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# Assessing the Capability of Indigenous Microorganisms in the Bioremediation of Hydrocarbon Contaminated Soil

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**Abstract:** *This study appraised the capability of indigenous microorganisms in a location with no history of hydrocarbon contamination. The experiment was conducted using microcosms and particle size of the soil was manipulated to arrive at about 9 different types of soils using the soil textural triangle. For operational convenience, nine types of soil were considered, and an undisturbed soil was considered a control experiment. Samples were artificially spiked with 3.4% (w/w) diesel and all nine samples were stimulated with 4% (w/w) NPK fertilizer. These treatments were prepared in three replicates and samples were drawn for extraction and microbial monitoring every 14 days and the study lasted for 56 days. Extraction was done using Soxhlet Extractor and analysis was performed with gas chromatography installed with mass spectrometry. The result of the diesel degradation is significant at the p = 0.05 levelwith little variation in microbial activities except in clay where the organisms experienced excessive stress leading to complete mortality of the degraders at the end of the experiment. Highest bioremediation rate was noted in silt with 75% diesel removal while the lowest bioremediation efficiency was in clay with about 18% and is significantly lower than control with 35% diesel removal. It has been concluded that clay soil bioremediation will require textural amendment to minimize effects of adsorption of nutrients making it unavailable for degraders and, its textural disadvantage for aerobic degradation of contaminants. There is high probability of increasing bioremediation rates as the texture moves away from clayand in this experiment;biostimulation wasthe most significant factor that improves remediation efficiency.*

**Key Words:***Bioremediation, degradation, texture, diesel, indigenous microorganisms.*

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## I. Introduction

One of the major environmental problems today is hydrocarbon contamination due to the activities associated with petrochemical industry through accidental or deliberate releases of petroleum products (Das et al., 2011).On the shore, the pollution arising from either extraction or application of the petroleum products is more obvious on the soil and subsequently, the water ecosystem and air is least impacted. Several studies have documented the impacts of oil spills pollution on ecological variables of the water (both marine and rivers) and soil (land, river and ocean sediments) at the vicinity of oil exploration, mining, refining, transportation and storage (Aghalino and Eyinla, 2009; Kadafa, 2012).Hydrocarbon spills on the land adversely affect both the population and diversity of microorganisms irrespective of the soil matrix (Sutton et al., 2013). The effect of crude oil pollution on soil properties, germination and growth properties of maize has been studied. This was conducted in pot experiments consisting of germination test under crude oil using high pollution levels of 5-40 mL $Kg^{-1}$ and plant growth test using lower pollution levels of 1-2.5 mL $Kg^{-1}$ of soil. The results showed that high levels of pollution inhibited both the germination and growth of maize due to modification of soil properties (Ogboghodoet al., 2004).

According to Falciglia and Vagliasindi (2016), the texture of hydrocarbon polluted soils affects the cost of physical remediation noting that fine textured soil is more expensive to remediate. However, application of appropriate bioremediation technology may upturn this assumption. The bioremediation of lindane from soils of different textures and bioaugmentation with a quadruple Streptomyces consortium has been studied (Raimondo et al., 2019). The result showed significant removal of lindane in bioaugmented sandy soil, followed by silty-loam and clay soils, and concluded that the rate of bioremediation was dependent on soil texture. On the contrary, similar study by Maqboolet al. (2012) assessed the impact of soil texture on the rhizodegradation of crude oil by the Sesbaniacannabina plant. After a 120-day phytoremediation, crude concentration reduced by 26%. Furthermore, analyses of root morphological characteristics and microbial biomass have shown insignificant differences with their controls. It was concluded that Sesbaniacannabina's fibrous root structure is not robust enough to support constricting and stretching forces of clayey textured soil and recommended that textural amendment is indispensable for enhancing rhizodegradation of crude oil in soil. The results reported by García et al. (2019) support the findings of Rainondoet al. and Maqbool et al. substantiating the impacts of variation in bioremediation due to differences in soil texture.

