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EVALUATION OF DIFFERENT TREATMENT OPTIONS IN BIO-AUGMENTATION OF CRUDE OIL POLLUTED SOIL

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ABSTRACT

This work is designed to evaluate different treatment methods applied in bio-augmentation of Crude oil impacted soil. The aim of the study is to ascertain the most efficient among all the methods used in the bio-augmentation process. To achieve this aim, initial parameter values of the soil from the study area were determined using standard methods and the result showed high level of depletion in the parameters because of the pollution. Different treatment Options A: Application of indigenous microorganisms and tilling, Option B: Application of locally sourced NPK fertilizer and tilling and Option C: Combination of treatment Options A and B were applied on the polluted soil leaving option D as Control. The processes was conducted for approximately Eighty-Four days. The soil parameter values for p.H, Moisture Content, TPH, Organic, Carbon, Nitrogen Content and Phosphorus were determined. Biokinetic equations were applied in the derivation of k values with respect to time of treatment. The results from the treatment options revealed that combination of Indigenous microorganisms, NPK fertilizer and tilling “C” gave 90% Contaminant reduction, Application of microorganism only “A” gave 75% contaminant reduction, Application of NPK fertilizer “B” gave 65% contaminant reduction while control “D” gave 5% contaminant reduction. With the percentage reduction shown in all the treatment methods, Treatment method “C” Combination of Microorganisms and NPK Fertilizer is most suitable for treatment of soil contaminated with crude oil and is therefore recommended as most efficient for bio-augmentation process.

Index terms: Bio-augmentation; NPK fertilizer and tilling ; parameter; characterization; standard method; depletion; microorganism

1.0 Introduction:

The need for recovery of economically viable areas for different uses in the polluted areas has become necessary due to scarcity of land space for human activities, industrial operation and effects of pollution to the habitats (Nwkwasi, 2016 & Albert 2018). Bio- augmentation is a remediation technique in which nutrient supplements are added to a contaminated environment to facilitate the recovery of its depleted parameters (Ukpaka, 2013). Prior to bio-augmentation, controlled steps are taken to achieve the desired result, it includes characterization and determination of physical and chemical properties of the polluted soil in the study area to determine if the interested parameters are in friendly or in poisonous dose (Adams 2015 & Mohammed 2017). Application of key nutrients and cultured microorganisms that can be used in bio-augmentation is required to be water soluble and have capabilities of assisting in recovery of the contaminated environment (Akpan, 2014 & Adams 2015). The process involves the use of petroleum hydrocarbon as energy source for the growth of microorganisms, with locally sourced fertilizer as nutrient supplements. Studies have proved that when an environment gets polluted, the microorganisms in the environment are depleted as other essential parameters are affected (Siles, 2018). This damage causes serious reduction in number of active microbes that can utilize nutrient supplements to degrade the pollutants. To ensure increase in number of indigenous microorganisms in the area, inoculum was added to boost the process (Somtrakoon, 2014). At the initial

stage of bio-augmentation process, performances of the applied nutrients are mostly very slow due to high concentration level of the toxicants. Some of the affected parameters like nitrogen decreased further due to its utilization by the microorganisms in degrading the contaminants at the initial stage. Moisture contents of the environment also continued to reduce due to reduction in vegetative cover as a result of exposure to weather which gives rooms for soil drying up as a result of evaporation (Zulfigar, & Safia, 2012). Continuous tilling contributes immensely to quick remediation, reduction of contaminant concentration and helps in soil aeration (Smith, 2015, Thapa & Kumar 2012). Application of nutrient supplements are done in a controlled manner with specified quantity per period like weekly, fortnightly or monthly and soil parameter values are tested according to the period of nutrient application. The samples collected at these periods are done in random spots and at different depths like topsoil, .0.3m and 0.6m depending on the degree of contamination (Uwadiae & Obasi 2021). The handling of the collected samples was consciously done to avoid destruction of the sample parameters and the expected data values in the process (Onwuka, 2012).

This research examines different treatment methods applied in bio-augmentation of Crude oil impacted soil and established the most efficient among the methods used in the bio-augmentation process.

2.0 Materials and methods

The bio-augmentation process was conducted from September to November when rain could not affect the work negatively. The materials used in the study is of analytical grade (Xylene and Acetone) which are used for hydrocarbon extraction and as drying agent respectively. The fertilizer used for this work is obtained from the local market and the locally made organic manure. Soil samples from the affected area were characterized prior to the commencement of the exercise to establish the physicochemical characteristics of the soil parameters in the study area. When compared with the already characterized samples done by SPDC in the area, it was observed that the parameters values concerned were seriously affected as shown in Table 1. The sample parameter values from SPDC Characterization information was used to compare the weekly observation of the soil parameters in the study area. Different bio-augmentation methods were applied for a period of Eighty-Four days. After random spot collection at different depths, the samples were homogenized and packaged in a well labelled polythene bag and immediately taken to laboratory for analysis. The handling of the collected samples was carefully done to avoid destruction of the sample parameters and the expected data values in the process. The mean values are computed and recorded for the final analysis. To ensure toxicity in the process is reduced less toxic biodegradable and natural additive like locally sourced Nitrogen-Phosphorus-Potassium fertilizers which have low solubility in water and bind to hydrocarbon are used. First order kinetic equation ($\frac{dc}{dt} = -KC$) was redefined to $\frac{1}{C} \times \frac{dc}{dt} = -K$ to obtain k values in decreasing parameters like Total Petroleum Hydrocarbon and Organic Carbon, while pH, Nitrogen, Phosphorus and Moisture content are increasing parameters. These applied methods were compared to determine the efficiency and duration of each bioremediation method.

