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Effectiveness of NPK Fertilizer-Saw Dust Amendment on Biodegradation of Crude Oil in Polluted Soil

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ABSTRACT

Soil pollution by crude oil causes nitrogen imbalance and impedes the flow of air and nutrient. Biostimulation improves this nutrient and aeration and enhances the rate of biodegradation of the pollutants. This study was undertaken to use biostimulation to strategically biodegrade crude oil in polluted soil. Optimization of NPK fertilizer (61:15:15) and sawdust enhances optimum growth of hydrocarbon-degrading microorganisms with a resultant restoration of the soil. Nine treatment cells (A, B, C, D, E, F, G, H, I) and control (J) were subjected to various treatment combinations according to the experimental design. The resulting residual concentrations of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) are indicators of the soil restoration. Moreover, the degradation efficiency for TPH ranged between 98.14% and 99.05% and the biodegradation kinetics rate between -0.0419 and -0.0479 per day while microbial kinetics growth rate varied between 0.1840 and 0.1931 per day in a 105 day bioremediation time. However, the percentage utilization of nitrogen and phosphorus were between 85.1%, 81.5% to 92.3%, 92.8% respectively. Interaction between the process independent variables provided both nutrient and favorable medium suitable for microbial activities for optimum treatment. Sawdust combined with NPK fertilizer is therefore effective for treating and restoring a hydrocarbon polluted soil.

Key words: *Crude oil, Aeration, Biostimulation, Biodegradation, Residual concentration, Soil restoration.*

1. INTRODUCTION

Pollution associated with crude oil is prevalent where its exploitation, transportation and refining takes place. This comes with social, sanitary and economic impacts, decreasing the productivity and value of the affected sites. Its occurrence at sea or on land triggers serious concerns for sustainable remediation approaches that could speedily restore the impacted environment. The Niger Delta of Nigeria had experienced oil spill cases of diverse magnitudes on the environment since 1970, devastating the affected lands and swamps. The Bomu oil spill of 1970, Escravos oil spill of 1978 (0.3Mbbl), Faniwa-5 well blow out of 1980 (0.4Mbbl), Mogho oil spill of 2004, K-dere oil seepage of 2008 and Yorla oil spill of 2009 [39] are just but examples.

Vast land areas are impacted causing great damage to the ecology of the region through the toxicity and persistence of crude oil's constituents [33]. The aftermath is poor soil aeration, nutrients immobilization, loss of water holding capacity and inhibition of catalytic enzymes. It creates an unhealthy distortion of the carbon-nitrogen ratio resulting in nitrogen deficiency in the soil. This situation retards the growth of soil microorganisms and hydrocarbon degradation [10, 5]. Agricultural productivity and recreational activities in the region are affected. Over the years various methods have been devised to degrade hydrocarbon in contaminated soils under different approaches and conditions. Some of these techniques are: monitored natural attenuation [32], land farming [10, 28], joint biostimulation and bioaugmentation in soil biopile [38] and enhanced bioremediation [19].

However, improper and inadequate administration of remediation has been shown to render the soil more toxic, inhibiting microbial growth [30, 5] while the residual oil devastates the soil. The unfortunate persistence of petroleum hydrocarbon in the

soil at Ogoni, in the Niger Delta region of Nigeria above regulatory level long after a remediation exercise is a pointer to ineffective and unsustainable remediation approach. As a result, TPH persisted in the soil far above the regulatory target of 50 mg/kg and benzene 900 times above regulatory concentration in underground water, forty (40) years after an oil spill [40]. This necessitates the use of a biostimulation strategy that systematically optimizes NPK fertilizer and sawdust under the native environmental condition in the field to generate the needed nutrient, permeability and aeration of the soil for rapid biodegradation of the pollutants. The process directly encounters variation in climatic condition and biological competition in the field at reduced operational cost and throughput time, having high efficiency with no adverse effect on soil functionality and resources management.

Generally, biostimulation is achieved by introducing nutrient in the form of organic or inorganic fertilizer into the polluted medium to boost the population of indigenous microorganisms with metabolic capacity to degrade hydrocarbon [25, 3] to nontoxic forms. Applying nutrient amendment and woodchips on clayey-loam soil in the field [19] with proper engineering of the environmental and process factors [34, 41], crude oil degradation is achievable. It is effective at any time of the year [24] with suitable controls in the rainy and dry season. A number of studies have focussed on identifying more imaginative uses and applications of waste materials in line with the waste to wealth concept. Consideration of saw dust is predicated on its local availability, cost effectiveness, organo-structural property ([15] and numerous large pore spaces [44] in addition to its low biodegradability which sustains soil permeability to air and nutrients longer. The environmental nuisance potential of this waste matter is therefore reduced. Consequently, enzyme activity is enhanced on its surface [23, 6] raising the temperature of the medium [24] thus increasing hydrocarbon biodegradation rates. Besides, sawdust is not degraded simultaneously with crude oil, rather sequentially in a “diauxic” manner according to [18]. In line with this concept, crude oil (the primary substrate) is exhausted in the medium before sawdust (the secondary, less preferred) could be consumed. This in effect, lengthens the permeability function of saw dust, giving long term solution for ecosystem balance. But the saw dust could be biodegraded in the long run to form products that build soil structure and water holding capacity [37].

