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Bioremediation and Kinetic Process of Contaminated Soil with Hydrocarbon Using *Bacillus Substilis and Aspergillus Niger*

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ABSTRACT
In this work, the physico-chemical properties of Khana LGA crude oil contaminated soils, are experimentally determined with respect to ASTM standard, as well the crude oil contaminated soils are remediated using Bacillus substilis and Aspergillus Niger. The kinetic parameters of the soils are as well ascertained using a well-developed biochemical model; estimating the concentration of the contaminants at time bases. During the experimental process, crude oil contaminated soil samples (1kg each) were weighed into 7 different bioreactors. 25ml to 75ml of crude oil was added to each bioreactor containing Kpean and Buan non-contaminated soil samples of Ogonilands, and mixed thoroughly as an act of contaminating the soil samples. The set-up was allowed to stand for one week for assimilation. Then 50 ml of microbial broth of 1.0X106 cfu/g loads were added to 6 of the 7 bioreactors, the other left as control. Samples were kept under an incubation period of 56 days at room temperature (25^{o}C – 30^{o}C) during which samples were collected for GC analysis at 7-day intervals to determine the TPH content. The results of the study show that the degradation of the amended samples was high 92.9% for Kpean and 95.9% for Buan soil. The kinetic parameters were 00.0017hr-1 and 0.0016 hr^{-1}, respectively. These results are consistent with previous empirical findings of k= 0.00207 hr^{-1}, and k = 0.0013 hr^{-1}. The results of this study showed that this consortium of microbes is able to effectively degrade petroleum hydrocarbon, hence can be employed for large scale bioremediation process.

KEYWORDS: Contaminated soil, crude oil, Total petroleum hydrocarbons, Bacillus substilis, Aspergillus Niger bacteria, Bio-kinetic modeling, Ogonilands experimental remediation

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1. INTRODUCTION
Sustainability of environments and the world as large greatly relies on the functionality and activeness of soils. It is an important basin of nutrient for agricultural and industrial practices / growth. According to Hou *et al*. (2020), soil plays vital roles on environmental and ecological security. The Niger Delta regions of Nigeria which Rivers state is inclusive, consist of huge natural gas and crude oil deposits that are being supportive as energy source of the country for over 50 years (Rodrignes *et al*., 2018) with different negative incidents recorded over these years, which amongst all is the increasing level of the petroleum hydrocarbon environmental pollutants. These hydrocarbon contaminants are highly effective in destroying environmental and agricultural qualities (Castro *et al*., 2021; Ugi *et al*., 2023; Shahryar, 2017; Li *et al*., 2016; Johnson and Cushing, 2020; Raffa, 2020; Gospoderek *et al*., 2016; Basak *et al*., 2020; United State Environmental Protection Agency, 2004; Poi *et al*., 2018; Podder *et al*., 2019; Rashed & Vazi, 2017). The effects of crude oil contaminants are of anthropogenic origin due to oil spillage from crude transporting pipelines, vandalization, mining, production units or facilities, inappropriate or illegal waste treatment (Bosco *et al*., 2019; Li *et al*., 2016), etc. The Nigeria National Petroleum Corporation states an average of 300 individual spills; which is about 2,300 m^3 occurs yearly (Adelena *et al*., 2011), which has led to drastic

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expansion of complex hydrocarbons generation that are environmentally, economically and health-wise dangerous (Koolivand *et al*., 2019). For these reasons, special interest towards not just the remediation of these hydrocarbon polluted environments but as well on promoting health and ecological safety, has being attempted by various researchers over the years, some even focused on the recovery of the hydrocarbons from the polluted environments to be reused for satisfaction of human daily needs (Ossai *et al*., 2020; Lim *et al*., 2016; Tran *et al*., 2021).

