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Biostimulation - A Sustainable Remediation of Crude Oil Pollution in the Tropics

Ekpobari Neebee*, Emmanuel Emeka Oguzie

Department of Environmental Management, School of Environmental Science,
Federal University of Technology Owerri, Nigeria
*Corresponding author: ekpobari.neebee@gmail.com

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Abstract The autochthonous microorganisms in hydrocarbon polluted soil are aroused for biodegradation by introduction of external nutrient factors. This research aims to show the sustainability of biostimulation in enhancing crude oil degradation in a tropical soil. The contaminated soils (A, B, C, D, E, F, G, H, and I) were treated simultaneously with a combination of NPK fertilizer and saw dust amendment in designed proportions to offer nutrient and a permeable medium suitable for microbial growth and hydrocarbon degradation. The unpolluted soil, control and the treated soil were monitored for total petroleum hydrocarbon (TPH), soil pH, total organic carbon, total nitrogen, total phosphorus, soil texture, hydrocarbon degrading bacteria (HDB) and hydrocarbon degrading fungi (HDF) using standard analytical method and procedures to establish soil restoration efficiency and kinetics of the bioremediation process. The percentage utilization of nitrogen and phosphorus by microorganism were B (85.1%/81.5%), C (82.2%/83.8%), E (88.7%/90.0%), H (92.3%/92.8%) and I (91.5%/92.2%) and culminated in corresponding HDB growth rate of B (0.1840), C (0.1844), E (0.1882), H (0.1921) and I (0.1931). The resultant optimum TPH degradation efficiency of 99% in treatment H at a degradation rate of -0.0479 day^{-1} in 105 days indicated the effectiveness of the process. The biodegradation process followed first order kinetics with biomass doubling time 3.61 days and a degradation half-life of 14.5 days. Biostimulation is therefore applied to fuel the medium, sustain microbial growth, improve biodegradation rate and ultimately restore the impacted media without any adverse ecological effect.

Keywords: *biostimulation, nutrient factors, microorganisms, biodegradation, impacted media, ecological effect*

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1. Introduction

The spate of pollution by crude oil is on the increase following increase in its exploitation and production to meet the industrial and economic demands of the society. To mitigate the effect of contamination of ecosystems, biostimulation built on application of equitable amount of nutrient into a medium to promote the growth of microorganisms with metabolic capacity to degrade pollutants especially the hydrocarbon family is applied. This strategy modifies the polluted environment with rate limiting nutrients and electron acceptors as nitrogen, phosphorus and oxygen to stimulate existing bacteria capable of reversing the presence of spilt crude oil. The success of this technique depends on optimum concentration of nutrient and the population growth of the microorganisms. The process degrades the pollutant to carbon dioxide and water thereby returning the altered environment to its natural condition [1]. It is easy to operate in-situ without undue disturbance to the native ecosystem [2].

Biostimulation on the beach of Alaska in the Exxon Valdez oil spill of 1989 [3] mitigated the ecological impacts on affected localities [4] and offers a proof of concept. Optimisation of applied nutrient amendments and monitoring through periodic analysis of remediation indicators ensures timely restoration of the oil polluted soil. Furthermore, integration of sawdust with nitrogenous fertiliser, tilling, optimum temperature, pH and moisture content of the medium accelerates biodegradation process [5,6,7]. Figure 1 expresses the process for achievement of soil restoration through biostimulation as presented by [7].

The abundance of nitrogenous nutrients in the soil from fertilizer application enhanced the massive biosynthesis of hydrocarbon into structural cell components by the available microorganisms in its growth process leading to proliferation of more microbes as typified equation (1).

$$Biostimulation: C_xH_yO_z + NH_4 \xrightarrow{Microbes} more\ microbes(Growth). \quad (1)$$

Cell biomass is biosynthesised with the precursor metabolites of acetyl-CoA, succinate or pyruvate while other needed sugars for growth are synthesized through

gluconeogenesis [8]. Tilling the sawdust treated permeable soil improves aeration and effectively boosted the luxuriant population growth of hydrocarbon degrading bacteria [7]. This exponential growth of the microorganisms successively reduced the amount of applied nutrient and the pollutant in the soil logarithmically with a resultant improvement in the quality of the soil. Whether at the sorbed or desorbed states, the biochemistry of the hydrocarbon, its bioavailability to microorganisms and bioactivity of the microorganisms [9] enhanced the biodegradation experienced.