Table 1: Physicochemical Properties before bio-augmentation

Soil Nature	Physicochemical Properties							
	Density (g/cm ³)	pH (1:1)	Moist Content (%)	Oil and Grease (ppm)	TPH (mg/g)	Organic Carbon (%)	N ₂ (%)	PO ₄ (mg/kg)
Uncontaminated Soil	2.70	6.69	17.46	1.32	7.89	1.57	0.95	9.35
Contaminated Soil	1.96	4.73	7.35	8.0	13.56	5.12	0.02	3.49

Methods of bio-augmentation and procedure

The bio-augmentation involves the following procedure:

Plot A: Application of microorganisms (*Pseudomonas genera*) followed by tilling.

Plot B: Application of locally sourced NPK fertilizer and tilling.

Plot C: Combination of microorganisms (*Pseudomonas genera*), NPK fertilizer and tilling.

Plot D: No treatment. (Control).

Addition of microorganisms and tilling

“Indigenous” microorganisms (*Pseudomonas genera*) was extracted from the soil by placing the soil in contact with a 0.85% Sodium Chloride (NaCl) solution and shaken for 30± 5minutes using Standard method. The extracted indigenous microbes were applied to Plot A and tilled

Application of fertilizer and tilling

Locally sourced Nitrogen, Phosphorus and Potassium (NPK) fertilizer was applied to Plot B and tilled to a depth of 30±5cm. About 350 kg per hectare of fertilizer was applied per week for the first eight weeks and later, once per two (2) weeks for the last four weeks.

Addition of indigenous microorganisms, fertilizer and tilling

Indigenous microorganisms (*Pseudomonas genera*), (NPK) fertilizer was applied to Plot C and tilled. The approach in this case involved the combination of methods used in Plots A and B.

Soil sampling and characterization

Soil samples were obtained from the topsoil and from the depth of 0.5 and 1.0m at random spots. The samples were homogenized, put in a well labeled polyethylene bag and taken immediately to the laboratory for analysis. This was done in three replicates, and the mean result of the three replicates was used for statistical data analysis. Soil sampling and analysis was conducted every week for twelve weeks.

Enumeration and scaling up of microorganisms

The population of microorganisms present in the soil was estimated by placing the soil samples in contact with sterile normal saline (0.85% NaCl) solution. This mixture was placed in a shaker for about 30 ± 5 minutes to extract the microorganisms present in the soil. The total heterotrophic bacteria count was done using the surface spread method since the efficiency of any bioremediation process depends to a large extent on the amount of microorganisms present. The population of microorganisms at the spilled site was very low prior to bioremediation therefore, inoculum was added to boost the process. The inoculum was developed from hydrogen utilizing genera from the site, with the addition of crude oil as the source of carbon and appropriate nutrients.

Bio-augmentation results

The field data result of different bio-augmentation techniques applied to the delineated sites within 0 to twelve weeks duration were used to determine the k values using first order kinetics. The microorganism population was determined as shown in table 2.

Table 2 Total Heterotrophic Bacteria Count

Option	Sampling Period (weeks)						
	0	2	4	6	8	10	12
	(x10 ⁵ Cfu/g)						
D	80	200	1320	2500	3300	3950	5860
A	750	3500	14670	223000	368000	456000	102000
B	80	1280	8550	17,600	59500	104000	309000
C	750	6270	29000	360000	440000	738000	1460000

3.0 Results and analysis

Using First Order Kinetic Equation, the change in Concentration (dC), Change in time (dt) and k values for pH and other parameters of all the treatment options were calculated and tabulated as shown in table 3 to 8 below:

$$\frac{dc}{dt} = -kC \quad (1)$$

Equation (1) is applicable to decreasing Parameters

$$\frac{dc}{dt} = kC \quad (2)$$

Equation (2) is applicable to increasing Parameters

$$\frac{dc}{dt} = kC_{avg} \quad (3)$$

$$\frac{dc}{dt} = -kC_{avg} \quad (4)$$

$$\frac{1}{C_{(avg)}} \times \frac{dc}{dt} = -K_o \quad (5)$$

$$\frac{1}{C_{(avg)}} \times \frac{dc}{dt} = K_o \quad (6)$$

For treat Option A, K_o , K_1 , K_2 , K_3 , K_4 , K_5 , ..., K_{12} values were computed using Equation (5)

$$K_o = \frac{1}{C_{(avg)}} \times \frac{dC}{dt}$$

$$C_{(avg)} = \frac{C_0 + C_1}{2} = \frac{4.7699 + 4.8652}{2} = 4.81755$$

$$dC = C_1 - C_o = 4.865 - 4.7699 = 0.0953$$

$$dt = t_1 - t_o = 7 - 0 = 7$$

$$\frac{1}{C_{(avg)}} = \frac{1}{4.81755} = 0.207574$$

$$K_o = 0.207574 \times \frac{0.0953}{7} = 0.002825977$$

This procedure was applied for all the parameters, and the k values were determined as shown in the tables below:

