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ASSESSMENT OF HYDROCARBON LOSS IN BIOSTIMULATED AND BIOAUGMENTED CRUDE OIL CONTAMINATED ULTISOL OF SOURTHERN NIGERIA

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ABSTRACT

A pot experiment was conducted at the Rivers State University of Science and Technology, Port Harcourt, Rivers State, Southern Nigeria to assessed the effectiveness of poultry manure, urea and selected hydrocarbon degrading bacteria in the remediation of a crude oil contaminated Ultisol. Each pot weighing 3kg, was contaminated to 2, 5 and 10% $(^{w}/_{w})$ with Bonny light crude oil of 0.835 specific gravity with no contamination as a control. Seven (7) days after, each level was amended with urea and poultry manure (PM), thereafter the pots were seeded with *Acinetobacter clavatus*, *Bacillius subtilis, Pseudomonas aeruginosa* and *Corynebacterium diphtheriae.* Unamended and unseeded soils were also included as controls. Treatments were replicated three (3) times, completely randomized and arranged in a green house. Results showed that crude oil lost correlated negatively correlated with total seeded bacterial counts. In *Pseudomonas aeruginosa* inoculated soil, more percent crude oil lost were recorded except that at 2 and 5% pollution levels in the PM amended soil inoculated with *Bacillus subtilis* more crude oil (99.95 and 66.08%) was loss. This study suggests that at 2% contamination, provision of adequate PM for indigenous microbes is sufficient to remedy the soil while at 5 and 10% pollution levels, inoculation with *Bacillus subtilis* and *Pseudomonas aeruginosa* biostimulated respectively with urea are recommended as suitable biodegrading agents because greater percent loss in the crude oil were recorded in this options.

Keywords: *Bioaugmention , Biostimulation, Amendment, Seeded, Hydrocarbonoclatic, Biodegradation.*

INTRODUCTION

Crude oil is a complex mixture of hydrocarbons that form from the partial decomposition of biogenic material (Overton *et al*., 1994). It is constituted from thousands of compounds which are separated into saturates, aromatic, resins and asphaltenes. Saturates, especially those of smaller molecular weight, are readily biodegradable. Aromatics with one, two or three aroma tics rings are also efficiently biodegradable; however, those with four or more aromatic rings are quite resistant to biodegradation. The asphaltenes and resin fractions contain higher molecular weight compounds whose chemical structures have not yet been resolved. The biodegradability of these compounds is yet unknown (Harayana and Liv. 1999). Crude oil is used for the production of fuel and lubricants for transportation and energy needs and as a substrate for the petrochemical industry (Benka-Coker *et al,* 1995). Moller (1991) warned that these could bring about undesirable changes in the characteristic of the air, soil, water and food and can adversely affect the health, survival or activities of humans or other living organisms. He also observed that the role of soil microbes is geometrical in nature such that a sudden change in soil conditions will adversely alter the mineralization of organic carbon, nitrogen, sulphur and phosphorus for growth of higher plants and animals, Oil spills can also increase water holding capacity of