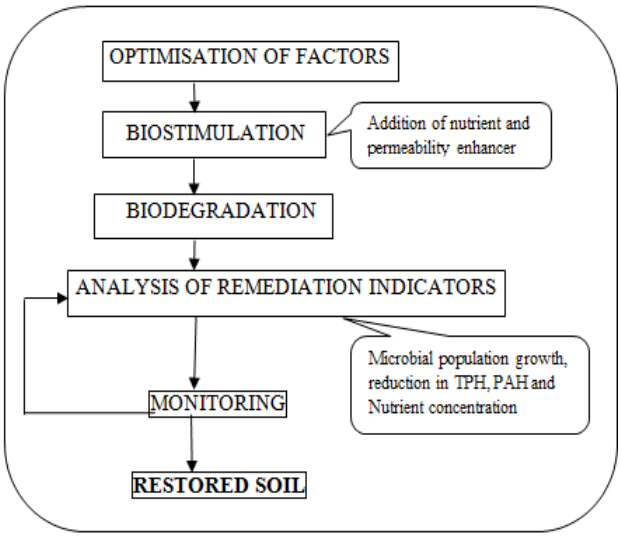

Figure 1. Framework of biostimulation for soil restoration [7]

In spite of toxicity by the initial high concentration of crude oil in the soil and its hydrophobic property with associated effect on bioavailability [6], its biodegradability, textural properties of the polluted soil and the treatment approach enhances the extent of biodegradation [10]. Consequently, biosurfactants released by the microbes facilitate emulsification [11], breaks the hydrophobicity and enhances bioavailability consummated by optimisation of the process factors. Optimisation and manipulation of the rate limiting factors reengineers the subsurface environment [12,13] with nutrient, aeration and temperature [9] and enhances the soil microbial potential [14]. The end result is a boom in microbial population and a concomitant degradation of crude oil in the polluted soil.

Biodegradation mineralises the crude oil into carbon dioxide, water, inorganic compounds and cell protein, a transformation of the complex organic structure into simpler ecologically compatible forms [8]. This process facilitates the decrease in concentration of petroleum hydrocarbon through depletion of the electron acceptors and mitigation of the migration, spread and risk of toxicity of the pollutant. The chemistry of the process is a microorganism mediated utilisation of organic compounds under aerobic condition to produce nontoxic carbon dioxide, water and heat energy which sustains the temperature required for biological activity as expressed by equation (2).

$$Biodegradation: C_xH_yO_z + O_2 \xrightarrow{Microbes} CO_2 + H_2O + Heat\ energy \quad (2)$$

Hydrocarbon degrading bacteria and fungi involved in the process, utilise the enzyme oxygenase and converts alkanes to carboxylic acid by β-oxidation and further to fatty acetate which enters the tricarboxylic acid (TCA) cycle. The rings of hydroxylated aromatic pollutants are equally cleaved to form catechol and degraded into intermediates of the TCA cycle [15]. The process is therefore sustainable with no net adverse environmental effect.

2. Materials and Methods

This research was conducted on a flat land located at Iriebe in Obio-Akpor local government area of Rivers state, Nigeria, close to the Oyigbo crude oil flow station operated by Shell Petroleum Development Company (SPDC). It is located on Latitude 4° 53' 20.35" and longitude 7° 06' 29.80" and an elevation of 76 feet above sea level.

It was executed in the field using analytical experimental design in a completely randomized block fitted into a 3^2 full factorial design with NPK (X_1) and Saw dust (X_2) as the independent variables. A 250 m^2 area was measured and nine cells of 1.0 m^2 each (A, B, C, D, E, F, G, H, and I) along with a control cell (J) were established, adequately ridged from each other to forestall treatment and nutrient migration. The physical (moisture content, particle size distribution and temperature), chemical (TPH, PAH, soil pH, total nitrogen, total phosphorus and total organic carbon) and biological (HDB and HDF) parameters of the test soil were determined for baseline information. Oil spill was then simulated with crude oil in all cells at the rate of 5 litres.m^{-2} of land. The control cell (J) was also polluted with five (5) litres of crude oil.

Batches of three cells were biostimulated consecutively with 1kg, 2kg and 3kg of the blended N.P.K fertilizer (61:15:15) followed simultaneously with 0.5kg, 1kg and 2kg of fine saw dust according to the experimental design. The fertiliser and saw dust were thoroughly mixed with the soil to a homogenous matrix. Samples were collected from each cell after treatment application for analysis. The cells were then re-tilled after sampling for aeration and homogeneity. This tilling helped to mix and optimize the contact among the microorganisms, hydrocarbon, moisture and nutrients for maximum oil degradation. The residual concentrations of TPH, PAH, Nitrate, Phosphate, HDB, HDF, pH and moisture were monitored periodically with analysis within 105 days.

2.1. Analysis of Physicochemical Parameters in the Soil

Soil pH was determined with a HACH multi-parameter pH meter fitted with both pH and reference electrodes. Soil particle size was analysed with Bouyoucos hydrometer method using BS 1377 (1990) and ASTM–D423/D-424-54T (1975) procedures. Moisture content was determined by gravimetry (ASTM D2216-66) and total organic carbon by the Walkley and Black method (APHA 5310B). Total Nitrogen was determined with the Kjedhal oxidation method using HACH digesdahl digestion apparatus/the peroxide method. However, total Phosphorus was

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

2.2. Analysis of Hydrocarbon Degrading Bacteria and Fungi

Microorganisms with hydrocarbon degrading ability were isolated, identified and their population determined before and within intervals of the soil treatment. Bacteria were enumerated using vapour phase transfer technique adopting spread plate method in a mineral salt medium according to APHA 9215C. Cell morphology and gram staining reaction was applied to identify and characterize bacterial isolates. Fungal colonies were also enumerated according to APHA 9610C but with antibiotics added to suppress the growth of bacteria. Fungi identification was by morphological characteristics and microscopic examination.