In this work, a remediation process using Bacillus Subtilis and Aspergillus Niger, which are commonly known as grass bacillus or hay bacillus and common or black mold respectively was carried out majorly for the determination of the various kinetic parameters for different soil locations within the Khana Local Government Area in Ogonilands. Bioremediation, be it the In-situ or ex-situ methods / types (Vidali, 2001), is the use of biological organisms such as fungi, bacteria, etc., to improve the soil performance in terms of nutritional value as a remediation exercise. These microbes facilitate the degradation of the toxic hydrocarbon atoms / pollutants by feeding on them spontaneously (Raffa *et al*., 2020). The possibility of remediating the environments using microbes happened to be very effective since hydrocarbons are classified to be biodegradable (Raffa *et al.*, 2020; Calvo *et al*., 2019). This remediating process can either be carried out naturally (natural attenuation) or enhanced by introduction of nutrient (biostimulation) or by introduction of microorganisms (bioaugmentation) (Bidja-Abena *et al*., 2016; Casale *et al*., 2018).

Bacillus Subtilis.

Bacillus Subtilis which is one of the microbes used in this work, is known to be an aerobic gram-positive soil heterogeneous protein producing bacterium (Earl *et al*, 2008), with abilities of secreting multiple enzymes that acts as biological catalyst in substrates degradation.

Aspergillius Niger.

Aspergillus niger which is used with B. subtilis in this work is known to be a filamentous ubiquitous ascomycete fungus, which degrades polysaccharides efficiently in the presence of an extracellular carbohydrate enzymes (deVries *et al*., 2017), yielding sugar which is further converted to energy that serves as biomolecule precursors via variety of metabolic pathways (Khosravi *et al*. 2015).

Vast number of current works have been successfully carried out using microorganisms including Bacillus Subtilis and Aspergillus niger, to reclaim different petroleum contaminated soils all over the world (Odili *et al*., 2020; Obumneme *et al.*, 2022; Ibietala *et al*., 2022; Eze and Orjiakor, 2020; Titah et al., 2021; Nimrat *et al*., 2019; Oyewole *et al.*, 2020) with some studies incorporating the microbial effects studied using kinetic models (Uba *et al*., 2019; Ukpaka, 2005; Ozyurek and Soyuer, 2023) and all come to acceptance based on their results that these microbes are effective in remediating hydrocarbons contaminated soils.

The Ogoniland regrettably over 50 years suffers various crude oil spills related incidents which include massive soil quality degradation that has led to poor agricultural practices and death of valuable wildlife and aquatic species. Different approach has been pronounced to assist the situation of the Ogoniland, such as the United Nations Environmental Programme (UNEP, 2011) plan though not yet accomplished since then. Although literature reviews few works on the soils, but none of these works either addressed the kinetic parameters of Kpean and Buan soils or the predictive models to determining the concentration of these contaminants, hence, the thrust for this study.

The objectives of this work were to:

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i. Determine the physico-chemical properties of the soils.
ii. Ascertain the type of microbes present in the soil using microbiological analysis.
iii. Conduct an experiment using Bacillus subtilis and Aspergillus niger for the bioremediation of the soils.
iv. Apply the kinetic rate equation to determine the kinetic parameters of the soils.
v. Develop predictive models to estimate the total petroleum hydrocarbon content at any time and validate the models with experimental data.

2. MATERIALS AND METHODS

2.1 Materials

The materials that were used in this research work are: Soil samples collected at depths of 20 cm from Kpean and Buan communities in Khana Local Government Area of Rivers State using a shovel, petroleum, a mixed culture of Bacillus Subtilis and Aspergillus Niger, spatula, 2.5 litre buckets, shovel, sieve, sack bags, handheld digital electrical conductivity meter, beakers, conical flaks, pipette, stove, crucible, desiccators, distilled water, Boric acid, measuring cylinders, kjeldahl flask, thermometer, Desktop pH meter, Inductively Coupled Plasma Mass Spectrometer(ICP-MS), electronic mass balance, indicator, test jar, KH_2PO_4, etc.