the soil, decrease mineralization, death of soil microorganisms due to toxic chemicals, retarded growth of nitrifiers result in ammonia accumulation, it adversely affect the life and productivity of soil and reduction in available plant nutrients (Amadi *et al*, 1993). Fortunately, Some varieties of bacteria have been implicated in the utilization of hydrocarbons (Odukuma and Dickson, 2003, Gerischer, 2008, Diaz, 2008, Ljungh, 2008). These varieties of bacteria are otherwise known as hydrocarbon utilizers or hydrocarbonscalstic microorganisms. They are capable of biodegrading a wide range of aromatic compounds and utilizing the carbon of petroleum as energy source. These oil utilizing bacteria are usually attracted and are more numerous in an oil contaminated environment (Okpokwasaill *et al*., 1986, Watanebe and Kasai 2008). They are found in a range of different ecological niches as soil, air, water, vegetable, sewage, skin etc. Moller (1991) warned that these could bring about undesirable changes in the characteristic of the air, soil, water and food and can adversely affect the health, survival or activities of humans or other living organisms. He also observed that the role of soil microbes is geometrical in nature such that a sudden change in soil conditions will adversely alter the mineralization of organic carbon, nitrogen, sulphur and phosphorus for growth of higher plants and animals, This is to say that in soil food web, microorganisms are essential in conservation either through storage of nutrients in living organisms or control of movement of nutrients between the biotic and interface. Oil spills can also increase water holding capacity of the soil, decrease mineralization, death of soil microorganisms due to toxic chemicals, retarded growth of nitrifiers result in ammonia accumulation, it adversely affect the life and productivity of soil and reduction in available plant nutrients (Amadi *et al*, 1993). In appreciation of the potential danger posed by oil spills and industrial effluents, countries world wide are encouraged to set up legislations to ensure proper compliance to effluent and oil spill clean up standards. Since then several researches have been undertaken aimed at effective clean up whenever it occurred. Apart from hydrocarbons spills, soils are frequently the recipients of many pollutants from agricultural production and other sources either accidentally or intentionally. This therefore means that soil contamination will occur from time to time.

Driesser (2004) reported that bioremediation holds promise in solving environmental pollution problems because of efficiency of the process, economic feasibility, legal requirements and the mechanisms involved in the remediation process. But a major setback to the effective use of bioremediation is that a particular strain of soil microorganisms is quite restricted in the range of hydrocarbons it can grow on (use as substrate). This is why the bioremediation of pollutants in the natural environment is known to be slow and a complicated process whose quantitative and qualitative aspects depend on the nature and the amount of pollutant present, the constitution and the seasonal environmental condition, the population density and the constitution of the indigenous microbial community (Leahy and Coleovell, 1990). Thus, the need to facilitate the rate of hydrocarbon biodegradation in the environment. In this regard bioaugmentation and biostimulation methods have often been employed (Atlas and Bartha, 1992). The rate of biodegradation also differs among individual bacteria and hence the need to evaluate effectiveness of bacteria. Most of the studies done are concentrated on the effects of crude oil pollution on the environment and soil biota.
Evaluation of pollutants degraders capabilities therefore become necessary because the finding could point to a more effective biodegradable agents and also help in the

development of perfect microbial cleanup culture for the bioremediation of contaminated soil. The objective this study was to identify the most effective hydrocarbon degrading hydrocarbonoclastic organism stimulated or without nutrient resources (organic and inorganic) to remedy a crude oil contaminated Ultisol.

MATERIALS AND METHODS

Experimental Site

The study site was Rivers State University of Science and Technology Teaching and Research Farm, Port-Harcourt, Rivers State of Nigeria. Rivers State has a projected population of 3.9 million (NPC, 2006) and lies within humid tropical rain forest zone of Nigeria located in latitude 4.5°N and longitudes 7.0l°E and on elevation of 18m above sea level (FAO, 1984). The study site Rivers State University of Science and Technology is situated at the Western corner of Port Harcourt within longitude 7°E and latitude 4.5°N on the coastal plain sand. The area experiences two distinct seasons rainy and dry seasons; the raining season starts from April and lasts till October with a brief period of dryness (August Break). The rainfall is' heavy with estimated annual range which may vary from 2000 to 2484mm (FAO 1984; MANR, , 2005). Rainfall pattern is bimodal with peaks in June and September (Ukpong, 1992). The highest temperature (31°C) is experienced during the months of February through March and coincides with the overhead passage of the sun (Enwezor, 1990; RISADEP, 1995).

Chemical Properties of Poultry Manure

Poultry manure was obtained from a local poultry farm and air dried, sieved and analyzed for chemical properties (pH, OC, total N, P, Ca, Mg, Na and K) by methods described by AOAC (1990)(Table 3.1). and applied according to Nyle and Ray (2007).