In a modelling study, Balseiro-Romero et al. (2019) found that there is high significance of bacterial density on biodegradation kinetics resulting from augmentation, and the importance of maintaining this parameter as high as possible to reduce the bioremediation time. However, several authors reported insignificant difference between augmented treatments and the control (Pala et al., 2018).

Even though, authors reported variable outcomes regarding the influence of texture, cost-effectiveness of bioremediation of contaminated soil was sustainably recognised (Clarkson and Abubakar, 2015; Clarkson and Clive, 2016).Roy et al. (2018) investigated the application of biostimulation and bioaugmentation on the indigenous microbial community in the bioremediation of oil refinery sludge. Their study found that nutrient-induced community dynamics of native microorganisms and their metabolic interplay within oil refinery sludge could be a driving force behind the accelerated bioremediation. Although, the study was able to focus on a chronic contamination site, it would be more useful if they focused on sites with no history of hydrocarbons contamination to ascertain whether some indigenous microorganisms in such sites are potential hydrocarbon degraders.An in-situ bioremediation experiment was conducted by Ayotamunoetal. (2006) and found that biostimulation (application of fertilizers) has resulted in the degradation of up to 50 to 95% hydrocarbons. This experiment has reported interesting outcomes;however, there may be a possibility of error due to loses from seepage and abiotic factors which may not have been considered. Elechiet al. (2018) compared the degradation kinetics of crude oil contaminated mangrove and clay soils and found that there is over 70% degradation in all samples at the end of 21 days experiment. It was concluded that there is correlation between soil texture and degradation rate. The present study will assess the capability of indigenous microorganisms to degrade hydrocarbons under different textures and biostimulation.

## II. Materials and Methods

### Soil Sampling and manipulation

Samples were collected from Orchard B of the Federal Polytechnic Bali, Taraba State Nigeria. The site had no history of contamination due to crude oil or any of its products prior to sampling. Sampling was randomly done at the topsoil going few inches below ground surface using a garden trowel. Precisely 10, 000 g of samples were shaken through 4-mm set of sieves and at the end of the particle separation, 6 particle sizes were obtained, and only particles less than 2 mm-size were considered in the manipulation. The bioremediation treatments used were the BS and natural attenuation (NA) as control. Sample pH, porosity and organic matter content were also determined.

### Preparation of microcosms

Exactly, ten soil types were determined by textural triangle and prepared by manipulating the content of different soil particles to be considered as different treatment each. Forty 100 ml flasks containing 50 g of the prepared samples were artificially contaminated with 3.4% (w/w) of diesel. Undisturbed soil without amendment but spiked with diesel was prepared as natural attenuation to investigate the natural degradation process. Precisely 4% (w/w) of NPK was added to the manipulated samples to stimulate the natural degradation. Deionised water was supplied to facilitate nutrient transfer and all the manipulated samples were inoculated with microorganisms isolated from a farmyard. The 10 treatments were prepared in 3 replicates and placed under room temperature in a fume cupboard. The flasks were covered with cotton wool to reduce evaporation and allow adequate aeration in the microcosms.

### Microbial inoculation

Microorganisms were isolated and grown overnight in 100 ml of Tryptic Soy Broth in shakers at 30$^{o}$C, and 0.5 ml of the turbid media was washed by a centrifuge at 3000 RPM for 15 minutes and suspended in ¼ Ringer’s Solution. The microcosms were inoculated immediately.

### Method of hydrocarbon extraction and analysis

At day 14 and 28, 36 and 48, 10 flasks were removed, and oven dried at 39$^{o}$C for extraction and subsequent analyses. Extraction was done using Soxhlet Extractor and methanol as solvent and the extracts were dried at 38$^{o}$C using a rotary evaporator. These were then mixed with known amount of solvent (methanol). All samples awaiting analyses were stored in refrigerator at 4 ±1$^{o}$C. Residual diesel was analysed by gas chromatography (GC) installed with mass spectrometry.

**Microbial Analysis**

Viable counts using spread plate method was used to estimate the growth of bacteria in the microcosms. These were carried out at 2, 4, 6 and 8weeks after treatments, by taking out 2 g of samples and suspended it in ¼ Ringer's Solution. Exactly 0.5 ml of the solution was plated on Nutrient Agar and CFUs were isolated using 8-fold serial dilution method. Plates were incubated at $30^{o}$C and counted 48 hours after plating.

