Bioremediation Potentials of Hydrocarbonoclastic Bacteria Indigenous in the Oil Impacted Sites of Ogoniland, Rivers State, Nigeria

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***Abstract:-*Hydrocarbon pollution Remediation by Enhanced Natural Attenuation method was adopted to remediate the hydrocarbon impacted site in Ogoniland Rivers State, Nigeria . The research lasted for 6 months. Samples were collected at monthly intervals . samples were collected intermittently between Feb 2019 to July 2019 . Mineral salt medium containing crude oil was used as a sole source of carbon and energy for the isolation of hydrocarbonoclastic bacteria. Samples were collected from the four (4) local government that made up Ogoniland and they includes Khana(k), Gokana (G),Tai (T), Eleme (E) and transported immediately to the laboratory for analysis. The microbial and physicochemical properties of the soil samples varied with the different local government areas. Seven bacteria genera were isolated from the samples from the four locations, viz, Pseudomonas, Lactobacter, Micrococcus, Arthrobacter, Bacillus, Brevibacterium and Mycobacterium were isolated and identified. the seven isolate were indigenous in the study area. Nutrient were added to identified plots of hydrocarbon pollution polluted site within the four local government and they were able degrade hydrocarbon within a short of period of time. Reassessment of physicochemical parameter impacted site was used to judge the bioremediation potentials of microorganism.**

***Key words:-* Bioremediation, Hydrocarbonoclastic, Bacteria, potentials, Ogoniland, Rivers state**

I. INTRODUCTION

Hydrocarbon pollution in ogoniland is a global issue,the land has been polluted with hydrocarbon which has greatly affected health and agricultural activities of the people, The extent of the pollution warranted the united nation environmental programme (UNEP) recommended the clean up of ogoniland.

Petroleum is at present Nigeria's and indeed, the world's most important derived energy source ((Moffat and Linden, 2005; Nigerian Environmental Study Action Team, 2006). However, the growth and activities of petroleum and petroleum associated industries in Nigeria and in other parts of the world has led to increased oil pollution in our environment. Petroleum in its natural state is called crude oil (Ukoli, 2003).

The greatest single environmental problem connected with crude oil exploration in Nigeria is oil spillage both on-shore and off-shore. The rate of oil spillage reported in the country has been rising with a corresponding increase in petroleum production.

The various genera that have been reported to contain hydrocarbon-degrading species include Pseudomonas, Vibrio, Corynebacterium, Arthrobacter, Brevibacterium, Flavobacterium, Sporobolomyces, Achromobacte, Bacillus, Aeromonas, Thiobacillus, Lactobacter, Staphylococcus, Penicillium and Articulosporium. These organisms have been isolated in large numbers from many oil polluted waters and soils, but are found in less numbers in uncontaminated environments.

Many studies have been conducted to isolate and characterize hydrocarbon degraders from oil spill sites but little have been done to determine the changes in soil mineral nutrients and total petroleum hydrocarbon as bioremediation of the spill site progresses. This study will provide information on the effectiveness of microorganisms in eliminating oil pollutants from the oil polluted areas of the Niger delta region in Nigeria. Specifically, the experiment was designed to; isolate and characterize hydrocarbon utilizing bacteria and fungi from crude oil impacted soil samples and also determine the extent to which the spilled crude oil has been degraded.

II. MATERIALS AND METHODS

Collection of Soil Samples

Soil contaminated samples used for the study were obtained from oil impacted sites in Khana, Gokana, Tai and Eleme Local Government Area of Rivers State, Nigeria. The sample were obtained between Feb 2019 to July 2019. The samples were collected aseptically using soil sampler to a depth of 20 cm, stored in sterile aluminum foils and transported to the laboratory immediately for analysis. The sample were coded using the first alphabet of the local government : K,T,G and E respectively.

Enumeration of total heterotrophic bacteria and in the soil samples 1 g of each sample was serially diluted (10^{-1} to 10^{-5}). And were plated in duplicate on sterile nutrient agar plates, using the pour plate method at 37°C for 24 h for the nutrient agar plate, however colonies who shows slow growth rate

were further allow for 48h at 37° and were afterwards enumerated

Enumeration of Hydrocarbonoclastic Bacteria

1 ml from dilutions of 10^{-5} were plated in duplicate on pre dried mineral salt agar using the spread plate technique, the 10-3 and 10-4 dilutions were plated . A filter paper saturated with sterile crude oil was aseptically placed on the inverted Petri dishes and the culture plates were incubated for 72h at 37°C . Plates yielding significant colonies were enumerated .

Isolation and Characterization of Hydrocarbon-Utilizing Bacteria

Colonies of different hydrocarbon eating bacteria were selected at random using a sterile wire loop and subcultured, by streaking on nutrient agar plates to obtain pure colonies. All the isolates were characterized using the techniques of Chesborogh (2000)

Determination of Physicochemical Properties of Contaminated Soil

Analyses of physicochemical properties of the soil samples were performed according to the American Society ASTM (1998).