3. Results and Discussion

The soil studied had some residual TPH likely due to its closeness to a crude oil production facility, with slightly low pH and low concentration of Nitrogen and Phosphorus. The texture was loamy sand with few bacteria and fungi colonies as shown in Table 1. However, these baseline characteristics changed due to external pollution by crude oil with significant impact on the physical, chemical and biological properties of the soil.

Crude oil pollution raised the TPH and PAH concentrations of the test soil to 11000mg/kg and 28mg/kg respectively. Microbial population became more significant after acclimatization and more luxuriant following nutrient application. Prior to treatment, organic carbon concentration overwhelmed the baseline concentrations of nitrogen and phosphorus. The resultant microbial growth from feeding on the nutrient (nitrogen and phosphorus) of the NPK fertiliser led to degradation of the organic carbon and the TPH from the pollutant, the energy source. However, the concentration of the nitrogen and phosphorus eventually reduces within the microbial growth process due to consumption as shown in Figure 2 and Figure 3.

Table 1. Baseline properties of the soil studied before spiking with crude oil

Parameters analysed	Value
TPH (mg/kg)	215.2
PAH (mg/kg)	3.15
HDB (cfu/g)	$2.10x10^2$
HDF (cfu/g)	$3.7x10^1$
Total Nitrogen (mg/kg)	9.10
Total Phosphorus (mg/kg)	49
TOC (%)	1.38
pH	5.28
Moisture Content (%)	4.62
Temperature (°C)	27

Source: Field data [7].

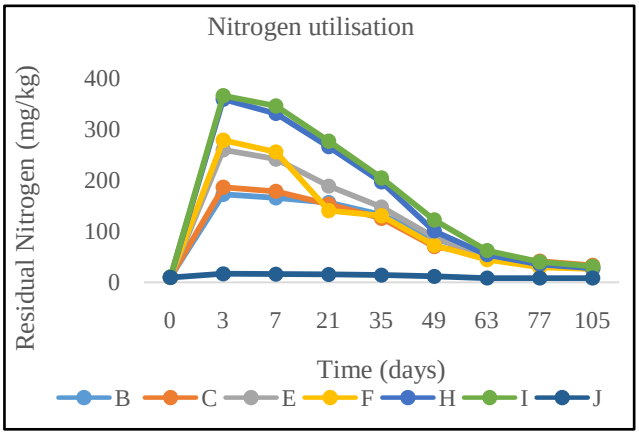

Figure 2. Nitrogen utilisation per treatment

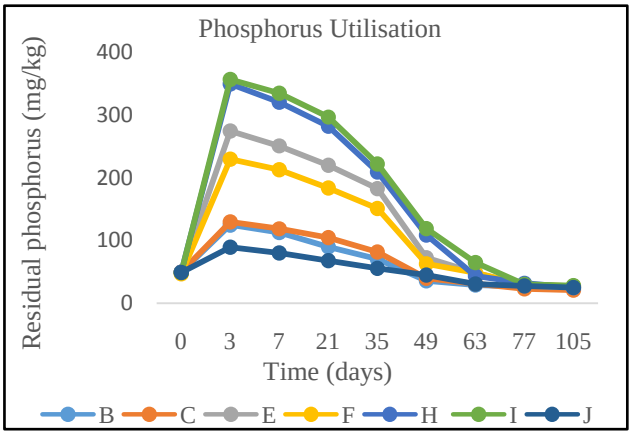

Figure 3. Phosphorus utilisation per treatment

The following table shows the characteristics of the treatments and the corresponding results.

Table 2. Table showing treatment factors, degradation constant, degradation half-life, microbial growth rate, nutrient utilization, 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)	**N/P Utilised (%)**	**Mean HDB** (CFU/g)	**μ** (day^{-1})	Degradation Efficiency (%)	Biostimulation Efficiency (%)
A	1	0.5	-0.0442	15.68	84.6/77.0	$4.18x10^5$	0.1837	98.61	28.19
B	1	1	-0.0452	15.34	85.1/81.5	$4.56\ x10^5$	0.1840	98.70	28.25
C	1	2	-0.0420	16.5	82.2/83.8	$4.55\ x10^5$	0.1844	98.23	27.91
D	2	0.5	-0.0441	15.72	89.6/88.3	$5.50\ x10^5$	0.1911	98.52	28.13
E	2	1	-0.0465	14.91	88.7/90.0	$8.33\ x10^5$	0.1882	98.73	28.28
F	2	2	-0.0425	16.31	90.6/88.3	$8.91\ x10^5$	0.1899	98.46	28.08
G	3	0.5	-0.0418	16.58	91.0/91.8	$9.49\ x10^5$	0.1889	98.35	28.00
H	3	1	-0.0479	14.47	92.3/92.8	$1.16\ x10^6$	0.1921	99.05	28.51
I	3	2	-0.0419	16.54	91.5/92.2	$9.52\ x10^5$	0.1931	98.14	27.85
J	0	0	-0.0126	55.01	-	$1.31\ x10^4$	0.0998	70.81	0

Where K is degradation constant, **μ** is bacterial growth rate and $T_{1/2}$ is degradation half-life. N and P are Nitrogen and Phosphorus respectively.