However, bioremediation by biostimulation applied directly with fertilizer on the beaches of Prince William Sound, Alaska accelerated natural degradation of oil by indigenous micro flora on the surface and subsurface soils [17]. Earlier studies by [32, 27, 2, 11, 26, 22, 4] adjudged biostimulation to be very effective in accelerating pollutant degradation. The present study evaluates the rate of hydrocarbon degradation in response to nutrient and microbial interaction in the field, identifies the optimum concentration of the treatment factors for maximum biodegradation of pollutant and evaluates the microbial growth kinetics and the predominant colonies in crude oil degradation.

2.0 MATERIALS AND METHODS

2.1 Description of study area

This study was conducted on about 250m^2 area of a flat land located at Iriebe in Obio-Akpor local government area of Rivers state, Nigeria. It is located on the Latitude; 4^o 53` 20.35`` and longitude; 7$^o$ 06` 29.80`` and an elevation of 76 feet above sea level.

2.2 Collection of research materials

NPK fertilizer (15:15:15%) was obtained from the Agricultural Development Program headquarters in Port Harcourt, Nigeria and blended with Urea (46%) from the same source into 61:15:15. Saw dust was obtained from a saw mill in Elelenwo, Port Harcourt, Nigeria while Crude oil (Amenam blend) was obtained from a certified crude oil export inspectorate firm in Port Harcourt, Nigeria. The polluted soil samples were collected in cores of 0-15cm deep in two replicates from the surface of each treatment cell

2.3 Soil characterization and physicochemical analysis

The native soil sample was analyzed for physicochemical characteristics like soil texture, pH, total petroleum hydrocarbon (TPH), total organic carbon, total nitrogen, and total phosphorus. These analyses were also carried out periodically on the polluted and treated soil to ascertain the bioremediation process. Soil pH was determined electrometrically with HACH multi-parameter pH meter fitted with both pH and reference electrodes. Particle size analysis was done using Bouyoucos hydrometer method with procedures that conform to BS 1377 (1990) and ASTM–D423/D-424-54T (1975). Moisture content was determined by gravimetric method (ASTM D2216-66) while total organic carbon was determined by the Walkley and Black method adopting high temperature combustion method (APHA 5310B). Total Nitrogen was determined using the Kjedhal oxidation method with HACH digesdahl digestion apparatus/peroxide method. However, total Phosphorus was determined by ascorbic acid-

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phosphomolybdate method and measured with HACH UV spectrophotometer. Total petroleum hydrocarbon (TPH) was extracted using dichloromethane according to USEPA 3550C, concentrated and analysed using gas chromatography fitted with flame ionisation detection (GC/FID) according to USEPA 8270. Polycyclic Aromatic Hydrocarbon (PAH) was likewise analyzed by gas chromatography fitted with mass Spectrometry (GC/MS) according to USEPA 8270.

2.4 Microbiological Analysis of hydrocarbon degrading Bacteria and Fungi

The indigenous soil microorganisms with hydrocarbon degrading abilities were isolated, identified and their microbial population determined before and within intervals of the treatment process. Bacteria were enumerated using vapor phase transfer technique adopting spread plate method in a mineral salt medium - APHA 9215C. Cell morphology and gram staining reaction was employed for identification and characterization of bacterial isolates. Fungal isolates were also enumerated using vapor phase transfer technique adopting spread plate method - APHA 9610C but antibiotics were added to suppress the growth of bacteria. Fungi identification was by morphological characteristics and microscopic examination.

2.5 Experimental design and analysis of data

This research was conducted in the field using analytical experimental design in a completely randomized block fitted into a 3^2 full factorial design. NPK fertilizer (X_1) and saw dust (X_2) were the independent variables. Nine cells of 1.0 square meter each (A, B, C, D, E, F, G, H, and I) in two replicates labeled A_n to I_n along with a control cell (J) were formed and adequately ridged from each other to forestall run off. Oil spill was then simulated in all twenty-seven cells using five (5) liters of crude oil to each. The control cell (J) was likewise polluted with five (5) liters of crude oil. Batches of three cells in each replicate were biostimulated with 1kg, 2kg and 3kg of the blended N.P.K fertilizer (61:15:15) followed simultaneously with 0.5kg, 1kg and 2kg of coarse saw dust according to the experimental design. The fertilizer and saw dust were thoroughly mixed with the soil to a homogenous matrix. Samples were collected from each cell according to sampling plan for analysis. Each cell was then re-tilled after sampling for aeration.

The residual concentration of TPH, PAH, Nitrate, Phosphate, HDB, HDF, pH and moisture were assessed within 105days through periodic monitoring. TPH was analyzed with GC-FID - USEPA 8270, PAH (GC-MS - USEPA 8270), Total Nitrogen (Kjeldhal method - APHA 4500), Total Phosphorus (Ascorbic acid method), Soil pH (Glass electrode pH meter), Moisture contents (Gravimetric method - ASTM D2216-66), Hydrocarbon Degrading bacteria and Fungi (HDB, HDF) - Vapor phase transfer and Spread plate method (APHA 9215C and APHA 9610C respectively). Soil restoration was determined by measuring the residual TPH and PAH as well as HDB and HDF populations at intervals to establish the efficiency and kinetics of the bioremediation process.

