



Bioremediation of Oil Polluted Arable Soil by Enhanced Natural Attenuation

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Abstract:

This study evaluated the efficacy of microorganisms in total hydrocarbon (THC) degradation under the influence of tillage, nutrient supply (NPK Fertilizer) and liming. The treatment options were: Reduced tillage + Application of fertilizer (treatment A); soil mixing (contaminated + uncontaminated soils) + Application of fertilizer + conventional tillage (Treatment B); Application of fertilizer + conventional tillage (treatment C); then control. The second segment of the treatment options consisted of replication of treatments A, B and C and the addition of lime. The findings show that treatment C1 (Application of fertilizer + convention tillage + liming) recorded the highest population of hydrocarbon utilizing bacteria (HUB) of $67,000 \times 10^3$ cfu/g with 93% reduction of THC. This was followed by treatment B1 (soil mixing (contaminated + uncontaminated soils)) + Application of fertilizer + conventional tillage + liming) with HUB count of $56,000 \times 10^3$ cfu/g and percentage THC reduction of 86%; and then treatment C having HUB of $48,000 \times 10^3$ cfu/g and THC reduction of 75%. Results of all the options were significantly ($P=0.05$) higher than the control.

Keywords: Bioremediation, Oil Pollution, Natural Attenuation, Degradation

1.0 Introduction:

One of the major polluters of the environment is the petroleum industry. In its production, processing and distribution of petroleum and its products, oil spillage occurs despite careful handling and containment (Atlas, 1981). The pollution is as a result of pipeline rupture, losses from shipping, illegal disposal of bilges and waste oil, accidental operations, natural seepages, well blowouts and sabotage resulting in the release of crude and refined oils into the aquatic and terrestrial environments. Oil pollution on land has negative impact on soils and plants (Gundlach et al., 1977; Odu and Nwoboshi, 1985; Ngobiri et al., 2007). This is the unfortunate situation confronting the indigenes of Nkali community in the oil-rich Niger Delta of Nigeria. Oil pollution has adversely affected their agriculture, public health and socio-economic wellbeing to an extent that a community once noted for high agricultural output is now experiencing decreased rate of crop production. The poor crop yield has forced most of their farmers out of their major source of livelihood, thus making them to migrate or engage in negative social vices that are

detrimental to their general development. In addition, oil pollution has denied the community of the aesthetic nature of their agricultural land which they much cherished. It is in an attempt to solve these problems that led to the concept remediation.

Remediation of oil impacted soil can be achieved through physico-chemical and biological methods (Long, 1993). Microbial remediation and how it works has been described by Shelton and Tiedje (1984); Scheibenbogen et al. (1994); stoner (1994); and Onwurah et al. (1998). This procedure is preferable because it causes less damage to the environment and results from such clean up technology show that this method is effective, safe to humans and environmentally friendly (Onwurah, 2000). However, the full benefits of bioremediation have not been realized because processes and organisms that are effective in controlled laboratory tests are not always equally effective in full scale applications (Forsyth et al., 1995). In part also, there is lack of understanding of most suitable environmental conditions for bioremediation processes to work in the affected area. Efforts were

therefore made in this study to determine the influence of some factors in microbial degradation of oil contaminants.

2.0 Materials and Methods:

2.1 Field Experiment:

The study was carried out in oil polluted medium textured soil in Nkali, Niger Delta, Nigeria. Eight experimental plots measuring 5m² were marked out in the polluted area for treatment in the presence of HUB. Four of the plots were treated with the following treatment options: A (Reduced tilling + fertilizer application); B (soil mixing (contaminated + uncontaminated) + fertilizer + conventional tilling); C (Fertilizer + Conventional tilling); control. The second segment involved the following treatments. A1 (Reduced tilling + fertilizer application + liming); B1 (soil mixing (contaminated + uncontaminated) + fertilizer application + conventional tilling + liming); C1 (Fertilizer application + conventional tilling + liming); control.

The treatment sections were separated with wooden planks to a depth of 60cm below the soil surface. Limed and un-limed areas were also separated by a two-meter gap. The experiment could not be replicated because of strict restriction placed by the community on space. Representative soil samples of

the area were collected at 30cm depth using soil auger. This was done prior to treatment to enable comparison of efficacy of treatment options. The soils were then sampled and analyzed for physico-chemical and biological properties.