**Statistical analysis**

All data generated were analysed using Two-Way ANOVA in the Microsoft Excel. Similarly, graphs and tables were prepared using the same software.

## III. Results and Discussion

### Effects of Manipulation of particle size distribution on some soil properties

Data in Table 3.1 show some of the physical and chemical parameters of undisturbed and the manipulated soil.Originally, the sampled soil has a pH of 7.27 and corresponding organic matter content of 12.8 mg $g^{-1}$. There is obvious deviation of these values after the soil has been artificially altered, there is a deviation range of about (-2.05) to (+1.20)in the pH. These corroborate the assertions of Clarkson and Clive (2016) that if large textural difference is considered, there will be a significant variation in the chemical composition of the soil types. The favourable range for a more efficient bioremediation is from neutral to slightly alkaline (Margesinet al., 2000), or specifically, at pH range of 7.2-7.8 was recommended as the optimum for diesel bioremediation, but substantial impediment of diesel degradation was noted at pH of 5.2 (Whanget al., 2009). Sometimes, modifying the texture of a soil is indispensable which is primarily to minimize constricting and stretching forcesof some types of soil or to facilitate aeration and reduce bioremediation time (Maqboolet al., 2012).

**Table 3.1**: Some chemical and Physical properties of undisturbed and manipulated soils used in the bioremediation

| Types of Soil | Soil properties | | | | | |
|---|---|---|---|---|---|---|
| | | | Soil particles (%) | | | |
| | pH | Organic Matter (mg/g) | clay | silt | sand | Porosity (%) |
| undisturbed soil (Control) | 7.27 | 12.81 | 47 | 39 | 14 | 0.49 |
| Clay | 5.22 | 14.54 | 70 | 20 | 10 | 0.15 |
| Silt | 5.89 | 13.82 | 87 | 13 | 0 | 0.22 |
| Loam | 7.58 | 12.59 | 42 | 41 | 17 | 0.52 |
| Silt Clay Loam | 6.11 | 13.04 | 30 | 70 | 0 | 0.46 |
| Silt Clay | 5.32 | 14.10 | 40 | 60 | 0 | 0.35 |
| Silt Loam | 5.75 | 13.00 | 10 | 70 | 20 | 0.39 |
| Loam-Sand | 8.33 | 9.28 | 10 | 10 | 80 | 0.41 |
| Sandy-Clay | 8.02 | 8.88 | 30 | 20 | 50 | 0.52 |
| Sandy-Loam | 8.47 | 8.07 | 10 | 30 | 60 | 0.43 |

The pH values for Clay and silt-clay are 5.22 and 5.32 respectively which is extremely low and may significantly retard the biodegradationormineralisation of diesel in the soil. On the contrary, soil types with high content of sand appeared to have shown increased pH of up to 8.47. An explanation to these results is likely because those soils whose pH became lower has much portion of finer particles of acidic nature thus increasing the pH while those with less finer particles became more alkaline. However, both pH extremes could adversely affect the effectiveness of bioremediation. Therefore, in order to achieve more efficient degradation, these pH extremes must be adjusted to minimize adverse effects of excessive acidity or alkalinity. Naturally, for high pH soils, the presence of diesel as contaminant will reduce the pH close to or up to optimum pH values and buffering of acidic soils may be desirable to improve process efficiency. Similarly, other soil behaviours such as the porosity and organic matter content have been significantly altered by the particle size manipulation (Table 3.1).