Table 3 k and t values for different treat Options after bioremediation for pH

Time (Days)	Control (D) k values of soil Ph	Treat Option A k values soil pH	Treat Option B k values of Soil pH	Treat Option C k values of Soil pH
0	0.0017	0.0028	0.0012	0.0019
7	0.0011	0.0033	0.0012	0.0023
14	0.0014	0.0034	0.0031	0.0027
21	0.0013	0.0055	0.0047	0.0032
28	0.0014	0.0066	0.0042	0.0027
35	0.0016	0.0075	0.0045	0.0028
42	0.0011	0.0081	0.0038	0.0029
49	0.0013	0.0051	0.0032	0.0027
56	0.0013	0.004	0.0029	0.0039
63	0.0013	0.0039	0.0032	0.0042
70	0.0012	0.0032	0.0034	0.0045
77	0.0015	0.0041	0.0037	0.0048
84	0.0017	0.0043	0.0045	0.0051

Table 4 k and t values for different treat Options after bioremediation for Moisture Content

Time (Days)	Control (D) k values	Treat Option A k values	Treat Option B k values	Treat Option C k values
0	0.0023	0.0063	0.0086	0.0086
7	0.0023	0.0070	0.0084	0.0068
14	0.0025	0.0076	0.0088	0.0078
21	0.0026	0.0074	0.0095	0.0085
28	0.0026	0.0088	0.0090	0.0092
35	0.0023	0.0088	0.0097	0.0096
42	0.0023	0.0088	0.0099	0.0110
49	0.0027	0.0097	0.0105	0.0112
56	0.0026	0.0113	0.0119	0.0123
63	0.0026	0.0119	0.0128	0.0146
70	0.0026	0.0112	0.0130	0.0148
77	0.0027	0.0125	0.0136	0.0146
84	0.0028	0.0131	0.0139	0.0149

Table 5 k and t values for different treat Options after bioremediation for TPH

Time	Control (D) k values	Treat Option A K values	Treat Option B k values	Treat Option C k values
0	0.0006	0.0118	0.0137	0.0185
7	0.0004	0.0119	0.0125	0.0151
14	0.0006	0.0108	0.0135	0.0108
21	0.0005	0.0111	0.0118	0.0098
28	0.0006	0.0091	0.0103	0.0076
35	0.0005	0.0096	0.0098	0.0084
42	0.0007	0.0081	0.0074	0.0071
49	0.0007	0.0071	0.0065	0.0056
56	0.0005	0.0042	0.0033	0.0045
63	0.0006	0.0021	0.0029	0.0038
70	0.0006	0.0023	0.0031	0.0039
77	0.0007	0.0027	0.0036	0.0039
84	0.0008	0.0034	0.0039	0.0041

Table 6 k and t values for different treat Options after bioremediation for OC

Time	Control (D) k values	Treat Option A k values	Treat Option B k values	Treat Option C k values
0	0.0007	0.0177	0.0192	0.0187
7	0.0007	0.0206	0.0193	0.0189
14	0.0007	0.0231	0.0173	0.0180
21	0.0007	0.0270	0.0192	0.0178
28	0.0006	0.0276	0.0167	0.0154
35	0.0007	0.0247	0.0124	0.0152

42	0.0008	0.0180	0.0066	0.0119
49	0.0007	0.0151	0.0062	0.0094
56	0.0007	0.0117	0.0050	0.0010
63	0.0008	0.0108	0.0037	0.0086
70	0.0008	0.0104	0.0038	0.0071
77	0.0008	0.0060	0.0031	0.0054
84	0.0009	0.0044	0.0028	0.0022

Table 7 k and t values for different treat Options after bioremediation for Nitrogen

Time	Control (D) k values	Treat Option A k values	Treat Option B k values	Treat Option C k values
0	0.0143	-0.0066	-0.0106	-0.0168
7	0.0066	-0.0220	-0.0238	-0.0295
14	0.0124	-0.0168	-0.0285	-0.0114
21	0.0058	-0.0295	-0.0168	0.0317
28	0.0110	0.0734	0.0571	0.0336
35	0.0102	0.0726	0.0220	0.0444
42	0.0308	0.0862	0.0432	0.0251
49	0.0408	0.0280	0.0389	0.0431
56	0.0030	0.0129	0.0435	0.0130
63	0.0057	0.0045	0.0397	0.0401
70	0.0082	0.0015	0.0498	0.0453
77	0.0307	0.0293	0.0467	0.0220
84	0.0083	0.2284	0.2389	0.2347