Table 1: Chemical Properties of Poultry Manure

Parameter Measured	Values
Ph	6.99
Total N (%)	3.01
Phosphorus (mg kg^{-1})	3893.12
Ca (cmol kg^{-1})	922.60
Mg (cmol kg^{-1})	578.40
Na (cmol kg^{-1})	1318.00
K (cmol kg^{-1})	1823.00

Table 2 Physico-chemical Properties of the Soil used for the study before the addition of treatments

Parameter Measured	Values
pH (H_2O) 1:2.5	5.80
Organic Carbon (%)	3.91
Total N (%)	0.12
Available Phosphorus (mg kg^{-1})	267
Exchangeable bases	

Ca (cmol kg^{-1})	3.07
Mg (cmol kg^{-1})	1.30
Na (cmol kg^{-1})	0.73
K (cmol kg^{-1})	0.86
Exchangeable acidity (cmol kg^{-1})	1.00
ECEC (cmol kg^{-1})	6.96
BS (%)	83.22
Sand (%)	85.82
Silt (%)	8.42
Clay (%)	5.76
Textural class	Sandy loam

Source of Crude Oil

The crude oil used as soil pollutant for the experiment was obtained from Shell Petroleum Development Company (SPDC) flow station at Korokoro, Tai Local Government, Rivers State. It was the fresh Bonny light crude oil with relative density (specific gravity) of about 0.835, determined by AOAC standard (1990).

Isolation, Identification and Cultivation of the Hydrocarbon Utilizing Bacteria (HUB)

Bacteria used in this study were the indigenous hydrocarbon utilizing bacteria. The study soil was contaminated with the Bonny light crude seven (7) days after the soil was collected, sieved for the purpose of isolation, identification. Characterization and cultivation of hydrocarbon utilizing bacteria (HUB) by methods described by Harrigan and McCane,(1990). Cowan, (1974), Buchanan and Gibbons, 1974).

Seeding of Bacteria into the Soil

Four most numerous species were isolated from the studied soil for the research. These were *Acinetobacter clavatus, Bacillus subitilis, Pseudomonas aeruginosa and Corynebacterium diphtheriae* having 1.80, 1.35, 2.00 and 1.30 x 10^4 cfu/g soil counts respectively. Nutrient broth culture of all the hydrocarbon utilizing genera isolated from the polluted soil were prepared and incubated for 48 hours. Nutrient broths were supplemented with Bonny light crude oil to optimize the organisms (Science Guardian, June 18, 2009; Mills *et al*, 1978). These nutrient broth cultures served as inocula for the treatment units. Twenty (20) millilitres (130 L/ha) of the 24 hours nutrient broth culture of each of the test organism were introduced into the respective units (Ekpo and Udofia, 2008).

The Experiment

Top soil (0-15cm) was collected from the uncultivated area of Rivers State University of Science and Technology Teaching and Research Farm for the experiment: Forty five (45) kilograms of soil were respectively polluted to 0, 2, 5 and 10% with Bonny light crude oil of 0.835 relative density and left undisturbed for seven days to enable it settled and then, fifteen (15) kilograms of each of the pollution level were respectively amended with 100

kg ha^{-1} N as urea, 10 tha^{-1} poultry manure and left unamended. Then, three (3) kilograms of each of the treatment options were transferred into five (5) polythene bags measuring 30 x 28 cm^3 and inoculated with *A. clavatus, B.subtilis, P. aeruginosa, C. diphtheriae*, left without inoculation and replicated three times, The aim was to simulate crude oil spill and emergency *ex-situ* clean up conditions. The experiment was kept moistened at field capacity prior to planting of maize using watering can (Akpan *et al*, 2006). The experiment was completely randomized and arranged in a green house (Alika, 1992), (Fig 3.2). There were one hundred and eighty bags (180). To ensure that all the experimental units received equal environmental condition, bags were relocated in the green house every week.