The degradation constants occurred in the range of -0.0418 to -0.0479 day^{-1}, half-life between 14 days and 16 days and restoration efficiencies between 98.14 and 99.05% for the treatments. Treatment H (3 kg NPK: 1 kg saw dust) had the highest degradation constant of -0.0479 day^{-1} with a corresponding lowest half-life of 14 days, restoration efficiency of 99.05% and biostimulation efficiency of 28.51%. Treatment I (3:2) had the least performance with a degradation constant of -0.0419 day^{-1}, half-life of 16 days and a restoration efficiency of 98.14%. The control J (natural attenuation) also had degradation constant of -0.0126 day^{-1}, half-life of 55 days and restoration efficiency of 70.81%. However, in order of decreasing degradation constants, the best treatments were obtained as H (-0.0479 day^{-1}) > E (-0.0465 day^{-1}) > B (-0.0452 day^{-1}). Similar trend was obtained when nutrient concentrations were considered implying that, increase in nutrient increased the growth rate of the organisms which fed on the pollutants as their energy source. The higher the growth rate, the higher the microbial population and the higher the degradation rate of the pollutant with a resultant speedy restoration of the soil at lower half-life. Beyond the optimum, regression in trend occurred.

The restoration efficiency, a measure of degradation of hydrocarbon and clean-up of the soil was determined by equation (3).

$$Y\% = \left(\left([TPH]_i - [TPH]_r\right)/[TPH]_i\right) x100 \qquad (3)$$

Where, [TPH]i and [TPH]r represents initial TPH and residual TPH concentrations respectively.

Similarly, the biostimulation efficiencies of the different treatments, a measure of the potential of the treatments to enhance microbial degradation were evaluated by equation (4).

$$\%BE = \%TPH_{(T)} - \%TPH_{(U)} / \%TPH_{(T)}) \; x100 \qquad (4)$$

Where; $\%TPH_{(T)}$ is crude oil removal in the treated soil, $\%TPH_{(U)}$ is crude oil removal in the untreated soil.

The bioremediation efficiency of each treatment option is also shown in Table 2.

Variations in the bacterial growth rate, hydrocarbon degradation rate constants and half-life in the treatments were due to the different concentrations of NPK fertilizer and saw dust applied in each treatment. This translated to the amount of nitrogen and phosphorus available in each case. Degradation rate increased with increase in amount of nitrogen available in conjunction with the concentration of saw dust in the medium. The increase in biostimulation efficiency and degradation rate across the NPK treatment levels was further influenced by increasing the concentration of saw dust by double strength from 0.5 $kg.m^{-2}$ (treatments A, D and G) to 1.0 $kg.m^{-2}$ (treatments B, E and H). Further increase to 2 $kg.m^{-2}$ (treatments C, F and I) showed decrease in outcome within each level owing to accumulation of excess water by the large mass of saw dust whose threshold was exceeded. Water retention in the medium affects oxygen flow, leading to suffocation and retardation of the growth of microbes for biodegradation. In spite of degradation efficiency increasing with nutrient concentration, 3 $kg.m^{-2}$ of NPK and 2 $kg.m^{-2}$ of saw dust produced a compound regressive and seemingly toxic effect that culminated in a less than proportionate degradation of the pollutant.

The highest crude oil degradation rate of -0.0479 day^{-1} was recorded in treatment H which gave rise to 99% hydrocarbon removal at a half-life of 14 days. The kinetics of bacterial growth and degradation of TPH in each treatment combination is shown in Figure 4 to Figure 10. Figure 11 show the trend of TPH degradation in each treatment influenced by the concentration of nutrient and the growth of microorganisms while Figure 12 shows the degradation efficiency resulting from each treatment.

The first order degradation kinetics was determined with the relation in equation (5);

$$InC_t = InC_o - kt. \qquad (5)$$

While first order biomass growth kinetics was determined by the relation in equation (6);

$$InX = InX_o + \mu t \qquad (6)$$

Where C_0 and C_t are initial and final pollutant concentrations at time **t** while X_0 and X are initial final biomass concentrations at time **t**.