Table 1: **Experimental design and treatment cells lay out**

J **Negative control**

R		
A x1y1	**B** x1y2	**C** x1y3
D x2y1	**E** x2y2	**F** x2y3
G x3y1	**H** x3y2	**I** x3y3

Where, R, R_1

R1		
D x2y1	**E** x2y2	**F** x2y3
G x3y1	**H** x3y2	**I** x3y3
A x1y1	**B** x1y2	**C** x1y3

and R_2

R2		
G x3y1	**H** x3y2	**I** x3y3
A x1y1	**B** x1y2	**C** x1y3
D x2y1	**E** x2y2	**F** x2y3

represent the treatment and replicates 1 and 2 respectively
x represents NPK fertiliser and x_1, x_2, x_3 is 1kg, 2kg and 3kg of the fertiliser respectively
y represents Saw dust and y_1, y_2 and y_3 is 0.5kg, 1kg and 2kg of the saw dust respectively

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3.0 RESULTS AND DISCUSSION

3.1 Results

Test samples from the treated soil were analyzed for physicochemical and biological parameters. Restoration of the soil and the kinetics of the process were determined through degradation of the hydrocarbon by microorganisms. The native soil had a slightly acidic pH and low concentration of nitrogen and phosphorus. However, initial soil moisture content was low and the soil texture loamy sand having some microbial colonies and a background TPH above Nigerian regulatory target of 50mg/kg by the department of petroleum resources (DPR) but below the intervention value of 5000mg/kg. These properties are expressed in *Table* 2.

Table 2: Physicochemical and biological properties of the native soil in the study area

Parameters	**Value**	**Nigerian DPR target**
TPH(mg/kg)	215.2	50
HDB (cfu/ml)	$2.10x10^{2}$	*
HDF (cfu/ml)	$3.7x10^{1}$	*
Total Nitrogen (mg/kg)	9.10	*
Total Phosphorus (mg/kg)	49	*
pH	5.28	*
Moist. Content (%)	4.62	*
Temperature (^{o}C)	27	*
PSD (%)	Sand: 83.40% Silt: 4.00% Clay: 12.60	
Predominant Bacteria Colonies	*Arthrobacter* spp., *Bacillus* spp., *Flavobacterium* spp., *Micrococcus* spp.	
Predominant Fungi Colonies	*Cladosporium* spp., *Penicillium* spp., *Aspergillus spp.*,	

Source: Field survey. **Note: No limit stated (*), DPR is Department of Petroleum resources**

TPH and PAH concentrations in the soil rose from the low baseline concentration (*Table 1*) to 11000mg/kg and 28mg/kg respectively. Moreover, microbial population had no significant growth at the initial phase of pollution but bloomed after acclimatization. Prior to treatment, organic carbon concentration overwhelmed the baseline concentrations of nitrogen and phosphorus as a result of the crude oil pollution as shown in *Figure1*.

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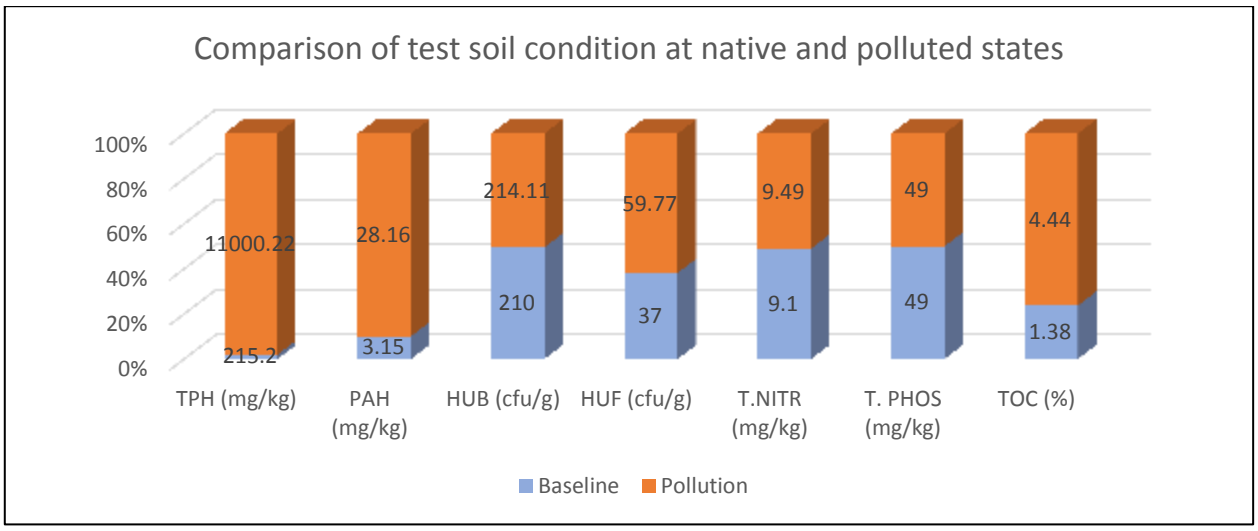

Figure 1: **Effect of crude oil on some native soil parameters before treatment**

Earlier pilot studies on sustainable remediation of crude oil pollution on similar site using only NPK fertilizer by the author in 2014 (unpublished), gave 93.3% degradation of TPH at a degradation rate of -0.0260day^{-1} with a half-life of 26.65 days. However, the restoration efficiency of each treatment in the present study, the combinations of process factors, degradation constants and the corresponding half-life time is shown in *Table 2.*

Table 3*: Degradation constant, half-life, microbial degradation characteristics and restoration and biostimulation efficiencies of the treatments *with NPK fertiliser and saw dust amendment

