BIOREMEDIATION OF A PETROLEUM-HYDROCARBON POLLUTED AGRICULTURAL SOIL AT VARIOUS LEVELS OF SOIL TILLAGE IN PORTHARCOURT, NIGERIA

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ABSTRACT

A combination of field cells involving a control and five treatment cells were evaluated under field conditions in the bioremediation of a petroleum- hydrocarbon polluted agricultural soil over a six-week period. Previous works have indicated that crude oil contamination of soils depletes oxygen reserves in the soils and slows down its diffusion rate to the deeper layers. Hence, this hypothesis was tested in the study by the treatments employed. The treatment option used was the application of mineral fertilizer, and different rates of oxygen exposure through various levels of soil tillage. In the experiments described in this paper, conditions of a major spill were simulated by sprinkling crude oil on the cells using perforated cans. The treatment applications were then resorted to and relevant soil physicochemical characteristics monitored at intervals. The results of the study showed an enormous increase in total heterotrophic bacterial (THB) counts in all the treatment cells. The percentage reduction in total hydrocarbon content (88% to 99%) experienced in the cells that received treatment were significantly different from the control. These results highlight the view that the availability of large amounts of oxygen in the soil profile induces an accelerated biodegradation of petroleum hydrocarbons in a polluted agricultural soil and implies that regular tillage of contaminated soils in the presence of nutrients could achieve the decontamination of such soils.

Keywords: *Bioremediation; petroleum pollution; oxygen exposure; soil physicochemical characteristics*

NOMENCLATURE

HUB	Hydrocarbon utilizing bacteria	C	Carbon
		N	Nitrogen
		CH_4	Methane
THC	Total hydrocarbon content, mg kg^{-1}	Cfu/ml	Colony forming unit per milliliter
THB	Total heterotrophic bacteria	NPK	Nitrogen, Phosphorus, Potassium
LSD	Least significant difference		
ANOVA	Analysis of variance	R	Correlation coefficient
EC	Electrical conductivity, mS cm^{-1}	%	Percent
		G level	Significance level
C/N	Carbon / Nitrogen	Vs	Versus

1.0 INTRODUCTION

Industrialization has brought about an ever-increasing dependence on petroleum hydrocarbons. This, in turn has resulted in the pollution of the environment in several dimensions. One common experience in areas where exploration and development activities are prevalent is the contamination of existing and potential agricultural lands with petroleum hydrocarbons. Oil spills create an imbalance in the carbon- nitrogen ratio at the spill site, leading to a nitrogen deficiency in an oil-soaked soil which retards the growth of soil bacteria and the utilization of carbon sources [1]. Also, large amounts of biodegradable organics within the soil profile deplete oxygen reserves in the soil and slow down the diffusion rate of oxygen to the deeper layers [2]. This leads to the recalcitrance of petroleum hydrocarbons in soils until remediation measures are undertaken. The current trend of heightened environment awareness demands concerted efforts in order to achieve the cleanup of contaminated sites in compliance with environmental quality standards. As a result of these environmental concerns, bioremediation of organic waste is becoming an increasingly important method of waste treatment due to its advantage which include inexpensive equipment, environmentally friendly nature of the process and simplicity [3]. This has occasioned several studies on various aspects of bioremediation. In Nigeria, there is a dearth of literature on the optimum conditions for effective biodegradation. Obahiagbon and Erhabor [4] compared with the effectiveness of treating refinery wastewater with pseudomonas and that of using Fusarium, and found the latter to be more effective. Analysis of biodegradation rate of crude oil contaminated soil using fertilizer or cow dung showed that fertilizer was a better nutrient source for biostimulation than cow dung [5]. It had been shown that oil is degraded efficiently by oil - oxidizing micro-organisms and their efficiency in degradation can be enhanced by application of special fertilizers [6, 7]. Field deployment of this biotechnology has also yielded good results in the treatment of oil spill in Exxon Valldez incident in Prince William Sound and in the gull of Alaska.s

