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Soil Remediation Analysis For Hydrocarbon Polluted Boggy Soils In Goi Community, Gokana LGA, Rivers State, Nigeria

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Abstract
The study carried out the bioremediation of crude oil impacted boggy soils in Goi community, Gokana LGA of Rivers state. Transect method of sampling was employed whereby soil samples were collected randomly from polluted and control soils for soil bioremediation treatments using separate experiments. Samples of polluted soils (P) and samples of substrates (cow dung (Cd) and poultry wastes (Pw)) were used for the bioremediation treatment procedures. Four (4) groups labeled A=control (CP only), B= (PCDPW), C= (PCD), and D= (PPW) were set up and replicated for bioremediation treatments and rate of degradation of total hydrocarbon contents (THC) in the soils. Samples were properly mixed for homogeneity and taking to laboratory for analysis at days 0-14, 28, 42, 56, 70, 84, 98, 112. The descriptive and inferential statistics using frequencies, mean, percentages, Tables and charts and ANOVA tool were employed for data presentation and analysis. The rate of degradation (%) of THC (mg/kg) in polluted soils (un-amended control (CP) and amended (PCDPW, PCD, PPW) at 14 days (2 weeks) were 2.4%, (CP), 5.7% (PCDPW), 3.0% (PCD) and 4.6% (PPW); while at the end of the 112 days (16 weeks) the degradation rate were 21.1% (CP), 46.3% (PCDPW), 34.8% (PCD) and 42.9% (PPW). Thus, higher % of THC was liberated from the polluted soils amended with nutrients than the polluted soil un-amended with nutrients after 16 weeks of observation. Research expansion is therefore needed in this area for effective bioremediation techniques in cases of crude oil pollution.

Keywords: *Crude oil polluted boggy soils; Soil bioremediation; Soil biodegradation; Total hydrocarbon content (THC); Substrates; Nutrients; Transect method; Goi community*

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I. Introduction

Bioremediation involves the processes where chemical substances are degraded by bacteria and other micro-organisms. The use of these microorganisms has been successfully applied for the treatment of waste and wastewater in controlled systems. The technique has been found to have a potential for broad applications in terrestrial and freshwater environments for treating soils and sediments contaminated with oil and other substances, as well as for coastal environments impacted by oil spills (Namkoong *et al*., 2002). Oil spills have been known to cause acute and long term damage to salt marshes and mangrove swamps. These impacts include; reduction in population and growth rate of the mangrove trees, acute and long term damage to salt marshes and mangroves, wide spread animal mortality as a result of the toxic effects of the oil and the disruption of the entire ecosystem (Xu & Obbard, 2004). For these reasons, microorganisms can be effective, economical, and non-disruptive tools for eliminating hazardous chemicals. Certain enzymes produced by microbes attack hydrocarbon molecules, causing degradation. The degradation of oil relies on having sufficient microbes to degrade the oil through the microbes' metabolic pathways (series of steps by which degradation occurs). Fortunately, nature has evolved many microbes to do this job. Throughout the world there are over 70 genera of microbes that are known to degrade hydrocarbons (Gray *et al*., 2000). These microbes usually account for less than 1% of natural population of microbes, but can account for more than 10% of the population in polluted ecosystems (Adebusoye *et al*., 2007).

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source. These microbes because of their adaptability and other biological systems can be used to degrade or remediate environmental hazards (Nwokoro & Okpokwasili, 2003). Evaluating the effectiveness of bioremediation technologies is complicated by several factors. First, biodegradation is only one of the processes at work removing petroleum from the marine environment; to understand the effect of this process on oil removal, one must know the effects of other processes. Biodegradation involves oil degradation and is a natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, eventually producing fatty acids and carbon dioxide (Hou & Laskin, 2006). Bioremediation is the acceleration of this process through the addition of exogenous microbial populations, through the stimulation of indigenous populations or through manipulation of the contaminated media using techniques such as aeration or temperature control (Hou & Laskin, 2006).