### Diesel degradation in clay, silt and loam soil types

At the end of the 56 days bioremediation experiment, highest degradation of diesel was obtained in silt followed by loam soil. Diesel decontamination rose to a high point and peaked inthe8th week with 75%, 72%, 18% and 36% in silt, loam, clay and control respectively (Table 3.2). On the overall, analysis of variance showed that there is a statistically significant difference within the treatments at 95% level of confidence ($P = 0.006$) but similar pattern was assumed by all treatments but with significant difference ($P = 0.004$) which can be seen clearly in Figures 3.1 and 3.2. This result is consistent with the result of Haghollahiet al. (2016) who reported highest decontamination of hydrocarbons in sandy soils. It was obvious that degradation in clay soil is almost half of the result for control, an indication that degradation has been impaired in clay despite stimulating the clay microcosms with fertilizers (Alrummanet al., 2015).Evidences suggest that the degradation has been credited to degrading microorganisms as reflected in Table 3.5. The microbial growth in clay was maintained at about $69 \times 10^{-4}$ CFU throughout the first 28 days but slightly declined and complete mortality was observed at the end of the 56 days study as no growth was noticed during the last viable count.Benyahia and Embaby (2016) reported much lower degradation (23%) for clay textured soil during bioremediation experiment.

**Table 3.2**: The degradation rate in clay, silt and loam soils

| Type of soil | Diesel Degradation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 2 | | Week 4 | | Week 6 | | Week 8 | |
| | (%) | (mg) | (%) | (mg) | (%) | (mg) | (%) | (mg) |
| Clay | 8.32 | 141.45 | 12.63 | 214.76 | 16.10 | 273.65 | 18.29 | 310.99 |
| Silt | 16.81 | 285.78 | 29.55 | 502.32 | 36.04 | 612.66 | 74.98 | 1274.61 |
| Loam | 16.68 | 283.57 | 43.58 | 740.85 | 52.81 | 897.71 | 71.66 | 1218.25 |
| Control | 5.35 | 90.90 | 18.98 | 322.63 | 26.43 | 449.35 | 35.39 | 601.71 |

Microbial growth peaked at 42 days after treatment in both loam and silt, but their population start to decline thereafter. This microbial growth pattern may be attributed to the soil properties such as porosity and pH while the degradation pattern may as well be attributed to pH and organic matter content (Figures 3.1 and 3.2). The presence of high concentration of organic matter in clay may provide alternative and adequate source of carbon, thus diesel is likely not the only carbon source.

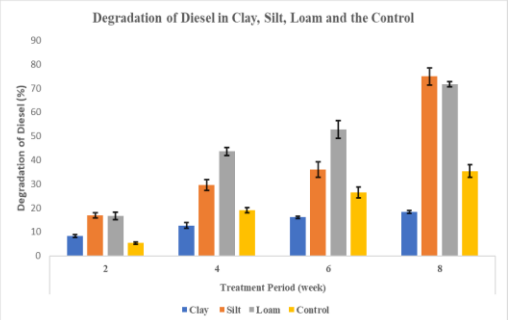

**Figure 3.1** Diesel degradation in clay, silt, loam textured soils at 14, 28, 42 and 60 days after treatment.

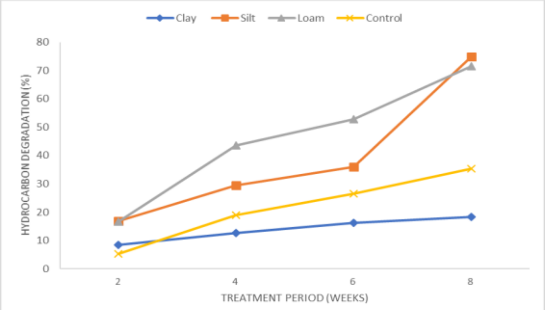

**Figure 3.2** Line graph showing degradation pattern in silt clay and loam textured soils

Obviously, the microorganisms in clay operated under stress and the availability of other carbon source may be responsible for low degradation which is surprisingly lower than that of the control microcosms. A recent study supported this assertion that removal of organic contaminants from fine-textured soil is avery challenging task and it was shown the substantial complexity of the contaminated clay soil under study was due to its hostilenature that hindered microbial growth (Covinoet al., 2016). The remedial activity for this anomaly is either buffering to raise the soil pH or textural amendment to dilute the chemical content of the soil towards neutrality. These results support the findings of Ayotamunoetal. (2006), Elechiet al. (2018) and Kogbaraet al. (2015).There was likely inhibition of the activities of microorganisms in the silt (as shown in Figure 3.2), but after the third week, the organisms might have overcome the diesel toxicity and textural stress and have adapted the situation. This is the likely explanation of the sharp rise in degradation after the 6th week. On the contrary, a sharp rise in degradation during the 2nd and 4th week of the experiment followed byslight but gradual decline in degradation and corresponding reduction of microorganism's population in loam. Again, looking at the results in table 3.1 and figures 3.1 and 3.2, it could be argued that biostimulation is another factor that facilitates the degradation making significant difference of 35-39% higher than control except clay. In clay, its adsorption properties may have delayed the release of nutrients to the organisms leading to poor bioremediation performance. Moreover, Moliterniet al. (2012) argued that bioaugmentation of native organisms is the best method for clay soil bioremediation.

