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A Novel Approach to Bioremediation of crude oil contaminated Land in the Niger Delta: A case Study of Ogoni Oil Polluted Area.

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Abstract: *This paper looks at the adverse effects of petroleum exploration, production, and transportation, resulting in frequent cases of oil spillage and environmental pollution in Ogoni area of the Niger Delta. Bioremediation is a means of restoring crude oil affected areas/environments by employing the diverse metabolic abilities of micro-organisms to convert any chemical, biological or physical substances to harmless products by mineralization, generation of carbon (IV) oxide and water. The ability of micro-organisms present in poultry wastes to break down the long chain hydrocarbon in crude oil to more useful molecules that are less harmful and friendly to the environment were studied using an experimental set up. Three containers of soil samples were collected and analyzed using Agilent GC equipment and gas Chromatography. The results obtained after two weeks of exposing the soil samples studied at different conditions showed a considerable reduction in the Total Petroleum Hydrocarbon (TPC) content of the poultry waste treated soil, when compared with a control soil sample subjected to similar conditions but without the poultry droppings treatment. As a result, this process offers an efficient and cost-effective way of treating contaminated ground water and soil with its advantages far outweighing the disadvantages.*

Keywords*-Bioremediation,environment, crude oil, Poultry waste, pollution*

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I. Introduction

Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which possesses measurable toxicity towards living systems [1]. The increase in demand for crude oil as a source of energy and primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment.

The sources of petroleum pollution include low-level discharge from oil wells, sabotage, cleaning operations as well as oil treatment. These sources together account for about 90% of total anthropogenic petroleum pollution. Similarly, oil pollution also comes from oil-well blowouts, seepage, and destabilizing operations, sale and use of petroleum products, pipeline overflow and breakage, and storage tank spills [2]. Addition of oil to the soil as a deliberate policy of waste disposal also leads to contamination of land. Omokaro and Amusan[3] reported a deliberate discharge of oilfield wastewater or effluent as a source of environmental contaminant.

Bioremediation is the process used to describe biological strategies applicable to repair of damaged environment using biological factors., or by conversion into microbial biomass (Baggott, 1993;[4]. Bioremediation may be employed in order to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken using multiple techniques including the addition of fertilizer to facilitate the decomposition of crude oil by bacteria. It is also the most effective management tool to manage the polluted environment and recover contaminated soil. A lot of experimental work has been carried out on bioremediation as a results of oil spillage.

Kamaludeen[5] had concluded in their work that bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water. Strong & Burgess [6] found out that bioremediation uses biological agents mainly microorganisms such as yeast, fungi or bacteria to clean up contaminated soil and water. These methods rely on promoting the growth of specific micro flora or microbial consortia that are indigenous to the contaminated sites, which are able to perform desired activities [7]. Establishment of such microbial consortia can be done in several ways. For example, by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions, among others (Hess et al., 1997; Agarwal, 1998; Smith et al., 1998). Establishment and maintenance of favorable conditions for microbial growth and process control are basic prerequisites [7]. Hess et al.[8]and Agarwal [7] conclude that microorganisms use contaminants as nutrient or energy sources in bioremediation processes.

Effect Of Oil Pollution On Production

Whenever there is a damage to oil pipelines or other facilities connected to the surface production facilities leading to oil spillage, the first and usually the most reasonable action is to shut-in the oil production. This not only helps reduce wastage of the precious resources but more importantly helps prevent further damage to the impacted environment (land, swamp or water).

In the Niger Delta region of Nigeria, millions of barrels of crude oil are shut in due to damages caused by sabotage, Negligence from the oil companies, equipment failure or human error. In 2009, it was reported that production losses represented more than 20 per cent of Nigeria's total production capacity of 2.5million bpd [9]. In the same year, The United States' Energy Information Administration was quoted to have estimated that from December 2005 to December 2007,. Nigeria lost an estimated $16 billion in export revenues due to shut-in oil production. Shell incurred the majority of shut-in oil production (477,000 bbl/d), followed by Chevron (70,000 bbl/d) and Agip (40,000 bbl/d). (ibid)

Fig 1: Oil spill impact on land and water area of the Niger Delta

Fig 2: Spill due to drilled hole on a flow pipeline in the Niger Delta

Fig 3: Part of spill impact at 20" Kolocreek-Rumuekpe Pipeline right of way at Ogbogoro (SPDC 2011).

