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DISCOVERY

Influence of microorganisms on Aluu Crude Oil polluted soil environment in Niger Delta

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ABSTRACT

Investigation was carried out to examine the effect of microorganisms on crude oil contamination of Aluu soil. The microorganisms studied were Total Heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF). The result obtained revealed the significant effect of microorganisms on the remediation process on the crude oil contaminated soil on the parameters investigated. The statistical analyses used were analysis of variance (ANOVA) and regression analysis. The time has effect on total heterotrophic fungi $P<0.05$ significant but has no effect ontotal heterotrophic bacteria $P<0.05$ not significant. There were high positive correlations between variables and time.

Keywords: Influence, microorganism, Aluu, crude oil, polluted soil, environment, Niger Delta

1. INTRODUCTION

Research work was conducted to examine the effects of microorganisms on the Aluu soil polluted with crude oil and the community Aluu is located in Ikwerre local government of Rivers State in Niger Delta area of Nigeria. The microbial population such as total

heterotrophic bacteria (THB) and total heterotrophic fungi were studied. Pure cultures of bacteria were obtained and subjected to various characterization procedures. The standard characterization tests performed included: Gram stain, motility test, catalase, methyl red and Vogues Proskauer test. Others are urease, indole, protease, nitrate reduction, starch hydrolysis and sugar fermentation tests. The isolates were identified on the basis of their cultural, morphological and biochemical reactions, and by reference to Cowan, 1974; Buchanan and Gibbons, 1994; and Winn *et al*., 2006.

Fungal isolates were characterized by microscopy by observing the colonial morphology, colour of colony, texture, shape, surface appearance, and colour on the reverse plates (Abbey, 1995); and microscopy using the wet preparation and slide culture by observing cultural characteristics to reveal the asexual and sexual reproductive structures like sporangia, conidial head, vegetative mycelia, septate and non-septate hyphae, and by reference to Barnett and Hunter, 1972; Abbey, 1995; Winn *et al*., 2006.

2. MATERIALS AND METHODS

Materials

The following materials were used in the research work; cylindrical tubes, clay soil, sandy soil, loamy soil, crude oil, saline, test tubes, glass rod and plates (figure 1 & 2).

