COMPARATIVE ANALYSIS OF SOME TECHNIQUES IN THE BIOLOGICAL RECLAMATION OF CRUDE OIL POLLUTED AGRICULTURAL SOILS IN NIGERIA

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ABSTRACT

Replicate field cells involving some techniques aimed at enhancing the bioremediation of crude oil polluted agricultural soils were used in a comparative study to determine the factors and environmental conditions that could optimize the bioremediation process on crude oil polluted soils in Nigeria. The treatment techniques involved the application of different levels of: nutrient, water, oxygen exposure, and the combined effect of different levels of oxygen, water and nutrient. These formed four options, A, B, C and D. Options E and F were Phytoremediation (using corn and elephant grass) and Biopile treatments respectively. The experiments involved the simulation of conditions of a major spill by pouring crude oil on the cells from perforated cans and the in-situ bioremediation of the polluted soils using the techniques that consisted in the manipulation of different variables within the soil environment. The analysis of soil characteristics after a six-week remediation period indicated that the total heterotrophic bacterial counts increased in all treatment options while the organic carbon and total hydrocarbon content (THC) of the soils decreased with time across the various options. Option C *(involving different levels of oxygen exposure) produced the highest hydrocarbon loss of* 94% *while Option E (phytoremediation using corn and grass) recorded the lowest level of hydrocarbon loss* (51%). *The THC losses recorded in the other options, which involved different levels of: nutrient application, water application; the combined effect of varying oxygen, water and nutrients and the use of biopiles ranged from* 67% to 91%. *Option A (the application of different levels of nutrients) had a hydrocarbon loss of* 78%, *Option* B *(involving different levels of water application) recorded a* 67% *hydrocarbon loss, the combined effect of different levels of oxygen, water and nutrients (Option* D) *recorded a hydrocarbon loss of* 91% *while the use of biopiles (Option E) had a hydrocarbon loss of* 51%. *These results were quite different from the control site which had an increased* THC *level* (14 316 - 14 *580 mg* kg^{-1}) *during the study period. The results of the study revealed that different levels of oxygen exposure, water and nutrient application induced different biodegradation rates with the implication that an accelerated bioremediation with the best biodegradation rates could be achieved when polluted soils are remedied with techniques that maintain optimum levels of these factors.*

Keywords*: Bioremediation, petroleum pollution, soil characteristics, comparative analysis.*

NOMENCLATURE / ABBREVIATION

THC	Total hydrocarbon content, mg kg^{-1}
THB	Total heterotrophic bacteria
HUB	Hydrocarbon utilizing bacteria
ANOVA	Analysis of variance
EC	Electrical conductivity, μScm^{-1}
C/N	Carbon / Nitrogen
OC	Organic carbon
TN	Total nitrogen
CH_4	Methane
Cfu/ml	Colony forming unit per milliliter
NPK	Nitrogen, phosphorus, Potassium
%	Percent
SiC	Silty clay
SiCL	Silty clay loam
d.f	Degree of freedom

1.0 INTRODUCTION

All things in nature ultimately succumb to degradation; as sequel oil spills whether on water or soil do disappear over time [1] but until recently little was known about what could be done to accelerate the process [2]. Different positions exist on methods to speed up the process just as there are different researchers. Atlas and Bartha [3] concluded that the disappearance of crude oil from sea water could be accelerated by the addition of deficient nutrients such as nitrogen and phosphorus or both. Others have suggested microbial seeding of oil spills since bacteria and fungi are the only biological species which have the metabolic capability of utilizing petroleum carbon for cell synthesis. On the other hand, Christofi et al. [4] posited that several agro technical methods including tilling and loosening, watering and the addition of organic materials (straw, compost etc) and mineral fertilizer could decrease the contamination level by 30 - 40% due to the oxidation of easily degradable petroleum components.

As these findings became common knowledge, biodegradation of petroleum hydrocarbons has become an increasingly important method of treatment of the contaminant in polluted soils due to its advantages which include inexpensive equipment, environmentally friendly nature of the process and simplicity [5] Hence the present study became necessary to broaden the horizon of existing knowledge on bioremediation and to investigate the factors that could be optimized for an accelerated biodegradation, and thus provide a veritable and cost effective approach in the clean up of contaminated soils in a low-income country like Nigeria where crude oil pollution of existing and potential agricultural lands is fast becoming a growing environmental problem.