### Diesel bioremediation in silt-clay-loam, silt-clay and silt loam

Surprisingly, there is highest diesel degradation of about 63% removal of contaminant in silt clay. It is followed by silt loam and silt clay loam with 55% and 45% degradation of the contaminant respectively (Table 3.3). ANOVA analysis at 95% level of confidence showed significant variation between the treatments (P = 0.01). An explanation of these findings is probably because the silt and sand portions in the three samples are much higher than contained in the clay and may have effectively improved other soil characteristics. The increase in the degradation rate of contaminant is alongside the pattern of growth of microorganisms with fungi prominently present and in substantial population (Table 3.5). These findingsare consistent with the results obtained by Moliterniet al. (2012) based on which they concluded that the best methodology to bioremediate silty soil is biostimulation of native organisms.

**Table 3.3** Degradation data for silt-clay-loam, silt-clay and silt loam at the through the bioremediation period

| **Type of Soil** | **Diesel Degradation** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Week 2** | | **Week 4** | | **Week 6** | | **Week 8** | |
| | **(%)** | **Mg** | **(%)** | **Mg** | **(%)** | **mg** | **(%)** | **mg** |
| Silt Clay Loam | 10.61 | 180.43 | 33.18 | 564.01 | 41.07 | 698.19 | 44.61 | 758.39 |
| Silt Clay | 15.27 | 259.60 | 32.59 | 554.09 | 48.04 | 816.70 | 62.88 | 1069.04 |
| Silt Loam | 7.79 | 132.38 | 22.77 | 387.10 | 25.19 | 428.15 | 55.21 | 938.59 |
| Control | 5.35 | 90.90 | 18.98 | 322.63 | 26.43 | 449.35 | 35.39 | 601.71 |

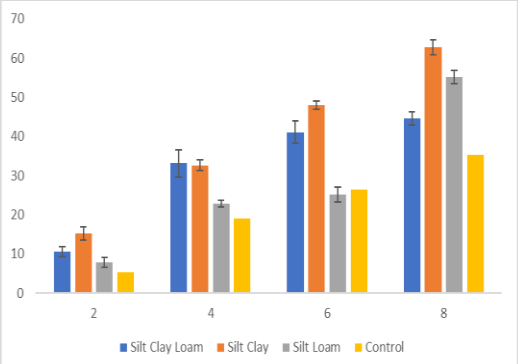

**Figure 3.3** Bioremediation of diesel from silt-clay-loam, silt-clay and silt-loam soil textures.

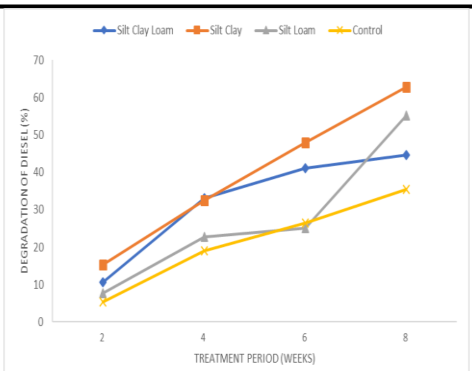

**Figure 3.4** Linear curve indicating degradation rate in silt-clay-loam, silt-clay and silt loam textured soils.

In silt loam, inhibition of activities of microorganisms was suspected during the first 6 weeks as shown in Figures 3.3 and 3.4. However, as the organisms gain acclamation, there was sharp rise in the degradation of diesel corresponding to appreciable microbial activities. It is likely that degradation efficiency in days after the termination of the experiment will be higher. Interestingly, the degradation curve for silt clay loam rises gradually and curved down, an indication of unhealthy developments in the microcosms.There is increasing degradation with time increase (days) where it peaked in the 8th week alongside high microbial population with highest fungal density than the bacterial degraders. Likely, microbial lag phase during the initial days in the treatments was more affected by the biostimulation than the soil texture. Generally, in these soils, microorganisms have responded effectively for nutrients than porosity or aeration except in clay where adsorption played significant role.

