination index of the guinea corn on the bioremediated soil was calculated using the formula [22] presented in Eq. (5).

$$\text{Germination index (\%)} = \frac{(\%\,SG)\times(\%GR)}{100} \quad \ldots \quad (5)$$

$$\%SG = \frac{(\%EG)}{(\%CG)} \times 100$$

$$\%GR = \frac{(GERm)}{(GERCm)} \times 100$$

where % SG = percentage seed germination, %GR = percentage growth of the root,

% EG = percentage germination on contaminated soil, %CG = percentage germination on control soil, GERm = elongation of root on contaminated soil, GERCm = elongation of root on control soil.

III. Results and Discussions

3.1 Isolation of Heterotrophic Bacteria

During the study period the bacteria isolates were stimulated using organic stimulants (cow dung and poultry litter), it is clear that the native bacteria namely *Pseudomonas* spp, *Klebsiella* spp, *Bacillus* spp, *Micrococcus* spp, *Proteus* spp isolated utilised the spent motor oil as their source of carbon and energy which agrees with similar findings [23, 24]. Detail of the microbial types and their colony morphology is shown in Table 3. In addition, it was observed that the non availability of nutrients toward the end of the study period make the microbial population enter dead phase.

Table 3.0: Bacterial Isolates and their Colony Morphology.

Colony Morphology	Bacterial Isolates
Mocoid, large colonies, umbonate elevation, diffusable green pigment and fruity odour.	*Pseudomonas* spp
Mucoid.grey round and shiny.	*Klebsiella* spp
Large colonies, lobated margin, dry and flat.	*Bacillus* spp
Circular, smaller colonies, convex with entire margin.	*Micrococcus* spp
Circular, entire, smooth, opaque colonies with swarming characteristics on MacConkey agar	*Proteus* spp

3.2 Physicochemical Properties of Soil and Organic Stimulants

The physical and chemical properties of the soil contaminated with spent motor oil including the organic stimulants used for the bioremediation were presented in Table 4. The high percentage of total organic carbon 8.19±0.31% in the contaminated soil was as a result of the spent motor oil in the soil which it oil and grease content was 18.83±0.03 %. This value of oil and grease content was above the safe limit of 500 mg/kg (0.05 %) set by the Nigerian Federal Ministry of Environment [25], therefore, the need for the bioremediation of the contaminated soil by spent motor oil. The soil pH of 6.83 ± 0.05 obtained was within the acceptable limit of 6.5 – 8.5 needed for effective bioremediation [6]. The soil moisture content was 1.70±1.02%), which fell out of the range of 12 – 25 % required for optimum growth and proliferation of microbes [5] hence the need for the moisture content adjustment.

The nitrogen content of the contaminated soil was not determined, since oil pollution concentration above 3 % has been reported to be increasingly deleterious to soil biota and crop growth [1], hence the reason for amendment with organic wastes (Poultry litter and cow dung), from Table 6 the THBC in the spent motor oil-contaminated soil was found to be 6.23E+06 ± 2.52E+05 cfu/g. The density of the indigenous bacteria in the contaminated soil was adequate for effective bioremediation since it exceeded the minimal value of 1.00E+5 required as reported in [26]. The nitrogen content of the poultry and cow dung was found to be 1.4 ± 0.00 % and 0.7 ± 0.00 % respectively which is one of the limiting nutrients in the contaminated soil required for efficient bioremediation of hydrocarbon contaminants. The average temperature within the period of study was 32±10℃ which is within the range required for effective bioremediation of as reported by Abdulsalam [26]. Details of the physical and chemical properties of the samples were presented in Table 4.

Table 4.0: Physicochemical properties of the samples

	Average/STDEV		
Parameter	Soil	Poultry litter	Cow dung
Texture	Sandy		
Total viable count (cfu/g)	6.23E+06±2.52E+05		
Organic Carbon (%)	8.19±0.31		
Moisture (%)	1.700±1.02		
Porosity (%)	47.98±20.14		
Water absorption capacity (%)	38.48±0.56		
Particle density (g/ml)	2.00±0.06		
Bulk density (g/ml)	1.04±0.37		
Total organic content (%)	26.00±1.36		
Oil and grease (%)	18.83±0.03		
pH	6.83±0.05		
Nitrogen (%)		1.4±0.00	0.7±0.00
Phosphorus (%)		0.24±0.00	0.04±0.01