Table 8 k and t values for different treat Options after bioremediation for PO₄

Time	Control (D) k values	Treat Option A k values	Treat Option B k values	Treat Option C k values
0	0.0015	0.0084	0.0086	0.0080
7	0.0011	0.0093	0.0083	0.0079
14	0.0011	0.0094	0.0094	0.0099
21	0.0011	0.0103	0.0132	0.0154
28	0.0012	0.0134	0.0141	0.0168
35	0.0014	0.0117	0.0136	0.0165
42	0.0026	0.0164	0.0170	0.0178
49	0.0022	0.0187	0.0197	0.0189
56	0.0034	0.0198	0.0186	0.0110
63	0.0045	0.0209	0.0218	0.0236
70	0.0035	0.0227	0.0266	0.0291
77	0.0037	0.0321	0.0342	0.0344
84	0.0033	0.0325	0.0362	0.0375

Table 9 Bacteria growth Rate with time in the treatment Options

Option	Sampling Period (weeks)						
	0	2	4	6	8	10	12
	(x10 ⁵ Cfu/g)						
D	80	200	1320	2500	3300	3950	5860
A	750	3500	14670	223000	368000	456000	102000
B	80	1280	8550	17,600	59500	104000	309000
C	750	6270	29000	360000	440000	738000	1460000

DATA PRESENTATION AND INTERPRETATION OF RESULT

Parameter values of pH, Moisture Content, Total Petroleum Hydrocarbon, Organic Carbon, Nitrogen, Phosphorus and Bacteria growth were plotted with respect to time and their trends of performance are as shown in Figure 1 to Figure 7.

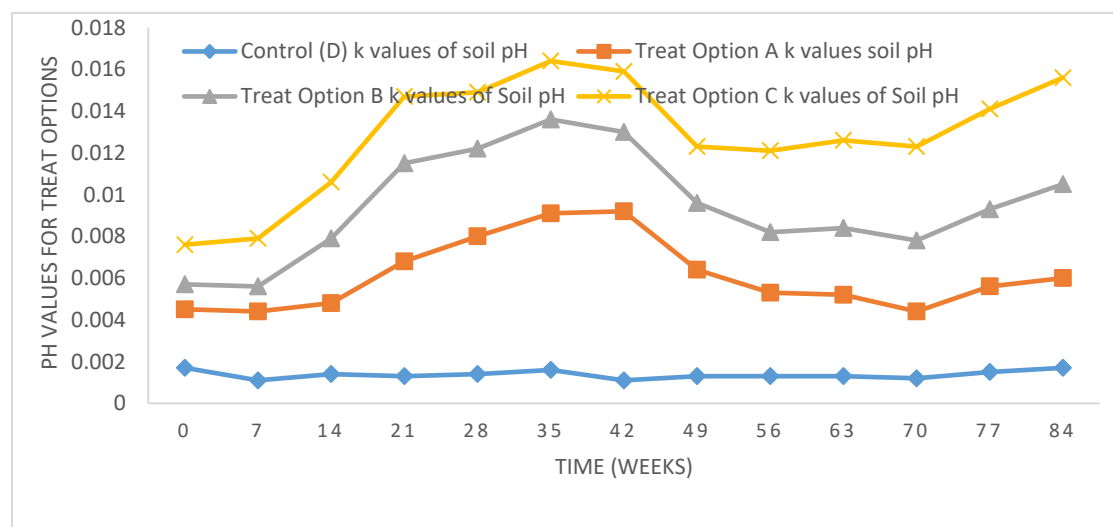


Figure 1: pH against time for all the Treatment Options

Figure 1 shows values of k with respect to time (t) for the soil pH after twelve weeks of the experiment. Application of Indigenous Microorganisms and tilling “A” started showing visible improvement from week four due to increase in the number of Pseudomonas genera which was low at the initial stage. “B” (Application of NPK Fertilizer) performance came lower after first week and later started improving from week three after more application of nutrient. More improvement was observed in treatment option C (Application of Pseudomonas, NPK fertilizer and Tilling) which responded better than other options from week three. The nutrient combination gave boost to the remediation unlike Treatment option D (Control) which showed low performance due to non-application of any kind of nutrient. Further to this, all the treatment options performed considerably well. Treatment options C yielded k value of (0.0051) followed by treatment Option B (0.0045), Option A (0.0043). This is attributed to the microbial

metabolism of *Pseudomonas* genera and the nutrient supplement of NPK fertilizer applied in the bioremediation process. D had the least performance of (0.0017) due to no nutrient application.