Treatments	NPK (kg)	Saw dust (kg)	K (day^{-1})	$T_{1/2}$ (days)	Restoration efficiency (%)	Biostimulation Efficiency (%)	Mean HDB (CFU/ml)	**μ**	**K_d**
B	1	1	-0.0452	15.34	98.70	28.25	4.56 x10^5	0.1841	-0.1692
C	1	2	-0.042	16.5	98.23	27.91	4.55 x10^5	0.1844	-0.1706
E	2	1	-0.0465	14.91	98.73	28.28	8.33 x10^5	0.1882	-0.1439
F	2	2	-0.0425	16.31	98.46	28.08	8.91 x10^5	0.1899	-0.1455
H	3	1	-0.0479	14.47	99.05	28.51	1.16 x10^6	0.1921	-0.1352
I	3	2	-0.0419	16.54	98.14	27.85	9.52 x10^5	0.1931	-0.1610
J	0	0	-0.0126	55.01	70.81	0	1.31 x10^4	0.0998	-0.0624

Source: Field survey

Where, $\mathbf{t_{1/2}}$ is biodegradation half-life time and **k** is biodegradation rate constant
μ is specific microbial growth rate and $\mathbf{K_d}$ is cell deactivation rate.
B is treatment with 1kg : 1kg of NPK fertilizer : saw dust
C is treatment with 1kg : 2kg of NPK fertilizer : saw dust
E is treatment with 2kg : 1kg of NPK fertilizer : saw dust
F is treatment with 2kg : 2kg of NPK fertilizer : saw dust
H is treatment with 3kg : 1kg of NPK fertilizer : saw dust
I is treatment with 3kg : 2kg of NPK fertilizer : saw dust and
J is crude oil polluted soil without treatment (natural attenuation)

Restoration efficiency of the soil is calculated as the difference between the initial and residual TPH in the soil as in Equation 1.

$$Y\% = (([TPH]i - [TPH]r) / [TPH]i) \times 100 \quad (1)$$

Where,

$[TPH]_i$ and $[TPH]_r$ represent initial TPH and residual TPH concentrations respectively.

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Besides, the biostimulation efficiency of the different treatments, a measure of the potential of the treatments to enhance microbial degradation was evaluated by the difference in percentage residual TPH of the treatments and the control as in the relation by [6] in Equation 2.

$$\%BE = \%TPH_{(T)} - \%TPH_{(C)} / \%TPH_{(T)}) \times 100 \quad (2)$$

Where,

$[TPH]_{(T)}$ and $[TPH]_{(C)}$ represent TPH of the treated sample and TPH of the control respectively

The growth rate and death rate of microorganisms in this auto catalytic biodegradation process followed first order kinetics thus; $InX = InX_o + \mu t$ for the growth phase and $InX = InX_0 - k_d t$ for the death phase.
Where, *InXo* is the initial biomass concentration and *InX* the biomass concentration at time *t.*

3.2 Discussion

3.2.1 Soil characterisation

The native soil's pH was slightly acidic (5.28). The pH range of 5.1 to 8.9 of the treated soil is capable of sustaining bioremediation [43]. With the initial soil moisture content as 4.62% and soil nitrogen, 9.10mg/kg, the low moisture content of the native soil however, encouraged high water absorption [2]. The soil texture was Loamy sand and the textural properties of the treated soil rose marginally with respect to clay after the biodegradation test. However, soil temperature in all the treatments ranged between 25^{o}C and 30^{o}C, optimum for effective bioremediation process but varies in no definite order**.** The native soil was not absolutely devoid of hydrocarbon presence possibly emanating from run-offs such that the baseline HDB and HDF were in hundreds and tens respectively. However, the soil's total nitrogen and phosphorus were significantly boosted to accommodate the pollution load.

3.2.2 Hydrocarbon degradation

The influence of NPK and saw dust amendment on degradation of TPH was investigated within the test period of February to May. Within this time, the progressive degradation of the hydrocarbon (TPH) and restoration of the soil in all treatments on days 7, 49 and 105 respectively as in *Table 2* and *Figures 2 and 3* were: A (34.1%, 91.06%, 98.6%), B (30.1%, 90.13%, 98.7%), C (30.46%, 90.96%, 98.2%), D (28.15%, 95.8%, 98.51), E (30.32%, 93.8%, 98.7%), F (30.87%, 88.19%, 98.45%), G (29.05%, 91.94%, 98.34%), H (33.35%, 92.22%, 99.05%), I (28.4%, 91.05%, 98.14%), and J (17.71%, 56.77%, 70.81%). NPK fertiliser as a biostimulation agent offered Nitrogen and Phosphorus to the microorganism [45, 28] while soil's porosity and transfer rate of water, oxygen and nutrients was enhanced by Saw dust [36, 46]. The percentage utilisation of nitrogen and phosphorus by microorganisms per treatment in course of hydrocarbon degradation under the influence of NPK fertilizer and sawdust were; A (84.6%/77.0%), B (85.1%/81.5%), C (82.2%/83.8%), D (89.6%/88.3%), E (88.7%/90.0%), F (90.6%/88.3%), G (91.0%/91.8%), H (92.3%/92.8%) and I (91.5%/92.2%) respectively as shown in *Figure 2*. The percentage utilization remained high according to the concentration of nutrient in the treatment combination and reflects the population increase of microorganisms in the treatment against the scanty growth in the control.