2.2 Laboratory Analyses:

The following parameters were analysed from the soil samples. Moisture content was determined according to the procedure described by Laverty (1977). pH was determined using a pH meter in a 1:1 soil water suspension. Total hydrocarbon content (THC) was done by spectrophotometric method. The organic carbon was determined by wet oxidation method (Walkley and Black, 1934). The procedure according to Brenner (1965) was adopted in determining total N. Available phosphorus was by Bray and Kurtz (1945) method while the hydrocarbon utilizing Bacteria (HUB) was also estimated. Liming procedure was as described by Miller and Donahue (1992).

3.0 Results and Discussion:

Table 1 shows the soil physico-chemical properties prior to treatment, while tables 2 and 3 are data generated twelve weeks after treatment for the un-limed and limed areas respectively.

Table 1: Soil physico-chemical properties of the study area prior to treatment.

Treatment	Moisture Content (%)	pH	THC (PPM)	Organic C(%)	Total N(%)	C/N Ratio	Available P(PPM)
A	26	5.42	11,480	4.61	0.04	90.25	3.02
B	26	5.01	12,610	3.02	0.06	50.33	2.60
C	25	5.42	13,109	4.81	0.12	40.08	2.51
Control	26	5.50	13,540	3.72	0.07	53.14	2.62

Table 2: Soil physico-chemical properties of the sample 12 weeks after treatment in un-limed section.

Treatment	Moisture Content (%)	pH	THC (PPM)	Organic C(%)	Total N(%)	C/N Ratio	Available P(PPM)
A	28	4.61	6641	2.41	0.17	20.06	4.21
B	27	4.86	4862	1.50	0.16	25.19	5.46
C	28	5.11	3254	2.14	0.18	17.44	5.72
Control	27	4.50	12,680	6.42	0.05	128.0	3.21

Table 3: Soil physico-chemical properties 12 weeks after treatment in limed section.

Treatment	Moisture Content (%)	pH	THC (PPM)	Organic C(%)	Total N(%)	C/N Ratio	Available P(PPM)
A1	29	6.5	3850	1.36	0.16	8.50	5.05
B1	30	6.7	1940	1.14	0.21	5.43	6.10
C1	29	6.7	950	1.84	0.30	6.13	6.55
Control	27	4.9	12820	6.25	0.04	131.25	3.29

There was a significant ($P=0.05$) reduction of total hydrocarbon content in both un-limed and limed sections at the end of the 12 weeks of the experiment, except for the controls. In plots A,B,C, and control of the un-limed section, the THC reduced by 45%, 61%, 75% and 6% in that order (table 4). Plot A (reduced tillage + fertilizer) degraded the hydrocarbon in the polluted site but did not have a good result probably due to poor aeration and low surface area which is not conducive for microbial processes. Treatment B which is soil mixing (contaminated + uncontaminated soils) + Application of fertilizer + conventional tilling) showed hydrocarbon degradation but the result was comparably low and this can be attributed to low rate of adaptation of the exogenous HUB to the new soil environment. The best result of HUB degradation was obtained from treatment C (Addition of fertilizer + conventional tilling) (fig.1). This is probably because of the use of indigenous HUB, provision of good surface area and adequate aeration.

Table 4: Percentage hydrocarbon reduction for the un-limed section.

Treatment	Sampling Period (weeks)			
	3	6	9	12
A	28%	35%	40%	45%
B	40%	48%	55%	61%
C	51%	64%	70%	75%
Control	1%	2%	4%	6%