Sampling

Soil

Before and after treatment application (Table 3.2) and at week nine, soil samples were analyzed for physico-chemical (total hydrocarbon content, % organic carbon, Available P., Calcium, Magnesium, Sodium and total nitrogen)(Table 2) Samples were collected from each of the treatments using sterile plastic tube.

Extraction of Total Hydrocarbon Content (THC)

Modified method of Toogood and McGill (1977) adopted by Ekpo *et al.,* (2008) was used for the extraction of THC in the soil. A preliminary extraction was carried out with 10ml of crude oil mixed with 50g of the soil. This was used to determine the percent recovery rate. 20ml of methlylene chloride (petroleum hydrocarbon contaminants extractor; USEPA Method 3345, 2010) was poured into each bottle containing 100g soil of each treatment option. It was shaken vigorously and decanted into sterile test tube. The extraction was repeated twice with 10ml each of the methylene chloride. The total extract was then heated for eight (8) minutes at 175^0C over a hot water bath for the evaporation of the methylene chloride. The residual crude oil left in the test tube was then weighed and the THC calculated based on the fractional recovery rate (FRR). Three (3) extractions were done for each set and the mean value recorded. It was discovered that the percent recovery rate was consistently 73.50 ± 2% (i.e. from 10g introduced into the soil, 73.50 ± 2g was recovered while the remaining 26.5 ± 2g absorbed to the soil particles.

From the above, it was possible to calculate the fractional loss per gram of the crude oil recovered as:

Amount of crude oil introduced	–	100g
Amount of crude oil recovered	-	73.50g
Recovery per gram of the crude oil	-	73.50/100 = 0.735g
Percent recovery	-	0.735x100 = 73.50%
Percent loss	-	26.50%

The fractional recovery rate of 0.735 is therefore a constant with respect to the method used here for crude oil residue recovery. The fractional loss of 26.50% needs to be calculated and added to the recovery crude before the quantity biodegraded is known.

Analytical Methods for Soil

The soil samples were processed for mechanical and chemical analyses. The soil samples were air-dried, crushed and passed through a 2mm sieve. For the determination of

organic C, total N, Ca, K, P, Na and Mg, the samples were further ground to pass through a 100-mesh sieve.

* pH was determined in water with glass electrodes in the 1:2.5 soil water ratio (Udo and Ogunwale, 1978).
* Organic matter was determined by wet oxidation method of Nelson and Summers (1982).
* Total nitrogen was determined using macrokjeldahl digestion and distillation method of Jackson (1970).
* Exchangeable bases (Ca, Mg,) were extracted with molar ammonium acetate, while K and Na concentration were determined by flame photometry (Thomas, 1982), and
* Magnesium and Sodium were determined by EDTA titration (Jackson, 1970).
* Exchangeable acidity (Al plus H) was extracted with molar KCL solution and acidity determined by titration (McLean, 1965).
* Effective cation exchange capacity (ECEC) was taken as the sum of individual exchangeable bases plus exchange acidity (Kamprath, 1984)
* Available P was determined by methods described by Page *et al.,* (1982) and Sparks (1996).
* Mechanical analysis was carried out by hydrometer procedures as described by Klute (1986).

Statistical Analysis

Analysis of variance according to the completely randomized design at 0.05% level was used to test the treatment effects. Soil physico-chemical properties and microbial analysis were compared using Least Significant Different (LSD) at 0.05% probability level.

RESULTS

Chemical Properties of the Soil used for the Study

Results of chemical properties of the soil used in the study are presented in Table 2. The soil was acidic in nature with a mean pH value of 5.80. Organic carbon values were 3.63, 4.11 and 3.99 with a mean value of 3.91%. Total nitrogen was 0.13, 0.13 and 0.10 with a mean value of 0.12% while available phosphorus mean value was 267mg kg^{-1}. Exchangeable bases examined include Ca, Mg, K and Na which had means values of 3.07, 1.30, 0.73 and 0.86 cmol kg^{-1} soil respectively with cation exchange capacity of 6.96 cmol kg^{-1} and 83.22% base saturation. The proportion of sand, silt and clay in the studied soil were 86.18, 84.94, 86.30 and 8.71, 8.39, 8.16 and 5.83, 5.83, 5.89, 5.56 with means values of 85.82, 8.42 and 5.76. Texturally, the soil was predominantly sandy loam.
on levels at week nine.