Bioremediation consists of biological and chemical processes; the biological process involves use of microorganisms which possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes and aromatics, others degrade both paraffinic and aromatic hydrocarbons. Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. The chemical process is characterized by the biodegradation of hydrocarbons by bacteria and fungi. This involves the oxidation of the substrate by oxygenases, for which molecular oxygen is required. Alkanes are subsequently converted to carboxylic acids that are further biodegraded via ß-oxidation (the central metabolic pathway for the utilization of fatty acids from lipids, which results in formation of acetate, which enters the tricarboxylic acid cycle).

There are several different bioremediation techniques. The underlying idea is to accelerate the rates of natural hydrocarbon biodegradation especially in swampy soils by overcoming the rate-limiting factors. Several techniques can lead to the desired results. If necessary, genetically altered bacteria can be used. Once the bacteria are chosen, the engineer must carefully meet their nutritional needs by choosing the correct mix of fertilizer (Head, 1998). Bioremediation is the most promising technology that is currently used in the degradation of oil in a contaminated environment. Apart from the use of microorganisms during bioremediation, other agents have been applied to clean-up an environment after an oil spill. These agents usually contain oil degradation bacteria and/or seed cultures of oil-degrading bacteria, organic or inorganic fertilizers (Gupta *et al*., 2016). The degradation rate is affected by temperature, pH and scarcity of nutrients like Nitrogen, Phosphorus and Oxygen (Ladousse & Tramier, 1991).

Therefore, to have these nutrients that are very essential for the effectiveness of bioremediation, some researchers have used some biological materials to enhance the rate of degradation and these include; food waste which is rich in carbon, organic nitrogen, phosphorous and mineral compounds required for growth of microorganisms (Riser-Roberts, 1998). Thus, addition of food waste provides required nutrients for enhanced biodegradation of petroleum hydrocarbon (Iwegbue *et al*., 2006). Additionally, EPA carried out a comprehensive, large-scale project applying different fertilizers to the contaminated shorelines in Prince William Sound. Its objective was to demonstrate the enhancement of biodegradation through the addition of nitrogen and phosphorus in the form of three different types of fertilizers: Inipol EAP2, an oleophilic fertilizer formulation, and Customble a granular slow-release fertilizer. Oleophilic means literally oil loving; Inipol contains surfactants as well as nutrients, and is designed to stick to oil on rocky substrates, providing nutrients at the oil/air interface where microbial degradation takes place. Several monitoring programs measured the effectiveness of these fertilizers in reducing crude oil contamination in polluted soils overtime (Xu and Obbard, 2004; Iwegbue *et al*., 2006). However, for this study, the application of nutrients in form of fertilizers (cow dung and poultry waste) was employed and the degradation rate of hydrocarbon in the polluted soils were determined over a period of 16 weeks. Thus, a study of this nature is required to show the need for proper remediation of crude oil polluted sites in Goi community because of its socio-economic impacts on residents. Also, based on the weak nature of the environmental laws that are supposed to compel organization to carry out proper clean up and in a bid to propose cheaper methods and ways of ameliorating the crude oil pollution effects of the polluted soils in the study area. The current study therefore focuses on soil bioremediation for hydrocarbon polluted boggy soils in Goi community. In view of this, the important questions for the study are: What is the rate of degradation of hydrocarbon content from polluted soils? Does the application of bioremediation lead to the significant reduction in hydrocarbon content of the crude oil polluted soils?