### Degradation in coarse textured soil

At the end of the 8 weeks bioremediation experiment, the results in Table 3.4 for coarse textured soil there is higher removal of contaminant than results obtained for fine textured soil and compared with the control.A two-way ANOVA analysis at 95% level of confidence showed a P value of<0.03, providing the basis that alternative hypothesis is accepted with significant difference across and within textures. In these textures, lag phase was not significant as was observed in the control suggesting that biostimulationstimulated the indigenous degraders. Similar finding was reported by Wu et al. (2016) in which populations of oil degraders in soil were generally greater for biostimulation than for any other treatment. Textural advantage may have contributed by providing adequate aeration close to the optimum oxygen requirement for highest efficiency. Clarkson and Sani (2015) argued that researchers were not unanimous on the optimum bioremediation $O_2$requirement, but their values are in the range of 10-40%. These textures may have provided enough void to supply oxygen in this range. This result supported by the findings of Clarkson and Clive (2016) during their studies on bioremediation of diesel from sandy loam and sandy soils where efficiencies of upto65-100% were reported.

**Table 3.4** Diesel decontamination in Loam-sand, Sandy-clay and sandy-loam textures

| Type of Soil | Diesel Degradation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 2 | | Week 4 | | Week 6 | | Week 8 | |
| | (%) | mg | (%) | mg | (%) | mg | (%) | mg |
| Loam-Sand | 8.29 | 140.99 | 15.85 | 269.53 | 34.18 | 581.05 | 64.26 | 1092.48 |
| Sandy-Clay | 8.15 | 138.54 | 22.68 | 385.48 | 29.56 | 502.59 | 50.74 | 862.64 |
| Sandy-Loam | 8.39 | 142.66 | 40.80 | 693.63 | 51.06 | 868.07 | 68.22 | 1159.71 |
| Control | 5.35 | 90.90 | 18.98 | 322.63 | 26.43 | 449.35 | 35.39 | 601.71 |

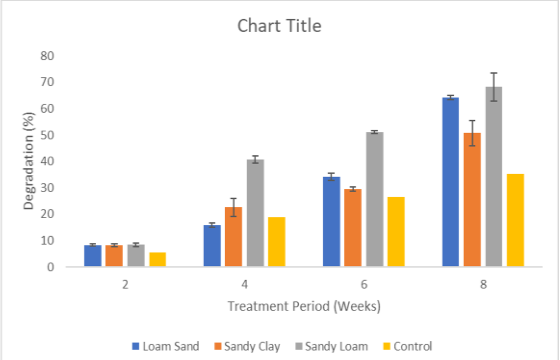

**Figure 3.5** Degradation data in Loam sand, Sandy Clay and Sandy loam

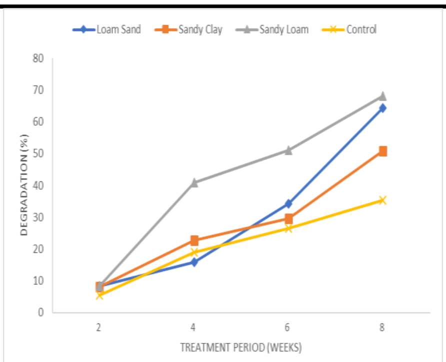

**Figure 3.6** Line graph indicating pattern assumed by the degradation of diesel in Loam sand, Sandy Clay and Sandy loam

The most interesting aspect of the curves in Figure 3.6 is the drawdown exhibited by loam sand which peaked at 8th week with 64% contaminant removal. In sandy loam, there is sharp rise in the first 4 weeks, but degradation remained gradually high throughout the experiment period. The degradation rates and contaminant removal are generally higher than the control with about 18% (17.26mg/g for sandy clay and 12.02 mg/g for control).