Total Hydrocarbon Content (THC)

Detailed results of THC in the study soil are presented in Table 3. The results showed that, THC values were higher in the uninoculated soil as compared to the inoculated soil.
After application of the crude oil, 58.97, 57.70, 56.61, 57.40 and 57.44g/kg soil THC values were recorded in the unamended soil without inoculation, soil inoculated with *A. clavatus, B. subtilis, P. aeruginosa* and *C. diphtheria,* respectively at 2% pollution level. These represent 1.72%, 2.17%, 5.65%, 4.33% and 4.26% THC loss. At 5 and 10%

pollution level, 0.60, 1.10, 2.28, 1.99, 1.65% and 0.26, 0.78, 1.00, 1.72, 1.00% hydrocarbon loss were respectively recorded in the above mentioned treatment options. In the urea amended soil at 2% pollution level, 57.38, 52.82, 51.77, 50.80 and 51.90g/kg THC were recorded in the uninoculated soil, soil inoculated with *A. clavatus, B. subtilis, P. aeruginosa* and *C. diphtheria,* respectively. These represent 4.37, 11.20, 13.72, 15.33 and 13.50% THC loss while in the PM amended soil (in above respectively soil), 3.43, 9.28, 13.17, 11.57, 10.48% hydrocarbon loss were recorded after treatments. At 5% pollution level, after treatment applications 149.10, 148.35, 146.55, 147.01, 147.52 and 147.52, 144.26, 142.26, 142.84, 141.93 and 148.23, 145.53, 144.10, 143.83, 145.02g/kg soil THC were recorded in the soil without inoculation, inoculated with *A. clavatus, B. subtilis, P. aeruginosa* and *C. diphtheriae* in the unamended soil, soil amended with urea and PM, respectively. Above represent 0.60, 1.10, 2.28, 1.99, 1.65% and 1.40, 3.83, 4.77, 5.38, 4.07% and 1.18, 2.98, 3.93, 4.11, 3.32% hydrocarbon loss. At 10% pollution level, 299.21, 297.66, 296.98, 294.83, 296.54g/kg and 296.74, 293.12, 291.69, 290.10, 292.60g/kg and 297.18, 292.14, 294.13g/kg soil THC were recorded in the soil without inoculation, inoculated with *A. clavatus, B. subtilis, P. aeruginosa* and *C. diphtheriae* in the unamended soil, soil amended with urea and PM respectively. Above represent 0.26, 0.78, 1.00, 1.72, 1.00% and 1.09, 2.29, 2.77, 3.30, 2.47% and 0.94, 1.75, 2.27, 2.62, 1.96% hydrocarbon loss.