2.2 Methods

2.2.1 Sample Preparation and Analysis

Soil samples of Kpean and Buan communities of Khana LGA of Rivers State, were measured (1 kg each) into 7 separate bioreactors, and labelled accordingly with vent spaces at the cover tops and sides well plugged with cotton wool to prevent cross contaminations. 25 ml to 75 ml of petroleum was added as contaminant and thoroughly mixed, then allowed for one week for proper assimilation. After then, 50 ml *Aspergillius Niger* and *bacillus substilis* microbial culture of 1.0 x 10^6 cfu/g were added to only 6 of the bioreactors, and the other left as control. The water level of the soils in the bioreactors were maintained by regular addition of distilled water to ensure good moisture. The soil samples were thoroughly mixed two times a week to avoid anaerobic situation. Samples were collected weekly (at 7-day interval) from each bioreactor within the 56 days incubation period to ascertain the Total Petroleum Hydrocarbon (TPH) Content of the soil samples

Plate 1: Soil Sampling and Weighing

2.2.2 Determination of Physico-Chemical Properties

The analysis of the soil samples was done to determine all relevant parameters defined below. The particle size distribution was done before the soil was contaminate with crude oil using hydrometer method taking sodium hexametaphosphate to be the dispenser (Ayotamuno, *et al*., 2011).

2.2.2.1 Determination of TPH

Precisely 5 g of the sieved soil sample under incubation was measured and introduced into a beaker, and then 25 ml of n-hexane was mixed

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with it in the flask, the mixture for a period of 5 minutes was thoroughly stirred. The resulting contents was later filtered and put into a test-tube. Part of the extract (a mixture of oil and n-hexane) was taken put on a cuvette and then determined its absorbance with a spectrophotometer. The resulting calibration curve was gotten through taking the absorbance of the dilute standard solution of crude oil by adding 0.2, 0.5, 0.8, 1.0, 1.5 and 2.0 g of the crude oil with 100 ml of n-hexane solution. Total petroleum hydrocarbon was ascertained through reading the absorbance of the extract from the spectrophotometer at a wavelength of 460 nm.

2.2.2.2 Determination of Potassium

5g of the soil sample was added with 10ml HCl. The sample was then taken into a volumetric flask and make up to mark with sterilized water. A standard KCl solution of varying concentration was prepared. The absorbance level of both the standard solution and sample were read using flame photometer through setting it at the wavelength of potassium.

2.2.2.3 Determination of Soil-pH and Electrical Conductivity (EC) (USDA 2003)

The soil pH levels were determined in the ratio 1:10 (soil to water extract). Five grams (5 g) of each soil was added to 50 ml of distilled water. The bulk of the soil sample was thoroughly stirred to make a homogenous slurry, thereafter a pH meter (Jenway 3015 model) and an EC meter (Jenway 4010 model) probes were connected respectively to the soil sample and made to be stabled at 30^{o}C and the pH value of the soil sample was recorded.

2.2.2.4 Determination of Nitrogen

Five grams (5 g) of the soil sample measured into a conical flask was treated with 20ml H_2SO_4 and then adding 5 g $CuSO_4$ catalyst at a hot plate maximum temperature for some l minutes till a visible solution was gotten. It was then allowed to cool and later diluted using 100ml distilled water. The content was thoroughly stirred using a glass rod and a tube holding the diluted soil sample was linked to the distillation unit and then a conical flask having 25 ml of boric acid was connected to the condenser. 50 ml of NaOH was measured into the conical flask and then a distillation process was done for about 10 minutes. The product (ammonium borate solution) gotten was then titrated against 0.1M HCl to produce a purplish – grey color (end point) with the use of methyl red indicator.

$$\%TN = \frac{(S-B)N(1.4007)}{w} \qquad (3.1)$$

N = normality of acid
S=titre value of soil sample
B=titre value of blank solution

2.2.2.5 Determination of Phosphorus

The phosphorus content was determined with the using vanadate colorimetric method (Gregory, 2005). Five grams (5g) of the sample was added to 20ml of distilled water. About 2ml of the solution of the sample was later measured into 100ml volumetric flask, and then the addition of 2.5ml of vanadate molybdate and thoroughly mixed. It was then increased with addition of distilled water and made to stand for period of 10 minutes. Another one was prepared as a control having 2ml distilled of water and 2.5ml vanadate in 100 ml volumetric flask and increased with distilled water. The absorbance level of the test and that of the control were read at a rate of 540 nm and the result compared with standard potassium dihydrogen phosphate (KH_2PO_4) curve.

$$\% \text{ phosphorus content} = \frac{A \; x \; 10}{W \; x \; V} \qquad (3.2)$$

Where,
A = concentration of dilute sample,
W = volume of originally prepared sample,
V = volume of 100ml of sample solution.