Fig 4: Spill due to failed clamp on 20" Kolocreek-Rumuekpe Pipeline right of way at Ogbogoro (SPDC 2011).

Approaches To Bioremediation

In-Situ Bioremediation

In-situ bioremediation involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and absorbed contaminants and use them as substrates for transformation [10]. This means there is no need to excavate or remove soils or water in order to accomplish remediation. In-situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater.

The in-situ technique includes conditions such as the infiltration of water containing nutrients and oxygen or other electron acceptors for groundwater treatment [11]. Most often, in-situ bioremediation is applied to the degradation of contaminants in saturated soils and groundwater. It is a superior method to cleaning contaminated environments since it is cheaper and uses harmless microbial organisms to degrade the chemicals. Chemotaxis is important to the study of in-situ bioremediation because microbial organisms with chemotactic abilities can move into an area containing contaminants. By enhancing the cells' chemotactic abilities, in-situ bioremediation will become a safer method in degrading harmful compounds.

Ex-Situ Bioremediation

Ex-situ bioremediation is a different approach that utilizes specially constructed treatment facility. It is more expensive than in-situ bioremediation. This process requires excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. This technique has more disadvantages than the advantages; one of which is that ex-situ bioremediation techniques involve the excavation or removal of contaminated soil from ground.

Depending on the state of the contaminant to be removed, ex-situ bioremediation can be classified as: solid phase system (including land treatment and soil piles) and slurry phase systems (including solid-liquid suspensions in bioreactors)

II. Materials And Methods

MATERIALS

The materials used for this work consistof3 Plastic containers of equal sizes,freshly collected garden soil,sample crude oil,Decomposing Poultry Droppings in a saw dust substrate and Agilent GC Equipment. The study area where samples were collected for this work is Kpean where Yorla oilfield is located in Khana Local Government Area of Rivers State. These areas had been deeply polluted as a result of neglect of SPDC equipment, sabotage of oil pipelines and blow-out from well head and Christmas trees.

METHODS

The methods used for restoring oil-polluted sites vary from complete removal of the affected soil to doing nothing at all and "letting nature take its course" [12]. Natural revegetation of an area affected by light crude oil spillage has occurred without any special treatment [13, 14]. At low levels of contamination of crude oil, cultivation of soil without nutrient amendment is possible.

Physical methods such as incineration may destroy indigenous organisms, including oil-degrading microbes, and increase the toxicity of the petroleum residue. Sinking the oil with heavy hydrophobic agents such as ground chalk merely removes the oil to anaerobic sediments or deep ocean floor, where long persistence of the oil pollutant is bound to occur. Large quantities of oil accumulating on the bottom foul the ocean floor and also tend to coalesce and rise again as large droplets. Mechanical removal of stranded oil from sand dunes or salt marshes is far more damaging than leaving it alone: Not only is the ecological balance disturbed, but the aesthetic effect may also be irreparable [15].

Chemical methods for removing or dispersing spilled oil from the environment were condemned by Nelson-Smith [1] because of their side-effects on the ecosystem and their toxicity, which is sometimes more pronounced than that of the oil itself. Chemical dispersants may inhibit microbial activity by damaging cell membranes or essential enzymes, or by altering the surface tension of the water in which microbes live. Furthermore, dispersed oil is never recovered from the environment, and its ultimate fate remains unknown.

Experimental Set-Up

The purpose of this work is to verify the ability of microorganisms to break down crude oil-based hydrocarbons in impacted soils and also to test the relative time it will take for remediation to take place. This involve 4 stages:

- Collection of local soil samples
- Contamination of the samples with collected crude oil.
- Treatment of selected test sample with poultry droppings
- Analysis of the samples for extent of petroleum related hydrocarbon degradation/ remediation using Gas Chromatography (GC).