In this bioremediation study, biostimulation option which entails the addition of appropriate microbial nutrients to a waste stream was utilized with the objective of stimulating the indigenous microbial flora of the waste to bring about its degradation [8]. A nitrogenous fertilizer was used to supply the much needed nitrogen while different levels of oxygen was applied to determine its degree of importance in the biodegradation process. Thus, the nutrient and oxygen deficiencies were corrected by the treatment applications. The objectives of the study were to determine the effects produced by different levels of oxygen in the soil profile during biodegradation and to assess the rate of tillage (or oxygen exposure) that would be optimum for the clean up of petroleum hydrocarbons in polluted agricultural soils.

2.0 MATERIALS AND METHODS

2.1 Field sites

The field cells were situated 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 3000 mm and an average temperature of 27° C. The vegetation cover is the tropical rain forest [9].

The field cells were made into beds of 40cm x 40cm dimension with depths of about 30 cm. The original idea behind the bed formation was to control the fate of the contaminant especially as regards run off to near by lands; since the experiment was

conducted amidst the rains (June to August 2005) .The beds gave room for the control of the depth and exposed surface area of the soil, and in turn the temperature, nutrient concentration and oxygen availability [10].

Cell 0, was the control (no treatment employed), cell A was tilled once a week, cell B was tilled three times per week, cell C received five times tillage per week, while cells D and E were labeled for a once-daily and twice daily tillage respectively.

2.2 Experimental procedure

Perforated cans were used to sprinkle crude oil on all the cells (including the control) at the rate of 0.8 liters of crude oil per 0.16 $m^2$of soil. The objective was to simulate conditions of a major spill. The cells were left undisturbed for three days. The treatment applications commenced after the three day period. The treatments involved the application of 50 g of 20-10-10 NPK fertilizer twice during the study period at an interval of two weeks. The broadcast method was used for fertilizer application, such that it was estimated that each cell received 1250 Kg ha^{-1} 0 nitrogen during the six-week remediation period. Tilling was done at the aforementioned rates with cutlasses and shovels to about 30cm depth. The objective was to control the levels of oxygen exposure in the cells and also to facilitate the mixing of nutrients and microbes with contaminated soil.

Soil samples were collected periodically for analyses. This was dam using a hand dug soil auger. The cells were augured to 30 cm depth at different random spots. The samples collected were bulked together (composite soil samples) and put in well labeled polyethylene bags and glass bottle: sealed with aluminum foil (samples for THC measurements) to guarantee quality results. The samples were immediately transferred to the laboratory for analyses

2.3 Laboratory / statistical methods

Relevant soil physicochemical characteristics including particle size analyses, moisture content, pH, electrical conductivity, total hydrocarbon content (THC), organic carbon and total nitrogen were determined using methods adapts from Black et al. [11] and Jackson [12]. The total heterotrophic bacterial count was performed on a nutrient agar (oxoide using the spread plate method [13] Methods derived from Wrenn and Venosa [14] were also employed in the enumeration process. Statistical method such as least significant difference (LSD Analysis of variance (ANOV A) and Correlation coefficient adapted from Finney [15] were employed to analyze data.

3.0 RESULTS AND DISCUSSION

The hypothesis that crude oil contamination of agricultural soils limits oxygen availability to soil biota has been substantiated by the findings of this study. This could be observed form the results of the study presented in Tables 1-4 The significant increase in bacterial counts during the experimental period (Table 5) shows that hydrocarbon utilizing bacterial (HUB) population are sensitive to different levels of oxygen in the soil.