3.3 Bioremediation Experiment

3.3.1 Microbial Types and Counts

The total heterotrophic bacteria count determined the extent of nutrient utilization by the microorganism, the trend observed in the microbial growth corresponds with the percentage degradation of the oil and grease contents in each microcosms hence, the findings of this study clearly show that the bacteria *Pseudomonas* spp, *Klebsiella* spp, *Bacillus* spp, *Micrococcus* spp and *Proteus spp* isolated were hydrocarbon degrading and utilised the spent motor oil as their source of carbon and energy [27, 28; 29], this agrees with

other similar works findings [23,24]. In addition, microbial population and their growth phases were monitored and weekly enumeration of the total heterotrophic bacteria count were performed, the maximum microbial counts were recorded in week 4 by sample 6 with THBC of 1.19E+09 cfu/g as showed in Figure 2. The THBC for the 14 treatments (S1 to S14) were 7.37E+08, 7.53E+08, 9.30E+08, 6.91E+08, 8.20E+08, 1.19E+09, 6.86E+08, 6.51E+08, 8.05E+08, 7.31E+08, 3.51E+08, 6.19E+08 and 4.36E+08 cfu/g. Among the 14 treatment options S11 gave a better growth pattern, depicting all the growth phases (lag, log, stationary and death) which is in line with literature [30].

However, there was fluctuation in the growth phases for the rest of the treatment options which could be attributed to non-uniformity in the distribution of nutrients within the microcosms. Hence, causes for fluctuations in microbial populations. This observation is in line with findings of Samuel [1], toward the end of the experiment the microbial counts started decreasing indicating exhaustion of nutrients by the microbes hence, entering dead phase.

3.3.2 Oil and Grease (O&G) Concentration (PPM)

Residual oil and grease content was used to determine the extent of the hydrocarbon degradation in the contaminated soil; the results obtained showed a decreasing trends as depicted in Figure 3. During the study sampling were made to determine residual oil and grease content and the results are shown in Figure 3 which shows changes in oil and grease concentration (content) with bioremediation time. After the completion of this study, level of hydrocarbon degradation in each microcosm was ascertained by finding the percentage (%) biodegradation and the extent of nutrient utilization by the microbes which was determined by calculating the biostimulant efficiency (%).

From the beginning of the bioremediation experiment, the oil and grease contents were in decreasing trend but after week 4 (the peak of hydrocarbon removals), then the O&G contents started fluctuating in increasing-decreasing trends as showed in Figure 3. This could be attributed to uneven distribution of nutrients within the microcosms. In week 4, the residual O&G contents in the 14 microcosms were 14,000.0, 14,666.7, 24,000.0, 20,000.0, 29, 333.3, 14,000.0, 193,333.3, 16,000.0, 24,000.0,18,000.0, 17,333.3, 13,333.3, 36,000.0 and 83,333.3 mg/kg respectively which correspond with 89.58, 86.67,86.23,85.42,67.74, 87.39, 75.00, 77.55, 71.43, 72.00, 88.97, 79.81 and 35.29% biodegradation. In addition, the biostimulant efficiencies for S1 to S14 were 60.40, 59.28, 59.07, 58.68, 47.81, 59.61, 58.61, 52.94, 54.49, 50.59, 50.98, 60.33, 55.78 and 0% respectively.

It was also observed that during bioremediation, the longer the remediation time, the higher the contaminant reductions. Based on the observed results, nutrient was a limiting factor to effective biodegradation of the contaminants, this agreed with similar findings [23]. In general, the microorganisms present in the test soil were able to consume carbon present in the contaminated soil as their energy and food source leading to biodegradation of the spent motor oil [1; 31]. It was also observed that the degradation of spent motor oil using blends of cow dung and poultry litter in various proportions resulted in effective bioremediation with S1 having the highest bioremediation response of 89.58 % and biostimulation efficiency of 60.40 %. In this study the highest degradation of 89.58% was achieved at the contaminant load of 1.0 – 13.0% (w/w) over a period of 56 days which is beyond the optimum value of 5-10% (w/w) for hydrocarbon degradation as reported by [6]. Also in another study by Abioye [8] not more than 92% degradation was achieved for the hydrocarbon contaminated soil with low contaminant load of 5% (w/w), this shows the effectiveness of the digital approach employed in this study over the conventional methods of bioremediation. The microbial counts increased in the first four (4) weeks (3.69×10^{6} – 1.19×10^{9}) cfu/g and later decreased from four weeks to eight (8) weeks (1.19×19^{7} – 1.72×10^{8}) cfu/g corresponding to the period of past and slow removal of O&G in all microcosms.