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2.2.2.6 Determination of Moisture Content

The soil sample moisture content was ascertained using drying method with aid of an oven at 110^0C for a period of 1 hour. Five grams (5 g) of soil sample l was measured into a crucible with the aid of an electronic mass balance. The combined weight of both the crucible and soil sample was taken to an oven at a temperature of 110 degrees. After a period of 10 minutes, the crucible with its content removed and reweighed to obtain a new weight for the combination. The heating was maintained until the weight of the soil sample was constant.

The total moisture content was evaluated by the equation:

$$\%Moisture = \frac{W_m - W_d}{W_m} \times 100 \qquad (3.3)$$

W_m = weight of moist soil sample
W_d = weight of dry soil sample

2.3 Model Development

2.3.1 Microbial Kinetics

Modeling microbial degradation rate of contaminants requires proper understanding of how microbes grow in an environment. At sufficient nutrient and favorable condition bacterial cultures generally multiply by binary fusion as indicated below:

$$O \longrightarrow OO \longrightarrow OOOO$$

While the cell growth rate is a differential as:

$$X = X_O e^{\mu t} \qquad (3.4)$$

Where,
X = Cell number or concentration
μ = Specific growth rate
X_O = Cell number when t = 0

The rate of disappearance of substrate can also be stated as:

$$\mu = \frac{1}{X}\frac{ds}{dt} \qquad (3.5)$$

Where,
S = Substrate concentration

For the relationship between substrate concentration and cell growth, we have,

$$\frac{dx}{ds} = -y\frac{ds}{dt} \qquad (3.6)$$

Where,
y = Yield factor

There are many kinetic models used to describe substrate-based growth e.g. logistic-growth and Monod-growth models

2.3.2 Monod Equation

The Monod equation is a mathematical expression describing growth of microbes. Tacques Monod propounded this equation as:

$$\mu = \frac{\mu_{max} S}{K_S + S} \qquad (3.7)$$

Where,
μ_{max} = Maximum specific growth rate
S = The concentration of the limiting substrate for growth
K_S = Half-velocity constant
μ_{max} and K_S are empirical coefficients to the Monod equation. These coefficients vary according to species and also depend on the ambient condition.

2.3.3 Substrate Kinetics

It is commonly observed that the rate of catalysis, which is the number of moles of product produced per second, varies based on the concentration of the substrate. Leonor Michaelis and Maud Leonora Menten propounded an equation called Michaelis-Menten kinetics to express enzymatic dynamics. The model explains how an enzyme brings about kinetics rate enhancement of chemical reactions and also describes how the rate of the reaction depend on the enzyme concentration and substrate.

The general reaction description of an enzyme-catalyzed reaction is:

$$E + S \xrightarrow{K_1} [ES] \xrightarrow{K_2} E + P \qquad (3.8)$$

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The amount of the product (P) produced per unit time is expressed as V, which is rate of catalysis. The ES complex is produced when enzyme (E) attaches itself with the substrate (S). The ES complex is left to either dissociate to produce free enzyme (E) and S or produce a product (P). The rate equation can be gotten in these two ways:

i. Rapid equilibrium approximation method
ii. Steady-state approximation method

2.3.3.1 The Steady-State Approximation

The formation and breakdown rate of the complex are as follows:

i. rate of formation of complex (ES) = K_1 [E] [S] (assuming that PZ = 0)
ii. rate of breakdown of complex (ES) = K_2 [ES] + K_3 [ES] = (K_2 + K_3) [ES]

At steady state:

$$\frac{d[ES]}{dt} = K_1[ES] + K_3[ES] = 0 \qquad (3.9)$$

2.3.3.2 Kinetic of the Bioremediation

The kinetics of the bioremediation process can be described with the model:

$$-r_i = \frac{-dc_i}{dt} = K.C_i^n \qquad (3.10)$$

Where,

C_i =Concentration of component i at time t

K = Reaction rate constant

In this study, the zero (n = 0) and the first order (n = 1).