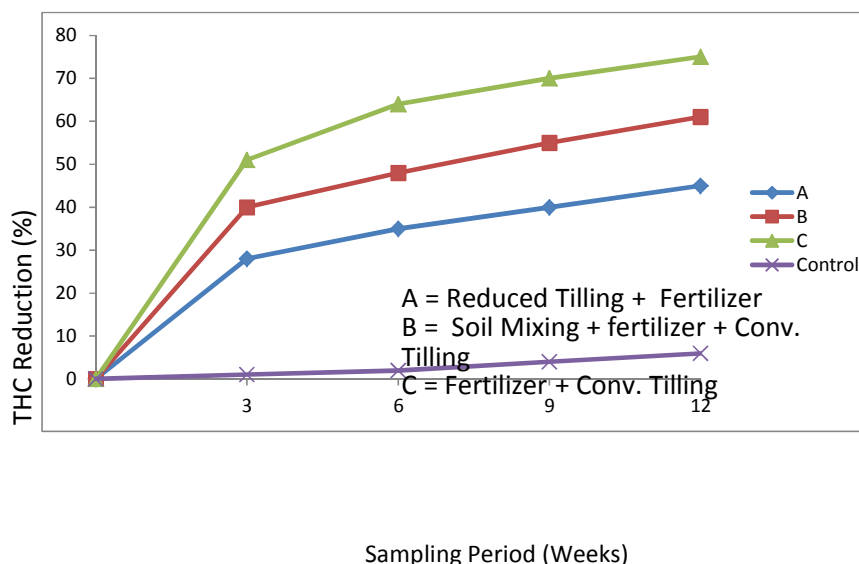


Fig 1: Relationship between sampling period and THC Reduction in un-limed section.

However, limed plots (A1,B1, and C1,) (Table 5) had higher percentage of THC reduction than the un-limed section at the end of the experiment. The data obtained were A1 (52%), B1 (85%), and C1 (93%) (Fig.2). These values were higher than that of un-limed treatment and control. The differences were probably as a consequence of liming. Liming raises soil pH thereby eliminating most major problems of acid soils, including excess (toxic) soluble aluminum and very slow microbial activity (Miller and Donahue, 1992).

The HUB showed a significant (P=0.05) increase in the un-limed and limed treatments than the controls. In the un-limed section, HUB population ranged from 4×10^3 cfu/g to $48,000 \times 10^3$ cfu/g (0-12wks) with plot C having the highest count. The

control varied from 4×10^3 cfu/g to 66×10^3 cfu/g for the same period (table 6). Similarly, the HUB values for the limed section ranged from 4×10^3 cfu/g at zero week to 67000×10^3 cfu/g at the 12th week. The control value were from 5×10^3 cfu/g to 65×10^3 cfu/g. Treatment C1, also had the highest population of 67000×10^3 cfu/g (table 7). High HUB values obtained from un-limed treatments compared to the control was probably due to the influence of tillage and nutrient. The higher values of the data from limed plots as against that of un-limed treatment could be attributed to the influence of lime application on soil pH and by extension to microbial count. With higher HUB, more of the THC will be reduced fast.

Table 5: Percentage hydrocarbon reduction for the limed section

Treatment	Sampling Period (weeks)			
	3	6	9	12
A1	33%	45%	58%	66%
B1	49%	61%	73%	85%
C1	61%	76%	85%	93%
Control	1%	2%	4%	5%

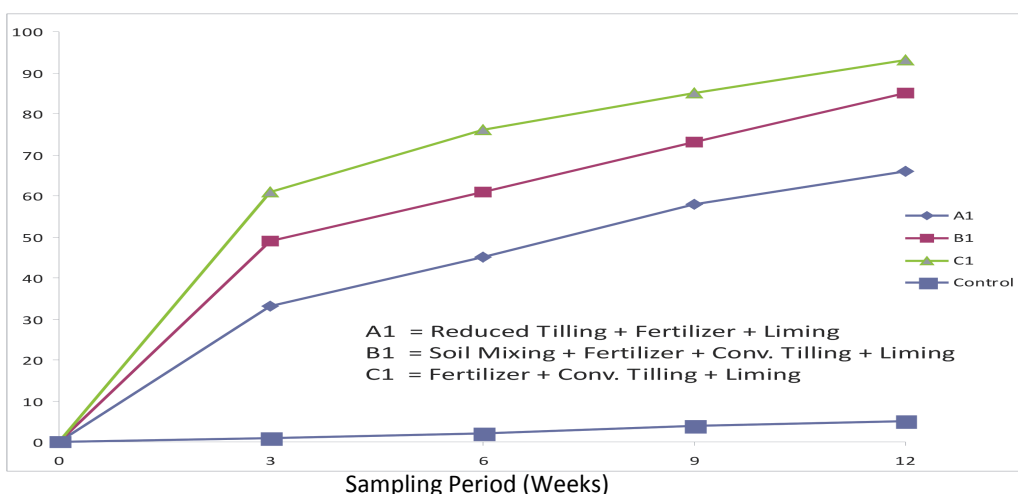


Fig 2: Relationship between sampling period and THC Reduction in limed section

Table 6: HUB Count of the soil samples from the un-limed plots.