This therefore indicates that in the unamended soil, the rate of THC degradation was greater in the soil inoculated with *B. subtilis* at 2 – 5% pollution levels and in the *P. aeruginosa* inoculated soil at 10% pollution level. Similar results were obtained in the PM anmended soil while in the soil amended with urea, THC reduction rate was greater in the soil inoculated with P. aeruginosa at 2- 10% pollution levels. The results also revealed that the rate of the degradation decreased with increase in crude oil concentration (2 > 5 > 10% pollution level). At week nine of the study period, 84.93, 99,50, 99.63 were the highest amount of hydrocarbon degraded in the unamended soil, soil amended with urea and PM, respectively in soil inoculated with *B. subtilis* at 2% pollution level. At 5% pollution level, highest percent hydrocarbon degradation (66.45, 91.69, and 73.45%) were recorded in the soil inoculated with *B. subtilis* in the unamended soil, soil inoculated with *P. aerginosa* in the soil amended with urea and PM, respectively. At 10% pollution level, 46.50, 74.58 and 61.44% highest hydrocarbon loss were recorded in the unamended soil, soil amended with urea and PM all in the soil inoculated with *P. aeruginosa.* The above data revealed that the rate of THC reduction was greater in urea amended soil, followed by the PM amended soil while the least values were obtained from the unamended soil. In the inoculated soil, THC decreased from 60g/kg soil to 57.70, 56.61, 57.40, 57.44g/kg and from 150 to 148.35, 146.55, 147.01, 146.53g/kg soil and from 300 to 297.66, 296.98, 295.83, 296.54g/kg soil in the unamended soil inoculated with *A.clavatus*, *B. subtilis, P.aeruginosa and C.diphtheriae* at 2, 5 and 10% pollution levels, respectively. These represent 3.83, 5.70, 4.30, 4.30% THC reductions at 2% pollution level, and 1.10, 2.30, 2.70, 2.31% THC reduction at 5% pollution level and 0.80, 1, 1.40 and 0.80% THC reductions at 10% pollution level after inoculation in the urea amended soil, 12, 13.70, 15.30% and 3.80, 4.80, 5.40, 4.07% and 2, 2.80, 3.30, 2.50% THC reductions were obtained in the soil inoculated with *A.clavatus, B. subtilis, P.aeruginosa and C.diphtheriae.* In the PM amended soil (in the above order) 9.30, 13.20, 9.90, 8.80% and 3.80, 4.60, 4.10, 3.10% and 1.80, 2.30, 2.60, 1.70% THC reductions

were obtained. This scenario implies that in the unamended soil, the rate of THC degradations was faster in the soil inoculated with *B.subtilis* at 2 - 5% pollution levels and in the *P.aeruginosa* inoculated soil at 10% pollution level. Similar results were obtained in the PM amended soil while in the soil amended with urea, THC reduction rate was higher in the soil inoculated with *P.aeruginosa* at 2 – 10% pollution levels. The results also revealed that the rate of degradation decreased with increase in crude oil concentration (2 > 5 > 10% pollution level). This indicated that the rate of crude oil loss decreased with increase in crude oil concentration. At week nine of the study period, 94.73, 99.50 and 99.63% THC were respectively degraded in the unamended soil, soil amended with urea and PM, all inoculated with *B.subtislis* at 2% pollution level. At 5% pollution level the highest percent degradation of (66.47, 91.63, 82.30%) were recorded in the soil inoculated with *P. aeruginosa*.

Table 3: Effect of treatments of soil THC (g kg^{-1})

	Week 0			Week 9		
Crude Oil Level /Bacteria	**Amendments**			**Amendments**		
	0	**1**	**2**	**0**	**1**	**2**
0%B_0	0	0	0	0	0	0
0%B_1	0	0	0	0	0	0
0%B_2	0	0	0	0	0	0
0%B_3	0	0	0	0	0	0
0%B_4	0	0	0	0	0	0
2%B_0	58.97	57.38	57.94	17.54	8.41	13.82
2%B_1	57.70	50.80	54.43	11.33	1.11	4.14
2%B_2	56.61	51.77	52.10	9.16	0.30	0.22
2%B_3	57.40	50.80	52.06	10.88	0.17	1.09
2%B_4	57.44	51.90	53.73	13.16	4.33	5.76
5%B_0	149.10	147.90	148.23	99.43	65.75	74.99
5%B_1	148.35	144.26	144.53	82.86	22.79	40.03
5%B_2	146.55	142.84	144.11	62.33	16.70	29.70
5%B_3	147.02	141.93	143.84	60.33	12.47	26.55
5%B_4	146.52	143.90	145.02	73.96	20.9	32.66
10%B_0	299.21	296.74	297.18	225.16	189.67	132.11
10%B_1	297.66	294.12	294.74	148.67	85.23	63.11
10%B_2	296.98	291.69	293.18	169.10	85.44	128.67
10%B_3	294.83	293.10	292.14	160.14	76.27	115.68