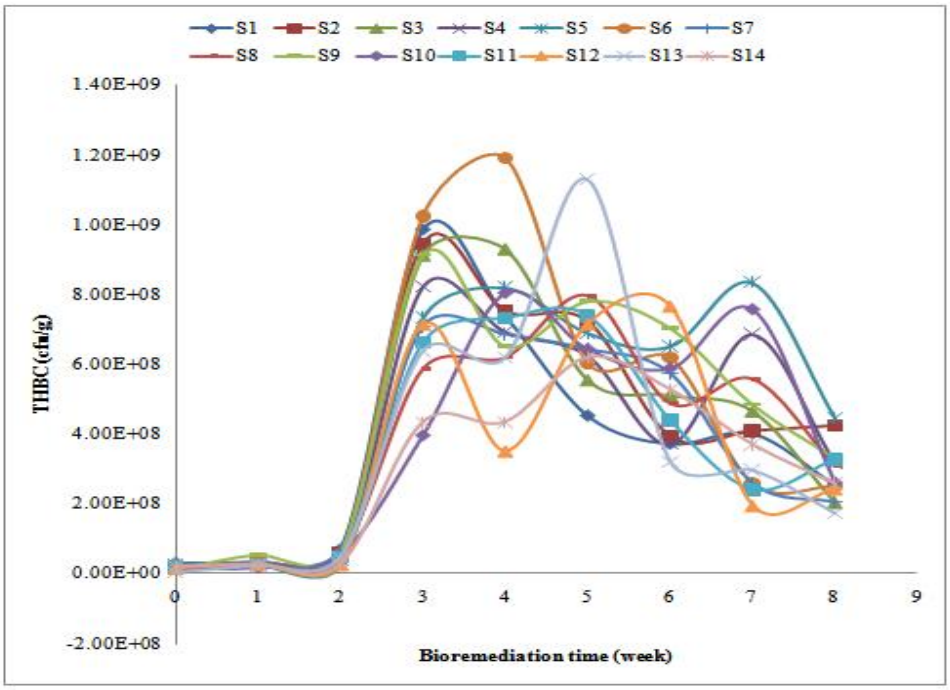

Fig. 2: Changes in total heterotrophic bacteria count (THBC) with bioremediation time.

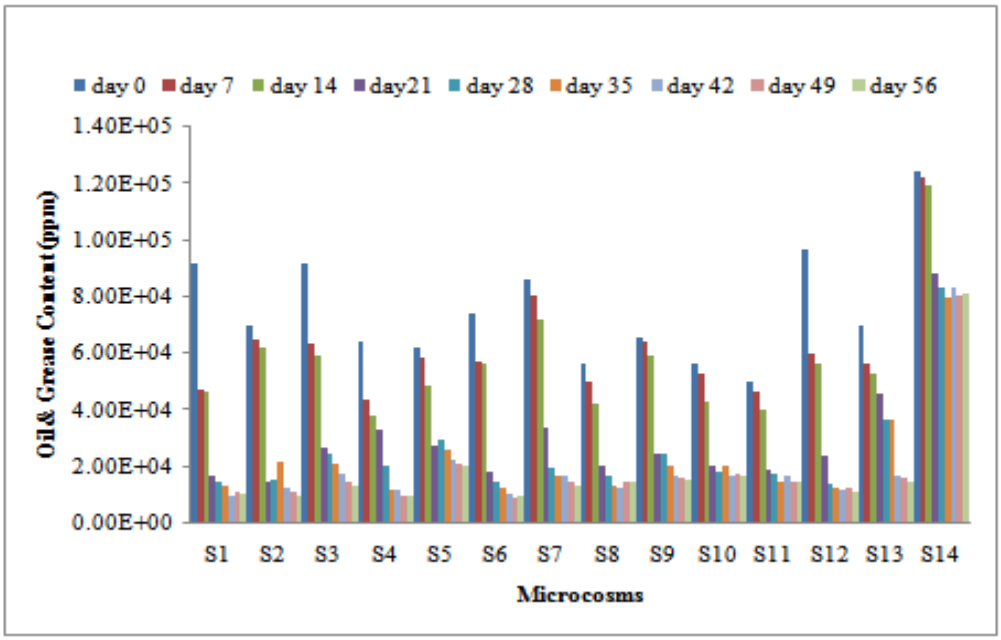

Fig. 3: Changes in oil and grease concentration (content) with bioremediation time**.**