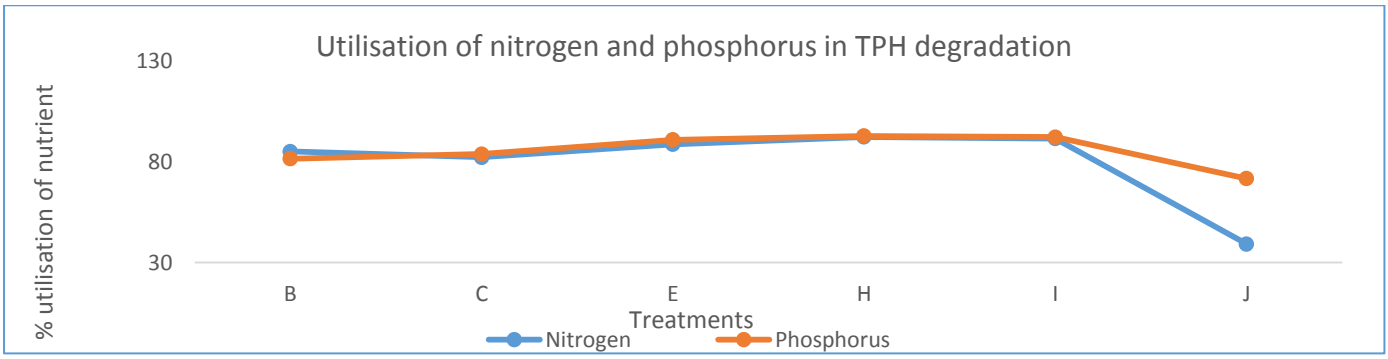

Figure 2: **Trend of nitrogen and phosphorus utilization arising from NPK and sawdust amendment**

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Microbial activities increased with population explosion on the saw dust surfaces [23, 6] resulting in reduction in TPH concentration at the range of efficiencies of treatments. Degradation constants were in the range of 0.0418 – 0.0479 per day, half-life; 14.47 -16.58 days and degradation efficiencies; 98.14% – 99.05% for the treatments. While treatment H (3:1; NPK: Saw dust) had the highest degradation constant of -0.0479 per day with a corresponding lowest half-life of 14.47 days and a restoration efficiency of 99.05%, treatment I (3:2) had the least performance with a degradation constant of -0.0419 per day, half-life of 16.54 days and a restoration efficiency of 98.14%. The control J (natural attenuation) had degradation constants of -0.0126 per day, half-life of 55.01 days and restoration efficiency of 70.81% as expressed in Table 3. Notably, biodegradation rate at specific NPK levels of 1, 2 and 3kgs corresponding in batches to: (A, B, C); (D, E, F) and (G, H, I) increased substantially between 0.5kg (A, D, G) and 1kg (B, E, H) of saw dust amendment but decreased as the amendment increased to 2kg (C, F, I) as in Table 3. This increase with saw dust concentration justified the role of saw dust in enhancing the biodegradation rate of crude oil. Moreover, comparing the optimum of 93.3% TPH removal using NPK fertilizer only (Neebee, 2014) on the same soil with the 99.05% optimum recorded when sawdust amendment was incorporated, the role of sawdust was vindicated. Saw dust in this regard offered surface for growth of microorganisms and adsorption of oil along with permeability and aeration enhancement [19, 6].

However, the 2kg amendment held excess soil moisture, restricting the flow of oxygen through the medium (USEPA 2009) at the respective NPK concentrations. This situation impeded microbial activities and reduced biodegradation rate thus a drop in TPH degradation in treatments C, F and I. It may be viewed that saw dust is a carbon source hence could serve as a substrate for microorganisms. The "Diauxic" concept of [18], by which substrates are degraded sequentially, projected crude oil as the primary substrate which must be exhausted before saw dust the secondary substrate could be degraded. This sustained saw dust's permeability and aeration enhancement capacity. The percentage degradation of hydrocarbon otherwise, restoration of the soil by each treatment option with remediation as outlined in Table 2 followed first order kinetics (equation 2) in agreement with the findings of [4]. Nevertheless, the degradation efficiency was determined by the same relation as in *Equation 1*.

The first order kinetics equation is expressed thus:

$$C_t = C_0 e^{-kt} \qquad (3)$$

And linearly;

$$InC_t = InC_o - kt \qquad (4)$$

And the half-life time determined thus:

$$t_{½} = \ln 2/k \qquad (5)$$

The trend of results in *Table 2* shows all treatment combinations to have capacity for treatment of the hydrocarbon pollution but treatment H is more outstanding. Degradation of TPH in each treatment (*Figure 3*) peaked on day 49 in response to the growth of hydrocarbon degrading bacteria and fungi, hydrocarbon being their energy source. It regressed after attaining a zenith on day 49 owing to depletion of nutrient concentration and death of the microorganisms.