Figure 2 is graph of k values with respect to time (t) for Percentage (%) Soil Moisture content after twelve weeks of bioremediation. Moisture content level was slow before the fifth week when improvement was noticed. This is attributed to consistent drying of the soil due exposure to weather as a result of poor vegetative cover until fifth week when the soil started growing some cover which also reduced its exposure to weather thereby reducing moisture evaporation. Treatment option B (Application of NPK fertilizer) started showing improvement in week three. This is attributed to the application of NPK fertilizer. Treatment option C (Application of *Pseudomonas* genera and NPK fertilizer) was lower at the second week before showing improvement from week three which later produced better performance among the other treatment options as shown in. This is attributed to the combination of the two-treatment options A and B. Treatment option D (Control) has the least performance due to no nutrient application. Treatment Options C yielded k value of (0.0149), treatment option B (0.0139), Option A (0.0131) This is attributed to the combination application of *Pseudomonas* genera and the nutrient supplement applied in Option C.

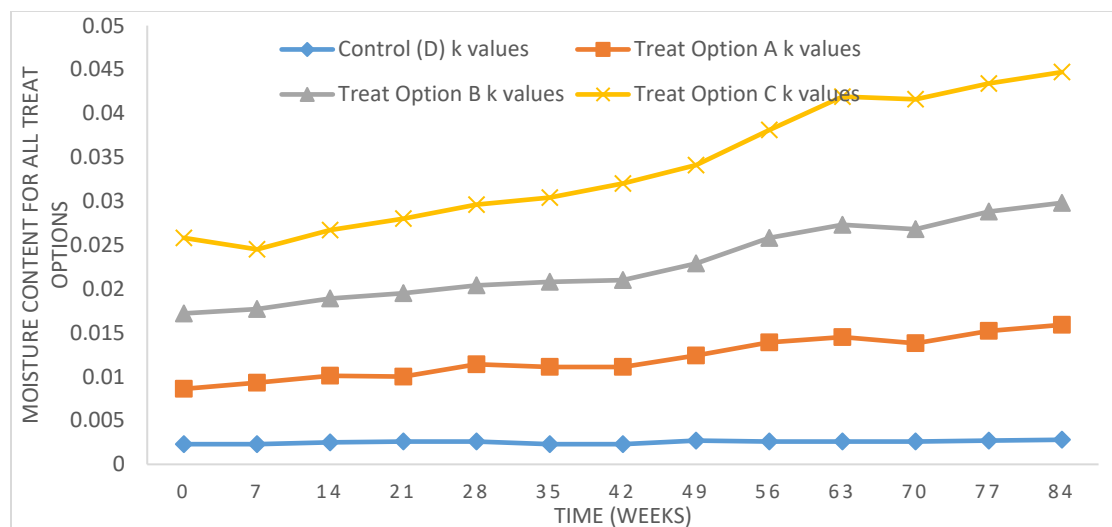


Figure 2 Moisture Content against Time for all the Treatment Options

Figure 3 is the graph of k with respect to time (t) for TPH after twelve weeks of bioremediation. TPH level in treatment option A (Application of microorganisms and tilling) did not show reduction until after three weeks this is because the soil was heavily laden with hydrocarbon from the crude oil spillage and there was much depletion in the number of microorganisms at that initial stage. Treatment Option B (Application of NPK fertilizer and Tilling) started showing gradual reduction in sixth week this is due to increase in application of NPK fertilizer. Treatment option C (Application of Microorganism, NPK Fertilizer and tilling) showed faster reduction of TPH but later went up in sixth week but started reducing again from seventh week. While Treatment option D (control) did not show much reduction in the TPH level. This is a confirmation that more microorganism is required to degrade toxicants faster. TPH in all

the treatment options reduced considerably with time except that of control D. Treatment Options C yielded k value of (0.0041), treatment option B (0.0039), treatment option A (0.0034). This is attributed to microbial metabolism by *Pseudomonas* genera and the nutrient supplement applied in treatment option C. while Control D yielded (0.0008).