Studies have shown that bacteria can exist within an extremely broad range of environmental conditions, and certain species called hydrocarbon utilizing bacteria (HUB) could achieve the clean up of crude oil contaminated soils when given a source of carbon, hydrogen, oxygen and nitrogen to stimulate their growth and multiplication [6-8]. Hence the hypothesis that HUB would be present in agricultural soils within the study area was utilized in the present study, since petroleum-hydrocarbon contamination of the soils has become common experience and such bacteria would have acclimated to the trend. This informed the use of techniques such as the application of different levels of nutrients, water and oxygen exposure and the combined effect of different levels of oxygen, water and nutrients. The other two treatment options, Phytoremediation and Biopile, slightly differ in principle from the above mentioned ones. The phytoremediation option (using corn and elephant grass) is based on the in situ use of plants and their associated microorganisms to degrade, contain or render harmless contaminants in soil or ground water [9], while the biopile treatment involves the use of piles or mounds of soil of about 50cm depth in which contaminated soil is spread over a large area and non-contaminated soil is added and mixed with the contaminated soil while ensuring the incorporation of sources of adequate oxygen, nutrients, moisture and pH. The biopile method is also called enhanced composting.

In the light of the hypothesis that HUB would be present in agricultural soils within the study area, the line of convergence between the above mentioned techniques is that they all consist in the stimulation of the indigenous microbial flora of the polluted soils to bring about its reclamation. Although reports by Wiltse et al. [10] have it that in addition to the biostimulation of soil bacteria, the phytoremediation mechanism also involves the translocation of petroleum hydrocarbons through plants and their subsequent transpiration to the atmosphere. The objectives of the study therefore were:

(i) To investigate the particular factors that mostly limits biodegradation and hence could be optimized for an accelerated bioremediation of the pollutant.

(ii) To determine the technique(s) that may yield the greatest utility, as well as other environmental conditions that may be optimized for the biological clean up of crude oil polluted agricultural soils.

2.0 MATERIALS AND METHODS

2.1 Field sites

The field cells were located at the Rivers State University of Science and Technology teaching and research farm in Port Harcourt, Nigeria. The ambient environment of the experimental area is characterized by an annual rainfall of about 2700 mm and an average temperature of 27°C. The vegetation cover is the tropical rainforest [11].

The field cells of five of the six treatment options (i.e. options A, B, C, D and E) and that of the control, used in the study were made into beds of 40 cm x 40 cm dimension with depths of about 30 cm. This was done in order that the depth and exposed surface area of the soil and in turn the temperature, nutrient concentration and oxygen availability could be controlled [12]. The beds also served to control the fate of the contaminant as regards run off to nearby lands, since the experiments were conducted amidst the rains (June to August,2005). The sixth option utilized piles of soil with a dimension of 1m x 1m.

Option 0, was the Control (no treatment employed), option A had different levels of fertilizer application, option B received different levels of water application, option C was the application of different levels of oxygen exposure, while option D had the combination of different levels of oxygen, water and fertilizers (nutrients). Options E and F received Phytoremediation (using corn and elephant grass) and Biopile treatments respectively.

3.0 EXPERIMENTAL PROCEDURE

Perforated cans were used to sprinkle Bonny light crude oil on all the cells (including the control) at the rate of 0.8 liters of crude oil per $0.16m^2$ of soil for all other options excepting option F which had 0.8 liters of crude oil per square meter of soil. The objective was to simulate conditions of a major spill. The soils were left undisturbed for three days. All treatment applications commenced after the three day period. The six treatment options had five cells each on which different levels of the treatments were applied. The detailed description of the treatment options are as follows:

3.1 Different levels of nutrient (fertilizer) application

The five constituent cells in this option received 50 g, 75 g, 100g, 150 g and 200 g of 20-10-10 NPK fertilizer. The afore-stated quantities of fertilizer were applied twice during the study period at an interval of two weeks.

3.2 Different levels of water application

The five cells involved in this option received 0.5 L, 0.75 L, 1 L, 1.5 Land 2 L of water once every three days during the six-week remediation period. Watering was done using perforated watering cans.

3.3 Different levels of oxygen exposure

The various cells in the option were tilled once, three times and five times in a week; once and twice daily respectively.