Determination of Total Petroleum Hydrocarbon

FTI Spectrophotometer was used for the test. Prior to analysis the soil samples were extracted with carbon tetrachloride and treated with 2% deactivated silica gel. The equipment was calibrated with isooctane/octane in carbon tetrachloride. TPH concentrations in the samples were determined by using the stored calibration graphmoisture Content determination

A weighed amount of the soil sample was placed in a weighed crucible and dried at 105°C in the oven until a constant weight was reached. From the difference in weight, the percentage moisture content was calculated.

Phosphate Content Determination

Samples were extracted with 25% acetic acid and the extract run on the Unicam UV/visible spectrophotometer at a wavelength of 700 nm. A spike sample was analyzed in every batch of analysis. A standard was analyzed after every batch of samples and the first value of the standard was used to plot the means control chart.

Nitrate and pH Determination

Soil samples were extracted with sodium acetate in the presence of sulphuric acid and measured at a wavelength of 470 nm using Unicam UV/visible spectrophotometer. To assess the pH, electrodes of a multimeter were dipped into a mixture of soil sample and deionized water. The pH values of the samples were subsequently read on the multimeter.

Statistical Analysis of Data

All the data obtained were subjected to statistical analysis of variance (ANOVA) using SPSS program.

III. RESULTS

Microbial Counts and Identification

Hydrocarbonoclastic bacteria isolated were, Brevibacterium Lactobacter, Arthrobacter, Bacillus, Pseudomonas, Mycobacterium and Micrococcus were isolated and identified.

TABLE 1: TOTAL HETEROTROPHIC BACTEIA THB

SAMPLE DILLUTIONS	10^{-3}	10^{-4}
KHANA (K)	$3.0X10^5$	$2.7X10^5$
GOKANA (G)	2.41X105	1.7X105
TAI (T)	1.90X105	1.5X105
ELEME (E)	2.30X1O5	2.50X105

TABLE 2 HYDROCARBONOCLASTIC BACTERIA ISOLATED FROM THE FOUR LOCATIONS

SAMPLE

ISOLATES	K	G	T	E
Mycobacterium	+	+	+	+
Pseudomonas	+	+	+	+
Lactobacter,	+	+	+	+
Micrococcus	+	+	+	+
Arthrobacter	+	+	+	+
Bacillus	+	+	+	+
Brevibacterium	+	+	+	+

TABLE 3. PHYSICOCHEMICAL PARAMETER OF OIL POLLUTED SOILBEFORE BIOREMEDIATION

Parameter Sample Location

PH	K	G	T	E
	7.00	7.01	6.50	6.90
Nitrogen	0.28mg/kg	0.26mg/kg	0.31mg/kg	0.33mg/kg
Phosphorus	0.33mg/kg	0.35mg/kg	0.30mg/kg	0.34mg/kg
Total Organic Carbon (TOC)	0.8%	0.7%	0.8%	0.7%
Total hydrocarbon content (THC)	2,845mg/g	2,885mg/g	2,950mg/g	2,740mg/g
Total Petroleum Hydrocarbon (TPH)	3,475mg/g	3,375mg/g	3,500mg/g	3,380mg/g
Polycyclic Aromatic Hydrocarbon(PATH)	15.65mg/g	15.40mg/g	15.35mg/g	15.29mg/g

TABLE 3. PHYSICOCHEMICAL PARAMETEROF OIL POLLUTED SOIL AFTER BIOREMEDIATION

PARAMETER SAMPLE LOCATION

PH	K	G	T	E
	4.00	4.01	4.50	3.90
Nitrogen	5.01g/kg	4.45mg/kg	3.22mg/kg	2.9.5mg/kg
Phosphorus	0.55mg/kg	0.50mg/kg	0.70mg/kg	0.61mg/kg

Total Organic Carbon (TOC)	115.5%	100.5%	200.1%	185.7%
Total hydrocarbon content (THC)	2,845mg/g	2,885mg/g	2,950mg/g	2,740mg/g
Total Petroleum Hydrocarbon (TPH)	70.5mg/g	75.5mg/g	80.7mg/g	68.0mg/g
Polycyclic Aromatic Hydrocarbon (PATH)	2.65mg/g	3.40mg/g	3.35mg/g	4.29mg/g

IV. DISCUSSION

The results of the study indicated that the total heterotrophic bacteria (THB) counts varied over a period of time. Result shows significant differences in physicochemical parameter before and after bioremediation. The differences in the physicochemical parameter was use to ascertain the degree or potentials of hydrocarbon utilizing effects of the isolated microorganism. The counts of hydrocarbonoclastic bacteria were higher in hydrocarbon polluted soil than non impacted soil, this may be as a result of the fact the hydrocarbon provides a source of carbon and nitrogen needed for the proliferation of the hydrocarbonoclast. The pH values of crude oil polluted soil were lower as compared to those of crude oil free soil. The decrease in pH value may be due to increased degradation of crude oil by microorganisms in the soil, res. Both nitrogen and phosphorus levels were high in hydrocarbon impacted soil than oil free soil. This agrees with the finding of (A.K. Onifade and F.A. Abubakar (2007)), who reported increase in nitrogen and phosphorus contents of a crude oil polluted soil.

Conclusively, the study shows that there was residual crude oil in the soil after 6 months of research. The study also shows that the physiochemical properties of the soil were significantly affected. Finally, the study has proven that Ogoniland has indeginous microbial population that has the potentials to remediate hydrocarbon pollution.

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