Soil moisture dropped in the various cells after crude oil contamination. This is expected because in heavily polluted soils water droplets adhere to the hydrophobic layer formed, and this prevents wetting of the inner parts of the soil aggregates. A similar observation was made by Odokuma and Dickson [8]. As the cells were remediated through the introduction of fertilizers and continuous tillage, the moisture content increased and later dropped again (Tables 3 and 4). The tilling of the soil gave way for continuous soil drying and evaporation and thus maintained an optimum moisture level for biodegradation. Statistical

analysis of these data indicated significance at the 1% probability level (Table 6).

Table 1: Soil physicochemical characteristics before crude oil contamination. (Results represent means ± standard deviation of treatment cells)

%				pH 1:2.5	EC $\mu Sc^{-1}m$	THC $mgkg^{-1}$	%		C/N ratio
Sand	Silt	Clay	Moisture by mass				OC	TN	
15.3 ± 0.6	45.2 ± 0.3	39.5 ± 0.5	17 ±1	4.73 ± 0.6	34 ± 6	46 ± 5	0.18 ± 0.02	0.36 ± 0.03	0.5 ± 0.02

Table 2: Soil physicochemical characteristics three days after contamination, prior to remediation. (Results represent means + standard deviation of three replicates).

Cell	**pH 1: 2.5**	**EC $\mu S\ cm^{-1}$**	**THC $mg\ kg^{-1}$**	**% Moisture by mass**	**%**		**C/N ratio**
					Organic C	**Total N**	
0	6.25±0.25	128 ± 4	16150±150	13 ± 1	0.87 ± 0.04	0.41 ± 0.03	2 ± 0.2
A	6.25±0.15	132 ± 3	16742 ± 50	12 ± 1	0.89 ± 0.04	0.43 ± 0.01	2 ± 0.6
B	6.24± 0.10	134 ± 4	13695 ± 150	4 ± 2	0.87 ± 0.03	0.56 ± 0.03	2 ± 0.5
C	6.22± 0.25	137 ± 3	13510 ± 30	12 ± 1	0.89 ± 0.05	0.58 ± 0.03	2 ± 0.3
D	6.28± 0.20	137 ± 2	18 601± 100	15 ± 2	0.90 ± 0.04	0.67 ± 0.02	1 ± 0.2
E	6.24± 0.20	138 ± 4	18650 ±50	15 ± 2	0.92 ± 0.05	0.69 ± 0.01	1 ± 0.4

Table 3: Soil physicochemical characteristics two weeks after remediation. (Results represent means ± standard deviation of three replicates)

Cell	**pH 1: 2.5**	**EC $\mu S\ cm^{-1}$**	**THC $mg\ kg^{-1}$**	**% Moisture by mass**	**%**		**C/N ratio**
					Organic C	**Total N**	
0	5.45 ± 0.20	47 ± 5	12 236 ± 210	10 ± 1	0.542 ± 0.01	0.13 ± 0.04	4 ± 0.5
A	5.30 ± 0.10	53 ± 15	3646 ± 160	18 ± 1	0.428 ± 0.02	0.14 ± 0.05	3 ± 0.5
B	5.59 ± 0.10	34 ± 8	1003 ± 120	16 ± 2	0.620 ± 0.06	0.30 ± 0.07	2 ± 0.4
C	5.40 ± 0.20	52 ± 7	952 ± 30	14 ± 2	0.573 ± 0.02	0.29 ± 0.06	2 ± 0.6
D	5.43 ± 0.25	49 ± 6	464 ± 50	22 ± 1	0.613 ± 0.04	0.27 ± 0.07	2 ± 0.4
E	5.41 ± 0.10	48 ± 7	339± 20	16 ± 2	0.810±0.06	0.46 ± 0.02	2 ± 0.4

Table 4: Soil physicochemical characteristics six weeks after remediation
(Results represent means ± standard deviations of three replicates)