Sampling Method

Equal volumes of garden soil, collected from the same source and initially contaminated with crude oil in container X are put into separate plastic bowls labeled Y and Z. From these, three separate test samples (X, Y and Z) are collected and tested for Total Petroleum Hydrocarbon (TPH) using Gas Chromatography (GC). The sample labels X, Y and Z has the following representation:

SAMPLE X: Portion of the Original Soil Sample impacted with heavy duty motor oil.

SAMPLE Y: Original impacted soil sample treated with poultry droppings and analyzed 2 weeks after preparation.

SAMPLE Z: Original impacted soil sample left to stand for 2 weeks as sample Y without any other form of treatment. It is analyzed at the same time with sample Y and is expected to serve as control.

Experimental procedure

About 4000cm^3 of freshly garden soil was collected in a container and pour into 600cm^3 of crude oil. It was allowed to completely percolateand then mixed properly with the aid of a scapula. Another 2000cm^3 of the

contaminated soil sample was measured into two separate plastic bowls labeled Y and Z respectively. The samples Y and Z above were allowed to stay up to 12 hours before it was mixed thoroughly using a spatula. Equal quantities were also collected with the aid of a spoon from both sample containers Y and Z and mixed in a small sample dish labeled X. Sample X was allow to stayed for another 12hours before taking for analysis to determine initial TPH.

Similarly, 600cm^3 of poultry droppings was put into sample Y and mixed thoroughly. Both samples Y and Z were allowed to stand for another 14 days while maintaining the moisture content, and turning them every 48hours to allow for aeration and encourage microbial activity. After 14 days, equal quantities of sand were measured out from the plastic bowls Y and Z, and was placed in small sample dishes labeled Y and Z respectively as was done with small sample dish X. Finally, both samples dishes Y and Z were taken for analysis to determine the TPH after 14 days.

Fig (5a): X - Fresh Soil Samples

Fig (5b): Contaminated Samples Y and Z

Fig (5c): Treated Sample Y

III. Results And Discussions

This paper aims to find out if all the introduction of microorganisms (contained in poultry) will accelerate the breakdown of the long chain hydrocarbons present in petroleum and its fractions. Three samples X, Y and Z containing soil samples subjected to different conditions were sent for analysis. The results obtained from the Gas Chromatography analysis are here presented and discussed.

Figure 6 shows the response of various carbon chains in the soil sample and the corresponding concentrations in parts per millions. As can be seen from the data generated by the analysis of the sample (Table 4.1), that up to 16 different carbon-chained compounds are identified (i.e C_9 C_{16}, $C_{19\text{-}27}$, $C_{30\text{-}33}$). The Total Petroleum Hydrocarbon (TPH) that is cumulative of $C_{19\text{-}38}$ which is indicated by their concentration in the extract is approximately **80.93182ppm**.

The concentration of the analyte in mg/kg can be determine using the relationship as:

Concentration in$\left(\frac{\mathbf{mg}}{\mathbf{kg}}\right) = \frac{\mathbf{A \times B}}{\mathbf{C}}$

Where: A = Data generated by the Agilent GC equipment in µg/ml.

B = Total volume of extract in ml.

C = Weight of sample extract in g.