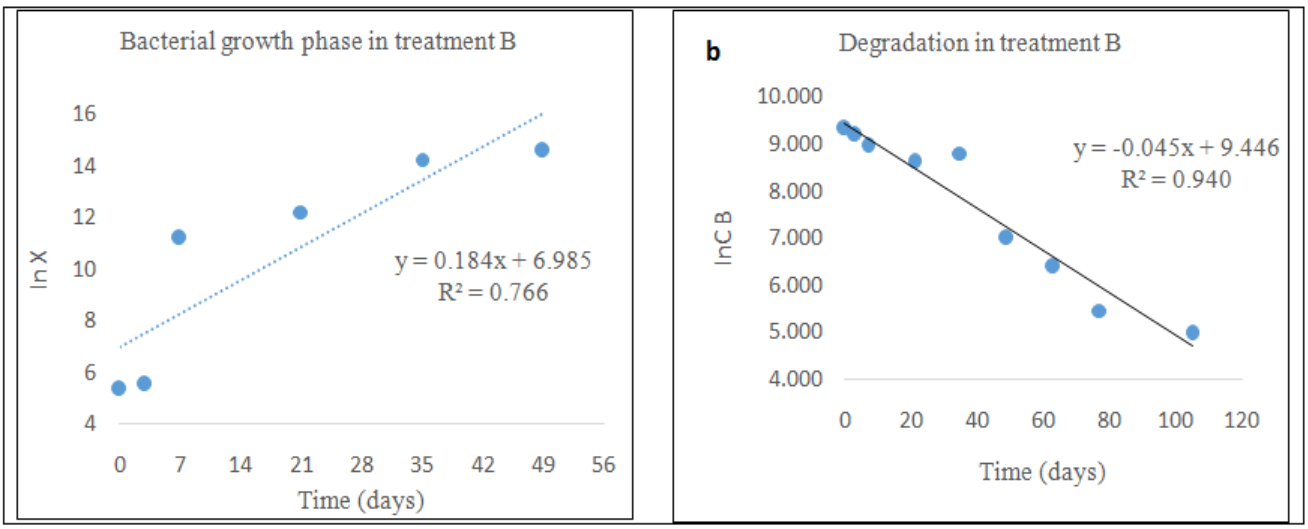

Figure 4. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in treatment B after fertiliser/saw dust application

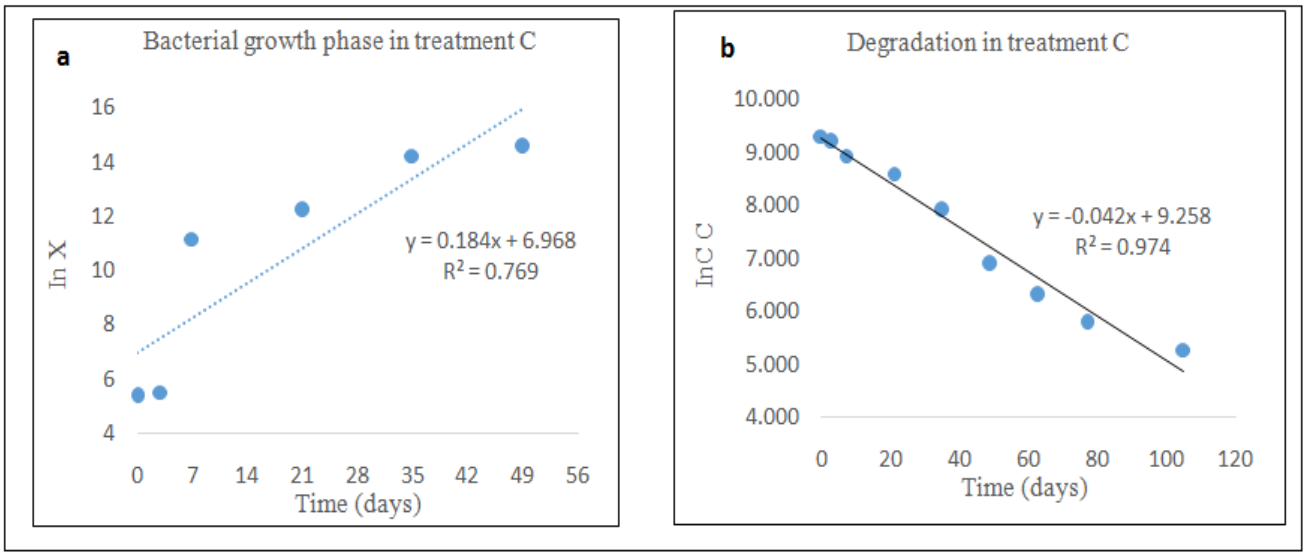

Figure 5. Kinetics of bacterial growth (a) and crude oil degradation (b) in treatment C after fertiliser/saw dust application