3.4 **Combination of different levels of oxygen, water and nutrients**

The first cell in the option received 50 g of fertilizer, 0.5 L of water and one time tillage per week, the second received 75 g of fertilizer, 0.75 L of water and three times tillage per week. In summary, each of the cells received the corresponding level of the three variables in options A, B and C above (for instance, option E received 200 g of fertilizer twice during the study period, 2 L of water once every three days and a twice daily tillage).

3.5 Phytoremediation using corn and elephant grass

The first cell had a combination of corn and elephant grass treatments, while two of the cells received corn treatment and the other two elephant grass treatments. All the cells had about ten stands of plants. In the course of the experiments

3.6 Biopiles

Each of the five soil piles (called biopiles) had different levels of liming (to reduce soil acidity), watering and fertilizer application. Two of the soil piles received 50g, and the other three, 100 g of 20-1010 NPK fertilizer twice during the study period. Oxygen was added by tilling the piles with cutlasses and shovels, while the fertilizer was added in solution to the piles and watering done at the rate of two times per week with watering cans. The top and bases of the piles were covered with polyethylene linings to facilitate the control of the moisture level within the pile.

It has to be noted that all the options received nutrients and tillage in order to provide the much needed nitrogen and oxygen for aerobic biodegradation. However, the application rate was constant for the option(s) in which the particular factor (nutrient or oxygen) was not varied. Thus, 50 g of 20-10-10 NPK fertilizer was added to all the treatment cells in the various options twice during the study period at an interval of two weeks. In exception of option E, the other relevant options in this context (i.e. options A, B and F) received two times tillage per week, using cutlasses and shovels.

4.0 LABORATORY/STATISTICAL ANALYSIS

Soil samples were taken periodically for analysis. The method employed was the auguring of different random spots and bulking them together to form composite samples. The samples were then analyzed for ~oil physicochemical parameters such as particle size analyses, total hydrocarbon content, organic carbon, total nitrogen, moisture content and soil pH/electrical conductivity using methods adapted from Black et al. [13] and Jackson [14]. Microbiological analyses were done following the procedure described by the American Public Health Association [15] and Buchanan and Gibbons [16].

Data analysis involved simple descriptive and univariate summary statistics (e.g. mean, range, standard deviation and percentage). A two-way analysis of variance (ANOVA) was also carried out in order to determine the relative effects of differences in treatment options and treatment application levels on soil THC with remediation period. The statistical methods were adapted from Frank and Althoen [17].

5.0 RESULTS AND DISCUSSION

The particle size analyses of the top 30cm of the soils before treatment indicated that the soil types consisted of silty clay and silty clay loam (Table 1). There was a distinct increase in soil pH when samples were taken three days after contamination. Brady and Weil [18] had previously reported such variability in soil pH which could arise during organic decay processes which may result in the formation of both acid and base forming chemicals. However, as remediation treatments were applied the mean pH value. for the options dropped from 5.81 to 5.55 at the end of the six week period (Tables 2-4). Tisdale and Nelson [19] made a similar observation and reported that the decrease in pH as remediation treatment began may have resulted from the production of acid radicals through the process of nitrification of the applied fertilizer. The pH range observed during the study highlighted the view that soils with pH on the acid side of neutrality are best suited for agriculture. In a related development the electrical conductivity (EC) increased generally in the treatment cells with values quite distinct from that of the control (Table 2 - 4). This may have been due to the soluble salt content in the soil induced by the introduction of the mineral fertilizer. Odu et al. [20] made a similar observation.

Table 1: Soil physicochemical characteristics before crude oil contamination.(Results represent mean values of the constituent cells)

Option	% by mass				pH 1 :2.5	EC μScm^{-1}	% moisture by mass	THC mg kg^{-1}	%		C/N ratio
	Sand	Silt	Clay	Texture					OC	TN	
0	18.1	45.1	36.8	SiCL	4.66	30	16	31	0.26	0.56	0.5
A	12.4	40.1	47.5	SiC	4.74	28	13	84	0.19	0.38	0.5
B	11.3	39.2	49.5	SiC	4.61	31	12	78	0.68	0.28	0.4
C	15.3	45.2	39.5	SiCL	4.73	34	17	46	0.18	0.36	0.5
D	16.1	55.1	38.8	SiCL	4.45	76	13	25	0.20	0.40	0.5
E	10.9	41.0	48.1	SiC	4.71	45	13	25	0.19	0.34	0.6
F	14.4	46.1	39.5	SiCL	4.54	65	14	25	0.22	0.61	0.4