II. Materials and Methods

i. Description of the Study Area

The study area is Goi community under Gokana LGA, Rivers state Nigeria. It lies geographically within latitude 4° 40′ 5″ N and 4° 43′ 19.5″ N and longitude 7° 22′ 53.7″ E and 7° 27′ 9.8″ E (Figure 1). Rainfall is present all year with an annual total of 200mm to 2300mm. However, a short spell of dry season usually occurs between the months of November and February. The vegetation of Gokana LGA falls within the rainforest vegetation belt. Trees found in this area are very tall, characteristics of the rainforest vegetation zone. These trees are about 30metres tall. These trees are evergreen with branches which interlace themselves to form canopies. The study area is predominated with sandy and loamy soil which is characteristic of coastal areas. The geology of the area and the adjourning areas is made up of the coastal plains due to the area’s proximity to the Atlantic Ocean. The area is generally underlain by the Benin formation, consisting of coarse sands interrupted by clay lenses of quaternary age. The study area is generally drained by the Goi River and its tributaries Crops cultivated include; yam, cassava, maize, cocoa, Oil palm etc.

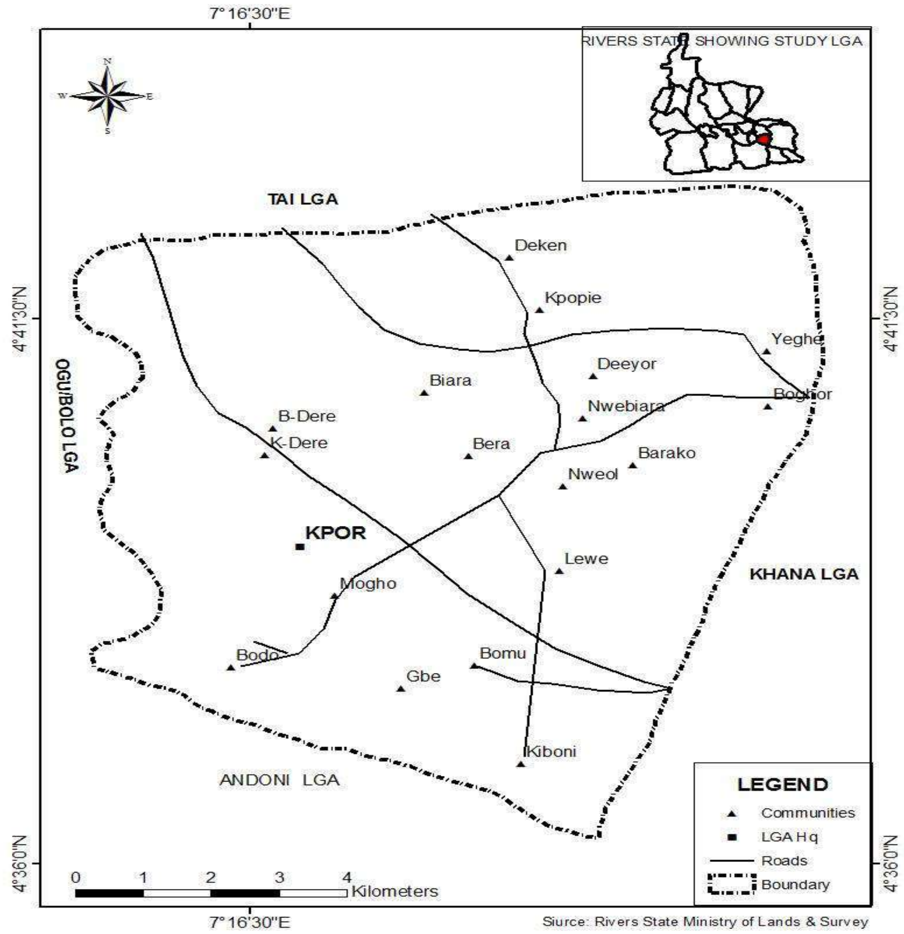

Figure 1. Gokana LGA in Rivers state (inset map)

ii. Data Acquisition

The primary data sources were employed for this study. The primary data sources included utilizing the point transect method whereby point samples were taken at random locations within the crude oil impacted boggy soils. This method was employed because the study is based on monitoring purposes that was easy to follow (Bonham, 1989; Poissonet et al., 1972). The point transect lines were established within the polluted soils (experimental site) area and the unpolluted soils area (control site). For the experimental site, within each established transect, 5 sub-transect lines were established whereby three sampling points were randomly selected in each sub-transect lines for collection of soil samples (see sample on Figure 2). This method was repeated in the control site for soil samples collection. Thus, a total of 30 soil samples were collected in the study area.