3.4 Seed Germination Index and Germination Toxicity Test

Prior to bioremediation the contaminant load was between 5 –13%, the result for the germination toxicity test were presented in Table 5.0. Guinea corn is one of the important tropical agricultural crop, it was observed that guinea corn is also sensitive to toxic chemicals (mostly hydrocarbon contaminants) when compared with the findings of [21; 32]. However, in a research conducted by [21], the results of toxicity test with lettuce indicated 90%, 70% and 60% germination in a soil contaminated with 10% amended with soy cake , potato skin and tea leaf. In addition, 40%, 20% and 20% seed germination were recorded in a soil contaminated with 20% diesel fuel and amended with soy cake, potato skin and diesel fuel. In this study, the experimental results obtained shows that microcosm S5 recorded the lowest germination index of 37.59% with

the corresponding percentage (%) biodegradation of 67.74% and this is due to higher residual oil and grease concentration compared to that of other treatment microcosms. The water amended controlled microcosm(S14) recorded) 0% germination index and this is due to the fact that it contained higher concentration of residual oil and grease content compared to other treatment microcosms signifying low biodegradation of spent motor oil contaminated soil, this finding is in line with the finding of [21;22]. It can also be seen that the microcosm with higher residual oil and grease content (S5) of 20 000.00 mg/kg recorded a decrease in the number of germinated seeds as shown in Table 5.0.

However, 100 percent (%) germination was recorded in uncontaminated control soil, this finding is also similar to other finding by [21; 32]. The germination index (GI) of the second microcosm (S2) recorded the highest value (77.76%) in soil amended by the blends of cow dung and poultry litter (Table 5.0) with the corresponding biodegradation of 86.67% at a contaminant load of 0.93 – *8*.07% (w/w). It's a well known fact that hydrocarbons may affect root surface of plants thereby preventing nutrient absorption by the plant roots, reducing gas and water exchange to the plant roots. Metabolic reactions may be altered and possibly the embryo when hydrocarbons enter the seeds, beside these aforementioned effects it also affect the plants cell membranes and altered the metabolic transport respiration, this finding is also supported by the research conducted by [21;32]. High percentage (%) biodegradation was recorded in microcosm S1 as shown in Table 6.0 due to high initial and final residual O&G contents of 96 000.00 mg/kg and 10 000.00 mg/kg respectively. It was observed that the germinated crop plant in the microcosms were lacking essential ingredients like sunlight and nutrient, the number of seeds that germinated in some microcosms were differ due to high number of seeds earlier planted which prevented free air movement to the plant for effective growth. Plate 1 is the images of germinated guinea corn (sorghum bicolor) seeds after two weeks of planting.

Table 5.0 Seed Germination (%) Index with Organic Stimulants (Cow Dung and Poultry Litter)

Contaminant Load of Bioremediated Soil (% of Residual Spent Motor Oil Pollution)	Microcosm	Percentage (%) %) Biodegradation	Germination Index (%)
	S1	89.58	70.26
0.93 – 8.07 %	S2	86.67	77.76
	S3	86.23	45.96
	S4	85.42	73.41
	S5	67.74	37.59
	S6	87.39	74.55
	S7	85.27	50.90
	S8	75.00	61.88
	S9	77.55	56.02
	S10	71.43	64.24
	S11	72.00	60.86
	S12	88.97	67.15
	S13	79.81	61.88
	S14	35.29	0.00
	S15	–	100.00

Plate I: Images of germinated guinea corn (Sorghum bicolor) seeds after two weeks of planting

IV. Conclusions

In this study, the biostimulation potentials of cow dung and poultry litter as organic stimulants were investigated in land farming microcosms at a soil contaminant load of 1.0 – 13.0% (w/w) during the bioremediation and carbon to nitrogen molar ratio in the range of 15:1 – 10:1. The result of microbiological

analyses of the test soil revealed that native microorganisms were present above the required quantity ($>10^5$ cfu/g) and the required type namely *pseudomonas* spp, *klebsiella* spp, *bacillus* spp, *micrococcus* spp, *proteus* spp. Bioremediation experimental studies via Box Behnken designed of experiment indicated that the native microorganisms in all the microcosms were able to utilize the oil and grease content in the soil contaminated with spent motor oil as their main source of carbon and energy. The germination index varied from 38-78% corresponding to biodegradation efficiency of 35 – 90% and biostimulant efficiency of 48 – 61%. Conclusively, the digital approach employed in the study is a more effective way of application of animal dung as stimulants in bioremediation.

Acknowledgements

The authors would like to acknowledge the support of Tertiary Education Trust Fund, Institutional Based Research (IBR) grant TETFUND/DESS/ATBU/BAUCHI/RP/VOL.V. Also, they would like to thank the management of Abubakar Tafawa Balewa University Bauchi-Nigeria for providing other necessary supports.

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