Cell	pH 1:2.5	EC $\mu S\ cm^{-1}$	THC $mg\ kg^{-1}$	% Moisture by mass	%		C/N Ratio
					Organic C	Total N	
0	5.34 ± 0.30	83 ± 10	9317 ± 105	9±3	0.36 ± 0.05	0.070 ±0.002	5 ± 0.6
A	5.22 ± 0.25	92 ± 9	1917 ± 120	10 ± 1	0.42 ± 0.04	0.094 ± 0.004	5 ± 0.8
B	5.34 ± 0.30	88 ± 6	1038 ± 90	17 ± 1	0.45 ± 0.02	0.141 ± 0.03	3 ± 0.8
C	5.29 ± 0.10	96 ± 8	744 ± 35	9±2	0.48 ± 0.04	0.147 ± 0.03	3 ± 0.7
D	5.36 ± 0.15	93 ± 6	156 ± 40	13 ± 1	0.51 ± 0.06	0.149 ± 0.04	3 ± 0.5
E	6.95 ± 0.15	120 ± 7	764 ± 10	9±2	0.36 ± 0.02	0.148 ± 0.02	2 ± 0.6

Table 5: Total heterotrophic bacterial count.

5	Sampling period [weeks]		
	0	2	6
	X 10^6[Cfu/ml]		
0	0.73	2.83	3.72
A	0.71	2.75	3.28
B	0.90	1.27	2.52
C	0.92	1.38	1.68
D	2.07	2.33	2.84
E	2.09	2.56	3.48

Table 6: The relationship 'between time and some measured soil characteristics.

Correlation regression factor	r	g Level	Significance equation
Time vs moisture	-0.68	**	Y=14.86+0.77x
Time vs PH	-0.68	**	Y=6.25-0.019x
Time vs THC	-0.078	ns	Y=12 046-315x
Time vs Organic C	-0.95	**	Y=1.30-0.01x
Time vs Total N	-0.85	**	Y=0.65-0.0095x

ns: not significant **: significant at 1% probability level

The mean pH values for the cells two .and six weeks after treatment ranged from 5.2 to 6.0. This implies that biodegradation treatments are favored more at pH values about the acid side of neutrality [16]. A major soil parameter that indicated substantial remediation of the cells was the enormous decline in the organic carbon content of the soil. The percent organic carbon increased significantly after crude oil contamination of the cells but later dropped as remediation treatment progressed. Statistical analysis showed that the relationship between remediation period and

organic carbon showed a negative correlation that indicated significance at the 1 % probability level. This suggests that organic carbon reduced with time.

The marked reduction in the percentage of THC reduction in all the treatment cells was quite different from the one observed in the control. This confirms the view that biodegradation is impeded by nutrient deficiency as well as the depletion of oxygen reserves in crude oil contaminated soils. This is evident from the results in Table 6. After six weeks of remediation the cells that received different levels of oxygen exposure in the presence of nutrients had percentage THC reductions of 88.5%, 92.4%, 94.5%, 99.2% and 95.4% (Table 7 and Figure 1). The implication of these results is that different levels of oxygen in the soil induce different biodegradation rates, and that oxygen is a major limiting factor in biodegradation. It was observed from the field cells that at certain levels of tillage the soil became compacted hence oxygen availability was limited. This was the case with cell E. After two weeks of constant tillage (twice daily), there was 98.2% reduction in THC but at the end of the six-week period the THC slightly increased thus yielding a 95.9% reduction (Table 7). This was probably due to the creation of anoxic zones in the soil such that the facultative microbes resumed anaerobic biodegradation in place of aerobic biodegradation. One of the products of anaerobic biodegradation, methane (CH_4) could most likely cause such slight increases in THC.

The microbiological analysis showed that bacterial types like Pseudomonas, Bacillus, Micrococcus, Alkaligenes, Flavobacterium, Serratia, Aerococcus and Corybacterium were the hydrocarbon utilizing bacterial population present in the degradation process. Table 7: Percentage reduction in THC

5	Sampling Period (weeks)	
	2	6
0	25.9%	45.6%
A	78.2%	88.5%
B	92.7%	92.4%
C	93.0%	94.5%
D	97.5%	99.2%
E	98.2%	95.9%

4.0 CONCLUSION AND RECOMMENDATION

From the results of the study it is evident that hydrocarbon utilizing bacterial population responds to the amount of oxygen available in the soil. Hence at higher and optimum levels of oxygen exposure they would effect accelerated biodegradation. These result showed that tillage rates ranging from to seven times a week are optimum an accelerated biodegradation.