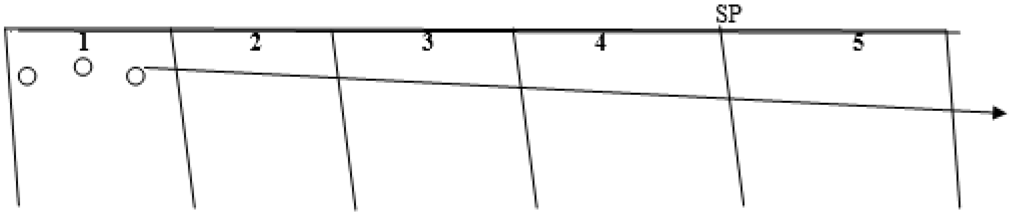

Figure 2: Point Transect Method for Collection of Soil samples

SP - Sampling Points of 0-30cm depths (3 x 5 = 15)

iii. Data Collection Techniques

The instruments for collection of the soil samples included clean and sterilized auger, a clean plastic which was used to collect 600gramms of the soil samples at each point of collection (that is, 200g per sample point), hand gloves for protecting the hands. Other instruments to be used include; autoclave, petri dishes, test tubes, pipette, centrifuge, incubator, Erlenmeyer flasks, Haemocytometer. All the apparatus and glassware were washed, dried and sterilized by autoclaving for 15 minutes at 121^{o}C. The collected soil samples were bulked together and set up into four (4) sub-groups A, B, C and D and were prepared for bioremediation treatments for the period of 16 weeks. Thereafter, treatments in the form of cow dung and poultry wastes were added by thoroughly mixing them with the soil samples (4000g of organic waste) which were set up as experimental designs in triplicates (B, C and D) and with a control (A) which is without the addition of cow dung or poultry waste as a form of organic waste. The samples were thoroughly mixed, and left untouched for 48 hr to reduce the amount of toxic components in the oil through volatilization. The cow dung and poultry wastes were collected from farm sites which have about 50-100 cows, and about 300 or more poultry chickens. Large quantities of the cow dung and poultry droppings are being generated daily on the ground and these substrates wastes were collected and used to carry out the analysis for the study.

iv. Data Analysis

The descriptive and inferential statistics using frequencies, percentages, Tables and charts and ANOVA tool were employed for data presentation and analysis. The Excel worksheet 2010 aided the study in data arrangement and presentation.

III. Results of the Analysis

Rate of Degradation of Total Petroleum Hydrocarbon Content (THC) in Polluted Soils (Amended & Un-amended soils)

The information for the rate of degradation of total petroleum hydrocarbon content (THC) (mg/kg) in sampled polluted soils from Goi community is displayed on Table 1. The variations in THC (mg/kg) were expressed in % for more clarification on the extent of degradation in the polluted treated and untreated polluted soils within the 16 weeks monitoring period. Their % reduction rates were computed at the end of each monitoring period (2 weeks each).

The results revealed that the first two weeks (14 days) of observation showed variations in reduction rates (rate of degradation) of total petroleum hydrocarbon content in treated and untreated soils and these variations were computed in percentages (%). The distribution revealed that for polluted soils, 2.4% reduction rate was recorded for CP; 5.7% reduction rate was recorded for PCDPW; 3.0% reduction rate was recorded for PCD; and 4.6% of reduction rate was recorded for PPW. Similarly, at the end of the 28 days (4 weeks) the reduction rate also varied among experimented soils showed that 7.7% (17.6 mg/kg) reduction rate was recorded for CP; 9.8% (22.2 mg/kg) was recorded for PCDPW; 6.7% (15.3 mg/kg) was recorded for PCD; and 6.7% (15.3 mg/kg) was recorded for PPW.