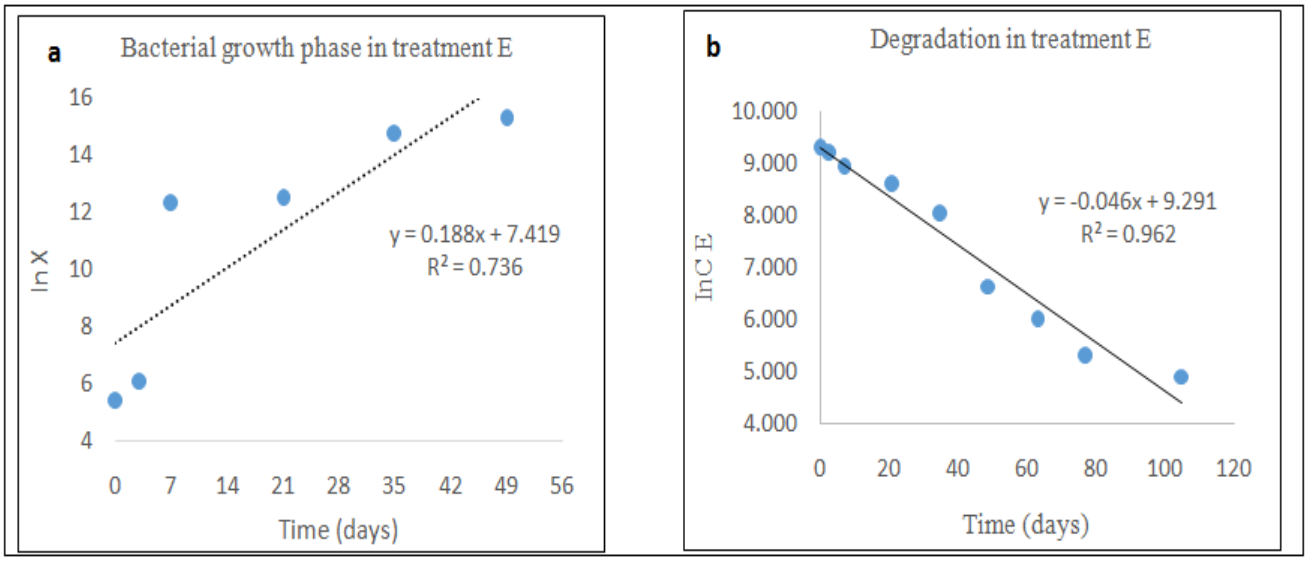

Figure 6. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in treatment E after fertiliser/saw dust application

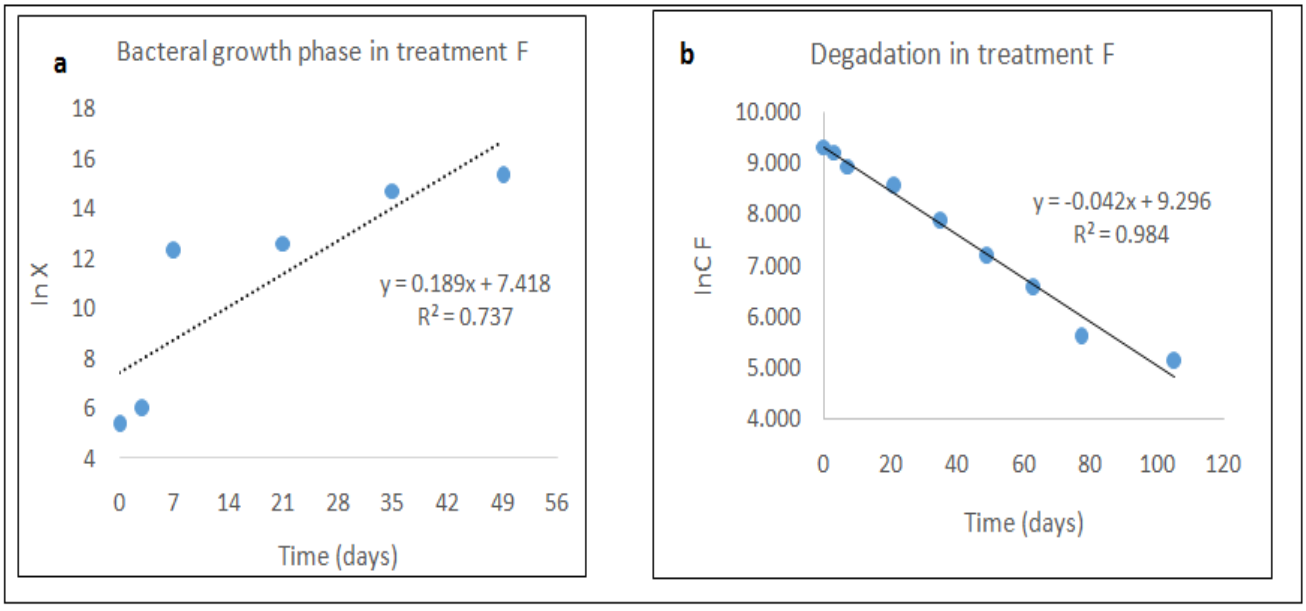

Figure 7. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in treatment F after fertiliser/saw dust application