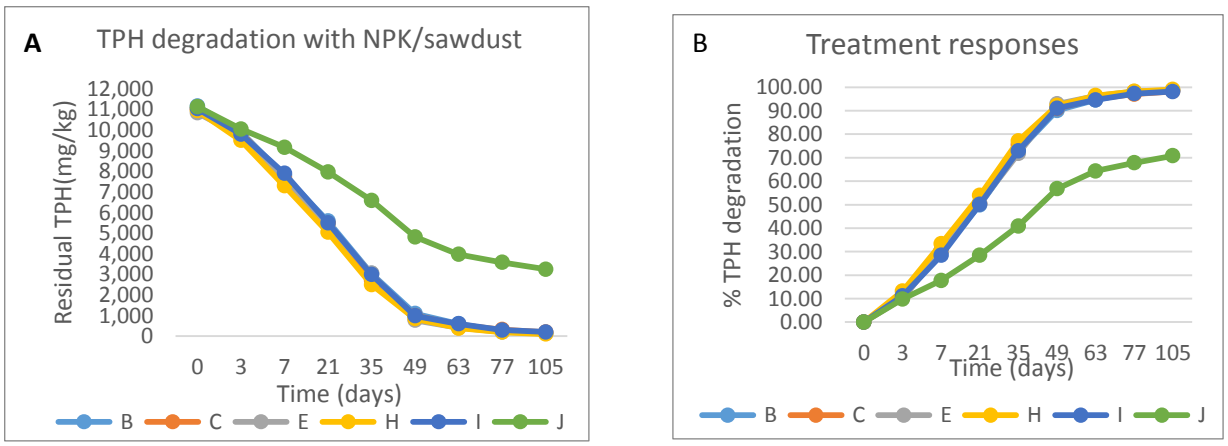

Figure 3 (a): **Trend of TPH degradation of crude oil in soil treated with NPK fertilizer and saw dust**
(b) **Restoration efficiency (response) of the polluted soil to treatments with bioremediation time**

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An optimum nutrient application rate was necessary because low concentration results in suboptimal biodegradation of oil [20], while high concentration leads to toxicity [14] with a resultant decrease in biodegradation efficiency. Treatment **H** had the best result due to optimum combination while treatment **I** had the least. The observed rapid loss of TPH concentration in all the treatments within the first week was due to photovolatilisation and abiotic losses of the lighter fractions of the hydrocarbon [21, 32] to the extent of 10% in J (control) and 13% in H (treatment) on the 3rd day prior to tilling and 17% in J and 33% in H by the 7th day with tilling as in *Figure 3*. By days 49 and 105, the degradation efficiency of H was (92.22%, 99.05%), respectively. The degradation of hydrocarbon influenced by only NPK fertilizer is shown in *Figure 4*.

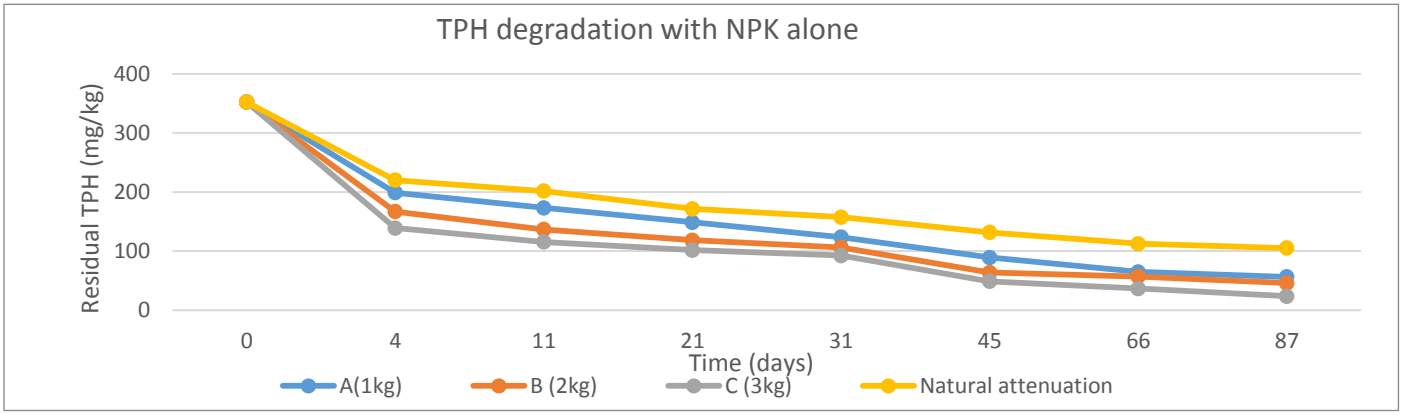

Figure 4: **Trend of hydrocarbon degradation in crude oil polluted soil treated with only NPK fertiliser as nutrient source**

3.2.3 Microbial growth

The total viable population of microorganisms (bacteria and fungi) in the treatments and control are presented in *Figure 5*. Observed increase in microbial population correlate with the decrease in TPH concentration at the stated time and treatment cell as in *Figure 3*.