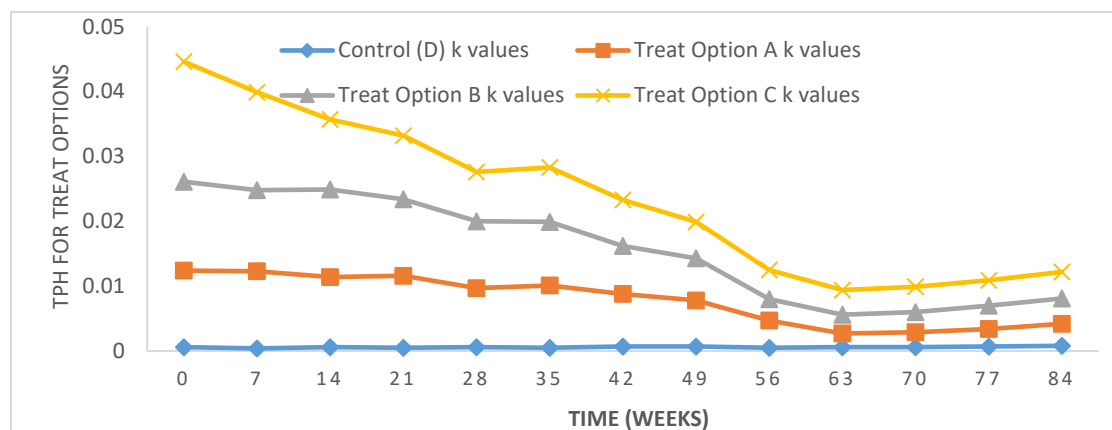


Figure 3 TPH against Time for all the Treatment Options

Figure 4 is the graphical representation of k with respect to time (t) for Organic Carbon after twelve weeks of bioremediation. Treatment Option A (Application of Microorganism and tilling) did not show reduction in organic carbon till sixth week before noticeable reduction was seen. This attributed to low quantity indigenous microorganism caused by high concentration of organic carbon in the soil. It took six weeks application of indigenous microorganism for the soil to start responding to the activities of the microorganisms. Treatment Option B (Application of NPK fertilizer and tilling) followed similar trend. Treatment Option C (Application of microorganism, NPK fertilizer and tilling) showed considerable reduction after fifth week of bioremediation. This is attributed to combined effort by Microorganisms and NPK fertilizer and the tilling. Organic carbon reduction in all the treatment Options with time is considered favorable except that of control D. Treatment options A yielded k value of (0.0044) treatment Option B (0.0028), Treatment option C (0.0022). This is attributed to the microbial metabolism by *Pseudomonas* genera applied in the study, NPK fertilizer and the tilling operation. While Control D yielded (0.0009) due to no treatment application.

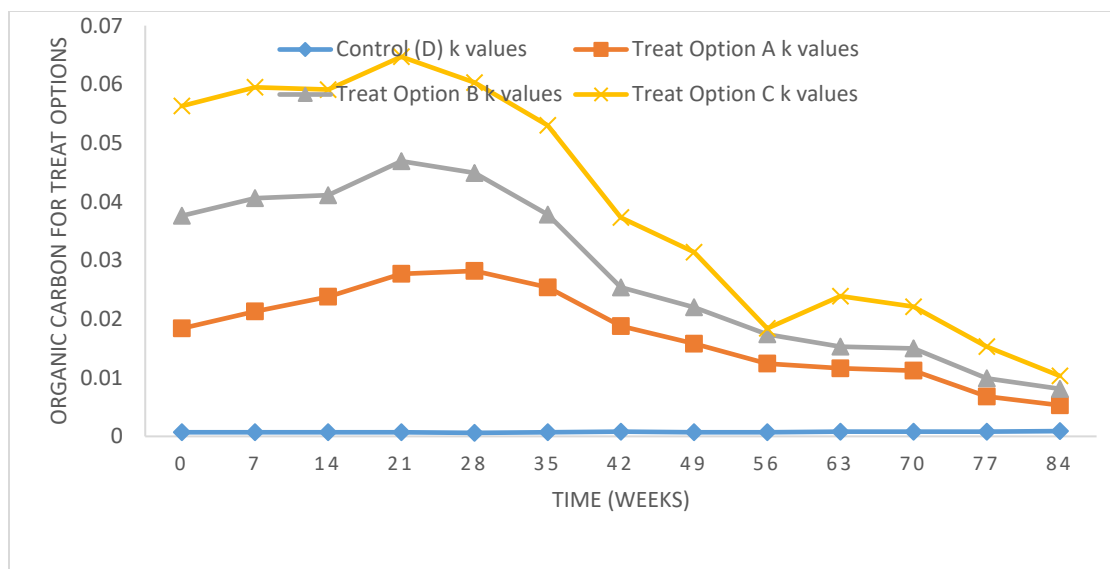


Figure 4 Organic Carbon against Time for the Treatment Options

Figure 5 is the graph of k with respect to time (t) for % Nitrogen after twelve weeks of the experiment. Nitrogen level of the soil was low and applied nitrogen nutrient was utilized by the organisms to degrade the pollutants which took up to five weeks of application before increment of nitrogen level was observed. Treatment option B (application of NPK fertilizer) followed same trend till week four of increment of nutrient application before it was noticed. Treatment option C (Application of microorganism, NPK fertilizer and tilling) showed improvement from third week and progressed till sixth week and came down again in week seven before improving again from week seven. Treat option D (Control) also shows little improvement which is attributed natural attenuation. This process of nitrogen slow rate is attributed to the utilization of nitrogen by degrading microbes to break down the pollutants. The noticed increment in Nitrogen after Five weeks was as result of more nutrient supply and the reduced concentration of the toxicants in the first four weeks except in Treatment option D (control) where there is only natural degradation.