Table 2: Soil physicochemical characteristics three days after contamination, prior to remediation. (Results represent means ± standard deviations of constituent cells)

Option	**pH 1:2.5**	**ECμScm-^{1}**	**THC mg kg^{-1}**	**% Moisture**	**%**		**C/N ratio**
					Organic C	**Total N**	
0	5.94±0.21	66± 47	14316±2322	11 ±2	0.52±0.22	0.31 ±0.18	2±0.41
A	5.91 ±0.06	74± 4	13508±2oo7	13±4	0.37 ± 0.04	0.21 ±O.02	2±0.00
B	5.76±0.14	72± 1	10 670 ± 2375	12±3	0.35 ± 0.03	0.21 ±0.01	2±0.00
C	.25 ± 0.02	136 ± 3	16236±2527	14±2	0.89±0.62	0.59±0.10	2±0.55
D	5.65 ± 0.27	69± 1	16541 ±4oo9	12 ±2	0.42±0.05	0.24±0.05	2±0.00
E	5.43±0.12	67± 8	22461 ±2259	17 ± 3	0.39±0.04	0.16±0.04	310.55
F	5.88±0.01	87± 5	11857± 1105	13 ±3	0.76±0.06	0.31 ±0.18	1 ±0.58

Table 3: Soil physicochemical characteristics two weeks after remediation
(Results represent means ± standard deviations of constituent cells)

Option	**pH I:2.5**	**ECμS cm^{-1}**	**THC mg kg^{-1}**	**% Moisture**	**%**		**C/N ratio**
					Organic	**Total N**	
0	5.59± 0.21	58± 25	12738±2847	1O± 1	0.47± 0.23	0.23 ± 0.16	2± 0.16
A	5.50± 0.13	96± 75	5462± 2476	17± 2	0.29± 0.02	0.08± 0.01	4± 0.45
B	5.32± 0.06	100±54	3316±764	15 ± 2	0.29± 0.02	0.07± 0.09	4 ± 0.55
C	5.43± 0.10	47± 8	1281 ± 1354	17± 3	0.54± 0.09	0.36± 0.26	2 ± 0.45
D	5.43 ± 0.10	52± 16	2461 ± 2907	15± 3	0.25± 0.13	0.09± 0.01	3 ± 0.55
E	5.43± 0.12	67± 8	5184± 1684	17± 3	0.39± 0.04	0.17± 0.04	2± 0.55
F	5.48 ± 0.07	96± 67	1075± 660	12± 3	0.63± 0.03	0.36± 0.06	2± 0.40

Table 4: Soil physicochemical characteristics six weeks after remediation. (Results represent means ± standard deviations of constituent cells)

Option	pH 1: 2.5	ECμScm^{-1}	THCmgkg^{-1}	% Moisture	% Organic C	% Total N	C/N ratio
O	5.53± 0.22	21 ± 9	14580±4593	12± 4	0.20± 0.11	0.08± 0.05	4±1.92
A	5.97± 0.09	121 ±76	2918± 2782	16± 2	0.18± 0.03	0.03± 0.00	6±0.45
B	4.98± 0.07	138± 30	3515 ± 2452	17±3	0.16± 0.04	0.03± 0.00	5±0.84
C	5.32± 0.06	98± 13	964±734	12± 3	0.44± 0.06	0.14±0.02	3 ± 1.10
D	6.45 ± 0.42	151 ± 138	1518± 1577	19± 3	0.22± 0.03	0.05± 0.01	4±0.55
E	5.21± 0.17	104±31	11054±4193	17± 1	0.27± 0.05	0.07± 0.01	4±0.71
F	5.38± 0.07	390±310	2788± 892	14± 2	0.38± 0.09	0.l4± 0.02	3±0.55