At the end of the 16 weeks (112 days) from the polluted soils, a total of 22.1% (50.8 mg/kg) of total petroleum hydrocarbons was degraded from the polluted soils (un-amended) used as the control; a total of 46.3% (104.8 mg/kg) of THC was degraded from polluted soils under the amended soils with cow dung and poultry waste at the same time (PCDPW); a total of 34.8% (79.0 mg/kg) of THC was degraded from the polluted soils amended with cow dung only; while a total of 98.2 mg/kg (42.9%) of THC was degraded from polluted soils amended with poultry waste only. Thus, it was discovered that the efficiency level of the amended polluted soils (PCDPW, PCD, PPW) were more significant in reducing total petroleum hydrocarbon (THC) in crude oil polluted soils when compared with the polluted un-amended soils (CP) which only contents were hydrocarbon utilizing bacteria because of the crude oil pollution. These hydrocarbon utilizing bacteria also feeds on the hydrocarbon contents in the polluted soil and biodegrades it as observed in the control soil experiment for the study.

Table 1: Rate of Degradation of THC in Polluted soils (un-amended) & Polluted soils (amended)

Observation period	Total Hydrocarbon Content				Degradation rate (cumulative %)			
	CP (mg/kg)	**PCDPW (mg/kg)**	**PCD (mg/kg**	**PPW (mg/kg)**	**CP (mg/kg)**	**PCDPW (mg/kg)**	**PCD (mg/kg**	**PPW (mg/kg)**
0 Day	229.6	226.3	227	228.9	0	0	0	0
14 Days (2 weeks)	224.1	213.3	220.2	218.4	5.5 (2.4%)	13 (5.7%)	6.8 (3.0%)	10.5 (4.6%)
28 Days (4 weeks)	212	204.1	211.7	213.6	17.6 (7.7%)	22.2 (9.8%)	15.3 (6.7%)	15.3 (6.7%)
42 Days (6 weeks)	208.3	193.5	201.1	203	21.3 (9.3%)	32.8 (14.5%)	25.9 (11.4%)	25.9 (11.2%)
56 Days (8 weeks)	201.4	181.6	192.3	184.7	28.2 (12.3%)	44.7 (19.8%)	34.7 (15.3%)	44.2 (19.4%)
70 Days (10 weeks)	198.1	167.3	179	173.3	31.5 (13.7%)	59 (27.1%)	48 (21.1%)	55.6 (24.2%)
84 Days (12 weeks)	193.4	152	168	160.4	36.2 (15.7%)	74.3 (32.8%)	59 (26.0%)	68.5 (29.9%)
98 Days (14 weeks)	185	139	157.4	146.2	44.6 (19.4%)	87.3 (38.7%)	69.6 (30.7%)	82.7 (36.1%)
112 Days (16 weeks)	178.8	121.5	148	130.7	50.8 (22.1%)	104.8 (46.3%)	79 (34.8%)	98.2 (42.9%)

CP – Polluted soils used as control; PCDPW- Polluted soils amended with cow dung and poultry waste; PCD-Polluted soils amended with cow dung only; PPW- Polluted soils amended with poultry wastes only

Variation in Hydrocarbon content before and after Remediation

The result on Table 2 revealed an F ratio of 56.730 at probability value of 0.05 (p-value of 95%) indicated a level of significance of 0.000. Since, the level of significance of 0.000 was lower than the p-value of 0.05, the null hypothesis H_0 was therefore rejected while the alternative hypothesis H_1 accepted. Thus, the application of bioremediation leads to the significant reduction in the hydrocarbon content of the crude oil polluted soils.