### Activities of microorganism in the microcosms

Table 3.5 presents the results obtained from the microbial viable counts of the microcosms during the entire experiment. What can be clearly seen in this table is low growth of microorganisms on some soil textures. There was growth of 45x10$^{-1}$ CFU in sandy loam, which was lower than the result for control. In the same manner, Microbial growth on silt clay, silt loam and sandy clay were the same with results documented for control (10$^{-2}$). It can therefore be assumed that toxicity of the diesel may be responsible for the delayed or low growth of microorganisms. During the 6th week, there was evidence of improved growth of microorganisms and this trend continued in all samples except clay. This, as earlier mentioned is attributed to nutrient adsorption by the clay mesoporous or microporous structure, and/or its acidic nature may have contributed to the impediment of fungal and bacterial activities. At the end of experiment, the lowest microbial growth was noticed on silt declining from 88x10$^{-5}$ to 110x10$^{-4}$ CFU during the last two weeks. This decline coupled with complete mortality on clay soil is attributed to lack of nutrients or textural stress. These results conform to the findings of Alrummanet al. (2015) where hydrocarbon contaminationinhibited enzymatic activities in all the soil samples and the extent of inhibition increased significantly with increasing levels of hydrocarbons and varied with the incubation period. The findings of this study are not consistent results obtained by Labudet al. (2007) wherediesel in clay soil stimulated enzyme activity, particularly at the higher concentrations.

**Table 3.5**Microbial growth in microcosms monitored every 14 days

| Texture | Microbial growth (CFU) | | | |
|---|---|---|---|---|
| | Week 2 | Week 4 | Week 6 | Week 8 |
| undisturbed soil (Control) | 49x10$^{-2}$ | 57x10$^{-3}$ | 68x10$^{-4}$ | 49x10$^{-4}$ |
| Clay | 41x10$^{-4}$ | 38x10$^{-4}$ | 65x10$^{-3}$ | NG |
| Silt | 61x10$^{-4}$ | 69x10$^{-5}$ | 88x10$^{-5}$ | 110x10$^{-4}$ |
| Loam | 70x10$^{-4}$ | 64x10$^{-4}$ | 156x10$^{-5}$ | 43x10$^{-6}$ |
| Silt Clay Loam | 48x10$^{-4}$ | 77x10$^{-3}$ | 112x10$^{-6}$ | 55x10$^{-7}$ |
| Silt Clay | 41x10$^{-2}$ | 35x10$^{-3}$ | 44x10$^{-5}$ | 84x10$^{-5}$ |
| Silt Loam | 96x10$^{-2}$ | 105x10$^{-4}$ | 90x10$^{-7}$ | 75x10$^{-6}$ |
| Loam-Sand | 68x10$^{-4}$ | 92x10-$^{5}$ | 85x10$^{-8}$ | 53x10$^{-7}$ |
| Sandy-Clay | 134x10$^{-2}$ | 63x10$^{-4}$ | 76x10$^{-5}$ | 55x10$^{-5}$ |
| Sandy-Loam | 45x10$^{-1}$ | 37x10$^{-3}$ | 78x10$^{-5}$ | 76x10$^{-6}$ |

NG = No growth

## IV. Conclusion

This study aims to assess the capability of indigenous microorganisms to degrade diesel. It has been found that both fungal and bacterial degraders exist and have quickly acclimated to the acute contamination of their environment with diesel. Textural manipulation employed in this study is found to affect all behaviours of soil ranging from physical, chemical to biological. It was found that moderate to coarse textured soils are more favourable to microbial activities and diversity compared to fine-textured especially clay where its adsorption properties and pH are thought to immensely contributed to delayed bioremediation and mortality of degraders towards the end of the experiment. It is also suspected that fine textured soil (clay) contains high organic matter. On this basis, this paper concludes that diesel is not the only carbon source and has partly contributed to low degradation efficiency.

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