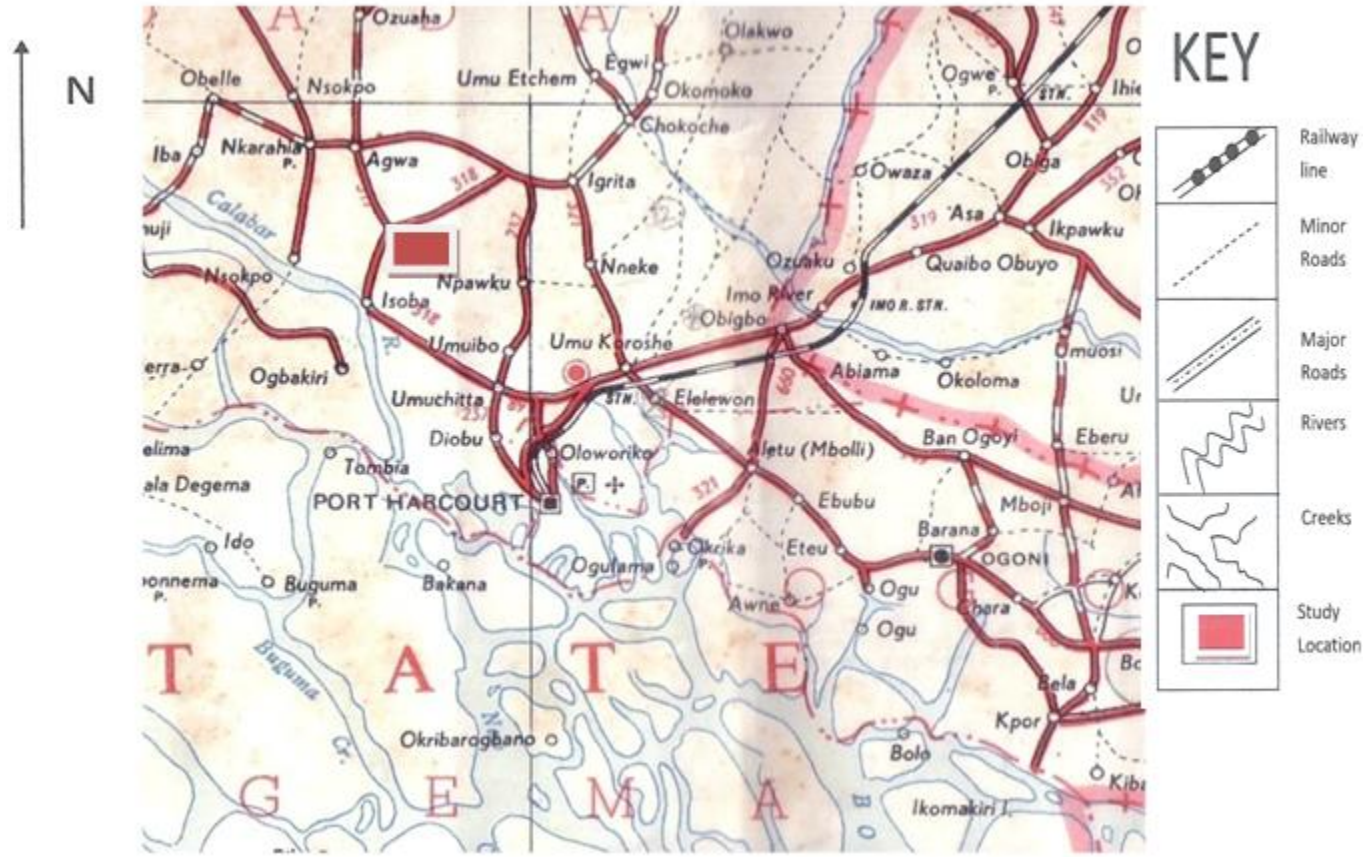

Figure 1 Schematic Drawing of Parts of Rivers State Showing the Study Site, Aluu in Ikwerre Local Government Area Rivers State.

Method

Sample bottles were used for the collection of the various soil samples from the collection points of Aluu community, such as the clay soil, sandy soil and loamy soil as well as packed into reactors labeled SS1- SS5, CS1-CS5 and LS1-LS5 for analysis of microbial growths of bacteria and fungi.

This involved enumeration and isolation of aerobic heterotrophic bacteria and fungi from the soil and crude samples. Ten-fold serial dilution of Harrigan and MacCance (1990); Ofunne (1999); and Nester and Nester (2004) was employed, in which 1g of soil and/or 1ml of crude oil was put into 9ml of normal saline (diluent) and diluted serially up to 10^{-3}dilutions. Aliquots (0.1ml) of appropriate dilutions were spread plated using a sterile bent glass rod onto the surfaces of fresh sterile dried nutrient agar plates for bacteria and sabouraud dextrose agar plates for fungi. The inoculated plates were incubated at 37^{O}C for 24 hours for bacteria and 2-

3 days for fungi. After incubation, plates that had significant growth were counted and the population of bacteria was recorded in colony forming units per gramme (cfu/g) while population of fungi was recorded in colony forming units per gramme (cfu/g) soil.

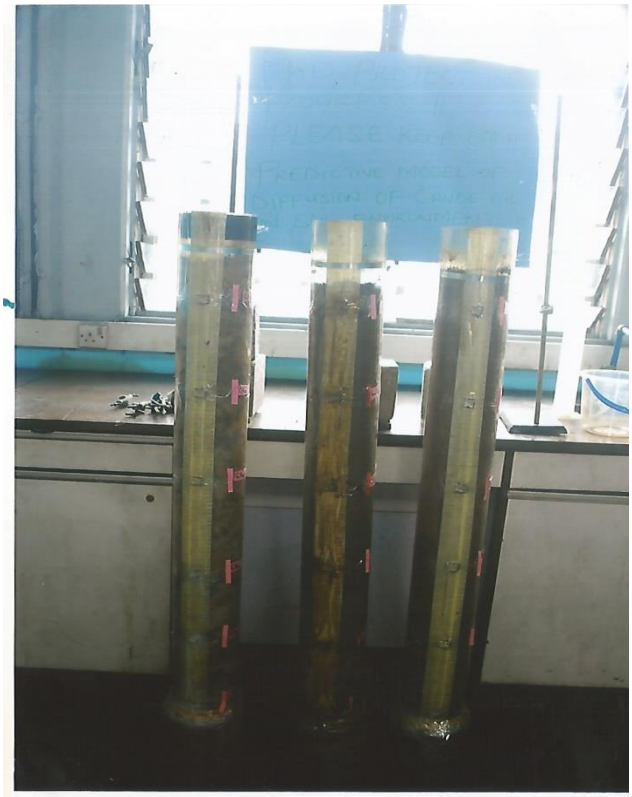

Figure 2 Experimental set-up for Diffusion of Crude Oil in the Three Soil Samples Obtained from Aluu Environment.

Representative discrete bacterial colonies were purified by sub culturing onto fresh sterile nutrient agar plates which were incubated at 37^{O}C for 24 hours and used as pure cultures for characterization of the isolates. Similarly, colonies of fungi were sub cultured onto SDA plates which were incubated at 28^{O}C for 3 – 5 days and the pure cultures used for characterization of fungal isolates (table 1 – 6).