A = 80.93182ppm = 80.93182 µg/ml; B = 30ml; C = 2g

Therefore,

Concentration (TPH) in sample X = 80.93182 x 30/2 = 1,213.97mg/kg

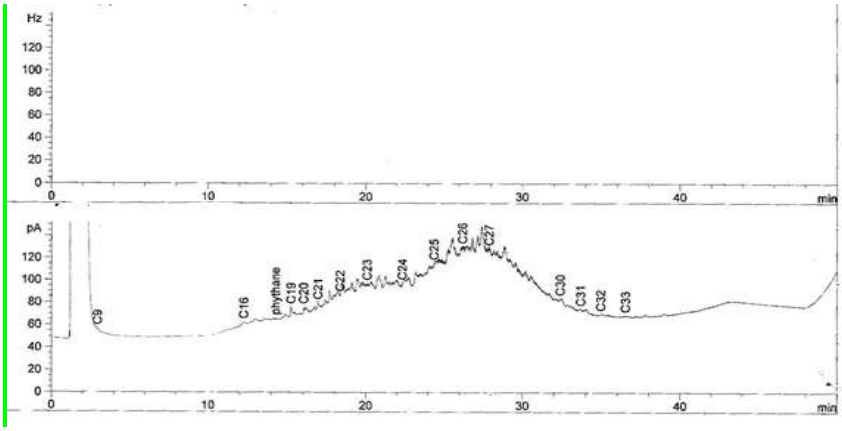

Fig 6: Showing 'Peak Area (pA) of the different hydrocarbon chains identified plotted against time (mins) in Test Sample X.

Similarly, Figure 7 shows graphical representation of the various carbon chains in the oil sample and their concentration as a function of time. From the graph (Figure 7), it is observed that sample B's analysis surprisingly showed a very low presence of petroleum related hydrocarbons compared to sample X as indicated on Figure 7 and summarized in Table 4.1. However, comparing sample Y to sample X, only about 30% of the original TPH are left intact after just 2 weeks of treatment with microbial infected poultry droppings. The concentration of TPH in the analyte as indicated by the GC equipment is **24.51786 ppm** or **367.76 mg/kg** respectively.

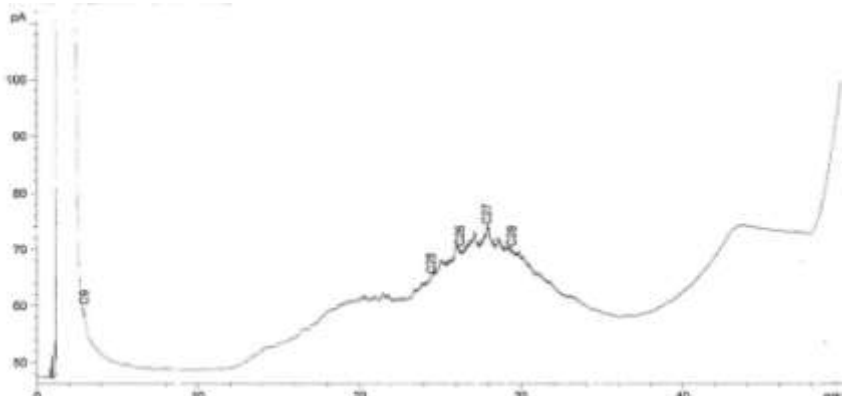

Fig 7; Showing 'Peak Area (pA) of the different hydrocarbon chains identified plotted against time (mins) in Test Sample Y.

Figure 8 shows the relationship of the response of various carbon chains in the soil sample and their concentrations in parts per million as a function of time. It is worthy to note that sample Z at the time of this analyses still contained much of the initial carbon-chained compounds found in the original sample, but noticeably absent however, is the C_{16}, $C_{32\text{-}33}$ compounds. Based on the analysis, the TPH at the time of this analysis with GC equipment is **78.40** ppm or 1176 mg/kg. Meanwhile, Table 4.1 summarizes the signaled HCs and their concentration in ppm. However, Figure 9 shows the comparison of the relationship of the average peak area as a function of time for the three samples.