10%B_4	297.73	292.40 294.93		177.24	89.58	150.17
LSD (0.05)	**45.04**	**20.32**	**16.34**	**18.42**	**12.72**	**16.56**

Key: B_0, B_1, B_2, B_3, B_4, = *Soil without inoculation, soil inoculated with A. clavatus, B. subtilis, P. aeruginosa and C. diphtheria, respectively.*
: 0, 1, 2, = Soil without amendment, soil amended with urea and PM respectively.

In the unamended soil, soil amended with urea and PM, respectively. At 10% pollution level, 64.60, 81.20 and 87.10% THC were degraded in the unamended soil, soil amended with urea and PM, all obtained in the soil inoculated with *P.aeruginosa.* this suggests that *B.subtilis* and *P.aeruginosa* can degrade more crude oil at the lower and the higher level of pollution, respectively.

Table 4: The relationship between THC and bacterial counts during the remediation as expressed by correlation coefficient values and regression (SE)

		Treatments Options		
Crude Oil Level (%)	**Bacteria**	**No Amendment**	**Amended with Urea**	**Amended PM**
2	B_0	r = -0.921, SE = 9.91 X+ 280.44	r = -0.990, SE = 10.152 X+ 11.67	r = -0.983, SE = 10.92 X+ 353.47
	B_1	r = - 0.995, SE = -19.629 X + 599.91	r = - 0.948, SE = -12.31 X + 34.46	r = - 0.927, SE = -12.39 X + 40.09
	B_2	r = - 0.991, SE = -12.83 X + 86.63	r = - 0.832, SE = -10.48 X + 234.53	r = - 0.996, SE = -16.94 X + 51.79
	B_3	r = - 0.975, SE = -93.59 X + 93.59	r = - 0.872, SE = -11.988 X + 159.63	r = - 0.984, SE = -15.76 X + 360.38
	B_4	r = - 0.958, SE = -12 X + 175.40	r = - 0.9748, SE = -12.06 X + 36.03	r = - 0.967, SE = -14.51 X + 23.35
5	B_0	r = - 0.910, SE = -6.16 X + 625.51	r = - 0.953, SE = -5.58 X + 376.68	r = - 0.956, SE = -0.145 X + 284
	B_1	r = - 0.978, SE = -8.66 X + 982	r = - 0.992, SE = -6.07 X + 166.71	r = - 0.962, SE = -6.550 X + 253.56
	B_2	r = - 0.309*, SE = -0X + 415.58	r = - 0.313*, SE = -1.29X + 332.75	r = - 0.996, SE = -7.83 X + 193.46
	B_3	r = - 0.276*, SE = -0X + 101.839	r = - 0.552*, SE = -5.62X + 45.95	r = - 0.987, SE = -7.91 X + 215.83
	B_4	r = - 0.716 SE = -0X + 281.80	r = - 0.995 SE = -5.06X + 110.38	r = - 0.911, SE = -3.54 X + 164
10	B_0	r = - 0.778, SE = -0.135 X + 3.71	r = - 0.789, SE = -1.78 X + 110.98	r = - 0.837, SE = -2.471 X + 503.07
	B_1	r = - 0.971, SE = -1.60 X + 316.88	r = - 0.497, SE = -0.0192 X + 136.50	r = - 0.956, SE = -2.237 X + 332.25
	B_2	r = - 0.945 SE = -2.00X + 349	r = - 0.985 SE = -0.533X + 103.62	r = - 0.894, SE = -0.030 X + 196.85
	B_3	r = - 0.942 SE = -1.74X + 376.86	r = - 0.938 SE = -0.179 X + 141.67	r = - 0.853, SE = -0.263 X + 302.65
	B_4	r = - 0.962 SE = -1.38X + 474	r = - 0.345 SE = -0.22 X + 95.226	r = - 0.919 SE = -2.69 X + 433.53