The findings of this study confirmed the position that crude oil contamination of agricultural soils limits the availability of oxygen in the soil layers and h impedes the biodegradation process. It stands to reason therefore that for an accelerated recovery of natural vegetation on contaminated agricultural soils must be regular exposure of the oxygen supply. This paper therefore advocates for regular tillage of crude oil contaminated agricultural soils in the presence of nutrients like NPK fertilizer since this could achieve the decontamination of such soils.

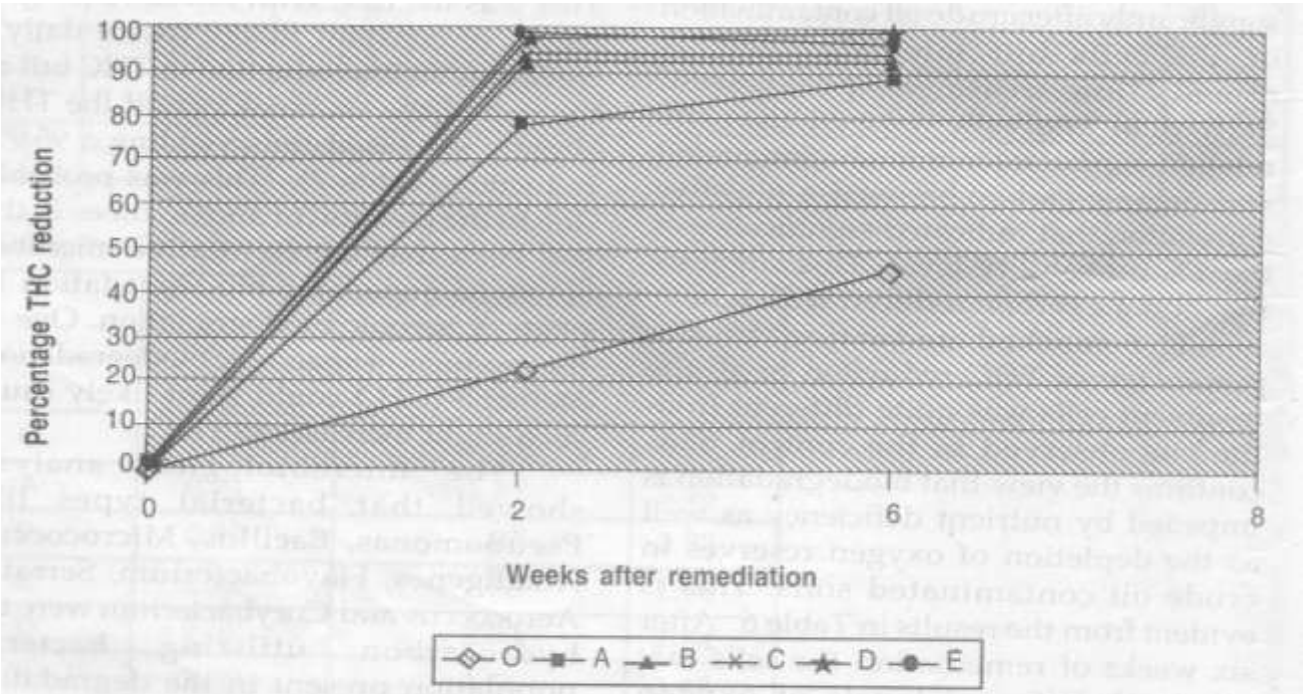

Fig. 1: Rate of hydrocarbon loss.

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