For zero order

$$-r_i = \frac{-dc_i}{dt} = K \qquad (n = 0) \qquad (3.11)$$

Integrating eqn. (3.11) between t = 0 and t

$$C_t = C_O - Kt \qquad (3.12)$$

At half-life, $C_t = 0.5\ C_0$ and $t = t_{1/2}$

Substituting into Eqn. (3.12) and arranging it

$$\text{Half-life, } t_{1/2} = \frac{C_O}{2.K} \qquad (3.13)$$

Where,

C_t =Concentration at time t

C_O = Initial concentration at time zero

$t_{1/2}$ = Half-life time

For first order, the reaction rate (-r) is defined as

$$-r_i = \frac{-dc_i}{dt} = K.C_i(n = 1) \qquad (3.14)$$

Integrating eqn. (3.37) between $t = 0$ and t

$$C_t = C_O.e^{-kt} \qquad (3.15)$$

At half-life, $C_t = 0.5\ C_0$ and $t = t_{1/2}$

Substituting and arranging in eqn. (3.15), we have

$$\text{Half-life time } t_{1/2} = \frac{\ln 2}{K} = \frac{0.693}{K} \qquad (3.16)$$

3. RESULTS AND DISCUSSION:

3.1 Variation in Total Petroleum Hydrocarbon in Samples

Table 1: Total Petroleum Hydrocarbon Experimental Results

Week	Days	A-Kpean (mg/kg)	A-Buan (mg/kg)	Kpean (Control) (mg/kg)
0	**0**	25,000	25,000	50,000
1	**7**	23750	22750	49625
2	**14**	17252	19752	49150
3	**21**	12251	12003	48932
4	**28**	9250	8007	48825
5	**35**	7872	6754	47877
6	**42**	4356	3502	46985
7	**49**	2825	2361	46310
8	**56**	1770	1033	46304

Using the experimental data displayed in Table 1, the plots of Figure 1 and 2 were obtained. Figure 1 depicts the decline in total petroleum hydrocarbon levels in the contaminated Kpean soil sample, where no microbial treatment was applied. The graph demonstrates a decreasing trend in hydrocarbon concentrations from 50000mg/kg to 46304mg/kg after 56 days of incubation period. This shows poor degradation efficiency where there is no amendment.

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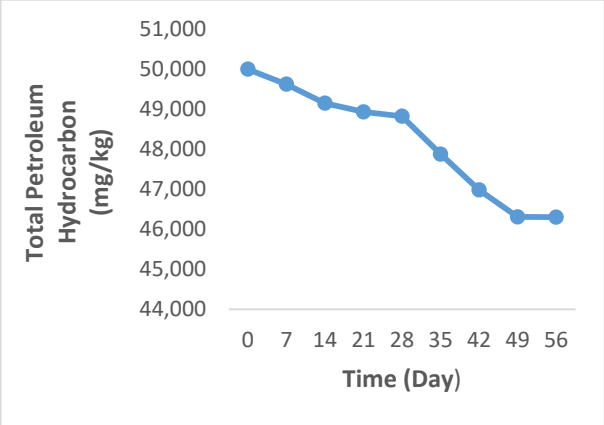

Fig 1: Variation of TPH levels in Control Sample

Figure 2 show the comparison of the degradation that occurred in equal samples of Kpean and Buan soils under the same experimental conditions and treatment. The graph displays a progressive diminishing trend from 1kg of soil with 25ml of crude oil, all with 50g of Aseragillus niger and Bacillus subtilus microbial broth. This trend proves the effectiveness of these organisms in remediating the petroleum hydrocarbon soils with respect to time. It also shows good degradation efficiency with amendment.