**** Not significant at 1 and 5%***
Key: *B_0, B_1, B_2, B_3, B_4 = soil without inoculation, soil inoculated with A.Clavatus, B.subtilis, P.aeruginosa and C.diphtheriae respectively.*
SE = Significance equation
PM = soil amended with poultry manure

DISCUSSIONS

The detailed data on the physic-chemical properties of the soil are presented in Table 2. Mechanical analysis of the studied soil as presented in Tables 2 showed that the silt contained more than 50% sand, less than 15% silt and clay. It is described as sandy loam (Esu, 1999) which give rise to typic Paleudult, a characteristic of soil derived from coastal

plain sand. Based on the soil taxonomy Soil Survey Staff, (1990) and the UNESCO (1998), the soil is classified as Ultisol. Crude oil lost in the soil are presented in Tables 3.. As at the end of the study (week nine) loss were recorded in the soil seeded with *B.clavatus* (99.95, 99.63, 66.08%) in the urea and in the PM amended soil at 2% pollution levels and in the unamended soil at 5% pollution level, respectively, while at higher levels (5 - 10%) with the exception in the unarnended soil at 5% pollution level, higher percent crude oil loss were recorded in the soil inoculated with *P.aeruginosa*. This therefore means that though *B.clavatus* can degrade more in the higher levels of crude oil concentration,.

The highest percent crude oil loss in the soil inoculated with *P.aeruginosa* at higher pollution levels is not surprising because *P.aeruginosa* species is known to possess a more competent and active hydrocarbon degrading enzymes than other biodegraders (Walter, *et al.,* 1976). It has also been reported that *P.aeruginosa* species, because of the ability to degrade wide ranges of pollutants exhibited an increase rate of the pollutants trichioroethylene (TCE) from groundwater (Munakata - Marr, *et al.,* 1996).

The amount of crude oil loss in the unamended soil was comparatively low and very low in the unamended soil without inoculation. This therefore means that inoculation was effective. Higher rate of biodegradation observed in the amended soil can be attributed to increase in the microbial biomass due to nutrient availability. This is in agreement with earlier report that added nutrient increased the rate of biodegradation (Dibble and Bartha, 1976, Jones and Greenfield.., (1991).

Higher percent of crude oil loss were observed in the area amended soil from week 0 – 4 and from week 4 -9 in the PM amended soil. This seems to suggest that inorganic nutrients are better than organic one in the short run and vis versa.

Finally, the significant negative correlation between THC and the microbial countsTable 4) in the soil suggests that the rate of crude oil degradation in the crude oil contaminated soil also depends upon the ability of the soil degrading microorganisms to multiply in large numbers.

CONCLUSION AND RECOMMENDATION

Greater percent loss in the total hydrocarbon was obtained in the soil inoculated with *B.subtilis* followed by that of *P.aeruginosa* in all the treatment options at 2 – 10% pollution levels. These bacteria also had greater population sizes which is generally regarded as the most efficient microorganisms which are well adapted to the remediation conditions as compared to *A.clavatus,* and *C.diphtheriae.* In this study, in the soil amended with urea, PM at 5% pollution level and in the soil without amendment at 10% pollution levels, inoculation with *B.subtilis* is recommended and in the unamended soil at 2 and 10% pollution levels and at 10% pollution levels in the amended soil, *P.aeruginosa* is recommended as the suitable crude oil degrading bacteria. In view of the above, it is not advisable to inoculate 2% crude oil contaminated soil but from 5% pollution levels with a thorough examination of bacteria nutrient pathways. This is because a well adapted bacterium could multiply in large numbers and assimilate the pollutants just to release them into their surrounding when they die. All that is needed is the application of the right types and quantities of feedstuff for indigenous microbial multiplication to enhance degradation especially at the lower level of contamination.

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