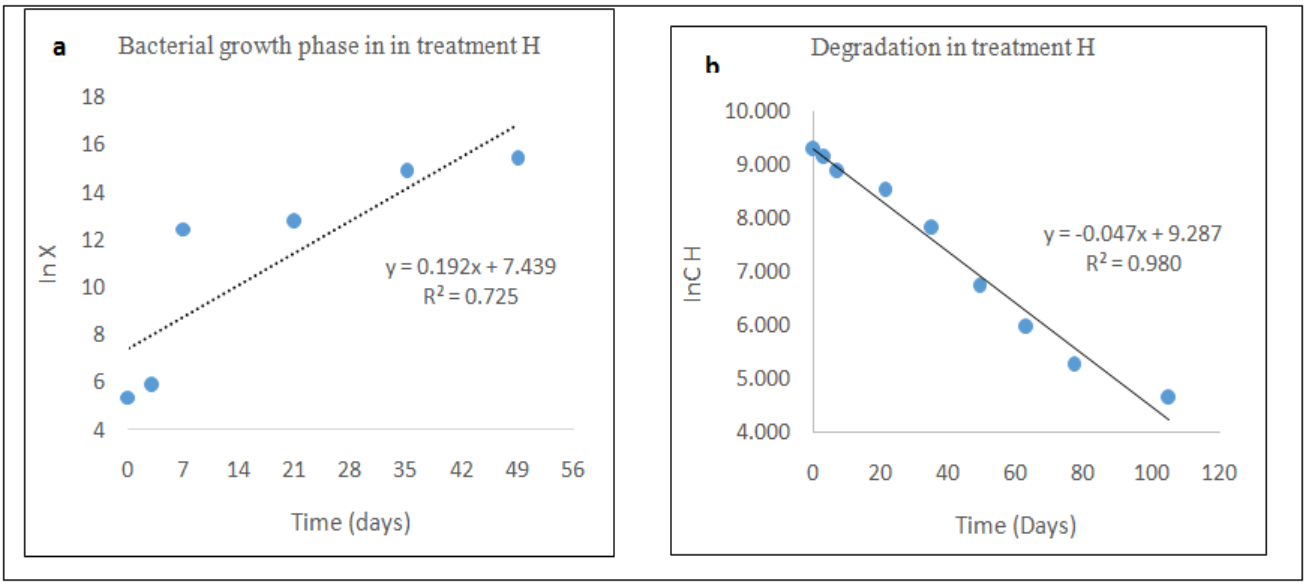

Figure 8. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in treatment H after fertiliser/saw dust application

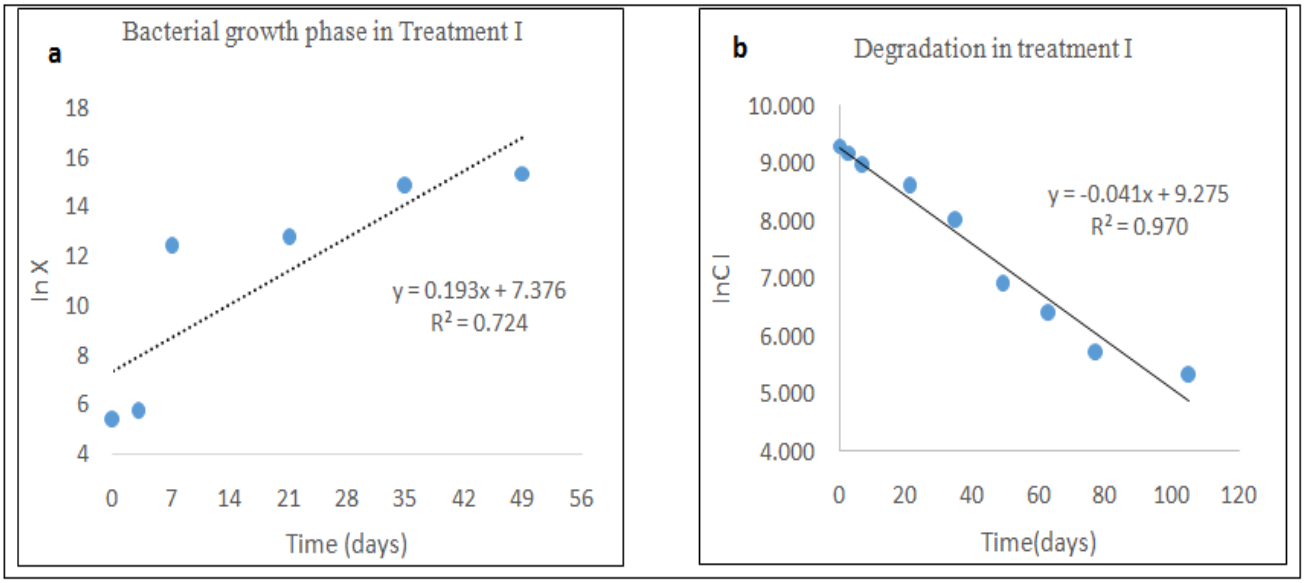

Figure 9. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in treatment I after fertiliser/saw dust application