Table 2: Variation in Hydrocarbon content before and after Remediation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21594.3025	1	21594.3025	56.370	.000
Within Groups	11492.505	15	383.0835		
Total	33086.8075	31			

IV. Discussion

The soil amendments carried out for polluted soils is a mixture of cow dung and poultry waste done in order to boost the capacity of the soil for bioremediation processes. Cow dung is a mixture of faeces and urine in the ratio of 3:1, which mainly consists of lignin, cellulose and hemicelluloses with other 24 different minerals like nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese (Gupta et al., 2016; Bhatt and Maheshwari, 2019); which makes them suitable for microbial degradation of pollutants (Umanu et al., 2013). Poultry wastes of chicken origin are very useful supplies of nitrogen, potassium, phosphorus, calcium and organic matter to soil. Poultry wastes help replenish the soil especially soils deficient of nitrogen, phosphorus and potassium (NPK) which are valuable nutrients determining soil's cation exchangeable capacity. The analysis of the rate of degradation of soil total petroleum hydrocarbon contents revealed significant changes at the end of the bioremediation experiments which lasted for 16 weeks (112 days). The significant changes were more evident under the soil samples amended with fertilizer (cow dung and poultry wastes) than under the polluted soils used as control which were un-amended. The polluted amended soils were efficient in the reduction of total petroleum hydrocarbon content in soils overtime. The study discovered a gradual reduction in the concentration of petroleum hydrocarbons at the end of each period of experiment. It was discovered that the amended soils (a mixture of cow dung and poultry waste; cow dung only; and poultry waste only) were able to reduce the hydrocarbon content in soils significantly especially after the 16 weeks period of observation. This could be attributed to the increase in the activities of soil bacteria which increased in polluted soils amended with fertilizer nutrients. The soil bacteria like Pseudomonas, Enterobacter, Bacillus, Flaviobacterium amongst others utilizes these hydrocarbon compounds as alternative carbon energy sources, especially in the absence of other soil substrates. Thus, the level of degradation of hydrocarbon compounds in the polluted soils is a function of the physiological activity of the soil bacteria present in the soil. These findings aligns with Mukherjee et al., (2017) and Song et al., (2017) who discovered that the diversity and population of soil bacteria as well as their physiological activities will to a large extent determine the level of degradation of hydrocarbons content in a polluted soil. Similar to this were the findings of Fayinminnu *et al.*, (2021) that cow dung and poultry waste are good source of hydrocarbon utilizing bacteria needed for effective bio-remediation processes. Similarly, on the basis of reduction capacity of the polluted un-amended soil, the study discovered that some level of degradation was observed which was attributed to the hydrocarbon utilizing bacteria (HUB) already present in the polluted soils due to the response of the soil to hydrocarbon pollution. Similarly, Pinholt et al., (1979) and Adebusoye et al., (2007) have earlier reported that hydrocarbon utilizing bacteria feeds on the hydrocarbon contents in the crude oil polluted soil and biodegrades it at an efficiency rate of between 0.13% and 50%.

V. Conclusion and Recommendation

The focus of the study which was based on bioremediation of polluted boggy soils from Goi community made some findings which were: the study discovered that the addition of nutrients to the polluted soils increased the soil bacteria counts and population of soil micro-organisms which are also known as hydrocarbon utilizing bacteria in the polluted soils. Thus, higher % of THC was liberated from the polluted soils amended with nutrients than the polluted soil un-amended with nutrients after 16 weeks of observation. Therefore, the addition of soil nutrients (fertilizer) of cow dung and poultry wastes was discovered to be effective in the bioremediation of the polluted boggy soils in Goi community under Gokana LGA, Rivers state. The study recommends that: research expansion is therefore needed in this area for effective bioremediation techniques in cases of crude oil pollution; research of this nature is very expensive and time consuming; thus, the government can support through adequate funding that will help to reduce cost and promote more accurate findings; the activities of the oil multinationals should be monitored at all levels in order to reduce their environmental impacts especially as it has to do with occasional crude oil spills in the study area.

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