The moisture content of the various options did not show conspicuous variations on the average level, however the mean value dropped slightly (13.7% - 13.5%) after crude oil contamination (Tables 1 and 2) and increased to a mean value of 15.8% after six weeks of remediation. This is expected because the experiments took place during the rainy season. On the other hand, the total nitrogen of all the treatment options and the control decreased throughout the study period thus producing unusual results in the C/N ratio (i.e. an abnormal increase instead of a decrease in the C/N ratio). Such results may not be regarded as phenomenal since soil properties are likely to change markedly across small distances, within a few hectares of farmland and even within a single soil individual [18]. The authors further submitted that during biodegradation an enormous loss of nitrogen can be experienced through a series of widely occurring biochemical reduction reactions brought about by denitrifying bacteria such as pseudomonas, bacillus and micrococcus, especially when localized micro sites of low oxygen exist in the center of soil aggregates. This could be supported by the findings of the microbiological analysis which revealed that the hydrocarbon utilizing bacterial population on the soil included pseudomonas, bacillus, micrococcus, flavobacterium, corynebacterium, alkaligenes etc, most of which are facultative in nature and could have induced the trend.

The hypothesis that HUB are present in agricultural soils within Nigeria was substantiated by the results of the total heterotrophic bacterial (THB) counts presented in Table 5 which showed a general increase in bacterial numbers as remediation treatments progressed. Slight exceptions to this were the values obtained in the control (option 0) in which the THB increased after the first two weeks (from 12.6 $\times 10^5$ to 19.9 $\times 10^5$ cfu/ ml) and dropped when sampling was done at the end of the six-week period (Table 5). This could be attributed to lack of optimal conditions that prevailed in these options. All in all, these results highlight the view that areas that have witnessed incessant spills contain HUB which would multiply in their numbers when conditions are optimized. At this juncture it is necessary to highlight the observation that the ability to degrade the contaminant is not necessarily proportional to the multiplicity of hydrocarbon utilizing bacteria in the soil; this is supported by the THB counts (31.1×10^5 cfu/ml, being the highest recorded after the six week remediation period) recorded in option E (Phytoremediation treatments) and its corresponding THC level (11 054 mg kg^{-1}, the option with the highest pollution level) at the end of the six-week period (Table 4 and 5). In addition, the THB counts recorded in option E was among the highest throughout the study period but its THC reduction did not follow suit. Previous reports by Cunningham et al. [9] has it that plants growing on oil-contaminated soils provide root exudates of carbon, energy, nutrients, enzymes and sometimes oxygen, that induce an enormous proliferation of microbial population than a soil not in contact with plant roots. However, an effective biodegradation is anchored on a number of factors than the mere proliferation of microbes

Table 5: Total heterotrophic bacterial count

Option	Sampling period [weeks]		
	0	2	6
	X 10^5[Cfu/ml]		
0	12.6	19.9	19.4
A	6.l	18.2	26.2
B	l3.2	24.4	25.0
C	l3.4	20.6	27.6
D	6.7	12.7	23.5
E	15.9	23.8	31.1
F	9.3	17.6	12.5

The utility of these techniques in were not receiving biodegradation was justified by the E was not tilled significant attenuation of the organic carbon (OC) and total hydrocarbon content (THC) in the various options. The organic carbon content of all the optics (including the control) decreased as time progressed. This is because bacteria need a source of carbon for cell synthesis in the course of their metabolism during the degradation process and since petroleum degrading bacteria were present in all the cells it is apparent that they utilized the organic carbon for their metabolism hence the general decline in organic carbon. After six weeks of remediation the THC level dropped from as much 16230 mg kg^{-l} to 964 mg kg^{-1} in option C which had the best degradation rate, and from 10 670 mg kg^{-l} to 3515 mg kg^{-l} in option B (which was next to the phytoremediation option, that had the poorest THC reduction level). Option (phytoremediation option) had an unusual trend in the sense that its THC level first dropped from 22 461 mg kg^{-l} to 5184 mg kg^{-1} after the first two weeks and later increased to 11 054 mg kg^{-l} after six weeks (Tables 2 - 4 and Figure 1) such a trend implied that as remediation treatment began environmental conditions were favorable but as time progressed there was a change in the soil environment which became unfavorable for biodegradation. Similar trends to that of the control were observed in options B and F (Figure 1). Comparing the results obtained in these options it can be deduced that the initial enormous reduction and subsequent increment in THC in the relevant options may be attributed to oxygen exposure since at the initial stage the soils contained adequate oxygen but as remediation progressed oxygen became limiting as these options were not receiving constant tillage (option E was not tilled in the course of the treatments), thus anoxic or anaerobic conditions became apparent. The products of anaerobic decomposition of organic materials, methane (CH_4) and carbon dioxide could most likely increase the THC since methane itself is a hydrocarbon.