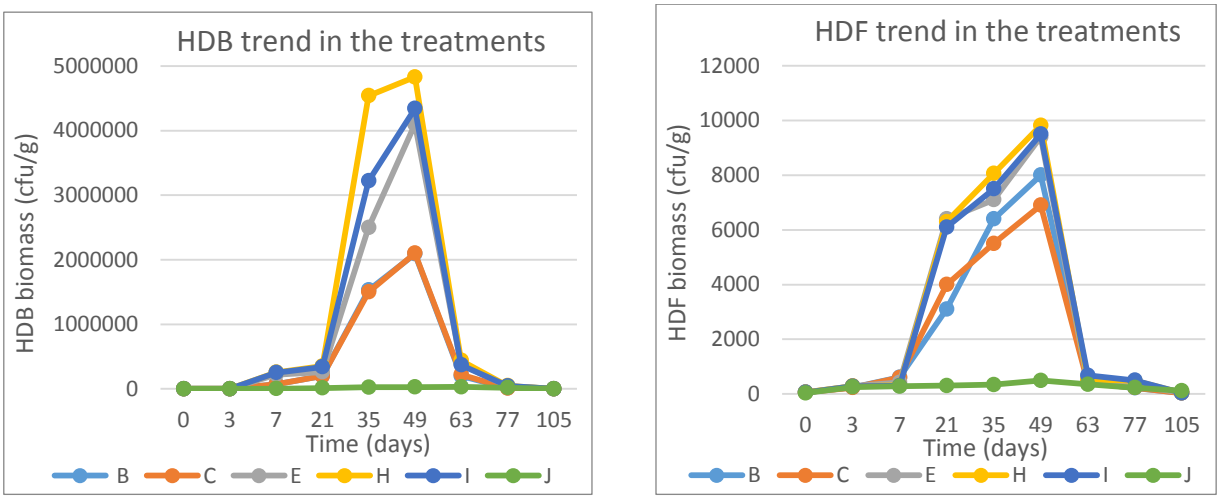

Figure 5 (a): HDB profile and (b) HDF profile in the researched soil treated with NPK fertiliser and Saw dust

With NPK fertilizer and improved permeability by saw dust, Nitrogen and Phosphorus were available to the microbes. Adequate moisture was retained for sustained microbial activities, even as the sawdust's surface constituted a platform for adsorption and growth of the microbes [6]. The experimental microbial growth profile shows that within the first seven days, there was no significant microbial activity, possibly due to adjustment to the new environment [2]. However, the lighter components of the hydrocarbon were photovolatilised, influenced by soil tilling. Progressive cell growth with a corresponding rapid degradation of hydrocarbon observed between days 7 and 49 resulted from the abundance of Nitrogen and Phosphorus from the fertilizer that enhanced proliferation of Bacteria and Fungi [31]. Likewise, permeability occasioned by saw dust enhanced oxygen availability for efficient bacterial activity [19]. Cell death set in after day 49 possibly due to exhaustion and competition and the rate of

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degradation slowed down gradually till day 105. However, cumulatively 99.05% optimum treatment was achieved. Microbial growth rate in the control - treatment J, was slow and low (0.0998/day) against 0.1922/day of treatment H (as in *Table* 4), underlining the importance of biostimulation.

Basically, the predominant hydrocarbon degrading bacteria colonies were identified as: Arthrobacter spp., Bacillus spp., Micrococcus spp., Flavobacterium spp., Norcadia spp., Rhodococcus spp., Pseudomonas spp., Alcaligens spp., Mycobacterium spp., Corynebacterium spp. The hydrocarbon degrading fungi species were: Cladosporium spp., Penicillium spp., Aspergillus spp., Trichoderma spp., Candida spp., Acremonium spp., Fusarium spp in agreement with the report of [42, 13, 1]. It indicates that the treatment given was favourable for the population increase of the microbes even as they respond to the presence of crude oil in the control.

3.2.4 Biodegradation kinetics

Biodegradation kinetics assesses biodegradation rate (*Equation 3*) and the half life time (*Equation 4*) to establish the performance of microorganisms in the soil. The high degradation rates for the treatments; A (-0.0442), B (-0.0452), C (-0.042), D (-0.0441), E (-0.0465), F (-0.0425), G (-0.0418), H (-0.0479) and I (-0.0419) per day results in the corresponding half-life times of; A (15.68), B (15.34), C (16.5), D (15.72), E (14.91), F (16.31), G (16.58), H(14.47) and I(16.54) days. Correlating with the control J(0.0126/day) and its half-life of 55days show that the combination of NPK fertilizer and saw dust were very effective in enhancing degradation of crude oil in the order of treatments H > E > B as in *Table 2*, *Figure 8* and *Figure 9*. The luxuriant growth observed showed the influence of NPK fertilizer and saw dust on the growth pattern.

Progressive degradation of crude oil from the adaptive proliferation of indigenous hydrocarbon degrading bacteria and fungi in the soil occurred through increase in the microbial growth rate (μ) and lower biomass doubling time (t_d) culminating in steady restoration or clean-up of the soil until cell deactivation set in.

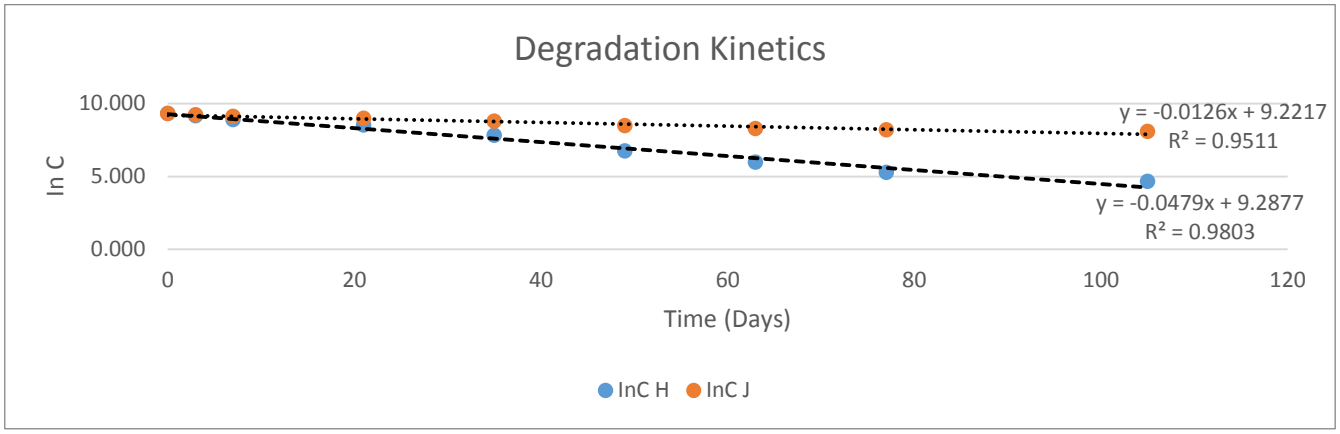

Figure 6: **Kinetics of bacterial degradation of crude oil in soil treated with NPK fertilizer and saw dust amendment.**