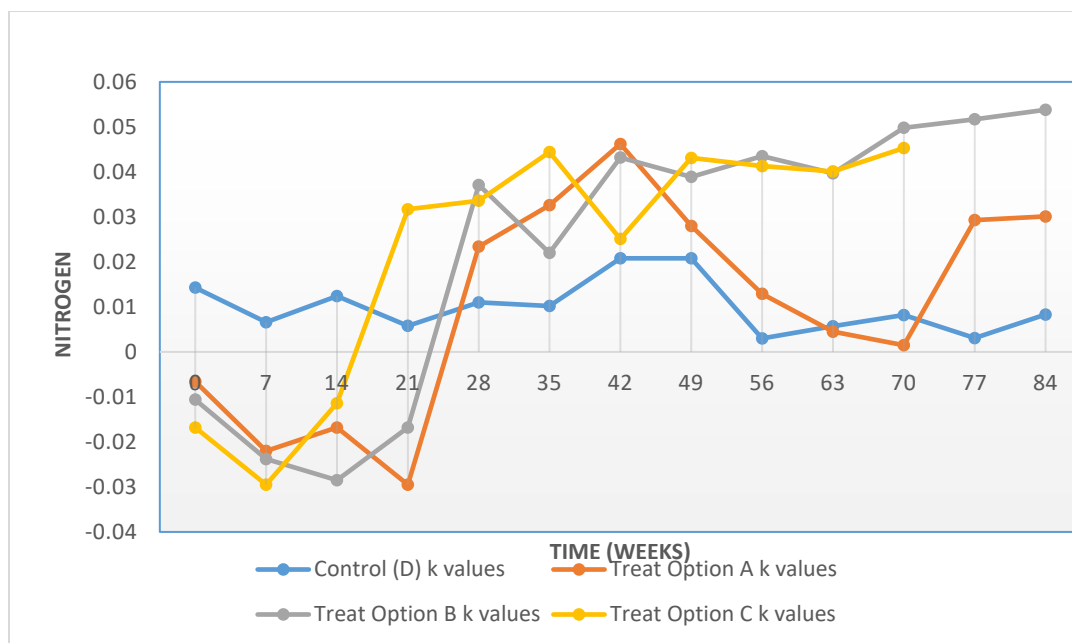


Figure 5 Nitrogen against Time for all the Treatment Options.

Figure 6 is the graphical representation of k with respect to time (t) for Phosphorous Content after twelve weeks of bioremediation. Treatment option A (Application of Microorganism and tilling) was slow at the initial stage until fifth week before improvement is seen. Treatment option B (Application NPK fertilizer and tilling) started showing improvement from week three. This is attributed to the increase in application of NPK fertilizer which boost the nutrient level. Treatment option C (Application of Microorganisms, NPK Fertilizer and tilling) showed gradual improvement from week three till week six when the trend slowed down and later improved again. This is attributed to the combination of microorganism and NPK fertilizer in moderate quantity except in Treat option D that have no treatment. The PO_4 (mg/kg) in all the treatment Options increased considerably with time except that of control D. Treatment Options C yielded k value of (0.0375) treatment Option B (0.0362), treatment option A (0.0325). This is attributed to microbial metabolism by *Pseudomonas* genera and the nutrient supplement applied in the study. While Control D yielded (0.0033) due to no treatment application.

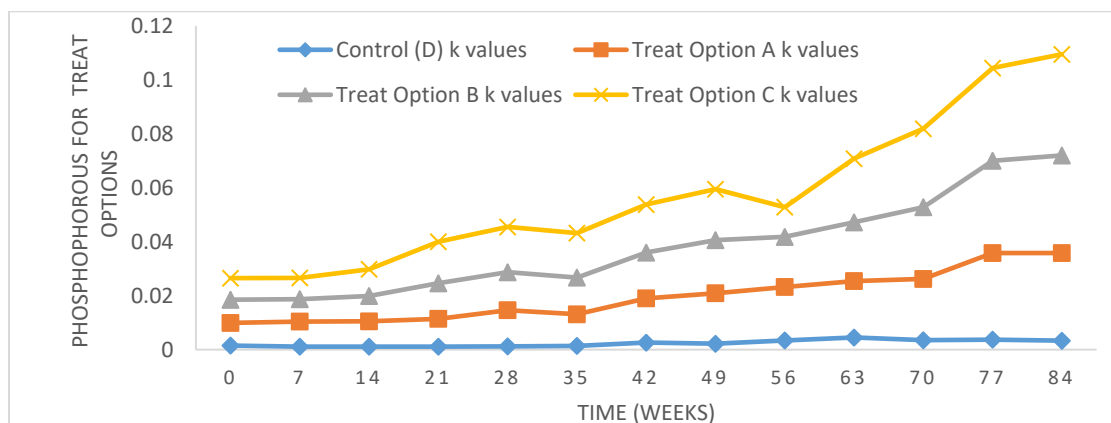


Figure 6 Phosphorus content against Time for all the Treatment Options.

Figure 7 shows the microbe growth with time. It was discovered that at the first three weeks of bioremediation, microorganism population was considerably low but after addition of inoculum to increase the population and application of nutrients for the first four weeks, there was accelerated growth of microbes with respect to time. The acceleration of growth of microbe during the bioremediation process is indicative of the ability of indigenous microorganisms to adapt to the presence of the contaminants and bring about their transformation to reduce levels of contamination in the soil. Maximum growth of microbes occurred at the eight weeks in treatment option A before reduction was noticed again in the tenth week. The early growth is attributed to the activities of the injected microorganisms while reduction in the tenth week is attributed to die off rate which occurs when microbes degrade contaminants. Treatment option B and C showed maximum growth at the tenth week, which came down on week twelve while treatment Option D did not show improvement due to no treatment application.