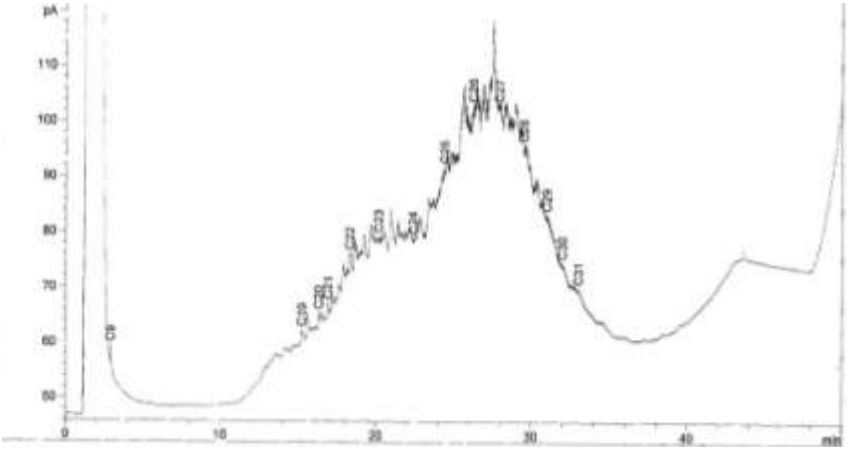

Fig 8: Showing 'Peak Area (pA) of the different hydrocarbon chains identified plotted against time (mins) in Test Sample Z.

Table 1: Summary of Concentrations of samples

Samples	Concentration of TPH (ppm)	Concentration of TPH (mg/kg)
X	80.93	1213.97
Y	24.517	367.76
Z	78.40	1176

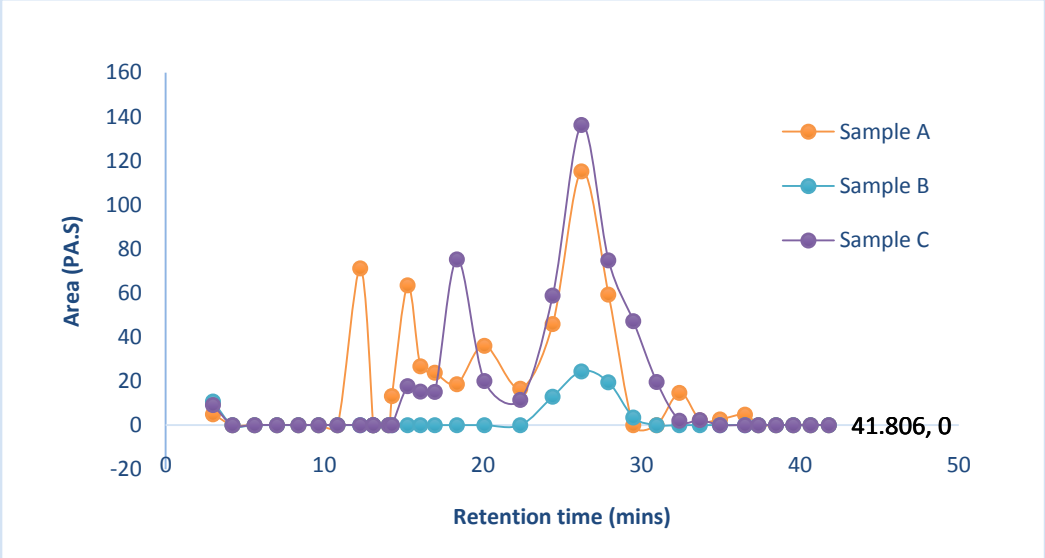

Figure 9: Comparison of average peak area (pA) of the different hydrocarbon chains identified plotted against time (mins) in for the three samples.

Table 1: Summary showing average signaled HCs and their concentration in ppm.