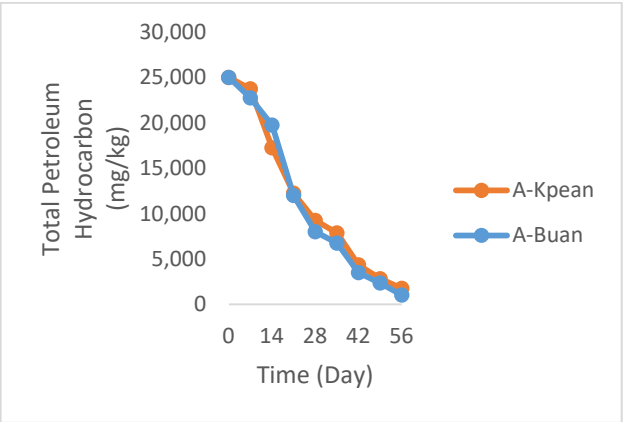

Fig 2: Variation of TPH levels in Samples Kpean and Buan with time

3.2 Variation in Percentage of TPH Degradation in Samples

Table 2: Percentage Total Petroleum Hydrocarbon Degradation

Week	Days	C-Kpean (%)	C-Buan (%)	Kpean (Control) (%)
0	**0**	0.00	0.00	0.00
1	**7**	6.30	3.57	0.75
2	**14**	17.50	30.87	1.70
3	**21**	31.77	58.47	2.14
4	**28**	40.30	65.51	2.35
5	**35**	58.13	78.17	4.25
6	**42**	69.14	83.75	6.03
7	**49**	75.93	87.34	7.38
8	**56**	86.24	91.52	7.39

Using the experimental data displayed in Table 2, the plots of Figure 3 and 4 were obtained. Figure 3 shows the percentage TPH degradation within the control sample. The graph displays an uptrend for degradation with respect to time, however attained a maximum TPH degradation of 7.39% after 56 days which shows poor degradation efficiency without amendment.

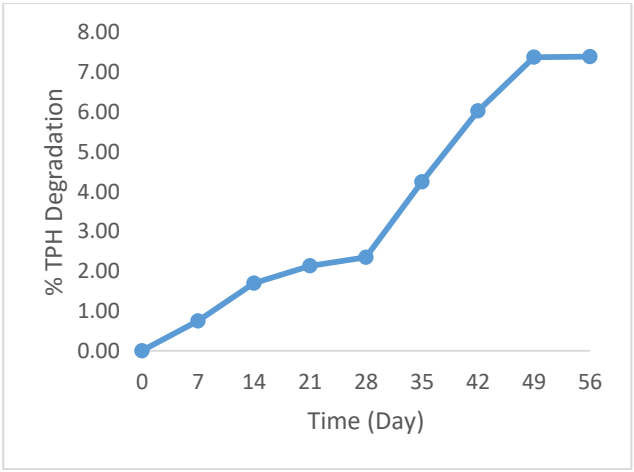

Fig 3: Change in Percentage TPH degradation in Control Sample

Figure 4 illustrate the percentage degradation of the hydrocarbons within the Kpean and Buan

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soil samples. The graph displays an uptrend for degradation with respect to time in the 1 kg samples of Kpean and Buan soil. The trend shows that the maximum TPH degradation attained were 92.92% for Kpean and 85.87%, for Buan samples respectively after 56 days which shows high degradation efficiency with amendment.

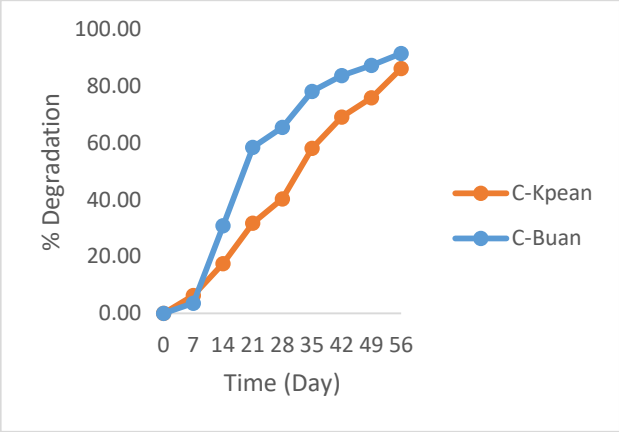

Fig 4: Percentage TPH degradation in Samples Kpean and Buan with time

3.3 Determination of Kinetic Parameters for the Bioremediation Process

Rearranging equation (3.15) gives;