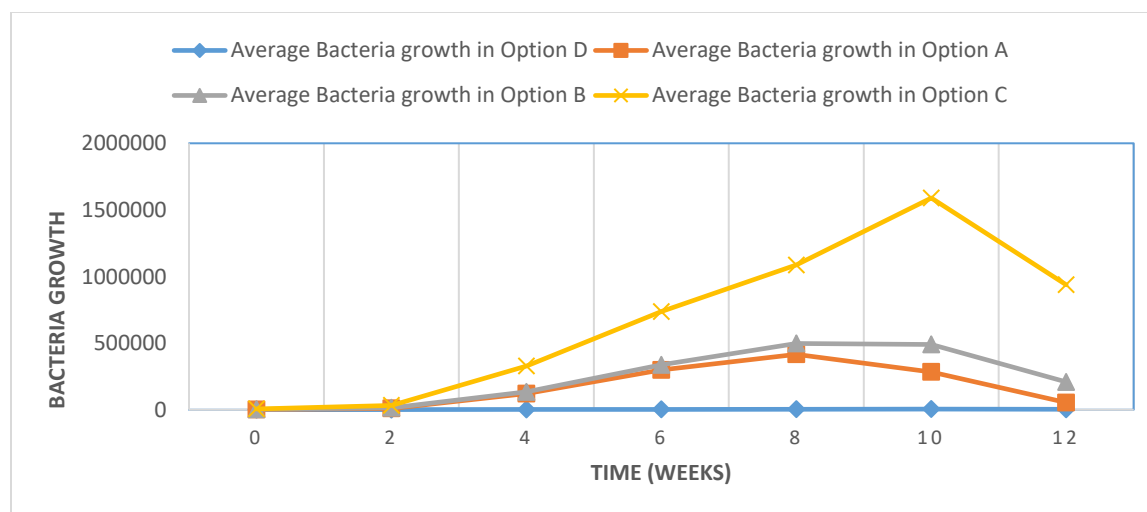


Figure 7 Bacteria growth with respect to time in all the treatment Option.

4.0 Conclusions

Following the bio-augmentation process painstaking, it is concluded that the increase in pH value is attributed to local decomposition of organic residue to acid or CO_2 evolution, as a result of bicarbonate accumulation during biodegradation of petroleum compounds by microorganisms. Bioremediation where nutrient was applied shows greater increase in pH because of the presence of Potassium Carbonate, Potassium hydroxide and Calcium Carbonate which when added to water increases alkalinity. The increase seen in the moisture contents of the soil in all the treatment options indicated that the microorganisms degraded hydrocarbons in the soil and water being one of the two products of degradation helped in producing carbon dioxide. Decrease in TPH is indicative of the ability of indigenous microorganisms to adapt to the presence of the contaminants and bring about their transformation to reduce levels of contamination in the soil. Reduction in Carbon content is attributed to microorganism degradation of major constituents of carbon and hydrogen to form CO_2 and H_2O . Escaping of CO_2 caused reduction in Carbon content of the soil. Percentage Nitrogen content decreased earlier and later improved with time because the microorganisms

utilized the nitrogen in degrading the toxicants. It was noticed that when the toxicants were degraded by the microbes, percentage nitrogen started improving. Many researchers opined that increase in microbial count by addition of substrates or increase in microbial population to soil contaminated with crude oil increases the rate of utilization of percentage Nitrogen (Adekunle et al., 2017, Ofoegbu et al., 2014, Yelebe et al., 2015, etc.). The increase in phosphorus content activated the increase in the biomass (growth pattern) of the organism. As biomass increases, soil phosphorous content increases because the organism possesses genes for phosphate solubilization at a reduced rate. However, variation of microbial growth with time showed considerable improvement. During the first three weeks of bioremediation, microorganism population was very low but after addition of inoculum, there was acceleration in growth of microbes with respect to time. The acceleration of growth of microbes during the bioremediation process is indicative of the ability of indigenous microorganisms to adapt to the presence of the contaminants and bring about their transformation to reduce levels of contamination in the soil. Maximum growth of microbes occurred at the eight weeks in treatment option A before reduction was noticed again in the tenth week. The early growth is attributed to the activities of the injected microorganisms while reduction in the tenth week is attributed to die off rate which occurs when microbes degrade contaminants. Treatment option B and C showed maximum growth at the tenth week, which came down on week twelve while treatment Option D did not show improvement due to no treatment application.

5.0 RECOMMEDATION: Following the result of the evaluations made on different bio-augmentation methods of the study, Combination of NPK Fertilizer, viable indigenous microbes and tilling is recommended for effective treatment of soil impacted with crude oil in similar capacity.

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