Treatment	Sampling Period (weeks)				
	0	3	6	9	12
	($\times 10^3$ cfu/g)				
A	4	70	360	21,000	32,000
B	4	110	420	36,000	44,000
C	4	182	522	42,000	48,000
Control	5	25	50	58	66

Table 7: HUB Count of the soil samples from the limed plots.

Treatment	Sampling Period (weeks)				
	0	3	6	9	12
	($\times 10^3$ cfu/g)				
A1	5	250	15,000	29,000	38,000
B1	4	570	25,000	42,000	56,000
C1	5	720	39,000	58,000	67,000
Control	5	28	47	61	65

Soil moisture for the microbial activity was suitable. It ranged from 26-27% in the control and 25 to 29% in other treatments. These values are within the limits (25-85%) stipulated by the United States Environmental Protection Agency (USEPA) (1989). The pH of the control treatment decreased from 5.6 at the beginning of the experiment to 4.9 by the 12th week (tables 1 and 4). This difference was probably as a result of CO₂ evolution and the release of organic acids following organic residue decomposition (Ranga Swami and Bagyaraj, 1993). Low pH is not a conducive condition for microbial activities. It also reduces the availability of phosphorus through the formation of insoluble iron and aluminum phosphates (Miller and Donahue, 1992).

Data from the un-limed plots indicate that phosphorus concentration increased significantly (P=0.05) except the control (table 2). This could be attributed to the addition of NPK fertilizer which released the element into the soil. Fertilizer addition in bioremediation process is important because many oil contaminated sites contain organic matter that are rich in carbon but deficient in Nitrogen and phosphorus (Atlas and Bartha, 1973). Effect of liming on phosphorus concentration is reflected in table 3. The phosphorus content was higher than control and un-limed treatments. Liming increases soil pH which increase the solubility and availability of phosphorus.

There was a significant (P=0.05) increase in organic carbon content in the control and a decrease in other treatments (tables 1, 2 and 3). The increase in the control could be attributed to the addition of carbon from the breakdown of hydrocarbon material, while the decrease observed in other treatments was probably due to increased immobilization of the element by high population of the microbes. The variations in carbon content affected the C/N ratio of the soil (tables 1, 2, and 3). The values increased in the control but decreased in

other treatments. The trend in the control could be as a result of more organic carbon from hydrocarbon degradation and low Nitrogen level in the control plots. In other treatments, the decrease observed could be because of higher utilization of carbon by high microbial population which reduced the carbon level while addition of fertilizer increased nitrogen concentration. Comparatively, limed treatments (table 3) had lower values of organic carbon and C/N ratio than the un-limed treatments (table 2). Liming is probably responsible through its influence on providing more suitable condition for microbial multiplication. Higher microbial population means more organic carbon will be utilized by the microbes.

4.0 Conclusion:

This study investigated the most appropriate conditions for microbial remediation of oil contaminated soils. The findings show that treatment option C1 (fertilizer application + conventional tilling + liming) recorded the highest hydrocarbon reduction. In addition, the treatment had the highest HUB build up which was the major active agent in hydrocarbon degradation. The results further showed that fertilizer application actually provided the soil with essential nutrients especially N and P needed to support microbial growth, while liming helped create a very conducive environment for their activities. It can therefore be concluded that nutrient-enhanced in-situ bioremediation is the most rapid, efficient, cost-effective, and environmentally friendly remediation option for all polluted soils. All that is needed is the application of the right types and quantities of nutrients, and providing the right environmental conditions for the indigenous HUB to multiply.

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