3. RESULTS AND DISCUSSION

Table 1 The Physiochemical Parameters of Aluu Crude Oil Polluted Soil Environment and Standard

Parameters	Clay Soil	Loamy Soil	Sandy Soil	Standard
Soil pHw 1:2:5	5.70	6.00	6.50	6.5-8.5
Temperature(°C)	25	25	25	30
Elect. Cond.(µs/cm)	123.10	49.60	110.6	300
Avail.phosphorus(mg/L)	45.61	1.05	7.12	45

Table 2 The Microbial Population at every Collection Point of the Reactor with Time

Sample Time(day) 2018	Sampling point	Total heterotrophic bacteria (THB)(cfu/g)	Total heterotrophic fungi (THF)(cfu/g)
14	CS 1	$2.2x10^5$	$2.4x10^3$
	SS 1	$8.5x10^5$	$1.0x10^3$
	LS 1	$9.5x10^5$	$0.3x10^3$

28	CS 2	$2.5x10^5$	$2.5x10^3$
	SS 2	$8.7x10^5$	$1.4x10^3$
	LS 2	$9.8x10^5$	$2.4x10^3$
42	CS 3	$2.6x10^5$	$0.4x10^3$
	SS 3	$8.8x10^5$	$2.7x10^3$
	LS 3	$10.1x10^5$	$1.5x10^3$
56	CS 4	$2.7x10^5$	$0.5x10^3$
	SS 4	$8.9x10^5$	$2.8x10^3$
	LS 4	$11x10^5$	$0.6x10^3$
70	CS 5	$2.8x10^5$	$2.9x10^3$
	SS 5	$10x10^5$	$1.8x10^3$
	LS 5	$11.5x10^5$	$0.7x10^3$

Table 3 Mean Values of THB and THF of Aluu Crude Oil polluted Soil with Time

Time(day)	Mean(THB)(cfu/g)	Mean (THF)(cfu/g)
14	$6.8x10^5$	$1.2x10^3$
28	$7x10^5$	$1.4x10^3$
42	$7.2x10^5$	$1.6x10^3$
56	$7.5x10^5$	$1.7x10^3$
70	$8.1x10^5$	$1.8x10^3$

Table 4 Variation of THB and THF in Aluu Crude Oil Polluted Soil Environment

Parameters	Mean ± SEM	Range	Overall mean
THB(cfu/g)	$7.31x10^5 \pm 0.21x10^4$	$2.3x10^5$-$11.5x10^5$	$7.31x10^5$
THF(cfu/g)	$1.54x10^3 \pm 0.08x10^3$	$0.3x10^3$-$2.9x10^3$	$1.54x10^3$

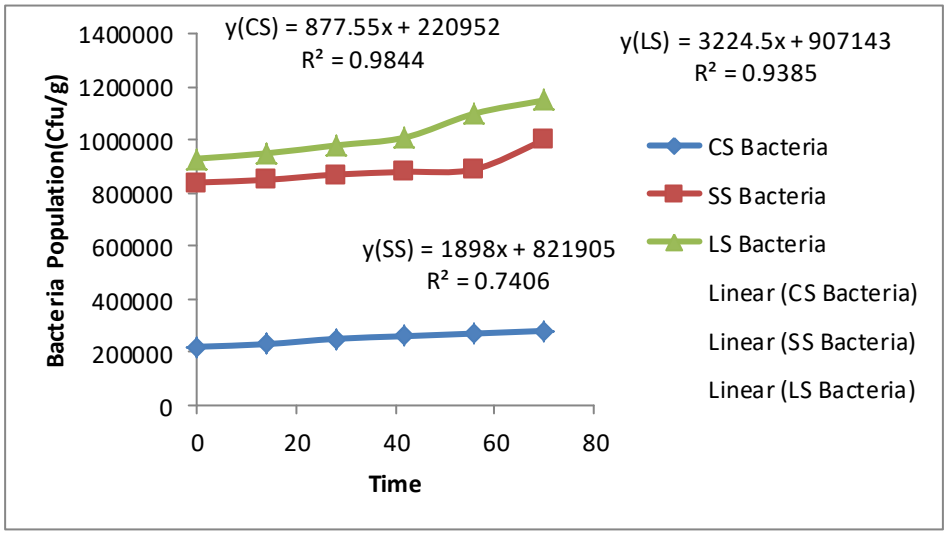

Figure 3 Graph of Total Heterotrophic Bacteria versus Time on Crude Oil Diffusion in Various Soil Samples

Figure 3 illustrates the significant of the graph of total heterotrophic bacteria (THB) versus Time on crude oil diffusion in various soil samples upon the influence of time. The equations of curves are expressed as $Y_{ss} = 1898X + 821905, Y_{cs} = 877.55X + 220952$, $Y_{ls} = 3224.5X + 907143$ with the square root of best fit given as $R^2_{ss} = 0.7406$, $R^2_{cs} = 0.9844$, and $R^2_{ls} = 0.9385$ respectively. Increase in the population of microorganisms was an indication that microorganisms acted on the polluted soil, bringing about the

decrease and disappearance of some of the hydrocarbons with increased in time as depicted in figure 3. The variation in the total heterotrophic bacteria can be attributed to variation in time as well as the concentration of the hydrocarbons.