$$\ln C_t = -Kt + \ln C_o \qquad (3.16)$$

Which is used to determine the kinetic parameter "K" that yields the predictive model (3.17);

$$C_{TPH} = C^0_{TPH}\, e^{-kt} \qquad (3.17)$$

3.3.1 Determination of Kinetic Parameter for Control Sample

Table 3: 1kg Soil Samples Contaminated with 25 g of Crude not Amended

Time (Week)	Time (Days)	Time (Hour)	B-Kpean (Control) (mg/kg)	ln (Kpean (Control)
0	**0**	**0**	50,000	10.82
1	**7**	**168**	49625	10.81
2	**14**	**336**	49150	10.80
3	**21**	**504**	48932	10.80
4	**28**	**672**	48825	10.80
5	**35**	**840**	47877	10.78
6	**42**	**1008**	46985	10.76
7	**49**	**1176**	46310	10.74
8	**56**	**1344**	46304	10.74

In Figure 5 is a scattered plot of ln Kpean control sample versus time which gives a straight line graph, showing that the degradation is a first order kinetics with the equation of the line $y = -0.000062x + 10.82$, and the coefficient of determination $R^2 = 0.942$. From the graph, the degradation rate constant K = 0.00006 2 hr^{-1} and the predictive model is $C_{TPH} = C^0_{TPH}\, e^{-0.000062t}$.

Where, C^0_{TPH} = initial TPH content in the soil, C_{TPH} = residual TPH content, t = time in hours. By comparing experimental and developed result model result, at the 56th day the residual TPH content is 46,304mg/kg (experiment) and 46,002mg/kg (model).

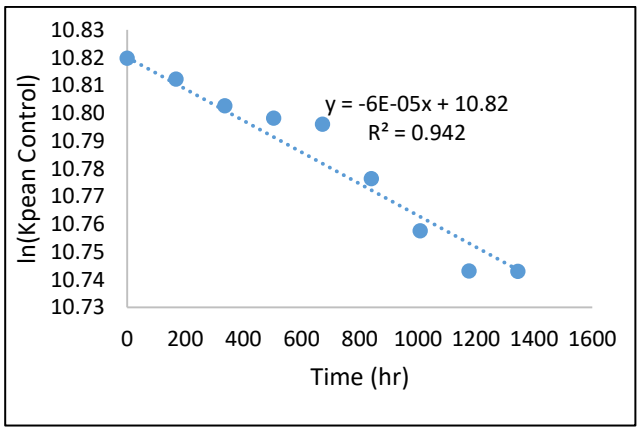

Fig 5: Scattered Plot for Control Sample

3.3.2 Determination of Kinetic Parameter for A-Kpean and A-Buan

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Table 4: 1kg A-Kpean Soil Sample Contaminated with 25 g of Crude

Time (Week)	Time (Days)	Time (Hour)	A-Kpean (mg/kg)	ln(A-Kpean)
0	**0**	**0**	25,000	10.13
1	**7**	**168**	23750	10.08
2	**14**	**336**	17252	9.76
3	**21**	**504**	12251	9.41
4	**28**	**672**	9250	9.13
5	**35**	**840**	7872	8.97
6	**42**	**1008**	4356	8.38
7	**49**	**1176**	2825	7.95
8	**56**	**1344**	1770	7.48

Figure 6 is a scattered plot of ln Kpean versus time, which gives a straight line graph, showing that the degradation is a first order kinetics with the equation of the line y = - 0.0017x + 10.13 and the coefficient of determination $R^2 = 0.9479$. Hence the degradation rate constant K = 0.0017hr-1 and the predictive model is $C_{TPH} = C^0_{TPH}\, e^{-0.0017t}$. By comparing experimental and developed model result, at the 42nd day, the residual TPH content is 4356mg/kg (experiment) and 4520mg/kg (model).