However, the kinetics of the growth and the death of microorganisms in executing biodegradation of hydrocarbon in the treatments are shown in *Figure 7a and b*. The specific biomass growth rates (**μ**) were between 0.1837/day and 0.1931/day for the treatments and biomass deactivation rate ($\mathbf{K_d}$) between -0.1352/day and -0.1706/day in the treatments. The μ for the control was 0.0998/day while the K_d was -0.0624/day. At the growth phase, the treatments had a biomass doubling time ($\mathbf{T_d}$) of 3.59 – 3.77days indicating luxuriant growth owing to nutrient availability and favorable environment offered by saw dust against the 6.95 days in the control.

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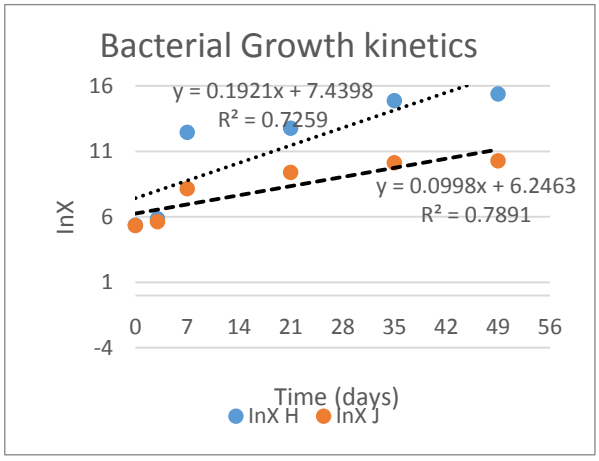

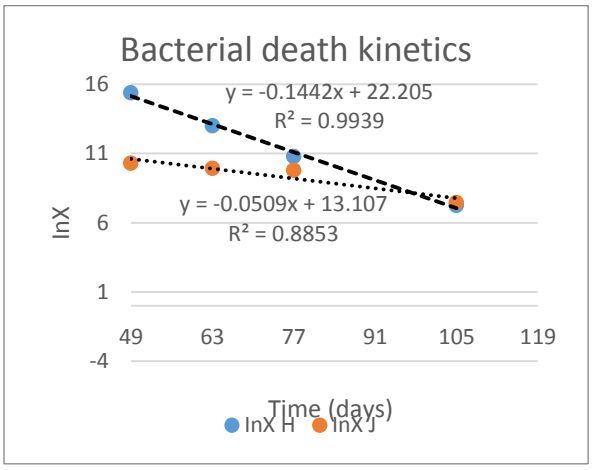

Figure 7(a): **Kinetics of bacterial growth phase (b) Kinetics of bacterial death phase in soil treated with NPK fertilizer and saw dust**

Treatment H had a growth rate of 0.1921/day, biomass doubling time of 3days, biomass deactivation (death) rate of -0.1352/day and biomass deactivation time of 5days. The high biomass growth rate (μ) and low doubling time (T_d) of the treatments imply favorable condition with the resultant high degradation of the hydrocarbon correspondingly. However, the control (J) had a biomass growth rate of 0.0998/day and doubling time of 6.95days.

Increasing the strength of combinations of NPK/saw dust gave higher biomass growth rate and lower doubling time due to the release of more nitrogen and phosphorus nutrients into the medium as well as the sustained aeration through saw dust. Treatment H has the highest biomass growth rate and a high frequency of replication at the least doubling time sequel to the optimum combination of the NPK/saw dust amendment. It is the best performer and has high biomass deactivation rate as a result of exhaustion. The hydrocarbon being the food and energy source for microbial growth is consumed in the luxuriant growth of the organisms in the test medium with the concomitant degradation witnessed.

4. CONCLUSION

This study has shown that biostimulation with NPK fertilizer (61:15:15)-saw dust amendment enhances the restoration of hydrocarbon polluted soil to an efficiency of 99.05% within 105 days. A combination of between 1kg and 2kg of NPK fertiliser with 1kg of saw dust per square meter of soil produces effective soil restoration. However, the population and metabolic capability of hydrocarbon degrading microorganisms is enhanced to a microbial kinetic growth rate of 0.1921 per day by the right mix of nutrient. About 92% of nitrogen and phosphorus nutrient is utilized for an optimum degradation of TPH at a kinetic rate of -0.0479 per day. Finally, NPK and saw dust both individually and by interaction enhanced TPH degradation positively. Biostimulation using NPK fertilizer and saw dust is therefore an effective bioremediation strategy for treatment and restoration of crude oil polluted soils especially in the Niger delta area of Nigeria. It eliminates the impact of crude oil pollution on the soil rapidly and naturally. In order to enhance consistent luxuriant microbial growth for rapid of soil restoration, NPK fertilizer and sawdust should be combined optimally.

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DECLARATION OF INTEREST

None

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