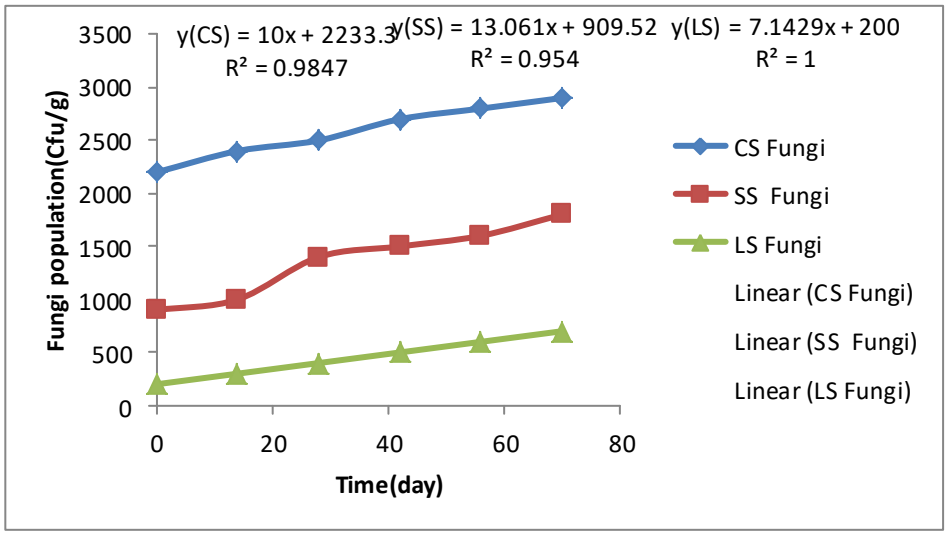

Figure 4 Graph of Total Heterotrophic Fungi versus Time on Crude Oil Diffusion in Various Soil Samples.

Figure 4 demonstrates the influence of the graphof total heterotrophic fungi (THF) in various soil samples upon the effect of time. The equations of curves are expressed as $Y_{ss} = 13.061X + 909.52, Y_{cs} = 10X + 2233.3$, $Y_{ls} = 7.1429X + 200$ with the square root of best fit given as $R^2_{ss} = 0.954$, $R^2_{cs} = 0.9847$, and $R^2_{ls} = 1$ respectively. Increase in the THF was observed with increase in time as shown in figure 4. The variation in the THF can be attributed to variation in time as well as the decrease inconcentration of hydrocarbons.

Statistical Analysis

Ho: T1 = 0: Time has no effect on the microbial growth of Aluu crude oil polluted soil environment.

Table 5 Analysis of Variation (ANOVA) of Total Heterotrophic Bacteria of Aluu Crude Oil Polluted Soil Environment.

Sources of Variance	df	SS	MS	F_{cal}	F_{tab}
Total	14	$1.77x10^{12}$			
Treatment	2	$1.73x10^{12}$	$8.65x10^{11}$	235.83	3.89
Error	12	$4.4x10^{10}$	$3.67x10^{9}$		

$F_{cal} > F_{tab}$ H_o: NS Accept

Table 6 Analysis of Variation (ANOVA) of Total Heterotrophic Fungi of Aluu Crude Oil Polluted Soil Environment.

Sources of Variance	df	SS	MS	F_{cal}	F_{tab}
Total	14	$1.23x10^{7}$			
Treatment	2	$2.01x10^{8}$	$1.004x10^{8}$	-6.39	3.89
Error	12	$-1.89x10^{8}$	$-1.57x10^{7}$		

$F_{tab} > F_{cal}$ H_o: Reject

4. CONCLUSION

The effect of microorganisms on Aluu crude oil polluted soil environment in Niger Delta was investigated. It was observed that in both total heterotrophic bacteria (THB) and total heterotrophic fungi (THF) as the time increases the microbial growth also increases. The variation in microbial activity may be attributed to the increased in population of the microorganisms as well as result of the consumption of the hydrocarbons in the polluted soil samples. Decrease in the concentration of the individual hydrocarbons was observed with increase in microbial concentration in each reactor.

Symbols

THB – Total heterotrophic Bacteria
THF – Total heterotrophic Fungi
SEM – Standard error of mean
CS- Clay soil, LS- Loamy soil and SS- Sandy soil

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