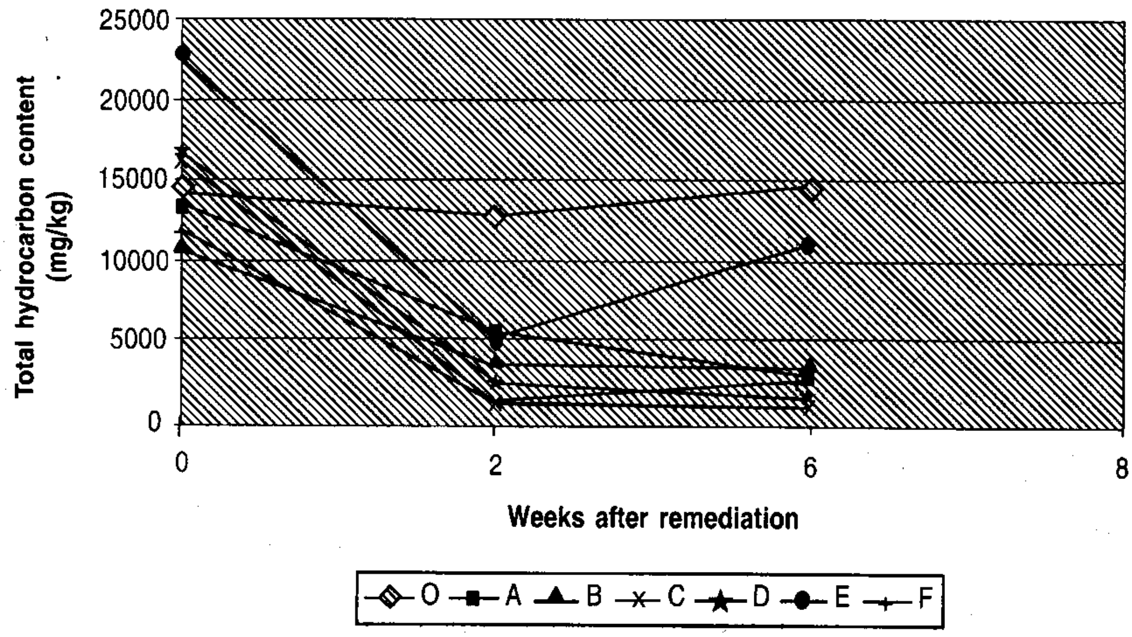

Fig. 1: Variation of THC with remediation period.