T(min)	HC	Sample X			Sample Y			Sample Z		
		Amt [ppm]	Area [pA*s]	Amt/ Area	Amt [ppm]	Area [pA*s]	Amt/ Area	Amt [ppm]	Area [pA*s]	Amt/ Area
2.97	C_9	2.17	4.91	0.44	2.36	10.76	0.21	2.30	9.08	0.25
4.21	C_{10}	-	-	-	-	-	-	-	-	-
5.60	C_{11}	-	-	-	-	-	-	-	-	-
7.01	C_{12}	-	-	-	-	-	-	-	-	-
8.36	C_{13}	-	-	-	-	-	-	-	-	-
9.64	C_{14}	-	-	-	-	-	-	-	-	-
10.83	C_{15}	-	-	-	-	-	-	-	-	-
12.26	C_{16}	4.29	71.23	0.06	-	-	-	-	-	-
13.07	C_{17}	-	-	-	-	-	-	-	-	-
13.09	Pristine	-	-	-	-	-	-	-	-	-
14.07	C18	-	-	-	-	-	-	-	-	-
14.26	Phytane	0.00	13.26	0.00	-	-	-	-	-	-
15.25	C_{19}	3.74	63.42	0.05	-	-	-	2.26	17.67	0.12
16.05	C_{20}	2.64	26.70	0.09	-	-	-	2.25	15.21	0.14
16.96	C_{21}	2.67	23.74	0.11	-	-	-	2.38	15.13	0.15
18.37	C_{22}	3.18	18.64	0.17	-	-	-	5.13	75.28	0.06
20.08	C_{23}	4.42	36.08	0.12	-	-	-	3.85	20.02	0.19
22.36	C_{24}	4.32	16.56	0.26	-	-	-	4.13	11.47	0.36
24.39	C_{25}	5.92	45.85	0.12	4.57	12.92	0.35	6.44	58.80	0.10
26.22	C_{26}	9.80	115.22	0.08	5.64	24.40	0.23	10.76	136.22	0.07
27.91	C_{27}	8.49	59.29	0.14	6.30	19.38	0.32	9.35	74.83	0.12
29.48	C_{28}	-	-	-	5.62	3.53	1.59	8.59	47.16	0.18
30.95	C_{29}	-	-	-	-	-	-	7.62	19.56	0.38
32.39	C_{30}	7.98	14.69	0.54	-	-	-	6.53	1.97	3.30
33.68	C_{31}	6.73	2.22	3.01	-	-	-	6.73	2.24	3.00
34.95	C_{32}	6.76	2.57	2.62	-	-	-	-	-	-
36.51	C_{33}	7.75	4.79	1.61	-	-	-	-	-	-
37.36	C_{34}	-	-	-	-	-	-	-	-	-
38.48	C_{35}	-	-	-	-	-	-	-	-	-
39.58	C_{36}	-	-	-	-	-	-	-	-	-
40.67	C_{37}	-	-	-	-	-	-	-	-	-
41.80	C_{38}	-	-	-	-	-	-	-	-	-
Total:		**80.93**			**24.51**			**78.40**		

IV. Conclusions

From the analyses of the GC (TPH) results in the preceding section, it is clear that microorganisms if well cultivated and properly applied can be very useful in remediation activities for crude oil impacted soils. The identification of only the $C_{25\text{-}28}$ group in sample Y, as against that of sample A is a testimony to this fact.

Unlike sample Z which like sample Y was left to stand for about 14 days (though without any microbial treatment), sample Y showed significant deterioration of the Petroleum Hydrocarbon Concentration in the soil samples after experimentation.

More important to note is the fact that this was achieved without any form of chemical treatment of the impacted soil sample and of course no environmentally unfriendly matters were generated in the process. Instead, at the time of taking the samples (Y and Z) for analyses, sample Y had a darker hue compared to Z and there was greater evidence of faunal and floral activities in Y explained by the presence of small earth worms, soil insects and fungal moulds; all pointing to a viable and perhaps thriving soil microbial population which are all pointers to soil fertility.

Also worthy of note is the structure of both soil samples after the standing period. Whereas sample Y showed a better crumb structure, sample Z at day 14 consisted mainly of rolled up balls of soil; an indication of the binding effect of oil and water coagulation of soil particles.

The microorganisms present in the poultry droppings used in this experiment can be improved upon in a real-life scenario. Proper inoculation of the desired agents unto suitable substrate e.g. saw dust can be useful in generating the desired quantity needed for in-situ remediation works. An application of this procedure can be very useful especially in restoring small fields where oil spillage has occurred, and with the relative shortness in time of remediation, local and communal land can be quickly freed up for agricultural purposes which have been the main bone of contention between oil firms and their host communities.

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