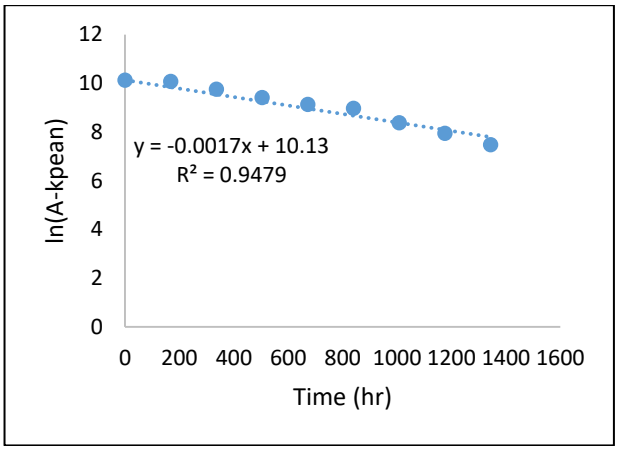

Fig 6: Scattered Plot for Kpean Sample

Table 5: 1kg A-Buan Soil Sample Contaminated with 25 g of Crude

Time (Week)	Time (Days)	Time (Hour)	A-Buan (mg/kg)	ln(A-Buan)
0	**0**	**0**	25,000	10.13
1	**7**	**168**	22750	10.03
2	**14**	**336**	19752	9.89
3	**21**	**504**	12003	9.39
4	**28**	**672**	8007	8.99
5	**35**	**840**	6754	8.82
6	**42**	**1008**	3502	8.16
7	**49**	**1176**	2361	7.77
8	**56**	**1344**	1033	6.94

Figure 7 is a scattered plot of ln Buan versus time, which gives a straight-line graph, showing that the degradation is a first order kinetics with the equation of the line y = - 0.002x + 10.13 and the R^2 value is 0.9232. Therefore, K = 0.002hr^{-1} and the predictive model is $C_{TPH} = C^0_{TPH}\, e^{-0.002t}$. By comparing the experimental and developed model result, at the 49th day, the residual TPH content is 2361mg/kg (experiment) and 2379mg/kg (model).

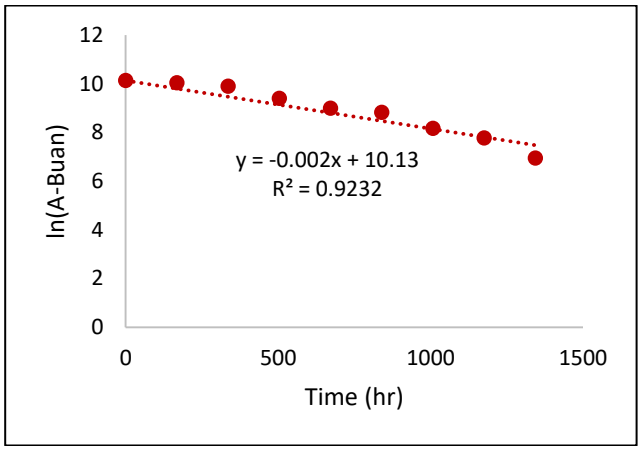

Fig 7: Scattered plot for Buan sample

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4. CONCLUSION

The following conclusions were drawn;

i. The values of the physic-chemical properties of Kpean and Buan soils showed that the quality of the soils were poor as compared with soil test standard.
ii. The polluted soils of same communities contained enough microbes that degrade hydrocarbon, such as pseudomonas A., E.coli, Microscoccus L., Bacillus Substilis, Aspergillus Niger.
iii. Bioremediation was successfully conducted for both Kpean and Buan soils with a consortium of Bacillus Substilis and Aspergillus Niger, and results showed high degradation efficiency with amendment.
iv. The first order kinetic equation was used to plot a graph to deduce the kinetic parameters: k=0.0017hr^{-1} for Kpean and k=0.0016hr^{-1} for Buan soils respectively.
v. The two predictive models developed and validated from this study are $C_{TPH} = C^0_{TPH}\, e^{-0.0017t}$ for Kpean soil and $C_{TPH} = C^0_{TPH}\, e^{-0.0016t}$ for Buan soil.

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