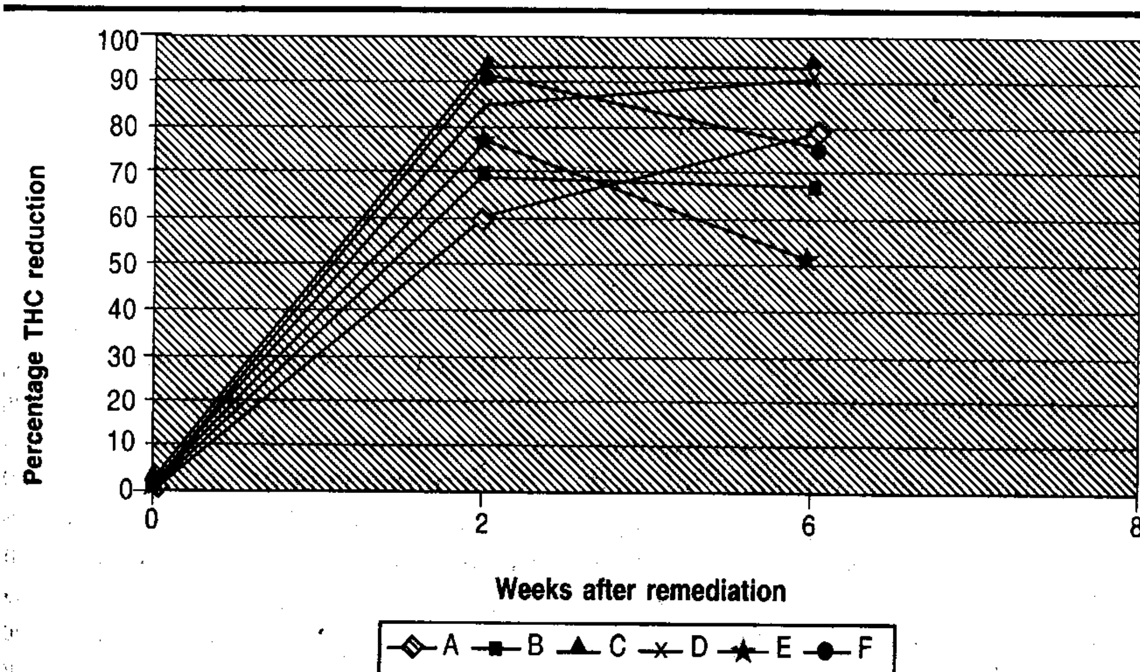

Fig. 2: Rate of hydrocarbon loss.

After the six-week remediation period, options A, B, C, D, E and F produced hydrocarbon losses of 78%, 67%,94%,91 %,51 % and 77% respectively (Figure 2). These results were markedly different from the ones obtained in the control, whose THC level increased from 14 316 mg kg^{-1} after crude oil contamination to 14580 mg kg^{-1}after the six-week period. From the results it is evident that oxygen and nutrients imposed the greatest limitation on the bioremediation of crude oil polluted soils. The THC values obtained in the control that received no nutrient treatment is an indication of the limitation imposed by the presence of nutrients (supplied by fertilizers) in biodegradation. Statistical analysis of these data indicated that the THC of the soils varied with different kinds of treatments and that as time progressed the variations in the THC could be attributed to the different kinds of treatment and various levels of application of the treatment options. These can be observed in the ANOVA summary table (Table 6) in which the calculated F values for column and interaction sources of variability were significant at 1% probability level the non significance of the row source of variability implies that there was no significant difference in the effect of remediation period on the mean values of THC in the various options

Table 6: Two-way ANOVA summary table showing the effect of treatments on soil THC

Parameter	Source of variation	d.f	F value (calculated)	p-value	F value (critical)
THC	Row	1	1.4	>0.15	= 4.05*
	Column	5	13.99	<0.0000 :	= 3.45 **
	Interaction	5	4.58	<0.005	= 3.45 **
	Error	48	*		

∗Not significant at 5%

** Significant at 1 % probability level

6.0 CONCLUSIONS AND RECOMMENDATIONS

Looking at the overall performance of the various options, it could be inferred that oxygen and nitrogen are the major factors that impose the greatest limitation on biodegradation. Soil water plays a lesser role compared to both factors in the biodegradation process. This is evident from the results of the study in which option C (different levels of oxygen exposure) recorded a hydrocarbon loss (94 %) far higher than that of option B (different levels of water application) which had 67%. The hydrocarbon loss in option C even exceeded that in option D (91 %) that combined the effects of oxygen, water and nutrient. It can also be deduced that phytoremediation treatments are less effective compared to the other options because it takes a longer time to remove large quantities of the contaminant. Previous reports by Cunningham et al. [9] support this view. It also requires a greater bioengineering than do the other techniques. Biopile treatment too has the same defect (i.e. a greater level of bioengineering has to be done to achieve the best results, especially as regards oxygen exposure since the piles are of greater depths). Therefore bioremediation techniques involving simpler methods of manipulating the soil environment that leads to the optimization of the levels of oxygen and nutrients in polluted soils yields the greatest utility.

From the statistical analysis, it is apparent that biodegradation rates respond to differences in the level of treatment applications, hence it is expedient that optimum levels are resorted to. As revealed by this study such optimums may include: a fertilizer application rate ranging from 75 - 200 g of fertilizer per 0.16 m? of soil (similar to 4.7 -12.5 to ha^{-1}), a pH range of about 5.5 to 6.5, moisture levels between 11 and 19% total moisture content by weight and tillage rates of about five to seven times per week.

This paper therefore advocates for the utilization of the findings of this study on a large scale basis with common agricultural machines like tractors, bottom ploughs and disks replacing the tools used in the study in order to achieve a cost effective reclamation of petroleum- hydrocarbon polluted agricultural soils.

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