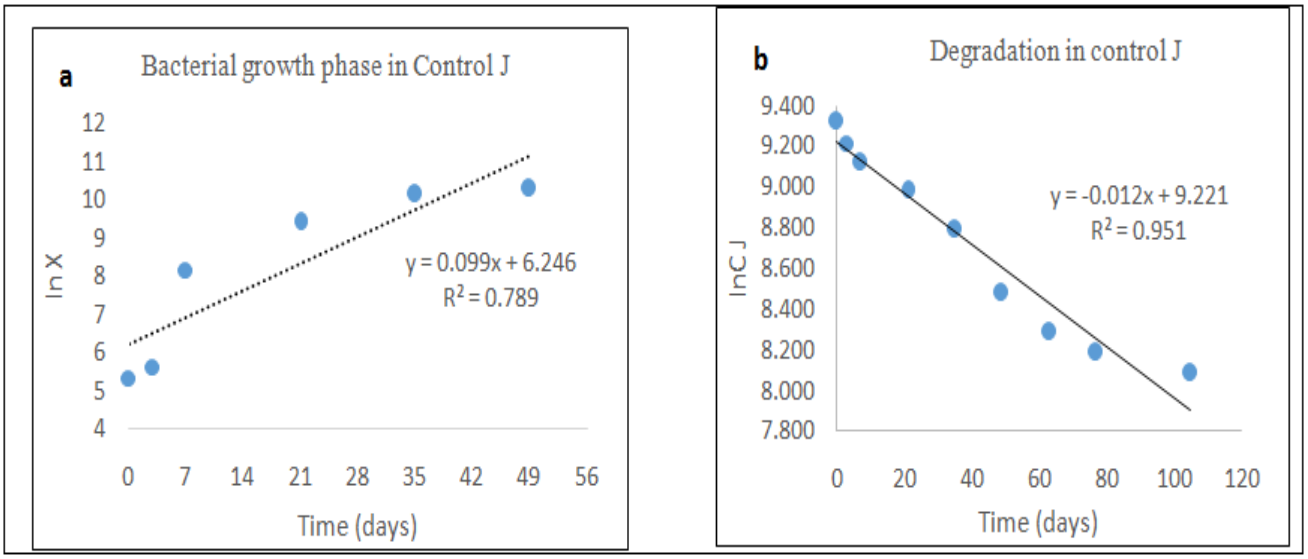

Figure 10. Kinetics of bacterial growth (**a**) and crude oil degradation (**b**) in control I after fertiliser/saw dust

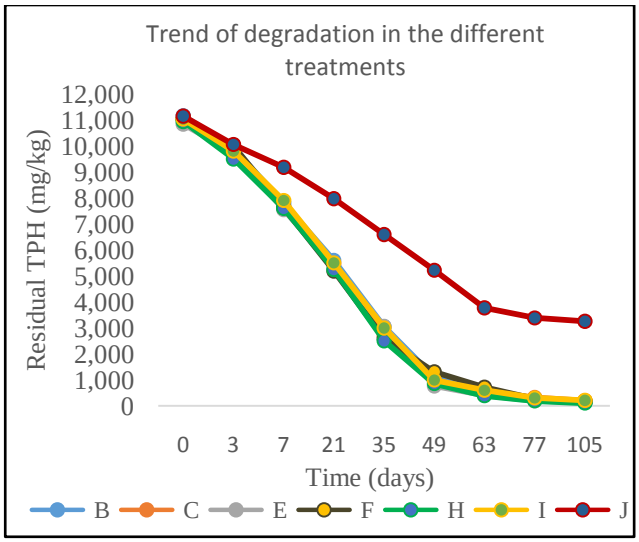

Figure 11. Trend of degradation in the treatments following application of corresponding nutrient proportions

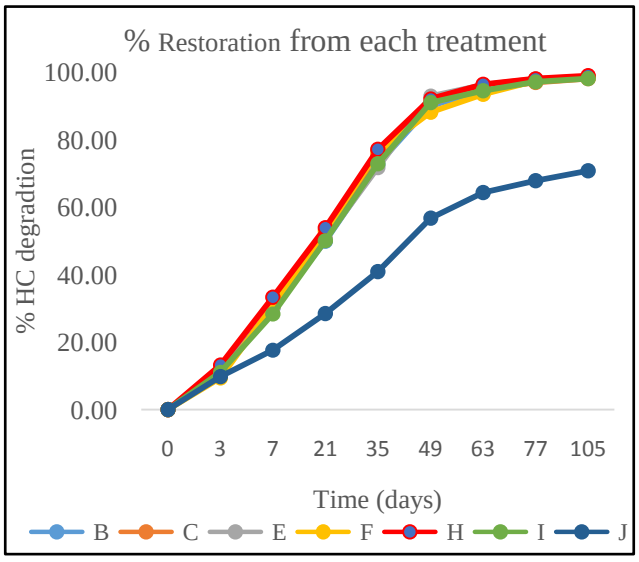

Figure 12. Degradation (restoration) eficiency of each treatment with NPK fertiliser and sawdust

All the treatment options influenced high bacterial growth rate and potential for effective soil restoration above 95% but treatment H became more efficacious thus, the order H > E > B. This result agrees with the report of [7], that biostimulation of the indigenous microbes with fertiliser accelerated the biodegradation of hydrocarbon contaminats in the soil. The polluted soil's functionality is therefore restored without adverse consequence on the ecology. The ultimate biological transformation of pollutants into nontoxic carbon dioxide, water and biomass acceptable into the food web, makes biostimulation an ecologically friendly and sustainable remediation strategy.

4. Conclusion

Biostimulation with NPK fertiliser in a suitably permeable environment enhanced by saw dust triggers luxuriant growth of autochthonous microbes for effective crude oil biodegradation. The synergistic metabolic relationship among the microorganisms enabled efficient natural degradation of soil pollutants in a sustainable and environmentally sound manner. Optimum amount of nutrient, temperature and aeration produced 99% restoration of the polluted soil. By this, nature was stimulated to solve its pollution problems with its internal mechanisms. Biostimulation should therefore be applied to fuel the medium, sustain microbial growth, improve biodegradation rate and ultimately restore the polluted media without adverse ecological effect. However, this strategy could be tried on a nontropical soil to compare effectiveness.

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Declaration of Interest

The authors have no competing interest.

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